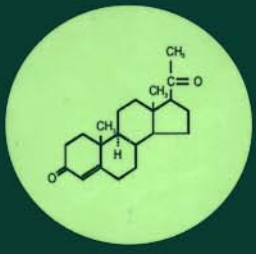
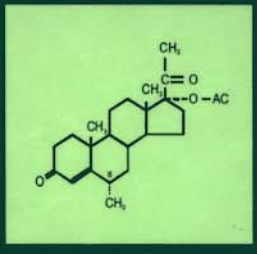
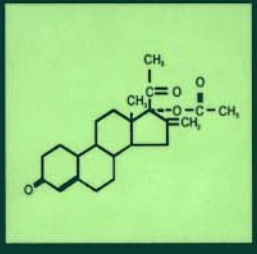
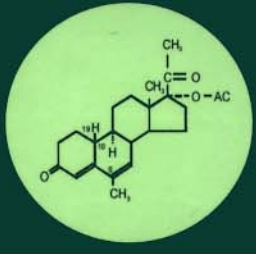
Progestins and Antiprogestins in Clinical Practice









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Foreword

As its name indicates, progesterone (from the Latin *pro gestare*) is an exceptionally important hormone, and when it was isolated in 1934 its discoverers were quite right to give it that name. Since its discovery, research has confirmed that it is indispensable for preparing for, allowing the onset of, and ensuring the continuation of pregnancy in the human species.

Numerous studies have dealt with progesterone's effects at different levels in the organism, particularly at the level of the uterus, where it allows implantation and the maintenance of pregnancy, and at the level of the hypothalamo-pituitary complex, where it regulates the secretion of pituitary hormones controlling both ovulation and the production of progesterone itself in the ovary.

In fact, one of the most important consequences of progesterone's properties is the development of hormonal contraception based on the antiovulatory power of the molecule. By modulating the doses and the type of synthetic steroid, as well as eventual hormone associations, a whole series of contraceptives make use of progesterone's properties. Moreover, its capacity to oppose ovulation having been established, its local properties in the uterus, endometrium, and/or cervix are being addressed with success, in the form of mini-pills, progesterone–intrauterine devices, or urgency contraception. Mastery over contraception is one of the greatest events of the twentieth century and progesterone has been in the front rank of contributors.

However, because all its functions are not yet known, questions still arise. Does ovarian progesterone influence the maturation and functioning of spermatazoa directly, and, if so, does it have a role in fertilization? How does the production of progesterone by glial cells (since progesterone is also a neurosteroid) influence the myelinization of the nervous system and perhaps even the trophicity of neurones? What regulates the production of metabolites of progesterone (such as 3α-hydroxy-5α-pregnan-20-one or allopregnanolone) that influence the activity of the $GABA_A$ receptor and probably the receptors of other neurotransmitters? What is the physiological anticorticosteroidal action of progesterone, which, as is known, binds to the receptors for the gluco- and mineralocorticosteroids?

Progesterone, a member of the family of nuclear receptors, principally acts via its own receptor and several discoveries of general interest have been made when studying it. There are several isoforms of the progesterone receptor: the association of the heat shock protein Hsp90 with steroid receptors was discovered during study of the progesterone receptor, and the regulation of the cell concentration of progesterone receptors was the prototype for showing that endocrinology is the study not only of hormones but also of their receptors; estrogens stimulate the synthesis of the progesterone receptor, which is physiologically important; and progesterone down-regulates its own receptor, which is also fundamental.

The decisive nature of the intervention of progesterone at the beginning of pregnancy led to development of the first antiprogesterone compound, RU486 (mifepristone), which is of considerable importance not only medically but also as a symbol of a true convergence between advanced research in biomedicine and efforts in favor of reproductive health and the feminine condition. The scandalous obstacles set up against the diffusion of this molecule are beginning to diminish. Not only should we soon be able to offer the choice of using RU486 to the women who so decide, throughout the world, but we must also develop the medical uses of this compound (for example, facilitating certain difficult deliveries or the treatment of uterine leiomyomas).

One can see the importance of a book about progesterone analogs, the progestins that allow the easier use of the natural hormone's principles of action, especially by offering oral, injectable, or even local in utero preparations. This book offers chapters that bring us up to date on all these derivatives, and also their antagonists at the receptor level, the antiprogestins. It will be considered important and useful in such varied domains as obstetrics and gynecology, endocrinology, certain aspects of neurology and psychiatry, oncology, and, as knowledge of the effects of agonistics and antagonistics steroids improves, the book will be useful for numerous general practitioners.

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Preface

Progesterone is a unique reproductive hormone that plays an essential role in the female reproductive system. Since the discovery and isolation of progesterone, several new progestins have been synthesized. Progesterone and other steroids with progestagenic properties have widespread applications. During the past several decades, scientists have gained a broad understanding of the mechanisms of action and the clinical application of many progestins. These aspects of progestins are reviewed in this book.

In Part I, Physiological Aspects, the molecular mechanisms of progesterone action are reviewed and the structure of the progesterone receptor addressed. Some distinctive tissue-specific progestational effects are thoroughly reviewed, including effects on the nervous system and breast tissue.

While several actions of progesterone are mediated through the progesterone receptor, this steroid and other progestins can act through other steroid receptors and produce a variety of effects. According to the steroid from which they are derived—either progesterone or testosterone—progestins may inhibit or potentiate the action of androgens, or they may exert some estrogenic, glucocorticoid, or mineralocorticoid effects. According to their interaction with the steroid receptors, different progestins would therefore exert different effects. Since all the progestins are not similar, it would be inappropriate to extrapolate from a single progestin to others as class effects. The major use of the progestins in humans is as hormonal contraceptives, and millions of women have been exposed to a variety of these agents.

Part II, Pharmacology of Synthetic Progestins, reviews the various categories of progestins from the first generation to the so-called third generation of these molecules. Newly synthesized progestins closer to the "ideal" progestational agent are described and could be called "fourth-generation" progestins. Some of them are already available in some countries; several are still under development and will be available to the clinician after the turn of the century.

Part III, Clinical Applications, reviews some of the newer methods of delivering steroids, devised to provide safer alternatives to the oral contraceptive pill. For this purpose, the antigonadotropic properties of progestins are essential.

With the increase in life expectancy, women will live for more than 30 years after the menopause. Hormonal substitution is currently used by a higher percentage of postmenopausal women than during the early 1980s, and this percentage will increase in the next century.

Several progestins have been utilized to oppose the estrogenic effects on the endometrium and new molecules are being developed. Part IV, Metabolic Effects of Progestins, reviews the key aspects of the risks and benefits of progestins in this population of menopausal women. The metabolic effects of progestins are specifically addressed, as well as their relevance and impact on cardiovascular risk.

Part V, Antiprogestins, describes the antiprogestins and their clinical applications. Part VI, Pharmacokinetics, discusses the pharmacokinetics of progesterone administered orally or parenterally.

Several contributors who are very knowledgeable in their field of research have addressed most of the effects of progesterone and its derivatives. According to the actions of several progestins on various tissues, it may be possible to select the most appropriate molecule to be prescribed in a given clinical situation. This volume is intended to clarify several of the aspects of progestins' actions and help the clinician in practicing the art of reproductive medicine.

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Structure of the Progesterone Receptor and Mode of Action of Progesterone

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I. INTRODUCTION

Progesterone is a major component in the regulation of normal female reproductive function. Its effects are mediated by an intracellular receptor, located in the nucleus of target cells. This receptor was discovered almost 30 years ago (1,2). However, its biochemical study was difficult because of its rarity in the cell and its sensitivity to proteolysis. The development of monoclonal antibodies (3,4) and the cloning of its cDNA in different species (5–8) have allowed the elucidation of the structural and functional properties of this receptor protein. After cloning, the progesterone receptor (PR) was further analyzed by transferring it to heterologous systems for large-scale production and in vitro testing. The receptors sequence was dissected by subcloning different regions to ascertain domains responsible for different receptor functions.

The mechanism of progesterone action in target tissues is complex, and a distinction has to be made between the direct targets of progesterone action and indirect consequences of progesterone regulation. For instance, because the effects of progesterone are mediated by PR, which is induced by estradiol in most target tissues, the delineation of specific progesterone effects is unclear. The recent development of a mouse model carrying a null mutation of the PR gene (9,10) has been used to answer many of the complex questions of progesterone action in vivo. Moreover, it has confirmed the importance and diversity of roles of progesterone in normal female development and reproduction.

II. STRUCTURE OF THE PROGESTERONE RECEPTOR

The progesterone receptor is a member of a superfamily of transcription factors, including receptor for steroid hormones, thyroid hormones, retinoic acid, products of intermediary metabolism, as well as other known transcription factors, and proteins of unknown function (orphan receptors). These proteins are characterized by an organization into specific functional domains that are conserved between species and family members (for review see Refs. 11,12). The most highly conserved region is in the center of the protein and corresponds to two "zinc finger" DNA-binding motifs. The carboxy-terminal domain is less conserved and is the ligand-binding domain. This domain behaves similar to a "molecular switch," controlling multiple receptor functions (13). It contains the "activation function 2" (AF-2). In the absence of ligand, it binds the 90-kDa heat-shock protein. After binding the ligand, it mediates the dimerization and the interaction with coactivators, allowing the modulation of transcription. The three-dimensional structure of the ligand-binding domain of different members of the steroid hormone receptor family (retinoic acid, thyroid hormones, estradiol, and progesterone receptors) has been solved recently (13–17). The crystal structure of this domain reveals a fold of an antiparallel α-helical sandwich. In the presence of the ligand, a conformational transition of the amphiphilic α-helix H12, containing the AF-2 domain, is observed. These conformational changes generate the surface that allows transcriptional mediators to bind. The structure of all hormone-bound ligand-binding domains characterized to date shows helix H12, hence, the AF-2 core, in the same position. Antagonists, such as raloxifen or mifepristone (RU 486), show a displacement of the helix H12 from this "active" position, providing structural evidence of the mechanism of antagonism (16,17). The amino-terminal domain of PR contains a ligand-independent activation function (AF-1). Both AF-1 and AF-2 activate transcription in a different promoter- and cell-specific fashion (18). They work in a synergistic manner and are required for full receptor activity in most cellular contexts.

PR has been cloned in different species, including human, rabbit, and chick (5–8,19). The chromosomal gene structure has been elucidated in the human and is a single copy of more than 90 kilobase pairs (kbp) composed of eight exons (20). It has been mapped to chromosome 11q22-23 (21,22).

In the human, two PR proteins, PR-A (85 kDa) and PR-B (115 kDa) have been described (23,24). PR-B differs from PR-A only in that it contains an additional amino-terminal fragment of 164 amino acids (25). Recently, a third activation domain (AF-3), which appears to function only in a restricted cell and promoter context, has been found (26). It is located within this PR-B-specific amino-terminal domain. PR-A and PR-B are encoded by a single gene, under the control of distinct promoters, each of which gives rise to a distinct subgroup of PR mRNA (25). An equimolar expression of PR-A and PR-B is observed

in human breast cancer cells in culture (24). In human breast tumors, the ratio of expression of PR-A and PR-B proteins differs markedly among women (27). The biological importance of these different ratios of PR expression has not been extensively explored, and little is known of their relative modulation in vivo.

III. MOLECULAR MECHANISM OF PROGESTERONE ACTION

In the absence of ligand, PR is in an "inactive" conformation. It forms large complexes with other proteins. The sedimentation coefficient of these complexes is between 8S and 10S. They are composed of a series of proteins of the heat-shock protein (hsp 90, hsp 70, and hsp 56) and the immunophilin families (28). Heat-shock proteins were identified first for their rapid induction after a thermic or chemical cellular stress. They have been proposed to function in normal cellular processes, such as protein folding. The binding of hsp 90 is required to maintain ligand binding by PR at 37°C (29). Immunophilins are characterized by their capacity to bind immunosuppressive agents, such as cyclosporin or tacrolimus (FK 506). They have a peptidylprolyl *cis–trans*-isomerase (PPIase) function. Their role in the inactive PR complex is unknown. The interaction with the ligand elicits a series of events: dissociation from the heat-shock proteins, activation of PR, and high-affinity binding to specific DNA elements (hormone responsive elements; HRE) located in the regulatory region of genes (reviewed in Ref. 30). HRE are short sequences of DNA. They are often present in an array with other elements and may be present in multiple copies. They are generally composed of two similar 6-bp sequences that are oriented head-to-head (palindrom) with a specific number of intervening bases. For the progesterone responsive element (PRE), the consensus sequence is TGTACAnnnTGTTCT. Glucocorticoid, mineralocorticoid, and androgen receptors bind and activate transcription from the same HRE as does PR. All these receptors bind this HRE as homodimers, one monomer binding one-half site. Amino acids involved in the specific binding of the HRE have been characterized. They are located at the basis of the first zinc finger of the DNA-binding domain. They define the P box. This P box is specific for the HRE and allows definition of different subfamilies of receptors. PR belongs to the same family as glucocorticoid, mineralocorticoid, and androgen receptors. Their P box is GSCKV.

This specific interaction of activated receptor with the DNA allows its interaction with the transcription machinery complex, inducing the modulation of transcription of the gene located in the region and, thereby, the modulation of the synthesis of the corresponding protein. The progress in the knowledge of transcriptional control in animal cells during the last 5 years has established a solid

foundation for the reformulation of the mechanisms of transcriptional control by steroid hormone receptors (reviewed in Refs. 31,32). Transcription requires two classes of transcription factors. One class comprises general factors (TFIIA, B, D, E, F, and H) and is responsible for basal promoter activity. The other class comprises modulators that either activate (coactivators; for instance SRC-1) or repress (corepressors; for instance, SMRT, N-CoR) transcription. Cointegrators, such as CBP/p300, have also recently been identified (33). They coordinate the transcriptional effects of simultaneous signals emanating from cell surface receptors and from nuclear receptors. Thus, they act as cointegrators of multiple competing, and perhaps conflicting, signals that can affect one promoter. The basal level of transcription of most genes appears to be maintained by histone deacetylation. Acetylation of chromatin in vivo is coupled with transcription, and specific histone acetyl transferases target histones bound to DNA and overcome the inhibitory effect of chromatin on gene expression. There is an equilibrium between histone acetylation and histone deacetylation toward the progressive accumulation of nucleosomes containing acetylated histones. The cointegrator CBP/p300, as well as the coactivator SRC-1, possess intrinsic histone acetylase activity (34,35). Furthermore, CBP/p300 recruits promoters' histone acetylases, for instance P/CAF (36). N-CoR and SMRT recruit histone deacetylases (37). Besides these transcription factors, proteins modifying the structural organization of the chromatin (for instance, proteins of the SWI/SNF family) regulate transcription.

Steroid hormone receptors act at all these levels. A direct interaction has been demonstrated in vitro between steroid hormone receptors and general transcription factors such as TFIIB or TFIID (31). Steroid hormone receptors recruit some coactivators in the presence of ligand (38–41). Moreover, a cooperation between certain steroid hormone receptors and proteins of the SWI/SNF family has been identified (42).

The current model for steroid hormone action is as follows: The binding of the ligand induces a sequence of events, i.e., positioning of the activated dimers on the HRE, modification of the position of the helix H12, interaction with different coactivators and cointegrators, recruitment to the promoter of histone acetylases, and activation of the transcription of the corresponding gene.

IV. REGULATION OF PROGESTERONE RECEPTOR EXPRESSION

The progesterone receptor is under the dual control of estradiol and progesterone, which act sequentially to regulate the cellular concentration of PR and, thereby, the likely cellular responsiveness to progestins. Estradiol increases PR transcriptionally in most target tissues. Conversely, progesterone down-regulates

PR transcriptionally and posttranscriptionally. This down-regulation of PR by progesterone is not observed in all cell types. In the uterus, it is observed only in epithelial cells and not in stromal and myometrial cells. Moreover, PR levels do not decrease in the normal breast between the follicular and luteal phases.

The main regulatory site in the rabbit PR gene is an intragenic estradiol responsive element (ERE) overlapping the initiation of translation (43). The same ERE confers progesterone down-regulation. PR does not interact directly with this ERE, but through an interaction with the estradiol receptor (ER; 43). In the rat PR, four imperfect ERE have been identified by in vitro techniques (44). One of these ERE appears to be biologically active (45). In human PR, the two PR isoforms are under the control of different promoters, both of which are estrogen inducible. No classic ERE was detected in the corresponding sequence (25).

In addition to sex steroids, other regulators of PR gene expression have been described. Retinoic acid decreases the transcription of PR gene. Growth factors and phorbol esters have been reported to decrease or increase PR mRNA concentration in a cell-specific manner. In the rabbit, PR repression by retinoic acid and AP1 is mediated by the same ERE as repression by progesterone. It is achieved by protein–protein interaction and not by a direct contact with the ERE (46). In the rat PR, induction by thyroid hormones is mediated by the same ERE as estradiol induction (45).

Progestin regulation of PR level is likely to vary among cell types, between species, and more importantly, in different physiological situations to regulate cellular sensitivity to progestins.

V. INTRACELLULAR TRAFFIC OF THE PROGESTERONE RECEPTOR

Steroid hormone receptors are intracellular proteins that may be found in both the cytosolic and nuclear fractions of a tissue homogenate. Until 1984–1986, the classic model for action of steroid hormones proposed that a cytoplasmic receptor protein undergoes nuclear translocation after interaction with its ligand (47,48). In 1984–1986, the development of monoclonal antibodies against steroid receptors (3,4,49) allowed immunocytochemical studies to be undertaken. This technology avoids the problem of the receptor redistribution during cell homogenization. Immunocytochemistry showed exclusive nuclear localization of ER (50) and PR (51), even in the absence of endogenous hormone. The nuclear localization of PR and ER have been demonstrated in a wide number of target tissues and in different species.

The nuclear localization of large proteins, such as steroid hormone receptors, is an active process: it is temperature-dependent, requires GTP, and displays

saturation kinetics. Active transport into the nucleus requires that proteins contain suitable nuclear localization signals (NLS). They are mostly short, basic sequence motifs, rich in arginines and lysines. Active nuclear import of proteins takes place in at least two steps. The first step is the interaction between the protein and the nuclear pore complex through the NLS. This step involves the binding to different cytosolic proteins. The second step is the translocation to the nucleus. Only this second step is an active process. It involves a small G protein, ran.

The cloning of receptors and in vitro mutagenesis studies led to the description of NLS for steroid hormone receptors (52–55).

The NLS of PR is a complex signal. It is formed of two subsignals, constitutive and hormone-dependent. It encompasses the hinge region between the DNA and the ligand-binding domains (containing the constitutive signal) and the second zinc finger of the DNA-binding region (containing the hormone-dependent signal). It bears several stretches of basic amino acids. All steroid hormone receptor NLSs are complex. If these receptors are aligned through their DNA-binding regions, sequences homologous with the PR constitutive NLS are found. These sequences are always implicated in the nuclear localization of these receptors.

There is indirect evidence that the nuclear localization of PR is the result of an equilibrium between the nucleus and the cytoplasm. The first indication is the observation of an in vivo interaction between receptor monomers during nuclear transport (53). Further evidence is the result of energy-depletion experiments. In these conditions, an efflux of the receptor from the nucleus is observed (56). The existence of a nucleocytoplasmic shuttling of the receptor was directly confirmed by the study of the migration of PR between nuclei in interspecies heterokaryons (56).

Thus, the residency of PR in the nucleus is a dynamic phenomenon, resulting from the continuous active transport into the nucleus, conterbalanced by diffusion into the cytoplasm. Similar experiments have been performed on ligand-bound glucocorticoid receptor (GR) (57) and on ER (58), showing the same results.

This mechanism of nuclear localization of receptors explains some previous observations: ligand-free PR or ER, which reside in the nucleus, are found in the cytosol after homogenization, even when nuclear structures have been preserved. Under these conditions, active transport is blocked by dilution and low temperature, and the exit of receptors from the nucleus to the cytoplasm is not counterbalanced by active entry into the nucleus. Association of receptors with nuclei after homogenization of cells at 25°C has been described (59). Moreover, localization of ligand-free GR in cytoplasm, or in both cytoplasm and nuclei, has been considered as completely different from that of ER or PR, which are located in the nucleus. However, if the receptor continually shuttles between

nucleus and cytoplasm, GR may be only quantitatively and not qualitatively different from sex steroid receptors. Less effective or masked constitutive NLS would lead to an increased time of residency of the receptor in the cytoplasm and to an apparent distribution between cytoplasmic and nuclear compartments.

The understanding of receptor function may also be modified by the fact that receptors shuttle between nucleus and cytoplasm. Receptors could thus interact with cytoplasmic components. Moreover, this mechanism is compatible with receptors exerting biological activities in the cytoplasm.

The mechanism by which proteins enter the nucleus is well understood, but the outward movement of nuclear proteins involved in the shuttling process is not. In the same type of energy-depletion and heterokaryon experiments, NLSs are also involved in the outward movement of PR through the nuclear envelope (60).

VI. MECHANISMS OF ANTIPROGESTINS ACTION

There is a great interest in the study of antiprogestins for two main reasons. First, they have a great therapeutic potential, such as interruption of early pregnancy, postcoital contraception, induction of labor, and treatment of hormone-dependent tumors. Second, antiprogestins have also been used as potent tools to investigate the molecular mechanisms of progesterone's action.

After binding to the receptor, antihormones can act at all the steps of hormone action, including activation, dimerization, or binding of the dimers to HREs. For antiestrogens, two types of antihormones have been described (61,62): type I that allows the binding of the activated receptor to the HRE; and type II (or pure) antiestrogens that prevent this binding. For antiprogestins, this type of classification has also been proposed. Mifepristone (RU 486) is the only antiprogestin currently used therapeutically. Its molecular mechanism of action on the PR has been largely studied by in vitro and in vivo experiments that have resulted in a quasi-consensus, i.e., it activates PR, it induces its in vivo oligomerization (53,63) and mifepristone–wild-type PR complexes do bind in vivo to HREs (64,65). Thus it acts at a distal step of hormone action (failure of transactivation by mifepristone–receptor complexes bound to HREs). Moreover, its agonistic effect has been described in certain cell and promoter configurations (65). This attests to the capacity for mifepristone–PR complexes to binding to HREs. After limited protease digestion of in vitro synthetized PR in the absence or presence of ligands, progesterone and mifepristone induced distinct conformational changes within the receptor protein. The use of specific monoclonal antibodies showed that these conformational changes occur at the extreme carboxy-terminus of the receptor (66). The 42 amino acids located in the carboxy-terminal region of PR are required for the receptor to bind pro-

gesterone (67). However, a mutant deleted of these 42 amino acids still binds mifepristone and fully activates transcription in its presence (67). Thus, the conformation of the receptor bound to the HRE is influenced by the nature of the ligand.

Recently, a different antiprogestin molecule, onapristone (ZK 98299) has been characterized. Studies of ZK 98299–PR complexes failed to detect in vitro binding to HRE (68). This has led to the suggestion that onapristone may be an example of a second class of antiprogestins that act by preventing the formation of DNA–PR complexes. In in vivo experiments it activates PR, and it induces its oligomerization (63,69). The results of in vivo competition with a constitutive PR for the binding to a PRE have shown that ZK 98299–PR complexes do bind to the PRE. In these experiments ZK 98299 differs only quantitatively and not qualitatively from RU 486, the latter being tenfold more potent than ZK 98299. This is correlated with their difference in affinity for the receptor, measured in vivo (69). However, the results of in vivo genomic footprinting experiments are completely the opposite, in that neither ZK 98299–PR complexes nor RU 486–PR complexes bind PRE (70). Proteolytic fragment analysis studies show that onapristone induces a fragment pattern intermediate between that induced by promegesterone (R 5020) and by mifepristone (71). Thus, it seems that, contrary to antiestrogens, it is difficult to identify two clear types of antiprogestins.

REFERENCES

1. YJ Topper, CS Freeman. Multiple hormone interactions in the developmental biology of the mammary gland. Physiol Rev 60:1049–1106, 1980.
2. CL Clarke, RL Sutherland. Progestin regulation of cellular proliferation. Endocr Rev 11:266–301, 1990.
3. F Logeat, MT Vu Hai, A Fournier, P Legrain, G Buttin, E Milgrom. Monoclonal antibodies to rabbit progesterone receptor: crossreaction with other mammalian progesterone receptors. Proc Natl Acad Sci USA 80:6456–6459, 1983.
4. WP Sullivan, TG Beito, J Proper, CJ Krco, DO Toft. Preparation of monoclonal antibodies to the avian progesterone receptor. Endocrinology 119:1549–1557, 1986.
5. H Loosfelt, M Atger, M Misrahi, A Guiochon-Mantel, C Meriel, F Logeat, R Benarous, E Milgrom. Cloning and sequence analysis of rabbit progesterone-receptor complementary DNA. Proc Natl Acad Sci USA 83:9045–9049, 1986.
6. OM Conneely, XP Sullivan, DO Toft, M Birnbaumer, RG Cook, BL Maxwell, T Zaruki-Schulz, GL Greene, WT Schrader, BW O'Malley. Molecular cloning of the chicken progesterone receptor. Science 233:767–770, 1986.
7. JM Jeltsch, Z Krozowski, C Quirin-Stricker, H Gronemeyer, RJ Simpson, JM Granier, A Krust, F Jacob, P Chambon. Cloning of the chicken progesterone receptor. Proc Natl Acad Sci USA 83:5424–5428, 1986.

8. M Misrahi, M Atger, L d'Auriol, H Loosfelt, C Meriel, F Fridlansky, A Guiochon-Mantel, F Galibert, E Milgrom. Complete amino acid sequence of the human progesterone receptor deduced from cloned cDNA. Biochem Biophys Res Commun 143:740–748, 1987.
9. JP Lydon, FJ De Mayo, CR Funk, SK Mani, AR Hughes, CA Montgomery, G Shyamala, OM Conneely, BW O'Malley. Mice lacking progesterone receptor exhibit pleiotropic reproductive abnormalities. Genes Dev 9:2266–2278, 1995.
10. JP Lydon, FJ De Mayo, OM Conneely, BW O'Malley. Reproductive phenotypes of the progesterone receptor null mutant mouse. J Steroid Biochem Mol Biol 56:67–77, 1996.
11. H Gronemeyer, V Laudet. Transcription factors. 3. Nuclear receptors. Rev Prot Profile 2:1173–1308, 1995.
12. MA Carson-Jurica, WT Schrader, BW O'Malley. Steroid receptor family: structure and functions. Endocr Rev 11:201–220, 1990.
13. W Bourguet, M Ruff, P Chambon, H Gronemeyer, D Moras. Crystal structure of the ligand-binding domain of the human nuclear receptor RXR-alpha. Nature 375:377–382, 1995.
14. JP Renaud, N Rochel, M Ruff, V Vivat, P Chambon, H Gronemeyer, D Moras. Crystal structure ot the RAR-γ ligand binding domain bound to all-*trans* retinoic acid. Nature 378:681–689, 1995.
15. RL Wagner, JW Apriletti, ME McGrath, BL West, JD Baxter, RL Fletterick. A structural role for hormone in the thyroid hormone receptor. Nature 378:690–697, 1995.
16. AM Bzozowski, ACW Pike, Z Dauter, RE Hubbard, T Bonn, O Engstrom, L Ohman, GL Greene, JA Gustafsson, M Carlquist. Molecular basis of agonism and antagonism in the oestrogen receptor. Nature 389:753–758, 1997.
17. DM Tanenbaum, Y Wang, SP Williams, PB Sigler. Crystallographic comparison of the estrogen and progesterone receptor's ligand binding domains. Proc Natl Acad Sci USA 95:5998–6003, 1998.
18. MT Bocquel, V Kumar, C Stricker, P Chambon, H Gronemeyer. The contribution of the N- and C-terminal regions of steroid receptors to activation of transcription is both receptor and cell-specific. Nucleic Acids Res 17:2581–2595, 1989.
19. H Gronemeyer, B Turcotte, C Quirin-Stricker, MT Bocquel, ME Meyer, Z Krozowski, JM Jeltsch, T Lerouge, JM Garnier, P Chambon. The chicken progesterone receptor: sequence, expression and functional analysis. EMBO J 6:3985–3994, 1987.
20. M Misrahi, PY Venencie, P Saugier-Veber, S Sar, P Dessen, E Milgrom. Structure of the human progesterone receptor gene. Biochim Biophys Acta 1216:289–292, 1993.
21. MF Rousseau-Merck, M Misrahi, H Loosfelt, E Milgrom, R Berger. Localization of the human progesterone receptor gene to chromosome 11q22-11q23. Hum Genet 77:280–282, 1987.
22. ML Law, FT Kao, Q Wei, JA Hartz, GL Greene, T Zaruki-Schulz, OM Conneely, C Jones, TT Puck, BW O'Malley, KB Horwitz. The progesterone receptor gene maps to human chromosome band 11q13, the site of the mammary oncogene *int*-2. Proc Natl Acad Sci USA 84:2877–2881, 1987.

23. BA Lessey, PS Alexander, KB Horwitz. The subunit structure of human breast cancer progesterone receptors: characterization by chromatography and photoaffinity labeling. Endocrinology 112:1267–1274, 1983.
24. KB Horwitz, PS Alexander. In situ photolinked nuclear progesterone receptors of human breast cancer cells: subunit molecular weights after transformation and translocation. Endocrinology 113:2195–2201, 1983.
25. P Kastner, A Krust, B Turcotte, U Stropp, L Tora, H Gronemeyer, P Chambon. Two distinct estrogen-regulated promoters generate transcripts encoding the two functionally different human progesterone receptor forms A and B. EMBO J 9:1603–1614, 1990.
26. CA Sartorius, MY Melville, AR Hovland, L Tung, GS Takimoto, KB Horwitz. A third transactivation function (AF3) of human progesterone receptors located in the unique N-terminal segment of the B-isoform. Mol Endocrinol 8:1347–1360, 1994.
27. JD Graham, C Yeates, RL Balleine, SS Harvey, JS Milliken, AM Bilous, CL Clarke. Progesterone receptor A and B protein expression in human breast cancer. J Steroid Biochem Mol Biol 56:93–98, 1996.
28. DF Smith, DO Toft. Steroid receptors and their associated proteins. Mol Endocrinol 7:4–11, 1993.
29. DF Smith. Dynamics of heat shock protein 90–progesterone receptor binding and the disactivation loop model for steroid receptor complexes. Mol Endocrinol 7:1418–1429, 1993.
30. M Truss, M Beato. Steroid hormone receptors: interaction with deoxyribonucleic acid and transcription factors. Endocr Rev 14:459–479, 1993.
31. M Beato, A Sanchez-Pacheco. Interaction of steroid hormone receptors with the transcription initiation complex. Endocr Rev 17:587–609, 1996.
32. KB Horwitz, TA Jackson, DL Bain, JK Richer, GS Takimoto, L Tung. Nuclear receptor coactivators and corepressors. Mol Endocrinol 10:1167–1177, 1996.
33. Y Kamei, L Xu, T Heinzel, J Torchia, R Kurokawa, B Gloss, SC Lin, RA Heyman, DW Rose, CK Glass, MG Rosenfeld. A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors. Cell 85:403–414, 1996.
34. AJ Bannister, T Kouzarides. The CBP co-activators is a histone acetyltransferase. Nature 384:641–643, 1996.
35. TE Spencer, G Jenster, MM Burcin, CD Allis, J Zhou, CA Mizzen, NJ McKenna, SA Onate, SY Tsai, MJ Tsai, BW O'Malley. Steroid receptor coactivator-1 is a histone acetyltransferase. Nature 389:194–198, 1997.
36. XY Yang, VV Ogrysko, J Nishikawa, BH Howard, YA Nakatani. A p300/CBP-associated factor that competes with the adenoviral oncoprotein E1A. Nature 382:319–324, 1996.
37. MJ Pazin, JT Kadonaga. What's up and down with histone deacylation and transcription? Cell 89:325–328, 1997.
38. V Cavaillès, S Dauvois, PS Danielian, MG Parker. Interaction of proteins with transcriptionally active estrogen receptors. Proc Natl Acad Sci USA 91:10009–10013, 1994.

39. B Le Douarin, C Zechel, JM Garnier, Y Lutz, L Tora, B Pierrat, D Heery, H Gronemeyer, P Chambon, R Losson. The N-terminal part of TIF1, a putative mediator of the ligand-dependent activation function (AF-2) of nuclear receptors, is fused to B-*raf* in the oncogenic protein T18. EMBO J 14:2020–2033, 1995.
40. S Halachmi, E Marden, G Martin, H MacKay, C Abbondanza, M Brown. Estrogen receptor-associated proteins: possible mediators of hormone-induced transcription. Science 264:1455–1458, 1994.
41. SA Onate, SY Tsai, MJ Tsai, BW O'Malley. Sequence and characterization of a coactivator for the steroid hormone receptor superfamily. Science 270:1354–1357, 1995.
42. C Muchardt, M Yaniv. A human homologue of *Saccharomyces cerevisiae SNF2/SWI2* and *Drosophila brm* genes potentiates transcriptional activation by the glucocorticoid receptor. EMBO J 12:4279–4290, 1993.
43. JF Savouret, A Bailly, M Misrahi, C Rauch, G Redeuilh, A Chauchereau, E Milgrom. Characterization of the hormone responsive element involved in the regulation of the progesterone receptor gene. EMBO J 10:1875–1883, 1991.
44. WL Kraus, MM Montano, S Katzenellenbogen. Identification of multiple, widely spaced estrogen-responsive regions in the rat progesterone receptor gene. Mol Endocrinol 8:952–969, 1994.
45. REM Scott, XS Wu-Peng, PM Yen, WW Chin, DW Pfaff. Interactions of estrogen and thyroid hormone receptors on a progesterone receptor estrogen response element (ERE) sequence: a comparison with the vitellogenin A2 consensus ERE. Mol Endocrinol 11:1581–1592, 1997.
46. JF Savouret, M Rauch, G Redeuilh, S Sar, A Chauchereau, K Woodruff, MG Parker, E Milgrom. Interplay between estrogens, progestins, retinoic acid and AP-1 on a single regulatory site in the progesterone receptor gene. J Biol Chem 269:28955–28962, 1994.
47. T Jensen, T Suzuki, T Kawashima, WE Stumpf, PW Jungblut, ER De Sombre. A two-step mechanism for the interaction of oestradiol with the rat uterus. Proc Natl Acad Sci USA 59:632–638, 1968.
48. J Gorski, D Toft, G Shyamala, D Smith, A Notides. Hormone receptors: studies on the interaction of estrogen with the uterus. Rec Prog Horm Res 24:45–81, 1968.
49. GL Greene, FW Fitch, EV Jensen. Monoclonal antibodies to estrophilin: probes for the study of estrogen receptors. Proc Natl Acad Sci USA. 77:157–161, 1980.
50. WJ King, GL Greene. Monoclonal antibodies localize oestrogen receptor in the nuclei of target cells. Nature 30:7745–7747, 1984.
51. M Perrot-Applanat, F Logeat, MT Groyer-Picard, E Milgrom. Immunocytochemical study of mammalian progesterone receptor using monoclonal antibodies. Endocrinology 116:1473–1484, 1985.
52. D Picard, KR Yamamoto. Two signals mediate hormone-dependent nuclear localization of the glucocorticoid receptor. EMBO J 6:3333–3340, 1987.
53. A Guiochon-Mantel, H Loosfelt, P Lescop, S Sar, M Atger, M Perrot-Applanat, E Milgrom. Mechanisms of nuclear localization of the progesterone receptor: evidence for interaction between monomers. Cell 57:1147–1154, 1989.

54. T Ylikomi, MT Bocquel, M Berry, H Gronemeyer, P Chambon. Cooperation of proto-signals for nuclear accumulation of estrogen and progesterone receptors. EMBO J 11:3681–3694, 1992.
55. D Picard, V Kumar, P Chambon, KR Yamamoto. Signal transduction by steroid hormones: nuclear localization is differentially regulated in estrogen and glucocorticoid receptors. Cell Reg 1:291–299, 1990.
56. A Guiochon-Mantel, P Lescop, S Christin-Maitre, H Loosfelt, M Perrot-Applanat, E Milgrom. Nucleocytoplasmic shuttling of the progesterone receptor. EMBO J 10:3851–3859, 1991.
57. AP Madan, DB De Franco. Bidirectional transport of glucocorticoid receptors across the nuclear envelope. Proc Natl Acad Sci USA 90:3588–3592, 1993.
58. S Dauvois, R White, MG Parker. The antiestrogen ICI182780 disrupts estrogen receptor nucleocytoplasmic shuttling. J Cell Sci 106:1377–1388, 1993.
59. AM Molinari, N Medici, I Armetta, V Nigro, B Montcharmont, GA Puca. Particulate nature of the unoccupied uterine estrogen receptor. Biochem Biophys Res Commun 128:634–642, 1985.
60. A Guiochon-Mantel, K Delabre, P Lescop, E Milgrom. Nuclear localization signals also mediate the outward movement of proteins from the nucleus. Proc Natl Acad Sci USA 91:7179–7183, 1994.
61. JC Reese, BS Katzenellenbogen. Differential DNA binding abilities of estrogen receptor occupied with two classes of antiestrogens: studies using human estrogen receptor overexpressed in mammalian cells. Nucleic Acids Res 19:6595–6602, 1991.
62. H Gronemeyer, B Benhamou, M Berry, MT Bocquel, D Gofflo, T Garcia, T Lerouge, D Metzger, ME Meyer, L Tora, A Vergezac, P Chambon. Mechanisms of antihormone action. J Steroid Biochem Mol Biol 41:217–221, 1992.
63. MT Bocquel, J Ji, T Ylikomi, B Benhamou, A Vergezac, P Chambon, H Gronemeyer. Type II antagonists impair the DNA binding of steroid hormone receptors. J Steroid Biochem Mol Biol 45:205–215, 1993.
64. A Guiochon-Mantel, H Loosfelt, T Ragot, A Bailly, M Atger, M Misrahi, M Perricaudet, E Milgrom. Receptors bound to antiprogestin form abortive complexes with hormone responsive elements. Nature 336:695–698, 1988.
65. ME Meyer, A Pornon, J Jingwei, MT Bocquel, P Chambon, H Gronemeyer. Agonistic and antagonist activities or RU 486 on the functions of the human progesterone receptor. EMBO J 12:3923–3932, 1990.
66. GF Allan, SY Tsai, MJ Tsai, BW O'Malley. Ligand-dependent conformational changes in the progesterone receptor are necessary for events that follow DNA binding. Proc Natl Acad Sci USA 89:11750–11754, 1992.
67. E Vegeto, GF Allan, WT Schrader, MJ Tsai, DP McDonnell, BW O'Malley. The mechanism of RU 486 antagonism is dependent on the conformation of the carboxy-terminal tail of the human progesterone receptor. Cell 69:703–713, 1992.
68. L Klein-Hitpass, ACB Cato, D Henderson, GU Ryffel. Two types of antiprogestins identified by their differential action in transcriptionally active extracts from T47D cells. Nucleic Acids Res 19:1227–1234, 1991.

69. K Delabre, A Guiochon-Mantel, E Milgrom. In vivo evidence against the existence of antiprogestins disrupting receptor binding to DNA. Proc Natl Acad Sci USA 90:4421–4425, 1993.
70. M Truss, J Bartsch, M Beato. Antiprogestins prevent progesterone receptor binding to hormone responsive elements in vivo. Proc Natl Acad Sci USA 91:11333–11337, 1994.
71. GF Allan, E Lombardi, D Haynes-Johnson, S Palmer, M Kiddoe, P Kraft, C Campen, P Rybczynski, DW Combs, A Philips. Induction of a novel conformation in the progesterone receptor by ZK299 involves a defined region of the carboxyl-terminal tail. Mol Endocrinol 10:1206–1213, 1996.

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Effects of Progesterone and Related Steroids in the Brain

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I. INTRODUCTION

Progesterone, its precursors, and its metabolites, which are present in the brain, originate from the gonads and adrenal glands, or are produced locally and, therefore, are named *neurosteroids*. They exert their actions on target cells by two distinct mechanisms: they increase the transcription of specific genes after binding to intracellular progesterone receptors, or they act directly on the cellular membrane. Their effects on the brain are diverse, ranging from the participation in the neuroendocrine control of reproduction and sexual behavior at the hypothalamic and pituitary levels, to the regulation of neurotrophicity and the modulation of behavior, including anxiety, stress, sleep, and memory.

II. ORIGIN OF BRAIN PROGESTERONE AND RELATED STEROIDS

A. Peripheral Sources and Brain Synthesis

Progesterone (PROG; 4-pregnene-3,20-dione), which is present in the brain, can be synthesized by the steroidogenic glands (gonads, placenta, and adrenal glands) and then penetrate into the brain, or it can be produced in the central nervous system.

In men, plasma PROG, mainly originating from the adrenals, is nearly undetectable (except in stress situations), whereas its concentration reaches 2–3 ng/ml in rodent males. In human and rodent males, the level of circulating pregnenolone (PREG; 3β-hydroxy-5-pregnen-20-one), the immediate precursor of PROG synthesis, is about 1 ng/ml. In female rodents during the follicular phase, circulating concentrations of PROG and PREG are similar to those of males. After ovulation, PROG is secreted by the corpus luteum and can be partly converted into 5α-pregnane-3,20-dione (5α-DH PROG) and 3α-hydroxy-5α-pregnan-20-one (3α,5α-TH PROG) in the ovary. During pregnancy, placental PROG, as well as 3α,5α-TH PROG (and PREG), reach very high blood concentrations. These steroids easily cross the blood–brain barrier.

The central nervous system also has its own source of steroids: indeed PREG, PROG, and 3α,5α-TH PROG belong to the neurosteroids, which can be synthesized from cholesterol, in the central and peripheral nervous system (1,2). Several years elapsed between the discovery of PREG in the brain of the rat (3) and many mammalian species, including the human, and the conclusive demonstration of its local biosynthesis.

The PROG precursors, PREG and its sulfate derivative (PREGS), are present at higher concentrations in the brain than in blood. Moreover, they remain in the brain for up to 1 month after gonadectomy and adrenalectomy. PREG can be formed by oligodendrocyte mitochondria incubated with [^{3}H]cholesterol (4), or by mixed glial cell cultures incubated with the sterol precursor [^{3}H]mevalonate (5). The side-chain cleavage enzyme cytochrome P-450scc is responsible for the first step of steroidogenesis, the conversion of cholesterol to PREG (Fig. 1). PREGS presence was demonstrated in brain homogenates and in cultured oligodendrocytes or type-1 astrocytes (but not neurons), using immunohistochemical techniques (6), in situ hybridization (7), and reverse transcription–polymerase chain reaction (RT-PCR) (8).

An alternative pathway for PREG synthesis has also been described (9). Rat C6 glioma cells were observed to produce high concentrations of PREG under $FeSO_4$ treatment, by the fragmentation of a hydroperoxide metabolite of cholesterol, even in the presence of a P-450scc inhibitor. Whether this pathway plays a physiological role in the brain remains unclear.

PREG can be converted to PROG by the 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase (3β-HSD) in mixed rat glial cell cultures (5) and cultures of type 1 astrocytes (10), but, apparently, not in embryonic neuronal cultures (11). Whether pure oligodendrocytes have the capability to synthesize PROG from PREG remains to be established. However, the presence of 3β-HSD mRNAs in all the cell types (7), including adult rat oligodendrocytes (N. Gago, personal communication) was shown by PCR experiments. The four isoforms of the enzyme are expressed in the brain. The type I isoform is predominant and is detected mainly in the olfactory bulbs, cerebellum, hippocampus, hypothalamus, cortex, and striatum (12).

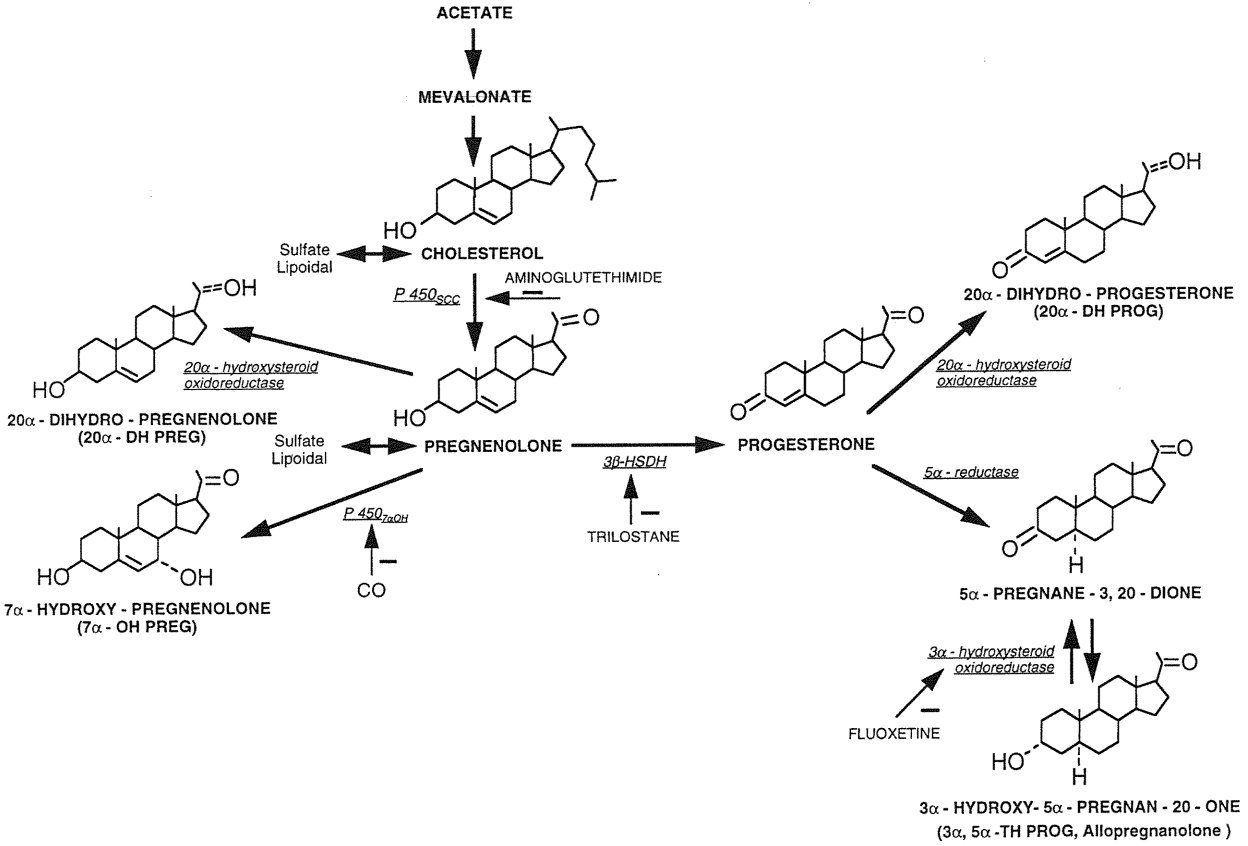

Figure 1 Biosynthesis of pregnenolone, progesterone, and their metabolites in the rat brain. Enzymes are underlined. *P450scc:* cytochrome P-450 cholesterol side chain cleavage. *3β-HSD:* 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4-isomerase. Inhibitors of enzyme activities are in capitals.

PROG is further metabolized to 5α-DH PROG by the type-1 5α-reductase, the most abundant isoform expressed in the brain (13,14). 5α-DH PROG, in turn, can be converted into 3β,5α-tetrahydroprogesterone (3β,5α-TH PROG; 3β-hydroxy-5α-pregnan-20-one) or 3α,5α-TH PROG (also named allopregnanolone), which is known as an allosteric modulator of the γ-aminobutyric acid type A ($GABA_A$) receptor. The biosynthesis of allopregnanolone is catalyzed by the 3α-hydroxysteroid oxidoreductase (3α-HSOR), which is present in the brain in at least two forms (cytosolic NADPH- and particulate NADH-dependent) (15). Both isoforms are widely and similarly distributed, with a predominance in the olfactory bulbs and cerebellum (16). 3β,5α-TH PROG is formed by a 3β-HSOR, especially in neuronal cultures (17). These three enzymatic activities are present in mixed glial cell cultures (5), in astrocyte cultures (11), in pure cultures of newborn and adult oligodendrocytes [N. Gago, personal communication, 1998], and in neurons (17).

Other reduced metabolites of unknown biological significance can be formed from PREG or PROG, namely 7α-OH PREG, 20α-dihydropregnenolone (20α-DH PREG) and 20α-dihydro-progesterone (20α-DH PROG) (10,17) (see Fig. 1). PREG is also found as sulfate and fatty acid esters. The formation of sulfate esters in the nervous system, although very likely, has not been completely demonstrated. Except in fetal brain, where they are relatively high, only low hydroxysteroid sulfotransferase activities are detected in rat brain homogenates (18). Therefore, these results are surprising, given the relatively high concentrations of PREGS (19 ± 6 ng/g tissue) in male as well as female rat brains (19) and the very low permeability of the blood–brain barrier to steroid sulfates.

In summary, PROG and related steroids present in the brain, are either synthesized locally from cholesterol, or are formed from in situ metabolism of bloodborne precursors, or synthesized by adrenals, placenta, or gonads, and transported into the central nervous system. The interaction between these steroids from different origins may be complex and difficult to elucidate in some situations such as stress, menstrual cycle, and pregnancy.

B. Regulation of Brain Steroidogenesis

The physiological stimuli that regulate neurosteroid formation are still unclear. Whether the steroidogenic factor-1 (SF-1), which regulates the transcription of the P-450scc gene in steroidogenic glands and which is also present in mouse embryonic forebrain (20), plays a role in neurosteroidogenesis during early development is unknown. However, this factor does not seem absolutely necessary, because C6 glioma cells, which convert cholesterol to PREG, do not express SF-1. Two other factors, steroidogenesis-stimulating protein (STP) and steroidogenic acute regulatory protein (StAR) stimulate PREG and PROG formation in

Sertoli cells and adrenal cortex, respectively (21,22), but until now, the brain expression and the role of these proteins in neurosteroid synthesis have not been determined.

Second-messenger systems influence the synthesis of neurosteroids. Cyclic-AMP (cAMP) increases the formation of [^{3}H]PREG from [^{3}H]mevalonolactone in rat glial cells cultures (4) and adult rat cortical minces (23). In C6 glioma cells, the β-adrenergic receptor agonist isoproterenol stimulates PREG formation, and its effect can be blocked by the Rp isomer of adenosine-3′,5′-cyclic monophosphorothioate (Rp-cAMPS), a competitive inhibitor of protein kinase A type-I and type-II (24). cAMP not only increases the activity of the cytochrome P-450scc, leading to PREG synthesis, but also stimulates the 5α-reductase, which converts PROG into 5α-DH PROG. On the contrary, the phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate (TPA) does not influence the 5α-reductase, suggesting that protein kinase A, but not protein kinase C, might be involved in the control of 5α-reductase activity (25).

The increase in PREG synthesis by activation of the peripheral-type benzodiazepine receptor (PBR) has been particularly well documented in glial cells (26). The rate of PREG formation depends on the rate of cholesterol transport from intracellular stores to the inner mitochondrial membrane. PBR may form a channel and increase the transfer of cholesterol to the inner mitochondrial membrane (27), leading to the activation of P-450scc and PREG synthesis. In glial cell cultures (28), rat brain mitochondria preparations (29), and in vivo experiments on rats (30), PBR agonists stimulate PREG synthesis.

Fluoxetine, the serotonin reuptake inhibitor, which is currently used in the treatment of depression, was recently reported to enhance brain neurosteroid content in adrenalectomized and castrated rats. In fact, PREG, PROG, and 5α-DH PROG levels fail to change, whereas fluoxetine increases 3α,5α-TH PROG accumulation in several brain structures, including the cortex, striatum, hippocampus, and olfactory bulbs. Paroxetine, another serotonin reuptake inhibitor is less potent in increasing 3α,5α-TH PROG levels in the brain (31). The conversion of 5α-DH PROG to 3α,5α-TH PROG, which is catalyzed by the same enzyme, is blocked by indometacin in vitro and in vivo (32).

An inhibition of central GABAergic transmission by isoniazid increases the concentration of PREG and PROG in rat brain and plasma, suggesting that GABA may exert a tonic inhibitory action on the synthesis and release of these neuroactive steroids in the central nervous system (33). Stress rapidly increases 3α,5α-TH PROG in the rat cerebral cortex, even after adrenalectomy (34).

A role for autocrine factors is suggested by experiments using primary cultures of astrocytes seeded at different densities. Indeed, a preferential conversion of PREG to PROG occurs at low cell density, whereas the 3β-HSD activity is inhibited in dense cultures, where only 7α-OH-PREG can be detected (10). The synthesis of neurosteroids in glial cells is also influenced by neurons. Thus,

the 5α-reductase activity is stimulated by the addition of neuron-conditioned medium to astrocyte cultures (35).

Genetically determined interindividual differences in the capacity to produce neurosteroids may also be important and may contribute to the variations in individual responses to stressors. Indeed, two psychogenetically selected Swiss rat sublines, which differ in emotional reactivity and anxiety, were analyzed for 5α-reduced neurosteroid levels in different brain regions, and the concentrations of 5α-DH PROG and 3α,5α-TH PROG were significantly higher in the frontal cortex of rats with low emotional reactivity (36).

III. MECHANISMS OF ACTION OF PROGESTERONE IN THE BRAIN

A. Genomic Effects

Classically, PROG binds to a selective intracellular receptor (PR). Thereafter, the activated receptor interacts with a specific progesterone-response element (PRE), which is generally located within the promoter region of target genes (37). Receptor activation, leading to a transcriptionally active form, seems to involve the dissociation of heat-shock proteins from the PR, phosphorylations, allosteric changes, and dimerization (which is needed for binding to DNA) (38) (Fig. 2[**1–3**]). The mechanisms by which the PR-DNA complex initiates mRNA synthesis are still much debated (39). In particular, the interaction of the activated receptor with other transcription factors (see Fig. 2[**4**]) and the mechanisms by which these complexes activate RNA polymerase II are poorly understood.

1. PR Ontogenesis and Distribution

The progesterone receptor appears in the rat brain of both sexes close to birth, increases during development in a region-specific manner, despite a low level of progesterone in blood, with a marked peak formation of the cerebral cortical receptors at postnatal days 7–10 (especially in females), whereas the PR level remains stable in the hypothalamus–preoptic area (40). PR exists in two forms: a larger molecular form B, and a smaller one, form A. PCR analysis of the levels of mRNA for the two forms show that the B form mRNA appears before the A form (at about birth and days 8–12, respectively) in the cortex as well as the hypothalamus. In each structure, the ontogenic patterns of the B form mRNA and of the PR protein are similar. In adult female rat brain, the two mRNA isoforms are expressed in the hypothalamus–preoptic area, pituitary gland, cerebral cortex, amygdala, and cerebellum. In the two latter structures, the A form is predominant (41).

2. Regulation of PR Concentration

The PR levels are differentially regulated according to age, sex, and brain structure. For example, hypothalamic PR concentrations are enhanced by estradiol in the adult male and female rabbit (42), enhanced by estradiol and lowered by PROG in the newborn mouse (43), whereas in the cerebral cortex of the two species, PR is not modulated by estradiol. Experiments in which the 5′-flanking regions of the two rat PR forms were linked to a reporter gene and transfected into MCF-7 breast cancer cells have suggested that form A, but not form B, may be inducible by estradiol (44). This observation may explain why, in rats as in mice, PR is induced by estradiol in the hypothalamus, where form A is predominant, but not in the cortex, where form B is the most abundant (45). In the hypothalamus of the chick embryo, the regulation is more complex. The early basal level of expression of PR is independent of both estradiol and progesterone, but above that constitutive basal level, PR is controlled by the two hormones: up-regulated by estradiol and down-regulated by progesterone (46). Likely by its conversion to estradiol, testosterone (but not dihydrotestosterone) increases the number of PR immunoreactive neurons in the hypothalamus of the chick embryo (47). Thyroid hormones also play an important role in the regulation of PR, particularly during development. When female rats are treated close to delivery with propylthiouracil, which induces a neonatal hypothyroidism, the levels of both forms of PR mRNAs and the concentration of cytosolic PR are markedly decreased in the cerebral cortex of the newborn rats compared with the control group, with no change in the levels of PR mRNAs in the hypothalamus–preoptic area (48,49).

3. Cellular Distribution

The cellular distribution of the receptor has been studied in the brain, using autoradiography and immunohistochemistry. PR is detected in neurons of the hypothalamus, cortex, amygdala, and cerebellum. In the hypothalamus, PR is absent from the neurons that secrete gonadotropin-releasing hormone (GnRH) (50), whereas surrounding dopamine, neuropeptide Y, catecholamines, galanin, GABA, opioid, tachykinins, and glutamate-containing neurons express them (51). As a consequence, PROG may not control reproduction at the hypothalamic level directly on GnRH neurons, but may modulate the release of neurotransmitters that, in turn, may regulate GnRH neurons.

Localization of PR is not restricted to neurons: indeed, PR is also detected in mixed glial cell cultures from male and female newborn rats. Indirect immunofluorescent staining of PR is observed mainly in oligodendrocytes (principally those prepared from female rats), but astrocytes (from females only) are also stained. In mixed glial cell cultures of both male or female origin, the binding of the specific PR ligand [^{3}H]Organon 2058 is greatly enhanced by

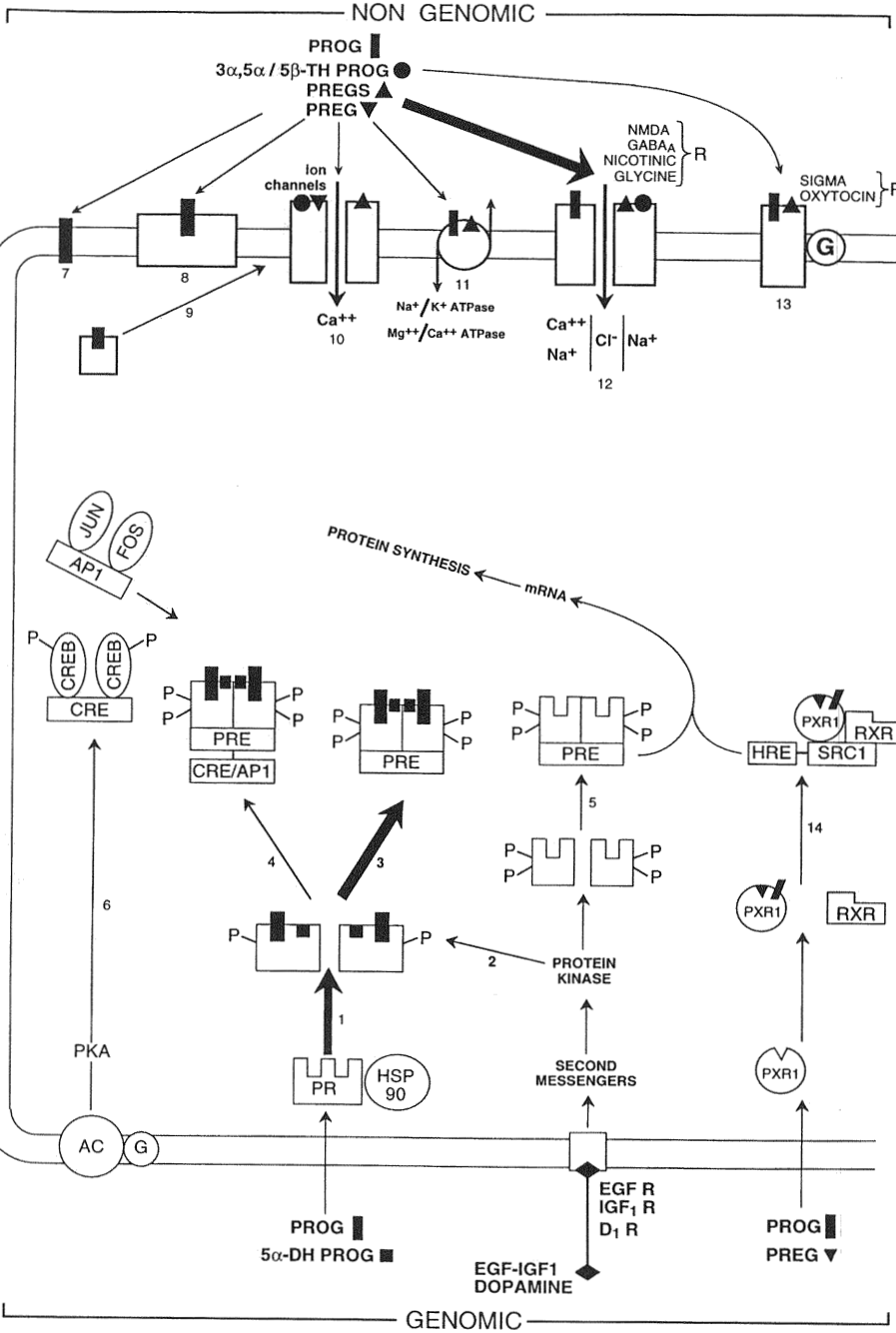
NON GENOMIC
PROG
3α,5α / 5β-TH PROG
PREGS
PREG
NMDA
GABA$_A$
NICOTINIC
GLYCINE
R
SIGMA
OXYTOCIN
R
ion channels
7
8
9
10
Ca++
11
Na+/K+ ATPase
Mg++/Ca++ ATPase
Ca++
Na+
Cl-
Na+
12
13
G
JUN
FOS
AP1
PROTEIN SYNTHESIS
mRNA
CREB
CREB
CRE
PRE
CRE/AP1
PRE
PRE
PRE
HRE
SRC1
PXR1
RXR
5
14
4
3
6
2
PROTEIN KINASE
1
PKA
PR
HSP 90
SECOND MESSENGERS
PXR1
RXR
PXR1
AC
G
PROG
5α-DH PROG
EGF R
IGF$_1$ R
D$_1$ R
EGF-IGF1
DOPAMINE
PROG
PREG
GENOMIC

Figure 2 Genomic and nongenomic actions of PROG and related steroids in the brain: *Genomic actions:* PROG or 5α-DH PROG binding to specific intracellular receptors (PR) leads to their activation, involving the dissociation of heat-shock proteins (HSP90) [**1**] and phosphorylation (P) [**2**]. The activated receptors then bind as dimers to specific response elements (PRE) of target genes [**3**]. The transcriptional activity of PR can be modulated by other transcription factors, such as c-Jun/c-Fos binding to AP-1 sites and cAMP-response DNA-binding proteins (CREB) binding to cAMP response elements (CRE) [**4**]. The activation of membrane receptors for neurotransmitters (dopamine) or growth factors (EGF, IGF-1) can also cause ligand-independent activation of PR, most likely by their phosphorylation [**5**]. By activating second-messenger systems, such as the cAMP-dependent signaling cascade, membrane actions of steroids may produce long-term changes in gene expression [**6**]. PROG and PREG may also bind to the pregnane X receptor type 1 (PXR1), which forms a heterodimer with a 9-*cis*-retinoic acid receptor (RXR). Both PXR1 and RXR interact with the activator protein 1 (SRC-1) [**14**]. Whether this receptor exists and plays a role in the brain remains uncertain. *Nongenomic actions:* Alternatively, PROG may intercalate into phospholipid bilayers [**7**] or bind to specific membrane receptors [**8**]. Intracellular PR may also act on the cellular membrane [**9**], but such a mechanism remains to be demonstrated. PROG and related steroids may modulate ion channels [**10**], ionic ATPase enzymes [**11**], or interact with ligand-gated ion channels ($GABA_A$ receptor, NMDA receptor, nicotinic acetylcholine receptor, glycine receptor) or G protein-coupled receptors for neurotransmitters (sigma receptor, oxytocin receptor) [**12**],[**13**]. PROG, (▮): 5α-DH PROG, (■); PREGS, (▲); PREG, (▼); 3α,5α/5β-TH PROG, (●).

estradiol. In pure cultures of astrocytes, PR immunoreactivity is weak, restricted to female cell cultures, and insensitive to estradiol (52). When studying the expression of steroid hormone receptors in human astrocytic neoplasms, Carroll et al. (53) showed that PR mRNAs are present in high-grade tumors, whereas androgen and glucocorticoid receptor mRNAs are constantly present, and estrogen receptor mRNAs are always absent.

4. *What Are the Target Genes of PROG in the Brain?*

Studies of changes in mRNA levels by RT-PCR, in situ hybridization and RNase protection assay have allowed the identification of PROG-regulated gene networks and have provided a large amount of information concerning the widespread actions of PROG within the central nervous system. PROG influences the activity of neuronal and glial genes, but the underlying mechanisms are unknown, and it is unsure whether the steroid influences the expression of these genes directly. For example, Weiland and Orchinik (54) described a regulation of $GABA_A$ receptor subunit mRNAs by PROG in the female rat hippocampus. Indeed, PROG suppressed α1-, slightly decreased α2-, largely increased γ2-, and had no effect on β1- and β2-subunit mRNA levels. Another example

of regulation of $GABA_A$ subunit expression by PROG is given by the "progesterone withdrawal syndrome," which corresponds to an increased seizure susceptibility and an insensitivity to benzodiazepine sedatives. This syndrome was very recently shown to be associated with increases in α4-subunit levels of $GABA_A$ receptors in pyramidal neurons from the rat CA1 hippocampus (55). The authors clearly demonstrated that reduced levels of 3α,5α-TH PROG, following PROG withdrawal, enhanced the transcription of the gene encoding the α4-subunit.

In vivo experiments performed in the ewe show a dramatic increase in the number of pituitary GnRH receptor mRNAs during luteolysis, when mean concentrations of PROG in the serum have decreased and before the increase of estradiol (6). In estrogen-primed rats, PROG upregulates the expression of medial preoptic μ-opioid receptors (57). In estrogen-primed monkeys, PROG decreases the expression of tyrosine hydroxylase mRNA in the ventral arcuate dopaminergic neurons (58).

Only very few observations have documented a direct effect of PROG on target genes. PROG increases mineralocorticoid receptor (MR) mRNA levels in the hippocampus in vivo and in vitro in cultures of hippocampal neurons. This stimulatory effect of PROG consists in a twofold increase in activity of the MR promoter transiently transfected into neuroblastoma and pituitary cells (59). Another example of a PROG-sensitive gene is the ovine follicle-stimulating hormone-β (FSH-β) gene, which contains six PRE-like elements in its 5′-flanking region. Band-shift experiments allowed discernment that each of the PREs can bind the human PR and enhance the expression of a reporter gene (60).

5. *Protein–Protein Interactions*

In fact, the action of steroid hormones on gene regulation is not a simple "one ligand–one receptor–one DNA response element" relation. PR and GR bind to identical response elements on the preproenkephalin gene, suggesting important competitive interactions in controlling the transcription of this gene (61). Interactions of estrogen receptor (ER) and thyroid hormone receptor (TR) have been described on an estrogen response element sequence identified in the PR proximal promoter. Because ER, TR, and PR are all present in hypothalamic neurons, these findings may be significant for endocrine integration and important for reproductive behavior (62).

DNA–protein interactions are further complicated by possible protein–protein interactions. For instance between PR and transcription factors such as AP-1 (homodimers and heterodimers of c-*jun* and c-*fos*), which mutually influence their activities (63) (Fig. 2[**4**]). A physiological example of such cross-modulation is given by observations of the uterine endometrial epithelium during

the cycle in which AP-1-dependent matrix metalloprotease secretion is down-regulated by PROG. The regulation is probably mediated through AP-1 (64). Interactions between PROG and AP-1 transcription factors also play an important role in the nervous system, in which long-lasting changes in neuronal functions can be caused by AP-1 signaling through the transcriptional regulation of downstream target genes (65). On the rat PR gene, interactions between the estrogen receptor and AP-1-binding proteins could underlie competitions and synergies between steroids and neurally signaled events (61).

B. Nongenomic Actions

The genomic mechanism of action of progesterone may explain some, but not all, its effects in the central nervous system. Indeed, some effects of PROG are too rapid to involve the PR. In electrophysiological studies, Smith et al. reported that locally applied PROG, but also its metabolites 5α-DH PROG and 3α,5α-TH PROG (which has no affinity for intracellular PR), altered neuronal responsiveness in the cerebellum (66). They observed that GABA-induced inhibition of Purkinje cell responses were markedly enhanced, whereas excitatory responses to glutamate were attenuated within less than 3 min after initiation of steroid application. In both cases, recovery to control levels of response was obtained 6–9 min after termination of pressure application of the steroid. The same results were obtained after systemic injection of PROG (67,66).

Finally, all the effects of PROG cannot be explained by a genomic mechanism. Moreover such a mechanism can neither account for the action of precursors or metabolites of PROG, which have no affinity for the PR. The rapid effects of neurosteroids suggest a membrane mechanism of action. How do PROG, its precursors (PREG and PREGS), and its metabolites (5α-DH PROG and 3α,5α or 5β-TH PROG) exert their effects at the membrane level?

1. Neurosteroids and Membrane Fluidity

It has been proposed that PROG, because of its lipophilic properties, may alter the fluidity of the cellular membrane by intercalating into phospholipid bilayers (see Fig. 2[**7**]). Progestagens and other steroids have indeed been shown to modify the viscosity of synaptic membranes (68), and changes in membrane fluidity have been reported to affect the activity of several neurotransmitter receptors and membrane-bound enzymes (69). However, this mechanism of action does not account for the stereospecificity of steroid membrane effects.

2. Specific Membrane Receptors

In radioactive hormone-binding experiments, specific membrane receptors (see Fig. 2[**8**]) have been sought with much difficulty, precisely because of the lipo-

philic structure of PROG and its nonspecific association with the cell membrane. Binding sites for PROG in synaptic membranes were first described by Towle and Sze (70). Then, it was shown that radioiodinated bovine serum albumin conjugated to PROG (PROG-BSA), which supposedly does not enter cells, can bind to a 40- to 50-kDA protein of synaptosomal preparations (71). In plasma membrane fractions from the hypothalamus–preoptic area, two binding sites for PROG were detected: a high-affinity–binding site with a K_d of 14 ± 1.3 nM and a B_{max} of 61.5 ± 2.7 pmol/mg protein, which is up-regulated by long-term PROG treatment, and a low-affinity binding site coupled to a cholera toxin-sensitive G protein (72) (see Fig. 2[**12**]). More recently, a cDNA encoding a PROG-binding protein of 194 amino acids, with a transmembrane segment, was sequenced from porcine vascular smooth muscle cells, and the corresponding mRNA was also detected by Northern blot analysis in the cerebellum (73).

3. *Possible Action of Intracellular PR at the Membrane Level*

Progesterone may exert nongenomic effects on neurotransmission not only by acting on membrane sites, but also by binding to cognate intracellular receptors (see Fig. 2[**9**]). Such a mechanism is suggested by the immunocytochemical localization of PR in dendrites and axon terminals. In these parts of the neuron, far from the nucleus, the receptors would not be expected to act as transcription factors, but instead, could influence neurotransmission by acting locally, possibly after association with the synaptic membrane (74,75). Such a mechanism remains to be demonstrated, however.

4. *Neurosteroids, Ion Channels, and Ionic ATPase*

Specific actions of neurosteroids on ion transport across the neuronal plasma membrane have been described (see Fig. 2[**10**]). In hippocampal CA1 neurons, Ffrench Mullen et al. (76) observed an inhibition by PREG, PREGS, and $3\alpha,5\beta$-TH PROG (but not PROG) of both the N- and L-type calcium channel currents, mediated by a pertussis toxin-sensitive G protein mechanism, associated with the activation of protein kinase C. PROG still has no effect on calcium channels of hypothalamic neurons from the ventromedial nucleus; however, the synthetic progestin megestrol acetate inhibits some high-threshold Ca^{2+} channel currents: not the N-type nor the P-type Ca^{2+} channel currents, but the residual current. Appetite enhancement induced by megestrol acetate might be partly due to the inhibition of these Ca^{2+} channel currents, and the attenuation of the firing of ventromedial nucleus neurons, involved in satiety mechanisms (77).

Neurosteroids can also interact with ionic ATPase enzymes (see Fig. 2[**11**]). For example, PREGS administered to rats decreases basal and calmodulin-stimulated Mg^{2+},Ca^{2+}-ATPase activity in synaptosomal membranes from the cortex, whereas it increases calmodulin-evoked stimulation of Mg^{2+},Ca^{2+}-

ATPase in cerebellar synaptosomes (78). Effects of neurosteroids on membrane ATPase are not restricted to neurons: indeed, PROG is able to inhibit the Na^+,K^+-ATPase pump in astrocyte cultures (79).

5. Interaction with Receptors for Neuromediators

A major breakthrough was the discovery that PROG, its precursors, and metabolites, interacted with membrane receptors for neurotransmitters (see Fig. 2[**12**], [**13**]). A now classic example is the modulation of $GABA_A$ receptors by the PROG metabolite, 3α,5α-TH PROG. This neurosteroid facilitates GABA action at nanomolar concentrations and opens the chloride channel at micromolar concentrations (in the absence of GABA). It potentiates both benzodiazepine and GABA binding, and inhibits the binding of the convulsant *t*-butylcyclophosphorothionate (TBPS) (80–82). The steroid interaction sites seem distinct from those of barbiturates and benzodiazepines. Transfection studies show that the effects of 3α,5α-TH PROG depends on the $GABA_A$ receptor subunit composition. Electrophysiological studies performed on embryonic kidney cells (HEK 293), transfected with various subunit combinations, demonstrate that the 3α,5α-TH PROG site is probably situated on the β-subunit, because steroid actions are still preserved using homo-oligomeric β1-subunit receptors (83). The β-subunit subtype does not seem to influence the response. For the α-subunit, the results vary according to the cells used (HEK 293 or oocytes), the origin of the subunits (mouse, human, or bovine) and the $GABA_A$ receptor function tested (currents, binding, or chloride flux; 84,85). In particular, discord exists concerning the role of α6-subunit. However, very recent experiments reveal the importance of the α4-subunit. Indeed a reduction in brain 3α,5α-TH PROG levels enhances transcription of the gene encoding the α4-subunit (55). In fact, the action of the synthetic analogue of 3α,5α-TH PROG, ganaxolone, which is under clinical investigation for treatment of epilepsy, does not seem to be influenced by the α-subunit subtype (86). The role of the γ-subunit was studied in electrophysiological experiments, which show a much greater effect of 3α,5α-TH PROG on the $\alpha1\beta1\gamma1$-subunit combination than on receptors composed of $\alpha1\beta1\gamma2$L- or $\alpha1\beta1\gamma3$-subunits (87). This observation has potential physiological importance because glial cells, which synthesize neurosteroids, possess $GABA_A$ receptors (88) and are known to express the γ1-subunit. Finally, the presence of the δ-subunit or the new ε-subunit seems to have no importance or to be inhibitory, depending on the type of effect (modulatory or mimetic) of the steroid (89,90). Other neurosteroids also enhance $GABA_A$ receptor function; namely, the sulfate esters of 3α,5α-TH PROG and 3α,5β-TH PROG (91). These interactions between neurosteroids and $GABA_A$ receptors may explain the anesthetic, hypnotic, and anxiolytic effects of some progestagens and make 3α,5α-TH PROG one of the most potent modulators of neuronal activity.

On the other hand, neurosteroids, such as PREGS and other 3β-hydroxysteroid sulfate esters (i.e., 3β,5α-TH PROG sulfate), are excitatory neurosteroids because, for example, at low micromolar concentrations, they decrease $GABA_A$ receptor-mediated $^{36}Cl^-$ uptake into synaptoneurosomes (91). PREGS enhances the binding of [^{3}H]muscimol to synaptosomal membranes from the rat cerebellum, cortex, hippocampus, and thalamus, but inhibits [^{3}H]muscimol binding to synaptosomes from the hypothalamus (92). PREGS slightly potentiates benzodiazepine binding, and inhibits the binding of [^{35}S]TBPS (93). PREGS antagonizes electrophysiological responses to GABA in cerebral cortical neurons of neonatal rats (94). Therefore, distinct sites for neurosteroids, mediating distinct allosteric modes of interaction, seem to exist on the $GABA_A$ receptor.

Neurosteroids can interact with the glycine receptors. Glycine represents the major inhibitory neurotransmitter in the spinal cord. In primary cultures of chick spinal cord neurons, PROG surprisingly inhibits glycine currents, whereas it produces an opposite effect on $GABA_A$ currents (probably by its conversion to 3α,5α-TH PROG). PREGS is a negative modulator of both $GABA_A$ and glycine receptors, and 3α,5α-TH PROG is inactive on the glycine receptor (95).

Neurosteroids also directly modulate excitatory glutamate receptor function. PREGS potentiates *N*-methyl-D-aspartate (NMDA)-mediated response and slightly inhibits the currents induced by kainate and α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) in cultures of spinal cord neurons from chick embryos. PREGS potentiation of the NMDA response is unchanged in the presence of maximal glycine, probably because PREGS does not act through the glycine modulatory site on the NMDA receptor (96). The interaction of PREGS with the NMDA receptor may partly explain the memory-enhancing effects of PREGS (97). On the contrary, some neurosteroids are negative modulators of glutamate receptors: 3α,5β-TH PROG sulfate inhibits the NMDA and non-NMDA receptor-mediated responses, whereas the free steroid is ineffective (98). Finally PREGS may exert excitatory effects by inhibition of $GABA_A$ receptors or stimulation of NMDA receptors, whereas 3α,5β-TH PROG sulfate may depress brain excitability by positive modulation of the $GABA_A$ receptor or inhibition of NMDA receptor activity.

However, NMDA receptor-mediated actions of PREGS may be indirect and opposite the direct ones. Indeed, Monnet et al. (99) observed that PREGS inhibits the release of [^{3}H]norepinephrine evoked by NMDA in rat hippocampal slices. This effect is indirect through sigma (σ) receptors, for the σ-antagonist haloperidol, inactive by itself, completely prevents the inhibitory effect of PREGS. Similarly, PROG enhances [^{3}H]dopamine release stimulated by NMDA in rat striatal slices (100), probably through a direct interaction with the NMDA receptor; whereas, similar to PREGS, it inhibits [^{3}H]norepinephrine release evoked by NMDA in hippocampal slices (99) and suppresses dehydroepiandro-

sterone-induced potentiation of the CA_3 pyramidal neuron response to NMDA (101), in both cases by $G_{i/o}$-coupled σ1-receptors.

Interactions of neurosteroids with other receptors for neuromediators have been described. For example, PROG inhibits the function of brain nicotinic acetylcholine receptors in a voltage-independent manner (102). PROG and 3α,5α-TH PROG inhibit nicotine-induced dopamine release and $^{86}Rb^{+}$ efflux in mouse striatal and thalamic synaptosomes, suggesting an inhibitory effect of these steroids on $\alpha 4\beta 2$- and $\alpha 3$-containing nicotinic receptors, respectively (103). In vitro experiments performed on hypothalamic and pituitary membranes showed an inhibitory effect of PROG on the binding of ligands to muscarinic receptors. PROG increased the K_d without altering the number of binding sites. This effect was rapid and reversible (104).

Very recently, Grazzini et al. (105) showed that PROG can directly interact with the oxytocin receptors from the rat parturient uterus. They also showed that PROG bound to recombinant rat oxytocin receptors expressed in CHO cells, and suppressed oxytocin-induced inositol phosphate production and calcium mobilization. Interestingly, in CHO cells transfected with the human oxytocin receptor, oxytocin binding and oxytocin-evoked inositol phosphate production were unaffected by PROG, but were altered by its metabolite 5β-DH PROG. The direct interaction of PROG (and 5β-DH PROG) with G protein-coupled oxytocin receptors, which may account for the reduction of uterine contractility, may also be important in regulating oxytocin receptor function within the brain.

Interestingly, some membrane effects of neurosteroids may be direct, some others may be indirect, and linked to a modulation of neurotransmitter release, through calcium channels or neurotransmitter receptors. Indeed, pulsatile administration of PROG or PROG–BSA rapidly stimulated dopamine (DA) release from striatal fragments of estrogen-primed rats, whereas continuous administration had no effect on spontaneous DA secretion, but potentiated L-DOPA-stimulated and inhibited amphetamine-evoked DA release (106). PROG also stimulated NMDA-induced DA release in striatal slices from rats in proestrus (107). 3α,5α-TH PROG reduced the depolarization-induced release of GABA and glutamate from hippocampal slices in a barbiturate-like manner (108). In hypothalamic neuronal cultures, 3α,5α-TH PROG potentiated GABA inhibition and reversed bicuculline-induced augmentation of somatostatin release (109). In vivo experiments have shown that intracerebroventricular injection of 3α,5α-TH PROG inhibited basal acetylcholine release and prevented the stress-induced increase of acetylcholine release from the prefrontal cortex and hippocampus of freely moving rats (110). PREGS administration can transiently correct memory deficits in aged rats, probably by a stimulation of acetylcholine release in the hippocampus (111).

C. Crosstalk Between PROG, PR, and Other Signaling Pathways

A series of experiments has given increasing evidence for a complex crosstalk between steroids and signal transduction pathways: steroids can influence second messengers, and the transcriptional activity of the steroid receptors, in turn, can be regulated by intracellular-signaling molecules and by phosphorylation cascades (see Fig. 2[**2**],[**4**]).

Several results suggest that neurosteroids may interact with G protein-coupled receptors or effector systems in neuronal membranes. In brain slices, physiological concentrations of PROG can inhibit norepinephrine-stimulated cAMP accumulation (112) or stimulate GTPase activity within several minutes of exposure to steroid, consistent with a PROG interaction with G protein-coupled receptors (113). PROG may also modulate the activity of transcription factors, such as the cAMP response element-binding protein (CREB) (Fig. 2[**6**]), which is present in most hypothalamic neurons and activates many neuropeptide genes. Indeed, acute administration of PROG to estrogen-primed rats rapidly decreased (within 30 min) and then increased (30 min later) the expression of phosphorylated CREB in the anteroventral periventricular nucleus (114).

Second messengers can also modify the activity of intracellular steroid receptors. For instance, in hypothalamic cell cultures, PROG enhances the levels of pro-opiomelanocortin, but only in the presence of elevated levels of cAMP (115). Moreover, activation of protein kinase cascades can affect the ligand specificity of steroid receptors. For example, in the presence of 8-bromo-cAMP, the PROG antagonist mifepristone (RU 486) behaves as an agonist and activates gene transcription in T47D breast cancer cells (116). Phosphorylation of PR may even lead to its activation in the absence of hormone (see Fig. 2[**5**]). Indeed, kinase activators and growth factors such as insulin-like growth factor-1 (IGF-1) can cause ligand-independent activation of the PR in uterine cells (117). In the brain, activation of neurotransmitter receptors can lead to such phosphorylation: treatment of monkey kidney cells expressing the chicken PR with a selective dopaminergic D_1 agonist activates the PR, as does treatment with the protein phosphatase inhibitor okadaic acid (118). Similar effects may play a role in the brain, regulating behavior, for the infusion of a D_1 receptor agonist into the third ventricle can mimic the PROG effect of facilitating lordosis in ovariectomized, estrogen-primed rats. Administration of a D_1 or a progesterone receptor antagonist or PR antisense polynucleotides reverses this effect (119). These observations suggest that dopamine may facilitate female sexual behavior by activating hypothalamic PR, possibly even in the absence of PROG.

D. Complexity of Neurosteroid Mechanisms of Action

Progesterone can exert long-lasting effects on the brain by the classic intracellular PR, but also act rapidly at the membrane level, by still hypothetic membrane receptors or via NMDA, glycine, or nicotinic acid receptors. Other neurosteroids, such as PREGS and reduced metabolites of PROG (5α-DH PROG and 3α,5α/5β-TH PROG) exert their effects in the brain by membrane mechanisms; for example, through receptors for neurotransmitters or ion channels.

Things are, however, more complex because the 5α-reduced metabolite of PROG, 5α-DH PROG (which is also the immediate precursor of 3α,5α-TH PROG, the positive modulator of $GABA_A$ receptors), was recently shown to regulate gene expression by the PR. In a neuroblastoma cell line cotransfected with the expression vector for the human or the chicken PR (hPR$_B$ and cPR$_B$) and a reporter plasmid encoding the mammary tumor virus (MTV) promoter coupled to the luciferase gene, 5α-DH PROG was able to activate both receptors. The transactivation induced by 5α-DH PROG required the ligand-binding domain of the PR. Also, radioactively labeled 3α,5α-TH PROG was rapidly converted to 5α-DH PROG, which bound to the cPR and the hPR, and induced gene expression (120,121). Thus, 5α-DH PROG may act rapidly by its conversion to 3α,5α-TH PROG and an interaction with $GABA_A$ receptors. It may also regulate gene expression by the PR.

The second remark concerns a new kind of nuclear receptors identified in the liver and intestine, the pregnane X receptors (PXR), which belong to the family of orphan receptors. Among the two isoforms, PXR_1 is preferentially activated by micromolar concentrations of PREG and PROG (see Fig. 2[**14**]). PXR_1 can bind as heterodimers with 9-*cis*-retinoic acid receptors (RXR) to hormone response elements (HRE) in the promoter region of CYP3A genes. These genes encode cytochrome P-450s, which are involved in the hydroxylation of various steroids, including corticosteroids and progestins (122). Whether this kind of receptor is present in the brain and whether it is involved in physiological actions of neurosteroids are still unknown.

IV. EFFECTS OF PROGESTERONE, ITS PRECURSORS AND METABOLITES, IN THE BRAIN

A. The Regulation of Gonadotropin Secretion

Although the negative feedback exerted by PROG on luteinizing hormone (LH) and follicle-stimulating hormone (FSH) release is well established, the positive influence of PROG on the hypothalamus and the pituitary gland remains a subject of extensive investigation (123). As early as 1948, Everett had suggested that

the effect of PROG on ovulation may be facilitatory or inhibitory, depending on the time and duration of administration during the cycle.

1. *Progesterone and the Gonadotropin Surge*

It now seems to be clear that the main event leading to ovulation in mammals, the LH surge, is triggered by estradiol acting not only at the pituitary, but also at the hypothalamic level (124). The participation of PROG, together with estradiol, in the induction of the gonadotropin surge is still debated, at least for humans, in whom no significant rise in circulating PROG can be measured before ovulation. However, several experiments performed mainly in rats favor the hypothesis of a complementary short-term effect of PROG in this induction. Indeed, when mifepristone (RU 486) or the progesterone synthesis inhibitor trilostane is administered to cycling rats on proestrus morning, the preovulatory LH surge is much attenuated (125), and the estrus behavior (lordosis and proceptivity) decreases (126). The source of PROG could be the adrenal gland, rather than the ovary. Indeed, adrenal vein cannulation studies in the rat have shown a rise in adrenal PROG in proestrus, that precedes a rise in ovarian PROG. Ovariectomy on the day of the expected surge in PMSG-primed immature female rats does not interfere with the gonadotropin surge, whereas the administration of mifepristone 2 h after ovariectomy, prevents it (51).

a. *PROG Actions on the Pituitary Gland*

Progesterone modulates the anterior pituitary responsiveness to GnRH in a biphasic manner. In rat pituitary cell cultures and pituitary glands of cycling rats superfused in vitro, brief treatment with PROG enhanced (127,128), whereas prolonged PROG treatment suppressed (129) the LH response to GnRH. Moreover, exposure of dispersed pituitary cells to PROG enhanced the release of LH induced by secretagogues that act distal to the GnRH receptor, such as protein kinase C activators (130). The ability of PROG to modulate GnRH receptors was also studied, and the results were discordant. However, it seems that PROG could exert a biphasic effect, with a short treatment increasing, but a prolonged treatment decreasing GnRH receptors (131).

Besides genomic effects of PROG on the pituitary gland through the PR, some rapid effects of steroids on gonadotropin release have been described. In ovariectomized rats injected with estradiol, a single injection of 3α,5α-TH PROG can significantly increase the release of LH. Estrogen priming is necessary but this effect, which is not prevented by the PROG antagonist mifepristone, appears to be mediated by the $GABA_A$ receptor (132). The release of FSH in the same animal model can be stimulated by 5α-DH PROG (133) through the same $GABA_A$ receptor, and inhibited in cultured pituitary cells by 3α-hydroxy-4-pregnen-20-one (a steroid that is electively synthesized in the pituitary), by

an interaction with the phosphoinositol-signaling cascade and Ca^{2+} channels involved in the GnRH transduction pathway (134). Although the slow alterations in gonadotropin levels can be controlled by the genomic action of steroids, the rapid oscillations may be the result of nongenomic actions (135).

b. Progesterone's Actions on the Hypothalamus

In the cycling rat, the gonadotropin surge is preceded by an initial increase in GnRH that may contribute to its self-priming effect on the pituitary (136). The second increase in GnRH release 2 or 3 h later, which initiates the surge (137), seems to be induced by acute PROG release, but not estradiol. Estradiol is necessary (ovariectomized rats have to be estrogen-primed), but not sufficient, and the surge of GnRH occurs only after acute administration of PROG (138).

In vitro studies show that PROG enhances GnRH mRNA levels (139), GnRH concentration (140), induces c-Fos in GnRH neurons (141), and stimulates GnRH release from rat mediobasal hypothalamus (142). In vivo studies, using push–pull perfusion of the mediobasal hypothalamus, demonstrate that an intermittent or acute administration of PROG to estrogen-primed rabbits (143) or monkeys (144) increases the amplitude of the largest GnRH pulse as well as the frequency of GnRH pulses. Moreover, PROG can also increase the availability of GnRH to the pituitary gland by inhibiting the activity of GnRH-degrading enzymes (145).

The genomic effect of PROG on GnRH release is indirect because GnRH neurons are devoid of PR. Thus, PROG may induce the release of neuromediators from surrounding neurons containing neuropeptide Y and norepinephrine (144,146), but also opioids, galanine, glutamate, or GABA which, in turn, may modify the spontaneous release of GnRH. The double-labeling of PR and Fos protein could provide a useful marker to aid in identification of neurons that respond to PROG (147).

Rapid effects of PROG and BSA-conjugated PROG, which does not cross cell membranes, have also been described in superfused mediobasal hypothalamus slices (148,149) and GnRH-secreting immortalized GT1-1 neurons (150). Moreover $3\alpha,5\alpha$-TH PROG, which can be metabolized from PROG by hypothalamic GT1-1 neurons, stimulates the release of GnRH (151). Two other PROG metabolites, 20α-DH PROG and $3\beta,5\beta$-TH PROG, infused in a pulsatile manner with push–pull cannulae into the hypothalamus of rabbits, are also effective in rapidly stimulating the release of GnRH in vivo (143). Except in experiments performed on GT1-1 cells, the effect of PROG, PROG–BSA, and PROG metabolites occurred only after a previous estrogen priming, suggesting that an estrogen-inducible binding component was also involved in these rapid, presumably nongenomic, actions of PROG. In GT1-1 cells, we demonstrated that the effect of $3\alpha,5\alpha$-TH PROG was clearly mediated by the $GABA_A$ receptor (151), whereas the action of PROG–BSA, which occurred with different kinetics, did

not involve the $GABA_A$ receptor, but was blocked by L-type calcium channels blockers and a protein kinase A inhibitor (150). The interaction between genomic and membrane effects of PROG in the stimulation of GnRH release should be further studied.

The complexity of the neuroendocrine regulation of gonadotropin surge is further enhanced by the new hypothesis of Levine (152), which states that estrogen may stimulate expression of PR which, in turn, may be transactivated (even in the absence of PROG) after stimulation of neurotransmitter receptors coupled to adenylate cyclase. Activated PR may thereafter regulate transcription of target genes that control transmitter synthesis and release in neural circuitries governing GnRH gene expression and/or pulsatile GnRH release.

2. Negative Feedback Action of Progesterone

A high rise in circulating PROG blocks the positive-feedback action of estradiol and prevents ovulation (153). The site of action of PROG in the blockade of the estradiol-induced gonadotropin discharge is mainly the hypothalamus (154) by an inhibition of the GnRH surge (155), but PROG can also decrease the responsiveness of pituitary cells to GnRH (156).

The electrophysiological manifestations of the GnRH pulse generator activity can be continuously monitored by radiotelemetry throughout the menstrual cycle in the monkey. During the LH surge, a dramatic decrease in pulse generator frequency occurs, presumably as a consequence of the rapid rise in estradiol. Then, the GnRH pulse frequency remains markedly reduced along the luteal phase until the early follicular phase of the next cycle (when GnRH pulsatility increases, probably owing to an escape from the inhibiting influence of PROG; 157). Similarly in ovariectomized ewes, a sustained elevation of circulating progesterone diminished or abolished the pulsatile secretion of GnRH (158). Finally, this progesterone-induced lowering of GnRH pulse frequency accounts for the absence of gonadotropin surges in the luteal phase of the menstrual or estrous cycle, despite occasional rises in circulating estradiol to a concentration sufficient for surge induction (159). The observation of this antigonadotropic action of PROG has led to the use of progestins as contraceptives.

B. Progesterone and Prolactin Release

In vivo, PROG actions on prolactin secretion depend on the time and the concentration administered. Indeed, estrogen-primed monkeys became hyperprolactinemic after the administration of physiological doses of PROG, and mifepristone fully reversed this effect (160). Consistent with this hyperprolactinemic effect, PROG decreased the expression of tyrosine hydroxylase (TH) mRNA in the monkey ventral arcuate dopaminergic neurons. The decrease in

TH mRNA may be associated with decreased dopamine reaching the pituitary cells and with the suppression of the inhibitory influence exerted by dopaminergic neurons on prolactin release (58). Similarly, in estrogen-primed rats, a single injection of PROG in the morning advanced and amplified the afternoon prolactin surge and decreased the activity of tuberoinfundibular dopaminergic neurons (161). Conversely, sustained high levels of circulating PROG inhibited hyperprolactinemia induced by estradiol implants in the arcuate nucleus of rats (162).

In vitro experiments demonstrated that 3α,5α-TH PROG inhibited the spontaneous release of prolactin from rat pituitary cell cultures by an interaction with the $GABA_A$ receptor (163).

C. Trophic and Neuroprotective Effects of Progesterone and Related Steroids in the Brain

1. *Trophicity and Development*

It has become clear that PROG has important effects on adult female brain structure and function and participates in the cyclic synaptogenesis in adult hippocampus. Indeed, PROG exerts biphasic effects on apical dendritic spine density in CA1 hippocampal pyramidal cells: it first rapidly potentiates the estrogen-induced spine formation, but then triggers the down-regulation of spines. Administration of PROG speeds up the decline, which is blocked by mifepristone administration. These steroid actions are dependent on the brain region: estradiol and PROG do not affect spine density of CA3 pyramidal cells or granule cells of the dentate gyrus (164). In the rat arcuate nucleus, estradiol and PROG also play a role in phased synaptic remodeling (loss and regain of axosomatic synapses during the 48-h period between the morning of proestrus and the morning of metestrus): estradiol lowers the number of axosomatic synaptic profiles; PROG, which has no effect alone, blocks the effect of estradiol on synapses (165).

Whether PROG that is locally synthesized by glial cells plays a role in neuronal development and survival is not well known. PROG affects the survival of neurons in vitro: indeed, pure cultures of neurons from the rat or mouse embryonic brain always survive longer when PROG is present in the medium (the currently used Bottenstein and Sato's medium contains 20 nM PROG) (166). During nerve cell development, the undulating process of cytoarchitectural emergence and retraction constitutes a normal growth pattern. Neurosteroids may play a role in this retraction–emergence process, which occurs normally within minutes; in vitro experiments performed on cultured fetal hippocampal neurons show that 3α,5α-TH PROG induces cytoarchitectural regression in nerve cells that have not yet established contacts with other nerve or glial cells within a relatively rapid time frame. In older more mature cultures, in which neurons

have established structural connections, 3α,5α-TH PROG protected cells from picrotoxin-induced nerve cell death (167).

2. Neuroprotection and Myelin Repair

In vivo studies have documented the role of PREG and PROG after brain injury. Thus, PROG reduces the consequences of contusion lesions in the rat frontal cortex, including cerebral edema, secondary neuronal degeneration, and behavioral impairment (168). PROG suppresses plasma extravasation within the meninges after electrical stimulation or substance P administration, probably by its conversion into 3α,5α-TH PROG and an interaction with the $GABA_A$ receptor (169). In vivo and in vitro studies have shown that 3α,5β-TH PROG sulfate (and its synthetic homologue 3α-hydroxy-5β-pregnan-20-one hemisuccinate), which is a negative modulator of NMDA receptors, could exert neuroprotective effects against cerebral ischemia in rats and NMDA-induced cell death in cultures of rat hippocampal neurons (170). PROG also protects the brain against lipid peroxidation after traumatic injury, and limits the damage caused by free radicals to the blood–brain barrier (171).

Moreover, PROG prevents gliosis and astrocyte proliferation: when gonadectomized rats are treated with PROG immediately after a longitudinal incision across the parietal cerebral cortex, the CA1 field of the dorsal hippocampus, or the dentate gyrus, the number of glial fibrillary acid protein (GFAP)-immunoreactive astrocytes decreases in the vicinity of the wound (172). In vitro, at physiological concentrations of potassium, PREG and PREGS increase the extension of GFAP-immunoreactive processes in cultured hippocampal slices, whereas both neurosteroids modulate the effect of high concentrations of potassium by rapidly inhibiting the extension of GFAP-positive processes, in agreement with in vivo observations after brain injury (173). Microglia also reacts to trauma or chronic diseases with proliferation and expression of cytokines associated with inflammatory events. PROG inhibits microglial proliferation in isolated cultures (174).

An interesting series of observations performed in our laboratory concerning the peripheral nervous system is worth reporting. We found that the concentration of PREG in the human sciatic nerve is more than 100-fold the plasma level of the steroid (175). In the rat sciatic nerve, PREG (176) and PROG (177) concentrations are higher than in the plasma and remain high after adrenalectomy and gonadectomy. Rat Schwann cells in culture can convert 25-OH cholesterol into PREG (176) but the synthesis of PROG, 5α-DH PROG, and 3α,5α-TH PROG occurs only if Schwann cells are exposed to a diffusible neuronal factor (177,178). We have recently demonstrated that PROG plays an important role during myelin repair in the peripheral nervous system. After cryolesion of the mouse sciatic nerve, axons and their myelin sheaths degenerate

quickly in the distal segments by wallerian degeneration. Schwann cells start to proliferate and myelinate the regenerating fibers after 1 week. Two weeks after production of the lesion, myelin sheaths reach one-third of their final thickness. In the damaged portion of the nerve, PREG and PROG levels remain high and even increase 15 days after lesion induction. The inhibition of PROG synthesis or action by repeated local applications of the 3β-HSD inhibitor trilostane (see Fig. 1) or of mifepristone, the potent competitive antagonist of PR, markedly decreases the thickness (number of lamellae) of myelin sheaths 15 days after lesion formation, whereas the inhibitory effect of trilostane is reversed by the simultaneous application of PROG. Moreover, application of 100 μg of PREG or PROG close to cryolesioned sciatic nerves increases the thickness of myelin sheaths after 15 days (177). Further experiments should explain the mechanisms involved in PROG action. It is likely that neurosteroids exert their trophic actions by acting in concert or even in synergy with growth factors, such as IGF-1 or nerve growth factor (NGF).

In the central nervous system, the effects of PROG on myelin repair are unknown. However, in primary cultures of rat glial cells (consisting of 60% oligodendrocytes and 40% astrocytes), PROG increases the expression of two myelin-specific proteins in the oligodendrocytes, the myelin basic protein (MBP) and the 2′,3′-cyclic nucleotide-3′-phosphodiesterase (CNPase), and a myelin-specific lipid, galactocerebroside (Gal C), as measured by immunofluorescence (179). These observations suggest that PROG might also play a role in myelin repair in the central nervous system.

D. Behavioral Effects

1. Sexual Behavior and Aggressiveness

Dehydroepiandrosterone (DHEA) and its 3β-methyl analogue inhibit the aggressive behavior of castrated male mice toward lactating female intruders. This inhibition is correlated with a profound decrease of brain PREGS concentrations (180). PREGS negatively modulates $GABA_A$ receptors. It is tempting to speculate that the GABA antagonist effect of PREGS is related to the development of aggressive behavior, for the potentiation of GABAergic control by a competitive inhibitor of GABA transaminase reduces this form of aggression (181). The PREGS decrease observed after DHEA administration may enhance the action of endogenous GABA and, thereby inhibit male aggression against lactating females. PROG can also reduce aggressive behavior in rodents of either sex, without depressing the general locomotor activity of the animals, indicating that this effect is not related to the sedative properties of the steroid. Alphaxalone induces sedation, but does not decrease aggressive displays (182).

Progesterone facilitates lordosis in hamsters of both sexes (182). In estrogen-primed female hamsters, the stimulation of both the ventral medial hypothalamus (VMH) and the ventral tegmental area (VTA) by PROG is necessary to facilitate sexual receptivity. The mechanism involved in PROG action is different in these two brain regions, genomic in the VMH, where many intracellular PR are present, and nongenomic in the VTA, which contains few PR. In the VTA, PROG has to be converted into 5α-reduced metabolites to be active through the $GABA_A$ receptor (183,184). When administered to male mice, the neurosteroid PREGS reduces their preference for the odors of estrous females. This inhibitory effect of PREGS on olfactory-mediated male sexual interest is not affected by $GABA_A$ antagonists, but seems to partly involve the NMDA receptors (185). Finally, neurosteroids seem to be involved in sex recognition: when male rats are exposed to females in estrus, the concentration of PREG electively decreases in their olfactory bulbs. This effect, caused by a pheromonal stimulus, is hormonodependent, for it is suppressed by castration, and the response is reestablished by testosterone administration (186).

2. *Sedative, Hypnotic, and Anesthetic Effects*

The central effects of PROG have been known for over half a century, when Selye demonstrated that PROG was capable of inducing sedation in rats (187). He also reported that 5β-DH PROG produced anesthesia in rats and observed a correlation between the structure and the sedative–anesthetic properties of steroids (188). In women, micronized PROG is usually administered at night because of its sedative or even hypnotic effect, depending on the dose (189). The soporific effects of PROG and 3α,5α-TH PROG have been examined on sleep electroencephalograms (EEG) in rats and humans: in both species, both steroids reduced the latency to non-rapid eye movement (non-REMS) sleep and increased the time spent in pre-REMS, an intermediate state between non-REMS and REMS (190,191). The effects of 3α,5α-TH PROG on sleep were similar to those elicited by larger doses of PROG, which produced comparable brain levels of 3α,5α-TH PROG in rodents. These effects closely resembled those of agonist modulators of $GABA_A$ receptors and, particularly, benzodiazepines (192). Conversely, PREGS, a negative modulator of $GABA_A$ receptors, antagonized barbiturate-induced hypnosis in rats (193). When administered to rats or to healthy male volunteers, PREG improved the quality of sleep and induced EEG changes compatible with inverse agonistic $GABA_A$ modulation (190,194).

By using the loss of the righting response (LRR) and the return of the righting response (RRR) test, Mok et al. demonstrated that 5α-DH PROG, PROG, and 3α,5α-TH PROG were able to induce anesthesia when injected into mice or added to the bath of frogs. They identified 3α,5α-TH PROG as the common metabolite responsible for the anesthetic effect (195–197). 3α,5α-TH PROG

acted more rapidly and at lower concentrations than 5α-DH PROG or PROG. The time course of brain concentrations of 3α,5α TH PROG, but not that of PROG nor 5α-DH PROG, correlated with the LRR and the RRR. Moreover, when PROG was administered with a 5α-reductase inhibitor (SKF 105111), the anesthetic activity of PROG was dramatically reduced (198). In humans, alphaxalone (3α-hydroxy-5α-pregnane-11,20-dione), which has a structure similar to that of 3α,5α-TH PROG, has been used for a long time as an anesthetic. 3α,5α-TH PROG itself has been administered to women with good tolerance, to induce anesthesia for delivery (199).

3. Anxiolytic Actions

Progesterone exerts its anxiolytic effect by a PR-independent mechanism (mifepristone does not prevent it), and only after its conversion to 3α,5α-TH PROG (the PROG effect is blocked by coadministration of a 5α-reductase inhibitor; 200). Thus, intracerebroventricular administration of 3α,5α(or 5β)-TH PROG to rats elicits anxiolytic effects (i.e., decreased locomotor activity and increased exploration in the elevated plus-maze). These actions are mediated by the $GABA_A$ receptor (201), but through a mechanism separate from that of the benzodiazepines (202). When administered systemically to rats, 3α,5α-TH PROG exerts anticonflict effects by interacting with the $GABA_A$ receptor, either at the site specific for the benzodiazepine receptor inverse agonist Ro-15-4513 or at the picrotoxin site, but not at the benzodiazepine site (203). When using the mirrored chamber behavior test in mice, Reddy et al. observed a differential anxiolytic effect of PREGS, PROG, and 3α,5α-TH PROG mediated by different mechanisms: PROG through its conversion to 3α,5α-TH PROG interacts with the $GABA_A$ receptor at or near the chloride channel, whereas PREGS seems to modulate a voltage-gated calcium channel of the L type (204). PREGS and 3α,5α-TH PROG also augment recovery from benzodiazepine withdrawal anxiety and hyperactivity (205).

Moreover, 3α,5α-TH PROG counteracts corticotropin-releasing hormone (CRH)-induced anxiety and alters the release and gene expression of CRH in the rat hypothalamus (206), suggesting that besides its sedative and analgesic activities, 3α,5α-TH PROG may also affect the neuroendocrine response to stress in a mode resembling that of corticosteroids. Indeed, pretreatment of rats with 3α,5α-TH PROG or PROG attenuates the elevation of plasma corticotropin (adrenocorticotropin; ACTH) and corticosterone after emotional stress and attenuates the increase in stress-related arginine–vasopressin mRNA levels in the hypothalamus. PROG (but not 3α,5α-TH PROG) also influences the transcription of corticosteroid receptors in the hippocampus (207).

Finally, the differences in the production of endogenous anxiolytic neurosteroids may be important in modulating individual responses to various stress-

ors. Indeed, the higher levels of 5α-DH PROG and 3α,5α-TH PROG observed in some brain regions, such as the frontal cortex and the bed nucleus of the striata terminalis, of hypoemotional rats compared with anxious rats, may account for the behavioral differences characterizing these two psychogenetically selected lines (37). In turn, genetic differences in sensitivity to 3α,5α-TH PROG may contribute to the differences observed in the behavioral responses to ethanol withdrawal (208).

4. *Analgesia*

Intracerebroventricular administration to mice of 3α,5α-TH PROG, and PROG itself, elicits significant, dose-dependent analgesia; the stereoisomer 3β,5α-TH PROG is ineffective. The analgesic properties of 3α,5α-TH PROG arises by mechanisms involving calcium channels, $GABA_A$ receptors, and endogenous opioid systems (209). PROG, 5α-DH PROG, and 3α,5α-TH PROG also attenuate pain sensitivity in rats submitted to the heat tailflick nociceptive test (210). Moreover, PROG–BSA, similar to PROG, is able to increase tailflick latencies. These effects are rapid and imply an interaction with the $GABA_A$ receptor (211). The synthetic structural analogue of 3α,5α-TH PROG, alphaxalone, produces preemptive analgesia in the rat formalin test, through its action at the $GABA_A$ receptor (the analgesic effect is antagonized by picrotoxin), whereas pentobarbital and propofol, which also modulate $GABA_A$ receptors *but at distinct sites*, are ineffective (212).

5. *Anticonvulsant Effects*

The frequency and intensity of seizures in women is altered in physiological states typically associated with cyclic changes in steroid hormone secretion. In 1976, Backstrom observed a negative correlation between the number of seizures in women and their plasma PROG levels (213). He also showed a significant decrease in EEG spike frequency during PROG infusion to women with partial epilepsy (214).

In rats, the anticonvulsant potential of 3α,5α-TH PROG has been clearly demonstrated against bicuculline, to a smaller extent against pentylenetetrazole (Metrazol) and picrotoxin, whereas no effect was observed against strychnine- and electroshock-induced seizures, thus supporting a GABA-mimetic profile (215). Intracerebroventricular injection of 3α,5α-TH PROG before the administration of pentylenetetrazole (PTZ) to rats delayed the onset of convulsions and reduced the percentage of animals showing seizures in a dose-dependent manner (216). Ethanol withdrawal enhanced susceptibility to seizures: 3α,5α-TH PROG protected rats against bicuculline-induced seizures during ethanol withdrawal (217). Among the different metabolites of PROG, those with 3-hydroxy in the α-position and the 5-H in the α- or β-configuration were highly

effective at potentiating GABA-evoked chloride currents and also showed potent anticonvulsivant activity in the PTZ seizure test (218). It is tempting to speculate that cyclic changes in the brain levels of 3α,5α-TH PROG and related 3α-hydroxylated PROG metabolites in women may contribute to changes in seizure susceptibility (e.g., catamenial epilepsy), although the comparison between plasma 3α,5α(and 5β)-TH PROG levels showed no difference in healthy women during the luteal phase and in women with partial epilepsy in the intercritical phase (219). An explanation to the enhanced seizure susceptibility and insensitivity to benzodiazepines following PROG withdrawal has been recently proposed: indeed, the decrease in 3α,5α-TH PROG levels down-regulated the expression of the α_4-$GABA_A$ receptor subunit in the rat CA1 hippocampus, and this subunit is responsible for increased seizure susceptibility (55).

Finally, a synthetic analogue of 3α,5α-TH PROG, ganaxolone (3α-hydroxy-3β-methyl-5α-pregnan-20-one), which is protected from metabolic attack of the 3α-position, prevented PTZ-induced convulsions (220). Ganaxolone is currently under phase II clinical investigation for therapy of epilepsy. Neuroactive steroids may constitute a novel class of antiepileptic agents, that may be used alone or at low doses together with benzodiazepines (221).

6. *Memory*

In the mid-1940s, Pincus believed that PREG might facilitate learning in humans (222). Experiments performed much later on male mice have shown that immediate posttraining intracerebroventricular administration of low doses of PREG and PREGS caused improvement of retention for footshock active avoidance training, while PROG did not (223). PREG and PREGS also improved acquisition and retention in a food search task (224). Immediate posttraining injections of low doses of PREGS into the limbic system structures, particularly the amygdala of mice (225), or into the nucleus basalis magnocellularis of rats, resulted in memory performance enhancement. Conversely, 3α,5α-TH PROG disrupted performance when injected into the nucleus basalis magnocellularis before an acquisition trial (226), suggesting a $GABA_A$-mediated mechanism. However, an intracerebroventricular injection of PREGS during pretraining also blocked NMDA antagonist-induced deficits in a passive memory task, suggesting an involvement of PREGS interaction with NMDA receptors (227).

A study by Vallée et al. on cognitive performances of aging rats has been particularly rewarding (111). PREGS levels were significantly lower in the hippocampus of aged (2-year-old rats) than in young male animals. Interestingly, individual concentrations of PREGS in the hippocampus of aged animals were significantly correlated with individual cognitive performances in two tasks for spatial memory, the Morris water maze and the Y maze. Low hippocampal levels of PREGS were correlated with poor performance in both tasks, whereas no

relation was found with the PREGS content of the amygdala, the prefrontal and parietal cortex, or the striatum. Moreover, the memory deficit of impaired aged rats was transiently corrected after intraperitoneal or bilateral intrahippocampal injection of PREGS. The mechanism underlying the memory-enhancing property of PREGS is unknown. PREGS is both a $GABA_A$ antagonist and a positive modulator at the NMDA receptor. It could be hypothesized that the neuromodulatory pathways of PREGS may reinforce neurotransmitter systems that decline with age. Central cholinergic neurotransmission which, in turn, is modulated by GABAergic and glutamatergic afferents represented a plausible candidate for the neurosteroid action. PREGS had already been shown to reverse the amnestic effect of scopolamine, a muscarinic receptor blocker (98). Vallée et al. demonstrated a stimulation of acetylcholine release in the hippocampus, following intracerebroventricular injection of PREGS (111). Thus, the hippocampal content of PREGS could play a physiological role in preserving or enhancing cognitive abilities in aged animals.

Much remains to be learned, including the study of the different types of memory deficits in young animals, the mechanisms of PREGS action, and the interaction with other factors controlling the memory pathways, such as calcineurin.

V. CONCLUSION

Progesterone, its precursors, and metabolites can be synthesized locally within the brain (neurosteroids), or originate from the endocrine glands. Whatever the sources, these steroids can exert their effects on target brain cells either by modulating transcription of specific genes through intracellular receptors, or by acting directly on the cellular membrane. The genomic effects are of great complexity, and most of the brain target genes, largely unknown, may include growth factor genes, and genes encoding neurotransmitter transporters. For example, PROG can up-regulate dopamine uptake sites in the nigrostriatal dopaminergic pathway (228), and reverse the down-regulation of serotonin transporters induced by castration in the hypothalamus of female rats, and these effects are probably genomic (229). Many aspects of genomic mechanisms have to be studied further in the brain; namely, the interactions between steroid receptors and other transcription factors, the binding of steroids to newly discovered intracellular receptors such as the pregnane X receptor PXR (which can be activated by PREG or PROG), or the crosstalk between steroids and signal transduction pathways.

The synthesis of neurosteroids within the brain raises the question of their mode of action and their biological significance. In contrast with the endocrine mode of action of gonadal and adrenal steroid hormones, neurosteroids have paracrine or autocrine activities. Most of their effects are membrane effects,

occurring through the $GABA_A$ or NMDA receptors. For example, the interaction of 3α,5α-TH PROG with $GABA_A$ receptors is probably *directly* responsible for its hypnotic and anxiolytic effects. However, membrane effects may be *indirect* and linked to a modulation of neurotransmitter release, through calcium channels or neurotransmitter receptors.

In addition to the problems set by the different mechanisms of actions, another critical question that remains to be explored concerns the interactions between the two pools of steroids: circulating steroids, which freely penetrate into the brain, and neurosteroids, which are synthesized within the brain.

Finally, apart from their involvement in reproductive function, the discovery of new actions of steroids in the brain has opened a new field of research. Among them, particularly promising are the studies of PROG effects on myelin formation and on memory, which could lead to therapeutical use in humans.

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REFERENCES

1. M Schumacher, EE Baulieu. Neurosteroids: synthesis and functions in the central and peripheral nervous system. In: GR Bock, JA Goode, eds. Non-reproductive Actions of Sex-Steroids. Ciba Found Symp 191:90–112, 1995.
2. EE Baulieu. Neurosteroids: of the nervous system, by the nervous system, for the nervous system. Recent Prog Horm Res 52:1–32, 1997.
3. C Corpéchot, M Synguelakis, S Talha, M Axelson, J Sjövall, R Vihko, EE Baulieu, P Robel. Pregnenolone and its sulfate ester in the rat brain. Brain Res 270:119–125, 1983.
4. ZY Hu, I Jung-Testas, P Robel, EE Baulieu. Neurosteroids: oligodendrocyte mitochondria convert cholesterol to pregnenolone. Proc Natl Acad Sci USA 84:8215–8219, 1987.
5. I Jung-Testas, ZY Hu, EE Baulieu, P Robel. Neurosteroids: biosynthesis of pregnenolone and progesterone in primary cultures of rat glial cells. Endocrinology 125:2083–2091, 1989.
6. C Le Goascogne, P Robel, M Gouézou, N Sananès, EE Baulieu, E Waterman. Neurosteroids: cytochrome P-450scc in rat brain. Science 237:1212–1215, 1987.
7. JL Sanne, KE Krueger. Expression of cytochrome P450 side-chain cleavage enzyme and 3β-hydroxysteroid dehydrogenase in the rat central nervous system: a study by polymerase chain reaction and in situ hybrydization. J Neurochem 65:528–536, 1995.

8. S Mellon, CF Deschepper. Neurosteroid biosynthesis: genes for adrenal steroidogenic enzymes are expressed in the brain. Brain Res 629:283–292, 1993.
9. C Cascio, VVK Prasad, YY Lin, S Lieberman, V Papadopoulos. Detection of P450c17-independent pathways for dehydroepiandrosterone (DHEA) biosynthesis in brain glial tumor cells. Proc Natl Acad Sci USA 95:2862–2867, 1998.
10. Y Akwa, N Sananès, M Gouézou, P Robel, EE Baulieu, C Le Goascogne. Astrocytes and neurosteroids: metabolism of pregnenolone and dehydroepiandrosterone. Regulation by cell density. J Cell Biol 121:135–143, 1993.
11. K Kabbadj, M El-Etr, EE Baulieu, P Robel. Pregnenolone metabolism in rodent embryonic neurons and astrocytes. Glia 7:170–175, 1993.
12. R Guennoun, RJ Fiddes, M Gouézou, M Lombès, EE Baulieu. A key enzyme in the biosynthesis of neurosteroids, 3β-hydroxysteroid dehydrogenase Δ5/4-isomerase (3β-HSD), is expressed in rat brain. Mol Brain Res 30:287–300, 1995.
13. RC Melcangi, F Celotti, L Martini. Progesterone 5α-reduction in neuronal and in different types of glial cell cultures: type 1 and 2 astrocytes and oligodendrocytes. Brain Res 639:202–206, 1994.
14. A Poletti, P Negri-Cesi, RC Melcangi, A Colciago, L Martini, F Celotti. Expression of androgen-activating enzymes in cultured cells of developing rat brain. J Neurochem 68:1298–1303, 1997.
15. X Li, PJ Bertics, HJ Karavolas. Regional distribution of cytosolic and particulate 5α-dihydroprogesterone 3α-hydroxysteroid oxidoreductases in female rat brain. J Steroid Biochem Mol Biol 60:311–318, 1997.
16. M Khanna, KN Qin, KC Cheng. Distribution of 3α-hydroxysteroid dehydrogenase in rat brain and molecular cloning of multiple cDNAs encoding structurally related proteins in humans. J Steroid Biochem Mol Biol 53:41–46, 1995.
17. P Robel, EE Baulieu. Neurosteroids. Biosynthesis and function. Trends Endocrinol Metab 5:1–8, 1994.
18. KM Rajkowski, P Robel, EE Baulieu. Hydroxysteroid sulfotransferase activity in the rat brain and liver as a function of age and sex. Steroids 62:427–436, 1997.
19. EE Baulieu. Neurosteroids: a new function in the brain. Biol Cell 71:3–10, 1991.
20. Y Ikeda, WH Shen, HA Ingraham, KL Parker. Developmental expression of mouse steroidogenic factor-1, an essential regulator of the steroid hydroxylases. Mol Endocrinol 8:654–662, 1994.
21. N Boujrad, SO Ogwuegbu, M Garnier, CH Lee, BM Martin, V Papadopoulos. Identification of a stimulator of steroid hormone synthesis isolated from testis. Science 268:1609–1612, 1995.
22. T Sugawara, JA Holt, D Driscoll, JF Strauss, D Lin, WL Miller, D Patterson, KP Clancy, IM Hart, BJ Clark, DM Stocco. Human steroidogenic acute regulatory protein: functional activity in COS-1 cells, tissue-specific expression and mapping of the structural gene to 8p11.2 and a pseudogene to chromosome 13. Proc Natl Acad Sci USA 92:4778–4782, 1995.
23. G Roscetti, C Ambrosio, M Trabucchi, M Massotti, ML Barbaccia. Modulatory mechanisms of cyclic AMP-stimulated steroid content in rat brain cortex. Eur J Pharmacol 269:17–24, 1994.

24. V Papadopoulos, P Guarneri. Regulation of C6 glioma cell steroidogenesis by adenosine 3′,5′-cyclic monophosphate. Glia 10:75–78, 1994.
25. RC Melcangi, F Celotti, P Castano, L Martini. Intracellular signalling systems controlling the 5α-reductase in glial cell cultures. Brain Res 585:411–415, 1992.
26. V Papadopoulos, H Amri, N Boujrad, C Cascio, M Culty, M Garnier, M Hardwick, H Li, AS Vidic, AS Brown, JL Reversa, JM Bernassau, K Drieu. Peripheral benzodiazepine receptor in cholesterol transport and steroidogenesis. Steroids 62:21–28, 1997.
27. V Papadopoulos, H Amri, H Li, N Boujrad, B Vidic, M Garnier. Targeted disruption of the peripheral-type benzodiazepine receptor gene inhibits steroidogenesis in the R2C Leydig tumor cell line. J Biol Chem 272:32129–32135, 1997.
28. P Guarneri, V Papadopoulos, B Pan, E Costa. Regulation of pregnenolone synthesis in C6-2B glioma cells by 4′-chlorodiazepam. Proc Natl Acad Sci USA 89:5118–5122, 1992.
29. LD McCauley, CH Park, NC Lan, JM Tomich, JE Shively, KW Gee. Benzodiazepines and peptides stimulate pregnenolone synthesis in brain mitochondria. Eur J Pharmacol 276:145–153, 1995.
30. A Korneyev, BS Pan, A Polo, E Romeo, A Guidotti, E Costa. Stimulation of brain pregnenolone synthesis by mitochondrial diazepam binding inhibitor receptor ligands in vivo. J Neurochem 61:1515–1524, 1993.
31. DP Uzunov, TB Cooper, E Costa, A Guidotti. Fluoxetine-elicited changes in brain neurosteroid content measured by negative ion mass fragmentography. Proc Natl Acad Sci USA 93:12599–12604, 1996.
32. MA Gallo, SS Smith. Progesterone withdrawal decreases latency to and increases duration of electrified prod burial: a possible rat model of PMS anxiety. Pharmacol Biochem Behav 46:897–904, 1993.
33. ML Barbaccia, G Roscetti, M Trabucchi, RH Purdy, MC Mostallino, C Perra, A Concas, G Biggio. Isoniazid-induced inhibition of GABAergic transmission enhances neurosteroid content in the rat brain. Neuropharmacology 35:1299–1305, 1996.
34. RH Purdy, AL Morrow, PH Moore Jr, SM Paul. Stress-induced elevations of γ-aminobutyric acid type A receptor-active steroids in the rat brain. Proc Natl Acad Sci USA 88:4553–4557, 1991.
35. RC Melcangi, M Ballabio, V Magnaghi, F Celotti. Metabolism of steroids in pure cultures of neurons and glial cells: role of intracellular signalling. J Steroid Biochem Mol Biol 53:331–336, 1995.
36. Th Steimer, P Driscoll, PE Schulz. Brain metabolism of progesterone, coping behavior and emotional reactivity in male rats from two psychogenetically selected lines. J Neuroendocrinol 9:169–175, 1997.
37. H Gronemeyer. Control of transcription by steroid hormone receptors. FASEB J 6:2524–2529, 1992.
38. M Truss, M Beato. Steroid hormone receptors: interaction with deoxyribonucleic acid and transcription factors. Endocr Rev 14:135–143, 1993.
39. M Beato, P Herrlich, G Schütz. Steroid hormone receptors: many actors in search of a plot. Cell 83:851–857, 1995.

40. J Kato, T Onouchi, S Okinaga, M Takamatsu. The ontogeny of cytosol and nuclear progestin receptors in male rat brain and its male–female differences. J Steroid Biochem 20:147–152, 1984.
41. J Kato, S Hirata, A Nozawa, N Mouri. The ontogeny of gene expression of progestin receptors in the female rat brain. J Steroid Biochem Mol Biol 47:173–182, 1993.
42. I Camacho-Arroyo, G Pérez-Palacios, AM Pasapera, MA Cerbon. Intracellular progesterone receptors are differentially regulated by sex steroid hormones in the hypothalamus and the cerebral cortex of the rabbit. J Steroid Biochem Mol Biol 50:299–303, 1994.
43. PJ Shughrue, WE Stumpf, W Elger, PE Schulze, M Sar. Progestin receptor cells in mouse cerebral cortex during early postnatal development: a comparison with preoptic area and central hypothalamus using autoradiography with [^{125}I]progestin. Dev Brain Res 59:143–155, 1991.
44. WL Kraus, MM Montano, BS Katzenellenbogen. Cloning of the rat progesterone receptor gene 5′-region and identification of two functionally distinct promoters. Mol Endocrinol 7:1603–1616, 1993.
45. J Kato, S Hirata, A Nozawa, N Yamada-Mouri. Gene expression of progesterone receptor isoforms in the rat brain. Horm Behav 28:454–463, 1994.
46. R Guennoun, JM Gasc. Estrogen-independent and estrogen-induced progesterone receptors and their regulation by progestins in the hypothalamus and pituitary of the chick embryo: an immunohistochemical study. Dev Brain Res 55:151–159, 1990.
47. R Guennoun, M Reyss-Brion, JM Gasc. Progesterone receptors in hypothalamus and pituitary during the embryonic development of the chick: regulation by sex steroid hormones. Dev Brain Res 37:1–9, 1987.
48. J Kato, T Onouchi, M Takamatsu. Decreased progestin receptors in the cerebral cortex of hypothyroid postnatal rats. J Steroid Biochem 20:817–819, 1984.
49. S Hirata, N Mouri-Yamada, J Kato. Effects of hypothyroidism on the gene expression of progesterone receptors in the neonatal rat brain. J Steroid Biochem Mol Biol 50:293–297, 1994.
50. RJ Sterling, JM Gasc, PJ Sharp, P Tuohimaa, EE Baulieu. Absence of nuclear progesterone receptor in LH releasing hormone neurones in laying hens. J Endocrinol 102:R5–R7, 1984.
51. VB Mahesh, DW Brann. Interaction between ovarian and adrenal steroids in the regulation of gonadotropin secretion. J Steroid Biochem Mol Biol 41:495–513, 1992.
52. I Jung-Testas, M Renoir, H Bugnard, G Greene, EE Baulieu. Demonstration of steroid hormone receptors and steroid action in primary cultures of rat glial cells. J Steroid Biochem Mol Biol 41:621–631, 1992.
53. RS Carroll, J Zhang, K Dashner, M Sar, PM Black. Steroid hormone receptors in astrocytic neoplasms. Neurosurgery 37:496–503, 1995.
54. NG Weiland, M Orchinik. Specific subunit mRNAs of the $GABA_A$ receptor are regulated by progesterone in subfields of the hippocampus. Mol Brain Res 32:271–278, 1995.

55. SS Smith, QH Gong, FC Hsu, RS Markowitz, JMH ffrench-Mullen, X Li. $GABA_A$ receptor $\alpha 4$ subunit suppression prevents withdrawal properties of an endogenous steroid. Nature 392:926–930, 1998.
56. AM Turzillo, CE Campion, CM Clay, TM Nett. Regulation of gonadotropin-releasing hormone (GnRH) receptor messenger ribonucleic acid and GnRH receptors during the early preovulatory period in the ewe. Endocrinology 135:1353–1358, 1994.
57. L Zhou, RP Hammer Jr. Gonadal steroid hormones upregulate medial preoptic μ-opioid receptors in the rat. Eur J Pharmacol 278:271–274, 1995.
58. SG Kohama, CL Bethea. Steroid regulation of tyrosine hydroxylase messenger ribonucleic acid in dopaminergic subpopulations of monkey hypothalamus. Endocrinology 136:1790–1800, 1995.
59. M Castren, VK Patchev, OF Almeida, F Holsboer, T Trapp, E Castren. Regulation of rat mineralocorticoid receptor expression in neurons by progesterone. Endocrinology 136:3800–3806, 1995.
60. JC Webster, NR Pedersen, DP Edwards. The 5′-flanking region of the ovine follicle-stimulating hormone-beta gene contains six progesterone response elements: three proximal elements are sufficient to increase transcription in the presence of progesterone. Endocrinology 136:1049–1058, 1995.
61. DW Pfaff, MM Freidin, XS Wu-Peng, J Yin, YS Zhu. Competition for DNA steroid response elements as a possible mechanism for neuroendocrine integration. J Steroid Biochem Mol Biol 49:373–379, 1994.
62. RE Scott, XS Wu-Peng, PM Yen, WW Chin, DW Pfaff. Interactions of estrogen- and thyroid hormone receptors on a progesterone receptor estrogen response element (ERE) sequence: a comparison with the vitellogenin A2 consensus ERE. Mol Endocrinol 11:1581–1592, 1997.
63. P Herrlich, H Ponta. Mutual cross-modulation of steroid/retinoic acid receptor and AP-1 transcription factor activities. Trends Endocrinol Metab 5:341–346, 1994.
64. E Marbaix, J Donnez, PJ Courtoy, Y Eeckhout. Progesterone regulates the activity of collagenase and related gelatinases A and B in human endometrial explants. Proc Natl Acad Sci USA 89:11789–11793, 1992.
65. JI Morgan, T Curran. Stimulus-transcription coupling in the nervous system: involvement of the inducible proto-oncogenes *fos* and *jun*. Annu Rev Neurosci 14:421–451, 1991.
66. SS Smith, BD Waterhouse, DJ Woodward. Locally applied progesterone metabolites alter neuronal responsiveness in the cerebellum. Brain Res Bull 18:739–747, 1987.
67. SS Smith, BD Waterhouse, JK Chapin, DJ Woodward. Progesterone alters GABA and glutamate responsiveness: a possible mechanism for its anxiolytic effect. Brain Res 400:353–359, 1987.
68. SGA Alivisatos, G Deliconstantinos, GP Theodosiadis. Specificity of binding of cholesterol, steroid hormones and other compounds in synaptosomal plasma membranes and their effect on ouabaine-sensitive ATPase. Biochim Biophys Acta 643:650–658, 1981.

69. DS Heron, M Shinitzky, M Hershkowitz, D Samuel. Lipid fluidity markedly modulates the binding of serotonin to mouse membranes. Proc Natl Acad Sci USA 77:7463–7467, 1980.
70. AC Towle, PY Sze. Steroid binding of synaptic plasma membrane: differential binding of glucocorticoids and gonadal steroids. J Steroid Biochem 18:135–143, 1983.
71. SA Tishkau, VD Ramirez. A specific membrane binding for progesterone in rat brain: sex differences and induction by estrogen. Proc Natl Acad Sci USA 90:1285–1289, 1993.
72. JD Caldwell, CH Walker, BM Faggin, RB Carr, CA Pedersen, GA Mason. Characterization of progesterone-3-[^{125}I] binding sites in the medial preoptic area and anterior hypothalamus. Brain Res 693:225–232, 1995.
73. E Falkenstein, C Meyer, C Eisen, PC Scriba, M Wehling. Full-length cDNA sequence of a progesterone membrane-binding protein from porcine vascular smooth muscle cells. Biochem Biophys Res Commun 229:86–89, 1996.
74. JD Blaustein, MN Lehman, JC Turcotte, G Greene. Estrogen receptors in dendrites and axon terminals in the guinea pig hypothalamus. Endocrinology 131: 281–290, 1992.
75. JD Blaustein. Estrogen receptors in neurons: new subcellular locations and functional implications. Endocr J 2:249–258, 1994.
76. JMH ffrench-Mullen, P Danks, KT Spence. Neurosteroids modulate calcium currents in hippocampal CA1 neurons via a pertussis toxin-sensitive G protein-coupled mechanism. J Neurosci 14:1963–1977, 1994.
77. AM Costa, KT Spence, CR Plata-Salaman, JMH ffrench-Mullen. Residual Ca^{2+} channel current modulation by megestrol acetate via a G-protein alpha s-subunit in rat hypothalamic neurones. J Physiol 487:291–303, 1995.
78. L Zylinska, E Rebas, E Gromadzinska, L Lachowicz. Neuroactive steroids modulate in vivo the Mg^{2+}/Ca^{2+}-ATPase activity in rat cortical and cerebellar synaptosomal membranes. Biochem Biophys Res Commun 212:178–183, 1995.
79. CL Fraser, RA Swanson. Female sex hormones inhibit volume regulation in rat brain astrocyte culture. Am J Physiol 267:C909–C914, 1994.
80. MD Majewska, NL Harrison, RD Schwartz, JL Barker, SM Paul. Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. Science 232:1004–1007, 1986.
81. MD Majewska. Neurosteroids: endogenous bimodal modulators of the $GABA_A$ receptor. Mechanism of action and physiological significance. Prog Neurobiol 38:379–395, 1992.
82. SM Paul, RH Purdy. Neuroactive steroids. FASEB J 6:2311–2322, 1992.
83. G Puia, MR Santi, S Vicini, DB Pritchett, RH Purdy, SM Paul, PH Seeburg, E Costa. Neurosteroids act on recombinant human $GABA_A$ receptors. Neuron 4:759–765, 1990.
84. NC Lan, JS Chen, D Belelli, DB Pritchett, PH Seeburg, KW Gee. A steroid recognition site is functionnally coupled to an expressed $GABA_A$–benzodiazepine receptor. Eur J Pharmacol 188:403–406, 1990.

85. R Shingai, ML Sutherland, EA Barnard. Effects of subunit types of the cloned $GABA_A$ receptor on the response to a neurosteroid. Eur J Pharmacol 206:77–80, 1991.
86. RB Carter, PL Wood, S Weiland, JE Hawkinson, D Belelli, JJ Lambert, HS White, HF Wolf, S Mirsadeghi, SH Tahir, MB Bolger, NC Lan, KW Gee. Characterization of the anticonvulsant properties of ganaxolone (CCD 1042; 3α-hydroxy-3β-methyl-5α-pregnan-20-one), a selective, high-affinity, steroid modulator of the γ-aminobutyric acid (A) receptor. J Pharmacol Exp Ther 280:1284–1295, 1997.
87. G Puia, I Ducic, S Vicini, E Costa. Does neurosteroid modulatory efficacy depend on $GABA_A$ receptor subunit composition? Receptors Channels 1:135–142, 1993.
88. A Chvatal, H Kettenman. Effects of steroids on γ-aminobutyrate-induced currents in cultured rat astrocytes. Pflügers Arch 419:263–266, 1991.
89. WJ Zhu, JF Wang, KE Krueger, S Vicini. δ Subunit inhibits neurosteroid modulators of $GABA_A$ receptors. J Neurosci 16:6648–6656, 1996.
90. PA Davies, MC Hannah, TG Hales, EF Kirkness. Insensitivity to anaesthetic agents conferred by a class of $GABA_A$ receptor subunit. Nature 385:820–823, 1997.
91. M El-Etr, Y Akwa, P Robel, EE Baulieu. Opposing effects of different steroid sulfates on $GABA_A$ receptor-mediated chloride uptake. Brain Res 790:334–338, 1998.
92. MD Majewska, JC Bisserbe, RL Eskay. Glucocorticoids are modulators of $GABA_A$ receptors in the brain. Brain Res 33:178–182, 1985.
93. MD Majewska, RD Schwartz. Pregnenolone sulfate: an antagonist of the γ-aminobutyric acid receptor complex in brain? Brain Res 404:355–360, 1987.
94. MD Majewska, JM Mienville, S Vicini. Neurosteroid pregnenolone sulfate antagonizes electrophysiological responses to GABA in neurons. Neurosci Lett 90:279–284, 1988.
95. FS Wu, TT Gibbs, DH Farb. Inverse modulation of γ-aminobutyric acid- and glycine-induced currents by progesterone. Mol Pharmacol 37:597–602, 1990.
96. FS Wu, TT Gibbs, DH Farb. Pregnenolone sulfate: a positive allosteric modulator at the *N*-methyl-D-aspartate receptor. Mol Pharmacol 40:333–336, 1991.
97. H Meziane, C Mathis, SM Paul, A Ungerer. The neurosteroid pregnenolone sulfate reduces learning induced by scopolamine and has promnesic effects in mice performing an appetitive learning task. Psychopharmacology 126:323–330, 1996.
98. M Park-Chung, FS Wu, DH Farb. 3α-hydroxy-5β-pregnan-20-one sulfate: a negative modulator of the NMDA-induced current in cultured neurons. Mol Pharmacol 46:146–150, 1994.
99. FP Monnet, V Mahé, P Robel, EE Baulieu. Neurosteroids, via σ receptors, modulate the [^{3}H]norepinephrine release evoked by *N*-methyl-D-aspartate in the rat hippocampus. Proc Natl Acad Sci USA 92:3774–3778, 1995.
100. RJ Cabrera, C Bregonzio. Turnover rate and stimulus-evoked release of dopamine by progesterone and *N*-methyl-D-aspartic acid in rat striatum during pregnancy. Eur J Pharmacol 317:55–59, 1996.

101. R Bergeron, C de Montigny, G Debonnel. Potentiation of neuronal NMDA response induced by dehydroepiandrosterone and its suppression by progesterone: effects mediated via sigma receptors. J Neurosci 16:1193–1202, 1996.
102. S Valera, M Ballivet, D Bertrand. Progesterone modulates a neuronal nicotinic acetylcholine receptor. Proc Natl Acad Sci USA 89:9949–9953, 1992.
103. AE Bullock, AL Clark, SR Grady, SF Robinson, BS Slobe, MJ Marks, AC Collins. Neurosteroids modulate nicotinic receptor function in mouse striatal and thalamic synaptosomes. J Neurochem 68:2412–2423, 1997.
104. B Klangkalya, A Chan. Inhibition of hypothalamic and pituitary muscarinic receptor binding by progesterone. Neuroendocrinology 47:294–302, 1988.
105. E Grazzini, G Guillon, B Mouillac, HH Zingg. Inhibition of oxytocin receptor function by direct binding of progesterone. Nature 392:509–512, 1998.
106. DE Dluzen, VD Ramirez. Progesterone effect upon dopamine release from the corpus striatum of female rats. II. Evidence for a membrane site of action and the role of albumin. Brain Res 476:338–344, 1989.
107. RJ Cabrera, CE Navarro. Progesterone increases NMDA-evoked [^{3}H]dopamine release from striatal slices in proestrus rats. Neuropharmacology 35:175–178, 1996.
108. E Tauboll, OP Ottersen, L Gjerstad. The progesterone metabolite 5α-pregnan-3α-ol-20-one reduces K^+-induced GABA and glutamate release from identified nerve terminals in rat hippocampus: a semi-quantitative immunocytochemical study. Brain Res 623:329–333, 1993.
109. L Tapia-Arancibia, M André, J Belmar, S Arancibia. Neuroactive steroids modulate GABA inhibition of hypothalamic somatostatin release. Neuroreport 6:1927–1931, 1995.
110. L Dazzi, A Sanna, E Cagetti, A Concas, G Biggio. Inhibition by the neurosteroid allopregnanolone of basal and stress-induced acetylcholine release in the brain of freely moving rats. Brain Res 710:275–280, 1996.
111. M Vallée, W Mayo, M Darnaudéry, C Corpéchot, J Young, M Koehl, M Le Moal, EE Baulieu, P Robel, H Simon. Neurosteroids: deficient cognitive performance in aged rats depends on low pregnenolone sulfate levels in the hippocampus. Proc Natl Acad Sci USA 94:14865–14870, 1997.
112. N Petitti, AM Etgen. Progesterone promotes rapid desensitization of alpha-1-adrenergic receptor augmentation of cAMP formation in rat hypothalamic slices. Neuroendocrinology 55:1–8, 1992.
113. R Ravindra, RS Aronstam. Progesterone, testosterone and estradiol-17 beta inhibit gonadotropin-releasing hormone stimulation of G protein GTPase activity in plasma membranes from rat anterior pituitary lobe. Acta Endocrinol 126:345–349, 1992.
114. G Gu, AA Rojo, MC Zee, J Yu, RB Simerly. Hormonal regulation of CREB phosphorylation in the anteroventral periventricular nucleus. J Neurosci 16:3035–3044, 1996.
115. Z Yang, AT Lim. Progesterone, but not estrogen, modulates the cAMP system mediated ir-beta-endorphin secretion and POMC mRNA expression from rat hypothalamic cells in culture. Brain Res 678:1251–258, 1995.

116. CA Beck, NL Weigel, ML Moyer, SK Nordeen, DP Edwards. The progesterone receptor antagonist RU486 acquires agonist activity upon stimulation of cAMP signaling pathways. Proc Natl Acad Sci USA 90:4441–4445, 1993.
117. SM Aronica, BS Katzenellenbogen. Progesterone receptor regulation in uterine cells: stimulation by estrogen, cyclic adenosine 3′,5′-monophosphate, and insulin-like growth factor I and suppression by antiestrogens and protein kinase inhibitors. Endocrinology 128:2045–2052, 1991.
118. RF Power, SK Manni, J Codina, OM Conneely, BW O'Malley. Dopaminergic and ligand-independent activation of steroid hormone receptors. Science 254:1636–1639, 1991.
119. SK Mani, JM Allen, JH Clark, JD Blaustein, BW O'Malley. Convergent pathways for steroid hormone- and neurotransmitter-induced rat sexual behavior. Science 265:1246–1249, 1994.
120. R Rupprecht, JMHM Reul, T Trapp, B Van Steensel, C Wetzel, K Damm, W Ziegelgänsberger, F Holsboer. Progesterone receptor-mediated effects of neuroactive steroids. Neuron 11:523–530, 1993.
121. R Rupprecht, B Berning, CAE Hauser, F Holsboer, JMHM Reul. Steroid receptor-mediated effects of neuroactive steroids: characterisation of structure–activity relationship. Eur J Pharmacol 303:227–234, 1996.
122. SA Kliewer, JT Moore, L Wade, JL Staudinger, MA Watson, SA Jones, DD McKee, BB Oliver, TM Wilson, RH Zetterstrom, T Perlmann, JM Lehmann. An orphan nuclear receptor activated by pregnanes defines a novel steroid signaling pathway. Cell 92:73–82, 1998.
123. VB Mahesh, DW Brann. Steroid control of hypothalamic function in reproduction. In: TW Stone, ed. CNS Neurotransmitters and Neuromodulators: Neuroactive Steroids. Boca Raton, FL, CRC Press, 1996, pp 177–209.
124. A Caraty, FJ Karsch, A Herbison, G Bruneau, B Delaleu, G Venier, C Fabre-Nys. Neuroendocrine control of ovulation in sheep. Ann Endocrinol 56:539–542, 1995.
125. IM Rao, VB Mahesh. Role of progesterone in the modulation of the preovulatory surge of gonadotropins and ovulation in the pregnant mare's serum gonadotropin-primed immature rat and adult rat. Biol Reprod 35:1154–1161, 1986.
126. C Beyer, O Gonzalez-Flores, G Gonzalez-Mariscal. Progesterone receptor participates in the stimulatory effect of LHRH, prostaglandin E_2, and cyclic AMP on lordosis and proceptive behaviours in rats. J Neuroendocrinol 9:609–614, 1997.
127. JL Turgeon, DW Waring. Rapid increase by progesterone of agonist-stimulated luteinizing hormone secretion by cultured pituitary cells. Endocrinology 127: 773–780, 1990.
128. LA Lesoon, VB Mahesh. Stimulatory and inhibitory effects of progesterone on FSH secretion by the anterior pituitary. J Steroid Biochem Mol Biol 42:479–491, 1992.
129. LC Krey, F Kamel. Progesterone modulation of gonadotropin secretion by dispersed rat pituitary cells in culture. I. Basal and gonadotropin-releasing hormone-stimulated luteinizing hormone release. Mol Cell Endocrinol 68:85–94, 1990.

130. LC Krey, F Kamel. Progesterone modulation of gonadotropin secretion by dispersed rat pituitary cells in culture. III. A23187, cAMP, phorbol ester and DiC8-stimulated luteinizing hormone release. Mol Cell Endocrinol 70:21–29, 1990.
131. G Emons, J Nill, R Sturm, O Ortmann. Effects of progesterone on GnRH receptor concentration in cultured estrogen-primed female rat pituitary cells. J Steroid Biochem Biol Mol 42:831–839, 1992.
132. DW Brann, CD Putnam, VB Mahesh. γ-Aminobutyric acid A receptors mediate 3α-hydroxy-5α-pregnan-20-one-induced gonadotropin secretion. Endocrinology 126:1854–1859, 1990.
133. LL Murphy, VB Mahesh. Selective release of follicle-stimulating hormone by 5α-dihydroprogesterone in immature ovariectomized estrogen-primed rats. Biol Reprod 30:594–602, 1984.
134. S Dhanvantari, JP Wiebe. Suppression of follicle-stimulating hormone by the gonadal- and neurosteroid 3α-hydroxy-4-pregnen-20-one involves actions at the level of the gonadotrope membrane/calcium channel. Endocrinology 134:371–376, 1994.
135. JP Wiebe. Nongenomic actions of steroids on gonadotropin release. Recent Prog Horm Res 52:71–101, 1997.
136. SP Kalra. Mandatory neuropeptide-steroid signaling for the preovulatory luteinizing hormone-releasing hormone discharge. Endocrine Rev 14:507–538, 1993.
137. JE Levine, V Ramirez. Luteinizing hormone-releasing hormone release during the rat estrous cycle and after ovariectomy as estimated with push–pull cannulae. Endocrinology 111:1439–1448, 1982.
138. SP Kalra, PS Kalra. Neural regulation of LH secretion in the rat. Endocr Rev 4:311–351, 1983.
139. K Kim, B Lee, Y Park, W Cho. Progesterone increases mRNA encoding LHRH level in the hypothalamus of ovariectomized estradiol-primed prepubertal rats. Mol Brain Res 6:151–158, 1989.
140. DW Brann, JK McDonald, CD Putnam, VB Mahesh. Regulation of hypothalamic gonadotropin-releasing hormone and neuropeptide Y concentrations by progesterone and corticosteroids in immature rats: correlation with luteinizing hormone and follicle-stimulating hormone release. Neuroendocrinology 54:425–432, 1991.
141. WS Lee, MS Smith, GE Hoffman. Progesterone enhances the surge of luteinizing hormone by increasing the activation of luteinizing hormone-releasing hormone neurons. Endocrinology 127:2604–2606, 1990.
142. K Kim, VD Ramirez. In vitro luteinizing hormone-releasing hormone release from superfused rat hypothalami: site of action of progesterone and effect of estrogen priming. Endocrinology 116:252–258, 1985.
143. WW Lin, VD Ramirez. Infusion of progestins into the hypothalamus of female New Zealand white rabbits: effect on in vivo luteinizing hormone-releasing hormone release as determined with push–pull perfusion. Endocrinology 126:261–272, 1990.
144. E Terasawa. Steroid modulation of pulsatile LHRH release in the rhesus monkey. Horm Behav 28:406–416, 1994.

145. JL O'Conner, VB Mahesh. A possible role for progesterone in the preovulatory gonadotropin surge through modulation of LHRH degrading activity. J Steroid Biochem 29:257–263, 1988.
146. DW Brann, VB Mahesh. Detailed examination of the mechanism and site of action of progesterone and corticosteroids in the regulation of gonadotropin secretion: hypothalamic gonadotropin-releasing hormone and catecholamine involvement. Biol Reprod 44:1005–1015, 1991.
147. AP Auger, JD Blaustein. Progesterone increases Fos-immunoreactivity within some progestin receptor-containing neurons in localized regions of female rat forebrain. Brain Res 746:164–170, 1997.
148. SV Drouva, E Laplante, C Kordon. Progesterone-induced LHRH release in vitro is an estrogen as well as Ca^{++}- and calmodulin-dependent secretory process. Neuroendocrinology 40:325–331, 1985.
149. FC Ke, VD Ramirez. Membrane mechanism mediates progesterone stimulatory effect on LHRH release from superfused rat hypothalami in vitro. Neuroendocrinology 45:514–517, 1987.
150. M El-Etr, K Shazand, Y Akwa, P Robel, EE Baulieu. Mechanism of progesterone-evoked release of gonadotropin-releasing hormone (GnRH) from GT1-1 neurons. Soc Neurosci Abstr 22, 625.3, p 1590, 1996.
151. M El-Etr, Y Akwa, RJ Fiddes, P Robel, EE Baulieu. A progesterone metabolite stimulates the release of gonadotropin-releasing hormone from GT1-1 hypothalamic neurons via the gamma-aminobutyric acid type A receptor. Proc Natl Acad Sci USA 92:3769–3773, 1995.
152. JE Levine. New concepts of the neuroendocrine regulation of gonadotropin surges in rats. Biol Reprod 56:293–302, 1997.
153. DJ Dierschke, T Yamaji, FJ Karsch, RF Weick, G Weiss, E Knobil. Blockade by progesterone of estrogen-induced LH and FSH release in the rhesus monkey. Endocrinology 92:1496–1501, 1973.
154. L Wildt, JS Hutchinson, G Marshall, CR Pohl, E Knobil. On the site of action of progesterone in the blockade of estradiol-induced gonadotropin discharge in the rhesus monkey. Endocrinology 109:1293–1294, 1981.
155. JZ Kasa-Vubu, GE Dahl, NP Evans, LA Thrun, SM Moenter, V Padmanabhan, FJ Karsch. Progesterone blocks the estradiol-induced gonadotropin discharge in the ewe by inhibiting the surge of gonadotropin-releasing hormone. Endocrinology 13:208–212, 1992.
156. SK Batra, WL Miller. Progesterone decreases the responsiveness of ovine pituitary cultures to luteinizing hormone-releasing hormone. Endocrinology 117: 1436–1440, 1985.
157. KT O'Byrne, JC Thalabard, PM Grosser, RC Wilson, CL Williams, MD Chen, D Ladendorf, J Hotchkiss, E Knobil. Radiotelemetric monitoring of hypothalamic gonadotropin-releasing hormone pulse generator activity throughout the menstrual cycle of the rhesus monkey. Endocrinology 129:1207–1214, 1991.
158. FJ Karsch, JT Cummins, GB Thomas, IJ Clarke. Steroid feedback inhibition of pulsatile secretion of gonadotropin-releasing hormone in the ewe. Biol Reprod 36:1207–1218, 1987.

159. FJ Karsch, SL Legan, KD Ryan, DL Foster. Importance of estradiol and progesterone in regulating LH secretion and estrous behavior during the sheep estrous cycle. Biol Reprod 23:404–413, 1980.
160. RF Williams, K Gordon, H Fung, P Kolm, GD Hodgen. Hypothalamo-pituitary effects of RU 486: inhibition of progesterone-induced hyperprolactinaemia. Hum Reprod 9(suppl1):63–68, 1994.
161. SH Yen, JT Pan. Progesterone advances the diurnal rhythm of tubero-infundibular dopaminergic neuronal activity and the prolactin surge in ovariectomized, estrogen-primed rats and intact proestrus rats. Endocrinology 139:1602–1609, 1998.
162. RW Caron, RP Deis. Estradiol implants in the arcuate nucleus induce lactogenesis in virgin rats. Role of progesterone. Life Sci 62:229–237, 1998.
163. M Vincens, C Shu, M Moguilewsky, D Philibert. A progesterone metabolite enhances the activity of the $GABA_A$ receptor complex at the pituitary level. Eur J Pharmacol 168:15–21, 1989.
164. BS McEwen, CS Woolley. Estradiol and progesterone regulate neuronal structure and synaptic connectivity in adult as well as developing brain. Exp Gerontol 29:431–436, 1994.
165. J Perez, S Luquin, F Naftolin, LM Garcia-Segura. The role of estradiol and progesterone in phased synaptic remodelling of the rat arcuate nucleus. Brain Res 608:38–44, 1993.
166. JE Bottenstein, GH Sato. Growth of a rat neuroblastoma cell line in serum-free supplemented medium. Proc Natl Acad Sci USA 76:514–517, 1979.
167. RD Brinton. The neurosteroid 3α-hydroxy-5α-pregnan-20-one induces cytoarchitectural regression in cultured fetal hippocampal neurons. J Neurosci 14:2763–2774, 1994.
168. RL Roof, R Duvdevani, L Braswell, DG Stein. Progesterone facilitates cognitive recovery and reduces secondary neuronal loss caused by cortical contusion injury in male rats. Exp Neurol 129:64–69, 1994.
169. V Limmroth, WS Lee, MA Moskowitz. $GABA_A$-receptor-mediated effects of progesterone, its ring-A-reduced metabolites and synthetic neuroactive steroids on neurogenic oedema in the rat meninges. Br J Pharmacol 117:99–104, 1996.
170. CE Weaver Jr, P Marek, M Park-Chung, SW Tam, DH Farb. Neuroprotective activity of a new class of steroidal inhibitors of the *N*-methyl-D-aspartate receptor. Proc Natl Acad Sci USA 94:10450–10454, 1997.
171. RL Roof, SW Hoffman, DG Stein. Progesterone protects against lipid peroxidation following traumatic brain injury in rats. Mol Chem Neuropathol 31:1–11, 1997.
172. J Garcia-Estrada, JA Del Rio, S Luquin, LM Garcia-Segura. Gonadal hormones down-regulate reactive gliosis and astrocyte proliferation after a penetrating brain injury. Brain Res 628:271–278, 1993.
173. LM Garcia-Segura, S Luquin, A Parducz, F Naftolin. Gonadal hormone regulation of glial fibrillary acidic protein immunoreactivity and glial ultrastructure in the rat neuroendocrine hypothalamus. Glia 10:59–69, 1994.
174. S Ganter, H Northoff, D Mannel, PJ Gebicke-Harter. Growth control of cultured microglia. J Neurosci Res 33:218–230, 1992.

175. R Morfin, J Toung, C Corpéchot, B Egestad, J Sjövall, EE Baulieu. Neurosteroids: pregnenolone in human sciatic nerves. Proc Natl Acad Sci USA 89:6790–6793, 1992.
176. Y Akwa, M Schumacher, I Jung-Testas, EE Baulieu. Neurosteroids in rat sciatic nerves and Schwann cells. C R Acad Sci Paris 316:410–414, 1993.
177. HL Koenig, M Schumacher, B Ferzaz, AN Do Thi, A Ressouches, R Guennoun, I Jung-Testas, P Robel, Y Akwa, EE Baulieu. Progesterone synthesis and myelin formation by Schwann cells. Science 268:1500–1503, 1995.
178. M Schumacher, P Robel, EE Baulieu. Development and regeneration of the nervous system: a role for neurosteroids. Dev Neurosci 18:6–21, 1996.
179. I Jung-Testas, M Schumacher, P Robel, EE Baulieu. Action of steroid hormones and growth factors on glial cells of the central and peripheral nervous system. J Steroid Biochem Mol Biol 48:145–154, 1994.
180. J Young, C Corpéchot, M Haug, S Gobaille, EE Baulieu, P Robel. Suppressive effects of dehydroepiandrosterone and 3β-methyl-androst-5-en-17-one on attack towards lactating female intruders by castrated male mice. II. Brain neurosteroids. Biochem Biophys Res Commun 174:892–897, 1991.
181. M Haug, S Simler, L Kim, P Mandel. Studies on the involvement of GABA in the aggression directed by groups of intact or gonadectomized male and female mice towards lactating intruders. Pharmacol Biochem Behav 12:189–193, 1980.
182. IG Fraile, BS McEwen, DW Pfaff. Comparative effects of progesterone and alphaxalone on aggressive, reproductive and locomotor behaviors. Pharmacol Biochem Behav 30:729–733, 1988.
183. CA Frye, JF DeBold. 3α-OH-DHP and 5α-THDOC implants to the ventral tegmental area facilitate sexual receptivity in hamsters after progesterone priming to the ventral medial hypothalamus. Brain Res 612:130–137, 1993.
184. CA Frye, EA Leadbetter. 5-alpha-reduced progesterone metabolites are essential in hamster VTA for sexual receptivity. Life Sci 54:653–659, 1994.
185. M Kavaliers, DM Kinsella. Male preference for the odors of estrous female mice is reduced by the neurosteroid pregnenolone sulfate. Brain Res 682:222–226, 1995.
186. C Corpéchot, P Leclerc, EE Baulieu, P Brazeau. Neurosteroids: regulatory mechanisms in male rat brain during heterosexual exposure. Steroids 45:229–234, 1985.
187. H Selye. The anesthetic effect of steroid hormones. Proc Soc Exp Biol Med 46:116–121, 1941.
188. H Selye. Correlations between the chemical structure and the pharmacological actions of steroids. Endocrinology 30:437–453, 1942.
189. ES Arafat, JT Hargrove, WS Maxson, DM Desiderio, AC Wentz, RN Andersen. Sedative and hypnotic effects of oral administration of micronized progesterone may be mediated through its metabolites. Am J Obst Gynecol 159:1203–1209, 1988.
190. M Lancel, TAM Crönlein, P Müller-Preuss, F Holsboer. Pregnenolone enhances EEG delta activity during non-rapid eye movement sleep in the rat, in contrast to midazolam. Brain Res 646:85–94, 1994.

191. E Friess, H Tagaya, L Trachsel, F Holsboer, R Rupprecht. Progesterone-induced changes in sleep in male subjects. Am J Physiol 272:E885–E891, 1997.
192. M Lancel, J Faulhaber, F Holsboer, R Rupprecht. Progesterone induces changes in sleep comparable to those of agonistic $GABA_A$ receptor modulators. Am J Physiol 271:E763–E772, 1996.
193. MD Majewska, MT Bluet-Pajot, P Robel, EE Baulieu. Pregnenolone sulfate antagonizes barbiturate-induced hypnosis. Pharmacol Biochem Behav 33:701–703, 1989.
194. A Steiger, L Trachsel, J Guldner, U Hemmeter, B Rothe, R Rupprecht, H Vedder, F Holsboer. Neurosteroid pregnenolone induces sleep-EEG changes in man compatible with inverse agonistic $GABA_A$-receptor modulation. Brain Res 615:267–274, 1993.
195. WM Mok, S Herschkowitz, NR Krieger. In vivo studies identify 5α-pregnan-3α-ol-20-one as an active anesthetic agent. J Neurochem 57:1296–1301, 1991.
196. WM Mok, S Herschkowitz, NR Krieger. Evidence that 5α-pregnan-3α-ol-20-one is the metabolite responsible for anesthesia induced by 5α-pregnanedione in the mouse. Neurosci Lett 135:145–148, 1992.
197. WM Mok, C Bukusoglu, NR Krieger. 3α-hydroxy-5α-pregnan-20-one is the only active anesthetic steroid in anesthetized mouse brain. Steroids 58:112–114, 1993.
198. A Korneyev, E Costa. Allopregnanolone (THP) mediates anesthetic effects of progesterone in rat brain. Horm Behav 30:37–43, 1996.
199. H Eriksson, J Haasio, K Kortila. Comparison of eltanolone and thiopental in anaesthesia for termination of pregnancy. Acta Anaestesiol Scand 39:479–484, 1995.
200. D Bitran, M Shiekh, M McLeod. Anxiolytic effect of progesterone is mediated by the neurosteroid allopregnanolone at brain $GABA_A$ receptors. J Neuroendocrinol 7:171–177, 1995.
201. D Bitran, RJ Hilvers, CK Kellogg. Anxiolytic effects of 3α-hydroxy-5α[β]-pregnan-20-one: endogenous metabolites of progesterone that are active at the $GABA_A$ receptor. Brain Res 561:157–161, 1991.
202. S Wieland, NC Lan, S Mirasedeghi, KW Gee. Anxiolytic activity of the progesterone metabolite 5α-pregnan-3α-ol-20-one. Brain Res 565:263–268, 1991.
203. MD Brot, Y Akwa, RH Purdy, GF Koob, KT Britton. The anxiolytic-like effects of the neurosteroid allopregnanolone: interactions with the $GABA_A$ receptors. Eur J Pharmacol 325:1–7, 1997.
204. DS Reddy, SK Kulkarni. Differential anxiolytic effects of neurosteroids in the mirrored chamber behavior test in mice. Brain Res 752:61–71, 1997.
205. DS Reddy, SK Kulkarni. Neurosteroid coadministration prevents development of tolerance and augments recovery from benzodiazepine withdrawal anxiety and hyperactivity in mice. Methods Find Exp Clin Pharmacol 19:395–405, 1997.
206. VK Patchev, M Shoaib, F Holsboer, OF Almeida. The neurosteroid tetrahydroprogesterone counteracts corticotropin-releasing hormone-induced anxiety and alters the release and gene expression of corticotropin-releasing hormone in the rat hypothalamus. Neuroscience 62:265–271, 1994.

207. VK Patchev, AH Hassan, F Holsboer, OF Almeida. The neurosteroid tetrahydroprogesterone attenuates the endocrine response to stress and exerts glucocorticoid-like effects on vasopressin gene transcription in the rat hypothalamus. Neuropsychopharmacology 15:533–540, 1996.
208. DA Finn, AJ Roberts, F Lotrich, EJ Gallaher. Genetic differences in behavioral sensitivity to a neuroactive steroid. J Pharmacol Exp Ther 280:820–828, 1997.
209. M Kavaliers, JP Wiebe. Analgesic effects of the progesterone metabolite, 3-alpha-hydroxy-5-alpha-pregnan-20-one, and possible modes of action in mice. Brain Res 415:393–398, 1987.
210. CA Frye, JE Duncan. Progesterone metabolites, effective at the $GABA_A$ receptor complex, attenuate pain sensitivity in rats. Brain Res 643:194–203, 1994.
211. CA Frye, KR Van Keuren, PN Rao, MS Erskine. Analgesic effects of the neurosteroid 3α-androstanediol. Brain Res 709:1–9, 1996.
212. I Gilron, TJ Coderre. Preemptive analgesic effects of steroid anesthesia with alphaxalone in the rat formalin test. Evidence for differential $GABA_A$ receptor modulation in persistent nociception. Anesthesiology 84:572–579, 1996.
213. T Backström. Epileptic seizures in women during the menstrual cycle. Acta Neurol Scand 54:321–347, 1976.
214. T Backström, B Zetterlund, S Blom, M Romano. Effects of intravenous progesterone infusions on the epileptic discharge frequency in women with partial epilepsy. Acta Neurol Scand 69:240–248, 1984.
215. D Belelli, MB Bolger, KW Gee. Anticonvulsivant profile of the progesterone metabolite 5α-pregnan-3α-ol-20-one. Eur J Pharmacol 166:325–329, 1989.
216. A Concas, MC Mostallino, C Perra, R Lener, G Roscetti, ML Barbaccia, RH Purdy, G Biggio. Functional correlation between allopregnanolone and [^{35}S]-TBPS binding in the brain of rats exposed to isoniazid, pentylenetetrazol, or stress. Br J Pharmacol 118:839–846, 1996.
217. LL Devaud, RH Purdy, AL Morrow. The neurosteroid 3-alpha-hydroxy-5-alpha-pregnan-20-one, protects against bicuculline-induced seizures during ethanol withdrawal in rats. Alcohol Clin Exp Res 19:350–355, 1995.
218. TG Kokate, BE Svensson, MA Rogawski. Anticonvulsant activity of neurosteroids: correlation with γ-aminobutyric acid-evoked chloride current potentiation. J Pharmacol Exp Ther 270:1223–1229, 1994.
219. R Galli, S Michelini, L Bartalena, R Massetani, L Pani, L Grasso, GB Cassano, E Martino, RH Purdy, L Murri. Circulating levels of anticonvulsant metabolites of progesterone in women with partial epilepsy in the intercritical phase. Ital J Neurol Sci 17:277–281, 1996.
220. M Beekman, JT Ungard, M Gasior, RB Carter, D Dijkstra, SR Goldberg, JM Witkin. Reversal of behavioral effects of pentylenetetrazol by the neuroactive steroid ganaxolone. J Pharmacol Exp Ther 284:868–877, 1998.
221. M Gasior, RB Carter, SR Goldberg, JM Witkin. Anticonvulsant and behavioral effects of neuroactive steroids alone and in conjunction with diazepam. J Pharmacol Exp Ther 282:543–553, 1997.
222. G Pincus, H Hoagland, CH Wilson, NJ Fay. Effects on industrial production of the administration of Δ5 pregnenolone to factory workers, II. Psychosom Med 10:347–352, 1945.

223. JF Flood, JE Morley, E Roberts. Memory-enhancing effects in male mice of pregnenolone and steroids metabolically derived from it. Proc Natl Acad Sci USA 89:1567–1571, 1992.
224. RL Isaacson, PE Yoder, J Varner. The effects of pregnenolone on acquisition and retention of a food search task. Behav Neural Biol 61:170–176, 1994.
225. JE Flood, JE Morley, E Roberts. Pregnenolone sulfate enhances post-training memory processes when injected in very low doses into limbic system structures: the amygdala is by far the most sensitive. Proc Natl Acad Sci USA 92:10806–10810, 1995.
226. W Mayo, F Dellu, P Robel, J Cherkaoui, M Le Moal, EE Baulieu, H Simon. Infusion of neurosteroids into the nucleus basalis magnocellularis affects cognitive processes in the rat. Brain Res 607:324–328, 1993.
227. C Mathis, SM Paul, JN Crawley. The neurosteroid pregnenolone sulfate blocks NMDA antagonist-induced deficits in a passive avoidance memory task. Psychopharmacology 116:201–206, 1994.
228. M Morissette, T Di Paolo. Effect of chronic estradiol and progesterone treatments of ovariectomized rats on brain dopamine uptake sites. J Neurochem 60:1876–1883, 1993.
229. G Attali, A Weizman, I Gil-Ad, M Rehavi. Opposite modulatory effects of ovarian hormones on rat brain dopamine and serotonine transporters. Brain Res 756:153–159, 1997.

3

Effects of Progestin on the Breast: Regulation of Cell Cycle Progression by Progestins

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I. INTRODUCTION

Control of the development and differentiated function of reproductive organs has multiple layers of complexity. Combinations of steroid and polypeptide hormones, growth factors, and cytokines provide the signals necessary to orchestrate these processes, eliciting a suite of cell–type-specific responses. Regulation of cell proliferation is an important component of these responses. In the mammary gland, the formation of a system of branched ducts at puberty, the subsequent development of alveoli during pregnancy, and lactation, all require estrogen and progesterone. Although estrogen appears to be the main drive to proliferation, particularly in the formation of the mammary ducts, progesterone acts in concert with other hormones, particularly prolactin, to cause the alveolar development necessary for subsequent milk production. Thus, progesterone can be viewed as balancing the proliferative effects of estrogen by directing the tissue toward its normal differentiated function. However, although proliferation and differentiation are often viewed as mutually exclusive processes, the differentiative role of progesterone does not necessarily imply a wholly antiproliferative effect. The stimulation of lobuloalveolar development is a prerequisite for subsequent lactation, and increasing evidence from studies of the murine mammary gland suggests that this lobuloalveolar development results from progesterone stimulation of epithelial cell proliferation. Nevertheless, the role of progesterone in

mediating breast epithelial cell proliferation in humans remains a controversial area.

It is perhaps not surprising that the steroid hormones with a role in the development of the normal breast have been implicated in the etiology of breast cancer, raising the possibility that exposure to exogenous steroid hormones through the use of oral contraceptives and hormone replacement therapies (HRTs) might influence the risk of developing such cancers. Indeed, there appears to be a slight increase in risk in recent or current users (1,2), but the risk of diagnosis of breast cancer associated with estrogen use does not appear to be reduced by the addition of progestin (3), in marked contrast with the protective effects of progestins against the risk of endometrial cancer. However, synthetic progestins have an established role in the therapy of both breast and endometrial cancers (4–6). The mechanism for the antitumor action of progestins is unknown, but inhibition of breast cancer cell proliferation is a likely contributor. Despite these issues and the role of progesterone in normal mammary development and differentiation, the effects of progesterone and synthetic progestins on cell proliferation have not been widely studied from a mechanistic viewpoint. However, recent research, summarized in the following, has focused on their regulation of critical cell cycle events that determine entry into, progression through, and exit from, the cell cycle.

II. PROGESTIN EFFECTS ON CELL CYCLE PROGRESSION

Several experimental models have been used in studies of the hormonal control of cell proliferation and differentiation. Early studies of the mouse uterus provided insight into the cell kinetic basis of progestin action. Whereas prior administration of progesterone or simultaneous administration of estrogen and progesterone completely blocked the mitogenic response to estrogen in uterine epithelium, no inhibition was seen when progesterone was given 2.5 h after estradiol (7,8). Because estrogen induces synchronous progression of resting—G_0 phase—uterine epithelial cells through the cell cycle, it was concluded that progesterone inhibited cell proliferation by an action early in the G_1 phase and was without effect on progression through late G_1, S, and G_2 phases. In contrast, in the uterine stroma progesterone appears to stimulate G_0 cells to enter the cell cycle where estrogen accelerates their passage through a round of replication by shortening G_1 phase. [Various phases of the cell cycle are: G_0, quiescence; S, DNA synthesis; M, mitosis (see Fig. 1).]

These observations identified critical issues for consideration in defining the molecular basis of progestin effects on cell proliferation. In particular, the complexity of the response was illustrated by observations on cell type specificity

of progestin responsiveness (i.e., between endometrial epithelium and endometrial stroma) and the dependence of the proliferative response on the temporal relation between administration of estradiol and progesterone. Perhaps more importantly from a mechanistic viewpoint, the data on cell cycle kinetics illustrated the inhibition of cell cycle progression in early G_1 phase in epithelial cells and activation of G_0 stromal cells, and identified that progestins have both stimulatory and inhibitory effects on target cell proliferation (9). These studies laid the foundation for later studies of breast epithelium in vitro and in vivo.

A. Breast Cancer Cells In Vitro

Studies with breast cancer cells in culture led to conclusions similar to those from studies of the endometrium, perhaps surprisingly, given the differences in the physiological response to progestin in the uterus and mammary gland. The earliest studies demonstrated direct growth inhibitory effects of synthetic progestins on proliferation in progesterone receptor-positive human breast cancer cell lines (10,11), consistent with the efficacy of progestins as therapy for breast cancer. Subsequently, it was demonstrated that the growth inhibition was accompanied by marked changes in cell cycle phase distribution during the first cell cycle of exposure (12). Little change was evident over the first 12 h of exposure to progestin, but from 12 to 24 h, cells accumulated in G_1 phase with a corresponding decrease in the proportion of cells in S and G_2 plus M phases. Cell numbers in both control and progestin-treated cultures approximately doubled after 24 h of treatment, although thereafter, the cell number in progestin-treated cultures increased little (12). Thus, the majority of cells were capable of progressing through the cell cycle and dividing once in the presence of progestin. In the short-term, these cells did not replicate further, although there was a concentration-dependent slow resumption of cell cycle progression after extended exposure (12). These data are compatible with an early G_1 site of action for progestin inhibition in breast cancer cells, such that cells that have passed the arrest point complete a round of replication, but are arrested upon reentry into G_1 phase (Fig. 1). However, because a decrease in S phase entry was not evident until after a time longer than the mean duration of G_1 phase, it was postulated that there was a delay of 4.5 h or more after addition of progestin before the inhibition of cell proliferation began (12). Subsequent experiments with synchronized cells have confirmed that there is a delay of several hours duration between administration of progestin and inhibition of cell cycle progression, although progestin effects on other parameters are apparent within 30 min. Thus, growth inhibition appears to depend on the completion of an initial progestin-induced process which, in turn, leads to actions on cell cycle regulatory pathways. The delay is unlikely to be due to induction of transforming growth factor-β (TGF-β), which is growth inhibitory for breast cancer cells, because

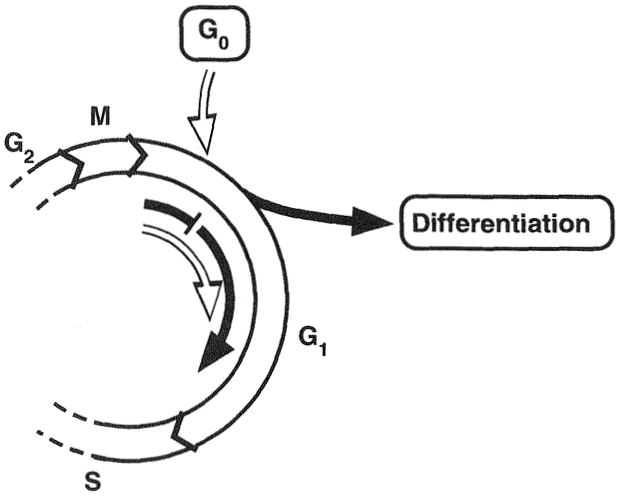

Figure 1 Cell cycle phase specificity of progestin effects on mammary epithelial cells. Four phases of the cell cycle—G_1, DNA synthesis or S phase, G_2, and mitosis (M)—are illustrated. Solid arrows indicate progestin inhibition of cell cycle progression in early G_1 phase, progestin acceleration of G_1 transit later in G_1 phase, and progestin-induced exit from the cell cycle to enter an irreversible program of cell differentiation. Open arrows indicate estrogen induction of reentry to the cell cycle from a resting G_0 state and estrogen-induced stimulation of G_1 phase progression.

there is no correlation between growth inhibition by progestins and production of, or sensitivity to, TGF-β (13). Given the physiological effects of progestins, one possibility for this, as yet undefined, process is initiation of a differentiation program.

Experiments using breast cancer cells proliferating under conditions for which either stimulation or inhibition could be detected, indicated that both responses to progestin treatment could occur in the one cell type (14). During initial exposure the proportion of cells entering S phase increased as a result of progestin-induced acceleration through G_1 phase (see Fig. 1), partially synchronizing a cohort of cells that then completed a round of replication. Consistent with the earlier experiments, cells originally in S or G_2 plus M continued through the cell cycle, but were arrested in G_1 phase at the beginning of the next cell cycle. Thus, the initial stimulation was transient, with the later inhibition predominating, and together, these responses resulted in a biphasic change in the proportion of cells undergoing DNA synthesis. Addition of an antiprogestin, mifepristone (RU 486), to progestin-treated cells completely inhibited both progestin-mediated stimulation and inhibition, provided the delay before

addition of antiprogestin was 3 h (14). With increasingly longer delay, the inhibition of progestin effects was reduced, such that after 7 h or more the addition of antiprogestin had little effect. Thus, within about 5 h, cells become committed to either stimulation or inhibition, and the presence of progestin is no longer required. Despite the presence of functional receptor and the rapid metabolism of progesterone, progesterone-inhibited cells are resistant to further stimulation by progestin for approximately 72 h (15), a time similar to that taken for spontaneous resumption of cell cycle progression in this experimental design (15). However, progesterone-inhibited cells become sensitive to the mitogenic effects of epidermal growth factor (EGF) much earlier, within 46 h (15), suggesting that this is a specific resistance to progestins. These data suggest that cyclic exposure to progesterone in vivo may lead to successive single rounds of proliferation.

Although some experimental protocols emphasize one component of the progestin response (i.e., in slowly proliferating cells stimulation is more pronounced, but in rapidly proliferating cells inhibition is more pronounced), the two effects display similar concentration dependence. It is as yet unclear whether the two effects are mediated by independent mechanisms. If this is true, the transient increase in cell cycle progression might arise from a necessity for DNA replication before full expression of a differentiated phenotype. Preliminary experiments using synchronized breast cancer cells indicate that arrest can occur in cells that have not completed S phase in the presence of progestin, arguing that there are indeed two distinct processes. If so, differential expression of the stimulatory and inhibitory pathways in different cell types may help explain the apparently paradoxical effects of progestins on cell proliferation in different target tissues. However, these data do not exclude the possibility that in vivo progestins stimulate proliferation to allow subsequent differentiation.

B. Breast and Mammary Epithelium In Vivo

The data outlined in the foregoing suggest that progestins may have only a transient proliferative effect on human breast epithelium in vivo, although this has yet to be demonstrated. Indeed, evidence for a proliferative effect in humans is somewhat circumstantial. Studies in which mitoses were evaluated histologically in breast biopsies showed that both mitosis and cell loss through apoptosis varied in a cyclic manner during the menstrual cycle, with mitoses being maximal on cycle days 23–26. The effect coincides with, or immediately follows, a rise on day 16 and a peak on day 21 in the serum concentrations of both estrogen and progesterone (16). The inference can thus be drawn that either or both of these hormones may be responsible, perhaps in a manner analogous to the synergism seen in the mature mouse mammary gland. Interestingly, the increased proliferation is followed by increased cell death through apoptosis in the late luteal phase, when hormonal levels are decreasing, suggesting that the apoptosis

may be a response to hormone withdrawal (17). In support of this conclusion, recent data have shown that in normal breast tissue transplanted into nude mice, increased apoptosis occurred in response to progestin withdrawal (18).

In experiments using techniques including organ culture, transplantation of normal human breast tissue into nude mice, and primary cell culture estrogen is capable of stimulating breast epithelial growth both in vitro and in vivo, but responses to progesterone are variable. Thus, no clear consensus on a stimulatory role for progesterone or an ability to inhibit the estrogen-mediated effect has yet emerged, although progestin treatment inhibits the proliferation of steroid-responsive breast epithelial cells in short-term culture (19). A more recent study has addressed the issue of which ovarian steroids stimulate normal human mammary epithelial cell proliferation, by implantation of normal breast tissue into nude mice followed by treatment with estradiol and progesterone. Estradiol stimulated the thymidine-labeling index, whereas progesterone had no effect either when administered alone or in combination with estradiol, despite the presence of estrogen-inducible progesterone receptors in this tissue (20). In another study, topical application of gel containing estradiol, progesterone, or both, for 10–13 days preceding breast surgery was used to increase the steroid accumulation within the breast tissue. The growth fraction of breast epithelial cells, as measured by proliferating cell nuclear antigen (PCNA) immunoreactivity, was reduced following progestin exposure (21). Although these results argue against a major proliferative role for progestins in breast epithelial cells, further experimentation in this area is critical to understanding the role of progesterone in the control of cell proliferation in the human breast, because the possibility that a short-term increase in proliferation precedes progestin-mediated inhibition cannot yet be excluded.

Despite the lack of clear evidence for progestin stimulation of breast epithelial cell proliferation in humans, stimulation of rodent mammary epithelium has been clearly demonstrated (22,23). The impaired mammary development in mice lacking progesterone receptor ($PR^{-/-}$; 24,25), indicating a requirement for progestin action for formation of the lobular alveoli during pregnancy, adds further support to these observations. However, progesterone is not the sole factor required for lobuloalveolar proliferation and differentiation, as illustrated by the similar phenotype of the mammary gland in $PR^{-/-}$ mice and mice lacking either prolactin or its receptor (24–28). Experiments in which $PR^{-/-}$ epithelium is transplanted onto wild-type stroma and vice versa indicate that, at least in the mouse, the effect of progesterone on lobuloalveolar development is mediated by the epithelium, although ductal development appears to depend on a progesterone-dependent signal from the stroma (25,29). The apparently direct effect of progesterone on lobuloalveolar epithelial cell proliferation is consistent with data from breast cancer cells indicating that growth factor-stimulated cells enter S phase some hours after cells stimulated by progestins, thus arguing

against progestin effects being mediated by paracrine or autocrine mechanisms (14). In contrast, the effect of estrogen appears to be mediated indirectly, by stromal, rather than epithelial receptors (30). The direct action of progestins on epithelial cell proliferation implies that progestin-regulated genes will include genes that either have a role in cell cycle control or are regulators of such genes. Recent experiments have thus concentrated on investigating the effects of progestins on cyclins and their associated cyclin-dependent kinases (CDKs), proteins that are regulators of key transitions during the cell cycle.

III. MECHANISMS OF CELL CYCLE CONTROL

The molecular controls of the cell cycle include as pivotal elements an evolutionarily conserved class of serine–threonine protein kinases, the CDKs, which are regulated by their association with cyclins, members of another well-conserved class of proteins. As might be expected for enzymes integral to the control of a fundamental biological process, the activity of CDKs is subject to multiple levels of regulation, such that cyclin binding is necessary, but not sufficient, for activation. Additional controls include phosphorylation at activating or inhibitory residues on the CDK and the abundance of CDK inhibitor proteins. Together these regulate the sequential activation of a series of CDKs to ensure passage through a series of checkpoints and, hence, orderly progression through the cell cycle.

The G_1 CDKs are cyclin D-Cdk4 and cyclin D-Cdk6, and cyclin E-Cdk2 (31), as illustrated in Fig. 2. Opposing the activation of these kinases are the CDK inhibitors p21 (Waf1, Cip1, Sdi1) and p27 (Kip1), which contact both the cyclin and CDK, causing multiple structural changes that interfere with kinase activation (32). However, despite these multiple modes of inhibition of CDK activity, not all cyclin–CDK complexes containing p21 or p27 are inactive. Recent data indicate that p21 and p27, as well as a related inhibitor, $p57^{Kip2}$, stabilize cyclin D–Cdk4 and cyclin D–Cdk6 complexes in vitro (33). High stoichiometry p21 or p27 binding appears to be required for inhibition of Cdk4 activity (33,34). Consequently, these molecules appear to have functions in addition to CDK inhibition, perhaps as adaptors that not only promote assembly of the cyclin–CDK complexes, but also target these complexes to specific intracellular compartments or substrates. An unrelated family of CDK inhibitors, characterized by an ankyrin repeat structure (including $p16^{INK4A}$ and $p15^{INK4B}$), more specifically target cyclin D-associated kinases (35). Although there is some evidence that they can inhibit preformed cyclin D–Cdk4/6 complexes, their inhibitory action appears to be largely due to competition with the cyclin for CDK binding.

The activity of CDKs is regulated not only by passage through the cell cycle, but also in response to various signals. Emerging evidence indicates roles

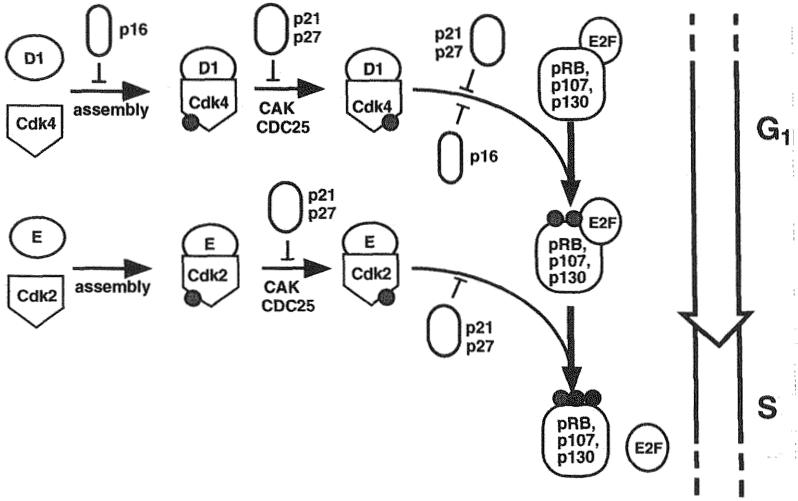

Figure 2 Cell cycle regulation during G_1 phase. Progress from G_1 into S phase depends on the actions of the molecular pathways illustrated schematically. The major G_1 cyclin complexes in breast cancer cells, cyclin D1–Cdk4 and cyclin E–Cdk2, are illustrated. The activity of these CDK complexes is regulated at several levels, including assembly and activation by kinases (CAK) and phosphatases (CDC25). Once active the CDKs phosphorylate substrates including pRB and the related "pocket proteins," p107 and p130, leading to the release of molecules including the transcription factor E2F and, in turn, to the transcription of genes necessary for entry into S phase. The CDK inhibitors p21 and p27 not only interfere with the phosphorylation steps leading to the activation of the CDK, but also inhibit active CDK complexes. Although the p16 CDK inhibitor may also inhibit holoenzyme complexes, a major function is to inhibit the assembly of cyclin D1–Cdk4 complexes. Phosphorylation of these proteins is indicated by (•).

in differentiation (36,37) and adhesion (38), which appear to be largely mediated by the phosphorylation of specific substrates, although recent data raise the possibility of CDK-independent functions for the D-type cyclins (39,40). The substrates for these kinases remain largely uncharacterized, although one key target in G_1 is the retinoblastoma protein, pRB. The "pocket proteins," pRB and the structurally and functionally related p107 and p130, exert an important part of their growth-inhibitory function by binding and inactivating proteins necessary for cell cycle progression, notably members of the E2F family of transcription factors (41). Pocket protein association with E2F is dependent on the phosphorylation state of the pocket protein which, in turn, is dependent on CDK activity. Thus, phosphorylation of pRb in late G_1 phase is necessary for entry in S phase.

The complexity of control of cyclin–CDK activity provides multiple targets through which physiological regulators of cell proliferation might mediate their effects. However, only a restricted range of these potential targets appear to be utilized. Thus, regulation of cyclin or CDK inhibitor expression is a frequent response to mitogens, including steroid hormones, peptide growth factors, and cytokines, and to growth arrest following induction of differentiation or treatment with inhibitory factors (e.g., TGF-β) (35,42). CDK activity is also regulated by phosphorylation of the kinase subunit at multiple regulatory sites. This phosphorylation is controlled by proteins including the Cdc25 family of activating phosphatases and the CDK-activating kinase CAK, itself a cyclin–CDK complex. However, regulation of the expression or activity of these CDK-regulatory kinases and phosphatases appears to be a relatively infrequent mechanism contributing to physiological regulation of cell proliferation. Consequently, examination of progestin effects on these pathways has focused on regulation of cyclins and CDK inhibitors.

IV. PROGESTIN EFFECTS ON CDK FUNCTION

A. Stimulation

Initial investigations of the possible effects of progestins on CDK function showed that the entry of progestin-stimulated T-47D human breast cancer cells into S phase was preceded by an increase in cyclin D1 mRNA and protein abundance (Fig. 3) (43). Cyclin D1 mRNA levels increased only transiently, however, returning to control levels as progestin-stimulated cells entered S phase (43). The induction of cyclin D1 protein led to an increase in the activity of Cdk4 (the major cyclin D1-associated CDK in T-47D cells). Although this increase was modest, the proportion of pRB in the hyperphosphorylated form also increased, indicating an increase in CDK activity following progestin treatment. Cyclin D1 expression is high in murine mammary epithelium during pregnancy, when serum steroid levels are high, but largely undetectable in mammary epithelium from virgin or lactating animals (44). In ovariectomized mice, estrogen increases cyclin D1 mRNA and protein expression in the mammary epithelium, and the addition of progesterone as well as estrogen further increases cyclin D1 levels (45). The correspondence between induction of cyclin D1 and subsequent proliferation suggests cyclin D1 induction as one of the elements coupling steroid hormone action with cell cycle control mechanisms. Experiments involving addition of the progestin antagonist mifepristone either at the same time as, or 3 h subsequent to, progestin treatment of breast cancer cells provide support for this proposition. Simultaneous addition of progestin and antiprogestin prevented both the progestin-mediated increase in cyclin D1 levels and progestin-induced

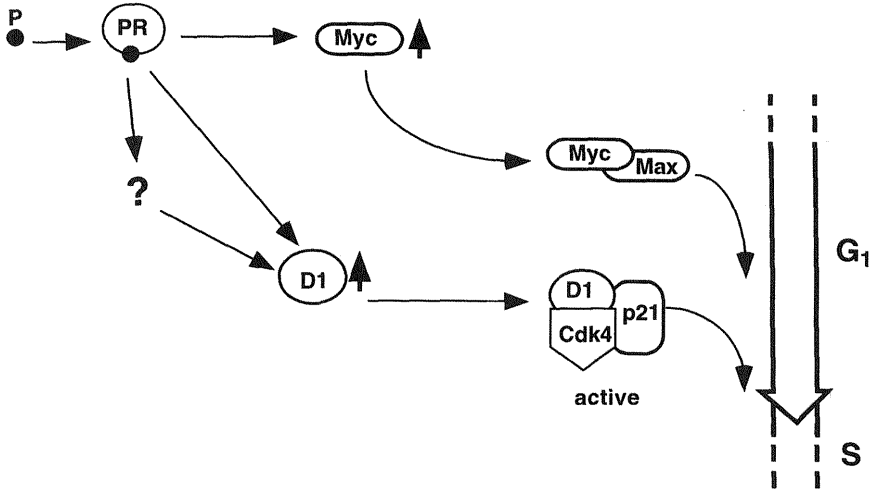

Figure 3 Progestin stimulation of cell cycle progression. Potential models of progestin molecular mechanisms underlying progestin stimulation of cell cycle progression are illustrated schematically. Progestin-stimulated entry into S phase is preceded by induction of the cell cycle regulatory transcriptional factor c-Myc and cyclin D1. c-Myc then interacts with its dimerization partner Max to activate transcription of target genes. Cyclin D1 induction is followed by activation of cyclin D1–Cdk4 and increased phosphorylation of pRB, leading to accelerated progress through G_1 phase. Progestins are indicated by P and the progesterone receptor by PR.

entry into S phase (14,43). Similarly, progestin antagonist addition within 3 h of progestin treatment prevented progestin stimulation of cell cycle progression (14). Although by 3 h after progestin treatment cyclin D1 mRNA abundance had already begun to increase, addition of antiprogestin rapidly reversed this increase (43), indicating that sustained cyclin D1 induction is necessary for acceleration into S phase following progestin treatment.

The conclusion that cyclin D1 is a critical component of the mitogenic response to progestins is strongly supported by experiments using T-47D breast cancer cells expressing cyclin D1 under the control of a inducible promoter, which indicated that cyclin D1 induction led to both an increase in the rate of G_1 transit and an increase in the proportion of the cell population engaged in active transit through G_1 (46). Thus, a progestin-induced increase in cyclin D1 abundance could account for the acceleration in G_1 phase progression. However, the induction of cyclin D1 is preceded by the induction of other genes (e.g., the cell cycle regulatory transcription factor c-*myc*) (14) (see Fig. 3), and the effect on cyclin D1 expression may not be direct, but rather, result from prior induction

of another gene or genes. Indeed, the 2 kb of the cyclin D1 gene promoter for which sequence is available does not contain a classic progesterone response element.

Studies in transgenic and knockout mice further support the conclusion that cyclin D1 induction is a critical component of the mitogenic response to progestins. Overexpression of cyclin D1 in the mammary gland of transgenic mice led to increased lobuloalveolar development, reminiscent of the changes associated with pregnancy, whereas conversely, development of the lobular alveoli did not occur in pregnant cyclin D1-null mice (47–49), indicating a very specific requirement for cyclin D1 in alveolar proliferation. In cyclin D1-null mice, ductal proliferation apparently occurs normally, allowing both pubertal development of the gland and some side-branching during pregnancy (48,49). Progesterone is also necessary for alveolar, but not ductal, proliferation (see Sec. I) suggesting that cyclin D1 action is essential for progesterone-driven mammary epithelial proliferation.

B. Inhibition

Progestin inhibition of entry into S phase is associated with decreased phosphorylation of pRB and p107 (15,50) and decreases in both cyclin D–Cdk4 (50) and cyclin E–Cdk2 activity (15,50), accompanying or slightly preceding the decrease in the fraction of S phase cells (Fig. 4). Down-regulation of kinase activity was accompanied by decreases in the expression of cyclin D1 and cyclin E, followed later by a decrease in cyclin D3 expression (15,50). Separation of whole cell lysates by gel filtration chromatography indicated that, in control T-47D cells, the cyclin E protein eluted as two overlapping peaks of approximately120 and 200 kDa (50). Most of the cyclin E-associated kinase activity eluted at approximately 120 kDa, with the 200-kDa form displaying little associated kinase activity, probably owing to association with the CDK inhibitors p21 and p27. Following progestin treatment, almost all of the cyclin E was in the low specific activity, p21- and p27-bound, form eluting at about 200 kDa (50). These data suggest conversion to this CDK inhibitor-bound form as a major mechanism for the decreased cyclin E-associated kinase activity following progestin treatment (see Fig. 4). The reduced number of cyclin E–Cdk2 complexes as a consequence of the decrease in cyclin E abundance is also likely to contribute to this effect.

The mechanisms for the decrease in Cdk4 activity following progestin treatment are less clearcut but a major factor appears to be a decrease in the abundance of cyclin D1–Cdk4 and cyclin D3–Cdk4 complexes, in combination with increased p27 association with the remaining complexes (50). However, it remains possible that other factors (e.g., induction of a p16-related inhibitor) might also contribute to the marked loss of Cdk4 activity. The increased relative association of p27 with cyclin–CDK complexes is apparent before there is any

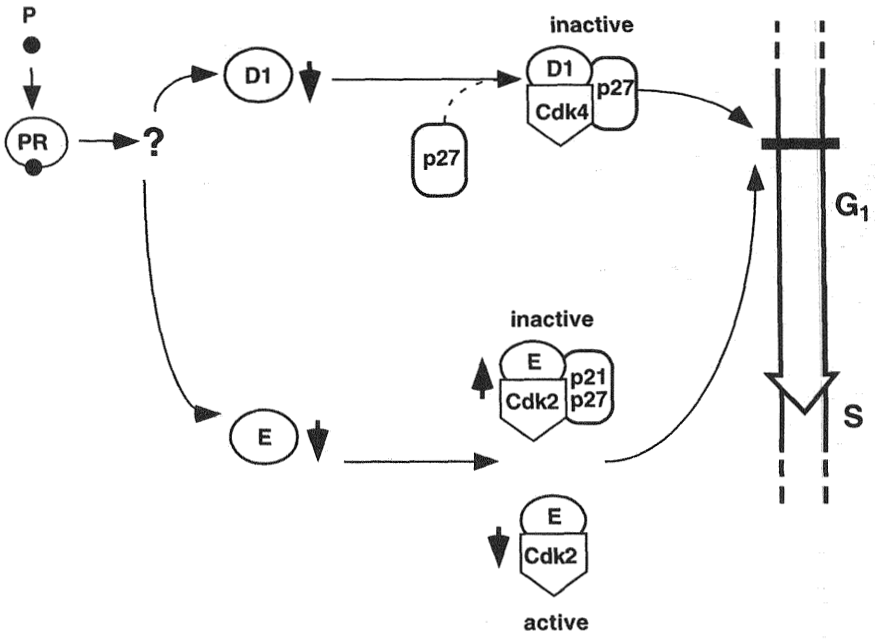

Figure 4 Progestin inhibition of cell cycle progression. Potential models of progestin inhibition of CDK activity and, hence, cell cycle progression are illustrated. Progestin inhibition of entry into S phase is preceded by decreased cyclin D1–Cdk4, cyclin D3–Cdk4, and cyclin E–Cdk2 activity, with consequent accumulation of underphosphorylated pRB. Several mechanisms appear to contribute to the decreases in kinase activity. These include decreases in expression of cyclins D1 and E and a later decrease in the expression of cyclin D3, in combination with increased CDK inhibitor association with the remaining complexes.

increase in p27 abundance (15,50). An early decrease in c-*myc* mRNA expression (50,51) raises the possibility that p27 is no longer sequestered by Myc-responsive proteins (52) following progestin treatment and is thus increasingly available to bind the remaining cyclin–CDK complexes. However, the decreased number of cyclin–CDK complexes appears to be important in progestin inhibition of CDK activity, for induction of ectopic cyclin D1 in progestin-inhibited cells leads to activation of Cdk4 and reappearance of the active, inhibitor-depleted form of cyclin E–Cdk2 preceding a resumption of cell cycle progression. Although clear effects on CDK activity are associated with progestin inhibition of proliferation, providing a likely explanation for the G_1 phase arrest, extended progestin treatment (>12 h) is required before these responses are manifest (12,15,50) and the basis of this delay remains to be defined.

V. CONCLUSIONS

Although it is apparent that substantial recent progress has been made toward understanding the potential molecular interactions leading to progestin regulation of cell proliferation, much remains to be learned. For example, further dissection of the proposed mechanisms underlying progestin inhibition of cell proliferation will involve identifying the basis for the delayed action on CDK activity, assessing the relative contributions of inhibition of Cdk4 and cyclin E–Cdk2 to the overall effect, and more fully testing the hypothesis that altered cyclin abundance is among the main initiators of the altered cyclin–CDK complex composition and activity. Potential models for progestin effects illustrated in Figs. 3 and 4 have largely been based on data obtained using breast cancer cells in tissue culture, particularly for progestin inhibition of proliferation. The degree to which the general features of these models can be applied to other progestin-responsive model systems is a critical issue. Of particular importance will be human breast epithelium, although it will be necessary first to more clearly define the effects of progestins on cell cycle progression in this tissue. In addition, the mechanisms that might contribute to apoptosis after hormone withdrawal are still not understood in this tissue. A further challenge arises from the interactions between progesterone and other hormones. For example, both progesterone and prolactin are required for the control of proliferation and differentiation in the lobular alveoli. Finally, the degree to which progestin-mediated growth inhibition or stimulation are the indirect results of induction of a differentiation program remains to be determined. Although progress toward answering these questions remains a goal of ongoing research, the evidence that cell cycle regulatory molecules, in particular cyclin D1, play a critical role in progestin action suggests that their aberrant expression may contribute to alterations in steroid sensitivity in breast cancer.

ACKNOWLEDGMENTS

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REFERENCES

1. Collaborative Group on Hormonal Factors in Breast Cancer. Breast cancer and hormonal contraceptives: collaborative reanalysis of individual data on 53,297

women with breast cancer and 100,239 women without breast cancer from 54 epidemiological studies. Lancet 347:1713–1727, 1996.
2. Collaborative Group on Hormonal Factors in Breast Cancer. Breast cancer and hormone replacement therapy: collaborative reanalysis of data from 51 epidemiological studies of 52,705 women with breast cancer and 108,411 women without breast cancer. Lancet 350:1047–1059, 1997.
3. GA Colditz, SE Hankinson, DJ Hunter, WC Willett, JE Manson, MJ Stampfer, C Hennekens, B Rosner, FE Speizer. The use of estrogens and progestins and the risk of breast cancer in postmenopausal women. N Engl J Med 332:1589–1593, 1995.
4. U Veronesi, A Goldhirsch, J Yarnold. Breast cancer. In: M Peckham, HM Pinedo, U Veronesi, eds. Oxford Textbook of Oncology. Oxford: Oxford University Press, 1995, pp 1243–1289.
5. RJ Santen, A Manni, H Harvey, C Redmond. Endocrine treatment of breast cancer in women. Endocr Rev 11:221–265, 1990.
6. PG Rose. Endometrial cancer. N Engl J Med 335:640–649, 1996.
7. RM Das, L Martin. Progesterone inhibition of mouse uterine epithelial proliferation. J Endocrinol 59:205–206, 1973.
8. L Martin, RM Das, CA Finn. The inhibition by progesterone of uterine epithelial proliferation in the mouse. J Endocrinol 57:549–554, 1973.
9. L Martin, CA Finn. Oestrogen–gestagen interactions on mitosis in target tissues. In: PO Hubinot, F Leroy, P Galard, eds. Basic Actions of Sex Steroids on Target Organs. Basel: Karger, 1971, pp 172–188.
10. F Vignon, S Bardon, D Chalbos, H Rochefort. Antiestrogenic effect of R5020, a synthetic progestin in human breast cancer cells in culture. J Clin Endocrinol Metab 56:1124–1130, 1983.
11. KB Horwitz, GR Freidenberg. Growth inhibition and increase of insulin receptors in antiestrogen-resistant $T47D_{co}$ human breast cancer cells by progestins: implications for endocrine therapies. Cancer Res 45:167–173, 1985.
12. RL Sutherland, RE Hall, GYN Pang, EA Musgrove, CL Clarke. Effect of medroxyprogesterone acetate on proliferation and cell cycle kinetics of human mammary carcinoma cells. Cancer Res 48:5084–5091, 1988.
13. E Kalkhoven, L Kwakkenbos-Isbrücker, CL Mummery, SW de Laat, AJM van den Eijnen-van Raij, PT van der Saag, B van der Burg. The role of TGF-β production in growth inhibition of breast-tumor cells by progestins. Int J Cancer 61:80–86, 1995.
14. EA Musgrove, CSL Lee, RL Sutherland. Progestins both stimulate and inhibit breast cancer cell cycle progression while increasing expression of transforming growth factor α, epidermal growth factor receptor, c-*fos* and c-*myc* genes. Mol Cell Biol 11:5032–5043, 1991.
15. SD Groshong, GI Owen, B Grimison, IE Schauer, MC Todd, TA Langan, RA Sclafani, CA Lange, KB Horwitz. Biphasic regulation of breast cancer cell growth by progesterone: role of the cyclin-dependent kinase inhibitors, p21 and $p27^{Kip1}$. Mol Endocrinol 11:1593–1607, 1997.
16. CL Clarke, RL Sutherland. Progestin regulation of cellular proliferation. Endocr Rev 11:266–302, 1990.

17. DJP Ferguson, PJ Anderson. Morphological evaluation of cell turnover in relation to the menstrual cycle in the ''resting'' human breast. Br J Cancer 44:177–181, 1981.
18. J Desreux, A Noel, JL Thomas, AM Bernard, J Paris, R Delansorne, H Van Cauwenberge, D Francart, V Heinen, JM Foidart. Progestin-induced apoptosis of normal human breast cells: an in vivo and in vitro study. Proceedings 8th International Congress on the Menopause 97, 1996.
19. A Gompel, C Malet, P Spritzer, JP Lalardrie, F Kuttenn, P Mauvais Jarvis. Progestin effect on cell proliferation and 17β-hydroxysteroid dehydrogenase activity in normal human breast cells in culture. J Clin Endocrinol Metab 63:1174–1180, 1986.
20. IJ Laidlaw, RB Clarke, A Howell, AWMC Owen, CS Potten, E Anderson. The proliferation of normal human breast tissue implanted into athymic nude mice is stimulated by oestrogen but not progesterone. Endocrinology 136:164–171, 1995.
21. K-J Chang, TTY Lee, G Linares-Cruz, S Fournier, B de Ligniéres. Influences of percutaneous administration of estradiol and progesterone on human breast epithelial cell cycle in vivo. Fertil Steril 63:785–791, 1995.
22. W Imagawa, Y Tomooka, S Mamamoto, S Nandi. Stimulation of mammary epithelial cell growth in vitro and interaction of epidermal growth factor and mammogenic hormones. Endocrinology 116:1514–1525, 1985.
23. SZ Haslam. Progesterone effects on deoxyribonucleic acid synthesis in normal mouse mammary glands. Endocrinology 122:464–470, 1988.
24. JP Lyndon, FJ DeMayo, CR Funk, SK Mani, AR Hughes, CA Montgomery, G Shyamala, OM Conneely, BW O'Malley. Mice lacking progesterone receptor exhibit pleiotrophic reproductive abnormalities. Genes Dev 9:2266–2278, 1995.
25. RC Humphreys, JP Lyndon, BW O'Malley, JM Rosen. Use of PRKO mice to study the role of progesterone in mammary gland development. J Mamm Gland Biol Neoplasia 2:343–354, 1997.
26. N Horseman, W Zhao, E Montecino-Rodriguez, M Tanaka, K Nakashima, S Engle, F Smith, E Markoff, K Dorshkind. Defective mammopoiesis, but normal hematopoiesis, in mice with a targeted disruption of the prolactin gene. EMBO J 16:6926–6935, 1997.
27. CJ Ormandy, A Camus, J Barra, D Damotte, B Lucas, H Buteau, M Edery, N Brousse, C Babinet, N Binart, PA Kelly. Null mutation of the prolactin receptor gene produces multiple reproductive defects in the mouse. Genes Dev 11:167–178, 1997.
28. CJ Ormandy, N Binart, PA Kelly. Mammary gland development in prolactin receptor knockout mice. J Mamm Gland Biol Neoplasia 2:355–364, 1997.
29. RC Humphreys, J Lyndon, BW O'Malley, JM Rosen. Mammary gland development is mediated by both stromal and epithelial progesterone receptors. Mol Endocrinol 11:801–811, 1997.
30. GR Cunha, P Young, YK Hom, PS Cooke, JA Taylor, DB Lubahn. Elucidation of a role for stromal steroid hormone receptors in mammary gland growth and development using tissue recombinants. J Mamm Gland Biol Neoplasia 2:393–402, 1997.
31. CJ Sherr. Cancer cell cycles. Science 274:1672–1677, 1996.

32. AA Russo, PD Jeffrey, AK Patten, J Massagué, N Pavletich. Crystal structure of the $p27^{Kip1}$ cyclin-dependent-kinase inhibitor bound to the cyclin A–Cdk2 complex. Nature 382:325–331, 1996.
33. J LaBaer, MD Garrett, LF Stevenson, JM Slingerland, C Sandhu, HS Chou, A Fattaey, E Harlow. New functional activities for the p21 family of CDK inhibitors. Genes Dev 11:847–862, 1997.
34. SW Blain, E Montalvo, J Massagué. Differential interaction of the cyclin-dependent kinase (Cdk) inhibitor $p27^{Kip1}$ with cyclin A–Cdk2 and cyclin D2–Cdk4. J Biol Chem 272:25863–25872, 1997.
35. CJ Sherr, JM Roberts. Inhibitors of mammalian G_1 cyclin-dependent kinases. Genes Dev 9:1149–1163, 1995.
36. K Walsh. Coordinate regulation of cell cycle and apoptosis during myogenesis. In: L Meijer, S Guidet, M Philippe, eds. Progress in Cell Cycle Research. New York: Plenum Press, 1997, pp 53–58.
37. J-Y Kato, CJ Sherr. Inhibition of granulocyte differentiation by G_1 cyclins D2 and D3 but not D1. Proc Natl Acad Sci USA 90:11513–11517, 1993.
38. X Zhu, M Ohtsubo, RM Bohmer, JM Roberts, RK Assoian. Adhesion-dependent cell cycle progression linked to the expresson of cyclin DI, activation of cyclin E–cdk2, and phosphorylation of the retinoblastoma protein. J Cell Biol 133:391–403, 1996.
39. RML Zwijsen, E Wientjens, R Klompmaker, J van der Sman, R Bernards, RJAM Michalides. CDK-independent activation of estrogen receptor by cyclin D1. Cell 88:405–415, 1997.
40. E Neuman, MH Ladha, N Lin, TM Upton, SJ Miller, J Direnzo, RG Pestelle, PW Hinds, SF Gowdy, M Brown, ME Ewen. Cyclin D1 stimulation of estrogen receptor transcriptional activity independent of cdk4. Mol Cell Biol 17:5338–5347, 1997.
41. X Mayol, X Graña. pRB, p107 and p130 as transcriptional regulators: role on cell growth and differentiation. In: L Meijer, S Guidet, M Philippe, eds. Progress in Cell Cycle Research. New York: Plenum Press, 1997, pp 157–169.
42. CJ Sherr. G_1 phase progression: cycling on cue. Cell 79:551–555, 1994.
43. EA Musgrove, JA Hamilton, CSL Lee, KJE Sweeney, CKW Watts, RL Sutherland. Growth factor, steroid and steroid antagonist regulation of cyclin gene expression associated with changes in T-47D human breast cancer cell cycle progression. Mol Cell Biol 13:3577–3587, 1993.
44. L Stepanova, X Leng, SB Parker, JW Harper. Mammalian $p50^{Cdc37}$ is a protein kinase-targeting subunit of Hsp90 that binds and stabilises Cdk4. Genes Dev 10:1491–1502, 1996.
45. TK Said, OM Conneely, D Medina, BW O'Malley, JP Lyndon. Progesterone, in addition to estrogen, induces cyclin D1 expression in the murine mammary epithelial cell, in vivo. Endocrinology 138:3933–3939, 1997.
46. EA Musgrove, CSL Lee, MF Buckley, RL Sutherland. Cyclin D1 induction in breast cancer cells shortens G_1 and is sufficient for cells arrested in G_1 to complete the cell cycle. Proc Natl Acad Sci USA 91:8022–8026, 1994.
47. TC Wang, RD Cardiff, L Zukerberg, E Lees, A Arnold, EV Schmidt. Mammary hyperplasia and carcinoma in MMTV–cyclin D1 transgenic mice. Nature 369:669–671, 1994.

48. P Sicinski, J Liu Donaher, SB Parker, T Li, A Fazeli, H Gardner, SZ Haslam, RB Bronson, SJ Elledge, RA Weinberg. Cyclin D1 provides a link between development and oncogenesis in the retina and breast. Cell 82:621–630, 1995.
49. V Fantl, G Stamp, A Andrews, I Rosewell, C Dickson. Mice lacking cyclin D1 are small and show defects in eye and mammary gland development. Genes Dev 9:2364–2372, 1995.
50. EA Musgrove, A Swarbrick, CSL Lee, AL Cornish, RL Sutherland. Mechanisms of CDK inactivation by progestins. Mol Cell Biol 18:1812–1825, 1998.
51. MSJ Wong, LC Murphy. Differential regulation of c-*myc* by progestins and antiestrogens in T-47D human breast cancer cells. J Steroid Biochem Mol Biol 39:39–44, 1991.
52. J Vlach, S Hennecke, K Alevizopoulos, D Conti, B Amati. Growth arrest by the cyclin-dependent kinase inhibitor $p27^{Kip1}$ is abrogated by c-Myc. EMBO J 15:6595–6604, 1996.

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Vaginal Progesterone: Direct Access to the Uterus, Physiological Hormone Replacement in Infertility and Gynecology

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I. INTRODUCTION

Progesterone, the natural hormone produced by the corpus luteum of the ovary, antagonizes the proliferative effects exerted by estradiol (E_2) on the endometrium. Progesterone also triggers a predefined sequence of secretory changes indispensable to the establishment and development of pregnancy. Because oral bioavailability of progesterone is notoriously poor, synthetic molecules have been designed to resist enzymatic degradation and remain active when ingested orally. Yet, these molecules collectively known as synthetic progestins are often the source of side effects (objective or subjective). Side effects, particularly psychological ones, can be so undesirable that long-term compliance of menopause treatment becomes problematic in affected individuals. Often the severity of side effects has been the basis for alternative treatments in these individuals. Also,

progestins antagonize some of the beneficial effects of estrogens. Finally, in infertility, only natural progesterone can be used, which has led to experimenting with all the various practical options for nonoral administration.

The purpose of the present chapter is to describe the recent evolution followed by vaginal progesterone and how it became a viable therapeutic option. Although originally selected merely as the simplest available form of nonoral progesterone, it is now recognized that vaginal progesterone provides an unexpected direct access to the uterus. This ''surprise'' finding has shed new light on the vaginal route and rendered it unique for administrating progesterone to the uterus which, precisely, is the primary target for the progestational effects. This direct transport has served to limit the side effects of treatments. Also, in the scope of this chapter, it is our desire to review the practical advantages of physiological progesterone replacement, particularly for women with chronic medical conditions, and to stress the low incidence of side effects encountered. For this we surveyed the advantages of natural progesterone treatments. In Sec. IV, we analyzed the uses of vaginal progesterone in infertility. In Sec. V, we reviewed new emerging indications for vaginal progesterone and the available options in gynecology. This field has been the site of recent developments, in part because new vaginal delivery forms now allow us to contemplate long-term treatments. In particular, a new vaginal gel of progesterone with sustained-release properties makes prolonged use, such as required in hormone replacement therapy (HRT), a practical and viable option. This should be of particular benefit for women intolerant to synthetic progestins, or those who suffer from cardiovascular conditions or other chronic medical ailments.

II. VAGINAL PROGESTERONE: THE BEST PRACTICAL NON-ORAL OPTION

Progesterone is readily absorbed when ingested orally in micronized form (1). Yet, because of an intense first-pass liver metabolism, the bioavailability of oral progesterone is poor (2). As illustrated in Fig. 1, the vast majority of progesterone absorbed orally is rapidly metabolized to 5α-reduced metabolites on reaching the peripheral circulation (2,3). Part of the large amount of 5α-reduced metabolites is further transformed into the 3-OH derivatives (α and β), which are potent stimulators of the γ-aminobutyric acid type A ($GABA_A$) receptor complex. This explains the side effects of sleepiness, loss of vigilance, and other psychological symptoms often observed after oral administration of progesterone. These central nervous system (CNS) effects induced by oral progesterone may be well tolerated by some women, whereas they are feared by others who would rather avoid these pharmacological effects. Also, as a result of the intense first-pass

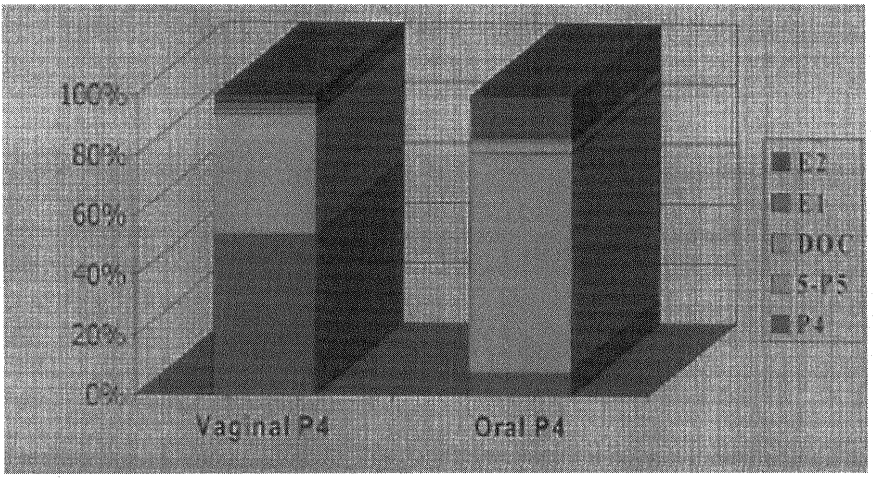

Figure 1 Plasma results after oral and vaginal administration. Oral progesterone induces an unphysiological elevation of progesterone metabolites, particularly the 5α-reduced ones that are the source of side effects on the CNS. The measured products are estradiol (E_2), estrone (E_1), desoxycorticosterone (DOC), 5α-dehydroprogesterone (5-P5), and progesterone (P4). (From Ref. 2, with permission.)

liver metabolism are the plasma progesterone levels, which are only minimally elevated after oral progesterone (3). In the light of findings reported by Nahoul et al. (2,3), it comes as no surprise that the endometrium shows only partial secretory transformation after oral administration of even large amounts of progesterone (4). On the contrary, vaginal administration of progesterone (4–7) reliably induced all the predecidual changes of the endometrial stroma (or "in-phase endometrium"). In these latter cases, findings were indistinguishable from those made during the late luteal phase of the menstrual cycle.

Originally, intramuscular (IM) injections (50 mg/day) were used for administrating progesterone and priming receptivity in women deprived of ovarian function. This practice stemmed from the nearly concomitant reports of Lutjen et al. (8) in Australia and Navot et al. (9) in Israel. Both authors described the possibility of priming endometrial receptivity with the sole help of exogenous E_2 and progesterone and achieve pregnancy through donor egg in vitro fertilization (IVF). In these early attempts at donor egg IVF, it was considered important that progesterone supplementation raised circulating levels of plasma progesterone to or above the physiological range for the menstrual cycle. This satisfied the then prevailing concept that endometrial effects were truly *controlled* by the

Table 1 Pregnancy Rates

	Vaginal gel of progesterone	IM progesterone
No. of fresh transfers	54	18
Implantation rate (%)[a]	23 (42/180)	18 (11/62)
No. of positive pregnancy tests[b]	29 (54)	7 (39)
No. of clinical pregnancies[b]	26 (48)	5 (28)
No. of miscarriages	7	1
No. of therapeutic abortions	2	—
No. of ongoing pregnancies[b]	17 (31)	4 (22)

[a]Values are percentages, with ratios in parentheses.
[b]Values in parentheses are percentages.
Source: Ref. 15.

circulating levels of ovarian hormones. Today, evidence has been gathered that indicates there is a "permissive" mode of endometrial control by sex hormones (10). In this alternative view, progesterone elicits preorganized actions or set of actions in the target organ, but does not truly control them.

In the nearly 15 years that followed the original donor egg reports, the efficacy of IM injections of progesterone has never been truly questioned. Moreover, donor egg IVF findings logically also led to adopt IM progesterone for luteal support in regular IVF. Yet it has been suggested that similar reliability on endometrial effects (5,11) and pregnancy rates (12) could be achieved with the more user-friendly vaginal administration. The practical advantages of the vaginal route for luteal support in regular IVF were also studied. In regular IVF, the objective is to restore a physiological sequence of changes in the endometrium that may be disrupted by the hormonal manipulations inherent to IVF treatments (13). When analyzing the quality of midluteal phase biopsies in controlled ovarian hyperstimulation (COH), Bourgain et al. (14) observed better endometrial quality (fewer signs of luteal phase defect) after vaginal administration of progesterone than seen after either no supplementation (controls) or even IM administration. More recently, Gibbons et al. (15) studied a new sustained-release vaginal gel (Crinone; Wyeth-Ayerst Pharmaceuticals, Radnor, PA) containing 90 mg of progesterone. Originally, the gel was administered twice daily to donor egg IVF candidates. These authors observed equivalent biopsy quality (in-phase late luteal endometrium) when comparing results obtained after IM supplementation. More importantly, in actual transfer cycles, pregnancy rates were also similar in women receiving progesterone from IM injection or from the vaginal gel (Table 1). Furthermore, in an extension of their first study, these authors observed that the amount of progesterone could be further reduced to

Table 2 Past Experience with Vaginal Progesterone in Gynecology[a]

Indication	Years	Effects
PMS	>20	Reported efficacy
IVF	12	Secretory endometrium
		Highly effective
		High pregnancy rates
		Subphysiological levels
Menopause	7	Predictable bleeding
		No side effects
		Favors continuance

[a]Vaginal suppositories of progesterone have been used in PMS for more than 20 years. In menopause, on the contrary, the experience is shorter with pioneers in the field reporting 7 years experience.
Source: Ref. 66.

once-daily vaginal application of the 90-mg gel (Crinone 8%) without affecting pregnancy rates (16).

Because of its proven efficacy, use of vaginal progesterone started as the most practical mode of nonoral administration of progesterone in infertility treatments (6,11). On the contrary, other nonoral options are less effective or are impractical (17). Transdermal administration, which became the primary nonoral route for E_2 (18) is ineffective for delivering progesterone (19). At least three factors account for this impossibility: (a) the amounts of progesterone produced daily during the luteal phase peak at 25 mg/24 h which exceeds by more than two orders of magnitude the amounts of E_2 commonly replaced transdermally in HRT (0.05–0.1 mg/24 h); (b) the skin is poorly permeable to progesterone; and (c) the skin is rich in 5α-reductase, an enzyme that inactivates progesterone. Alternative transdermal options have investigated circumventing these problems by administering synthetic progestins, such as norethisterone acetate (NETA). Yet, transdermal NETA is far from providing physiological hormonal replacement because of the synthetic nature of the NETA molecule. Side effects of NETA, as are those of other synthetic products, are molecule-dependent and route-independent. For synthetic molecules, the route of administration can only succeed in lowering side effects if the amounts administered can be decreased as in local deliveries on, or near, the target tissue. Other nonoral option, such as the transnasal route, have been tested (17), but are completely impractical. The duration of clinical experience with vaginal progesterone in gynecology is summarized in Table 2.

III. VAGINAL PROGESTERONE: A DIRECT VAGINA–TO–UTERUS TRANSPORT OR UTERINE "FIRST-PASS EFFECT"

The early experience with vaginal administration of progesterone for priming endometrial receptivity in women deprived of ovarian function has been rewarding. In women receiving vaginal progesterone, pregnancy rates most often exceeded results obtained in the respective regular IVF programs (6). However, it is only with the availability of a new sustained-release progesterone vaginal gel that a vaginal route paradox has become truly evident. Because of the low doses administered, the progesterone gel revealed endometrial effects that far exceeded the expectations normally drawn from the minimal elevation of plasma progesterone levels (Fig. 2). In a dose-ranging study (20), the sustained-release characteristics of the gel (Fig. 3) have been emphasized by decreasing the amount and

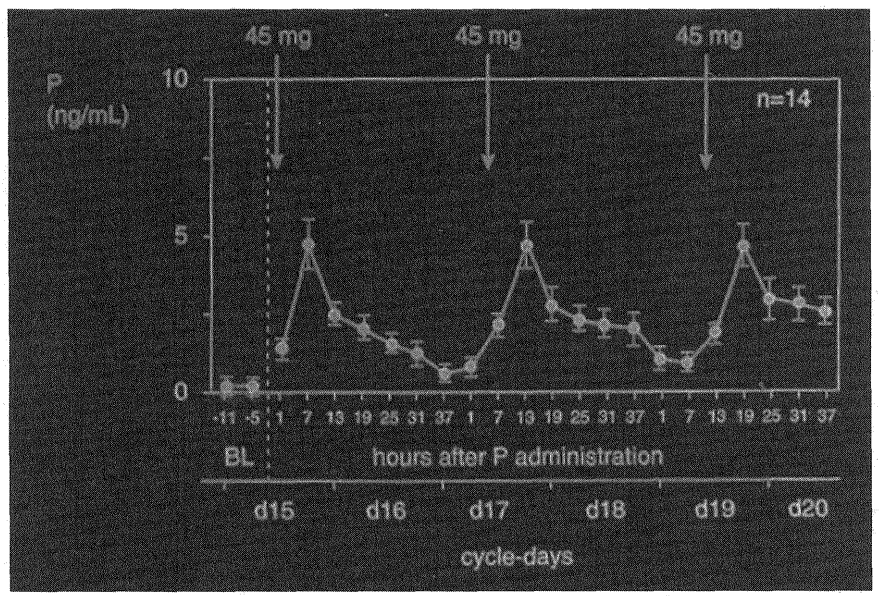

Figure 2 Plasma progesterone levels after vaginal progesterone gel 4% every-other-day. Every-2-day administration of the vaginal gel of progesterone, Crinone 4% (45 mg), raised plasma progesterone to "luteal phase defect" levels. Despite these low plasma progesterone levels, secretory changes of endometrial glands (day 20) and stroma (day 24) were no different from those findings made in the normal menstrual cycle. The discrepancy between high uterine efficacy and low plasma levels was the basis for the uterine first-pass effect hypothesis. (From Ref. 20, with permission.)

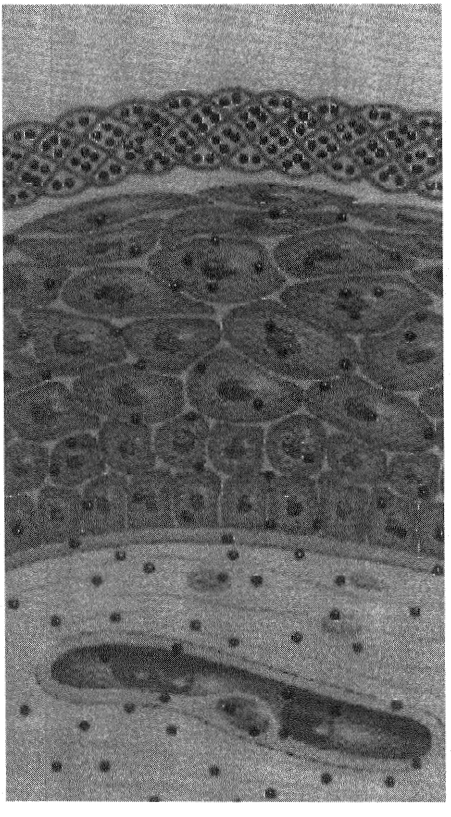

Figure 3 The sustained-release gel of progesterone. The vaginal gel of progesterone gains its sustained-release properties from its base. The base for this gel is polycarbophil, an inert substance that plays the role of mucin in mucus and provides bioadhesion. The gel, acting as an artificial mucus, adheres to the surface of the vaginal epithelial cells from where the release of progesterone is sustained over time.

frequency of administration. Studies were undertaken to determine the safety margins existing when the gel is used at the recommended dose (90 mg) and regimen (one application per day). In the dose-ranging study, infertile women deprived of ovarian function received physiological quantities of estrogen (ranging from 0.1 to 0.4 mg/day) and various amounts of the vaginal progesterone gel. In the lowest-dose group, as little as 45 mg of progesterone was administered every-other-day. In this treatment group, plasma progesterone levels (see Fig. 2) were determined every 6 h for the first 6 days (days 15–20). Results showed plasma progesterone values that were markedly subphysiological, being frankly

in the ''luteal phase defect'' range (with peaks under 5 ng/mL and troughs at or near 1 ng/mL). Despite these low progesterone levels, endometrial changes observed in endometrial glands (day 20 biopsies) and stroma (day 24 biopsies) were not different (20) from findings observed with either higher doses of progesterone (IM injections) or in the luteal phase of the menstrual cycle (21). This striking observation has been the basis for formulating the hypothesis that a fraction of progesterone administered vaginally is transported directly to the uterus through a local ''portal'' distribution system or uterine first-pass effect.

Also intrigued by the high efficacy of vaginal progesterone, Miles et al. (22) have observed higher uterine tissue concentrations after vaginal than after IM administration of progesterone, despite higher plasma progesterone concentration in the latter cases. Recently, similar findings were made when comparing the vaginal gel and IM progesterone administered before retrieving endometrial tissue from hysterectomy specimens (23). Confirming high uterine progesterone concentration, the latter experimental model was important because it excluded the possibility that endometrial specimens (nanogram amounts) might have been contaminated by progesterone still remaining in the vagina (milligram amounts).

With an original ex vivo model for perfusing the human uterus, Bulletti et al. (24) verified the veracity of the uterine first-pass effect hypothesis. These investigators applied tritiated progesterone on the rim of vaginal tissue remaining attached after hysterectomy and observed a direct transport of radioactivity from the cervical to the fundal end of the uterus. In their model, these authors showed that the wave of radioactivity spread from the cervical to the fundal area of the uterus in approximately 5–6 h. In contrast, absorption and elimination in the venous effluent collected in an ''open'' circulation system, peaked after only 1–2 h. At each time interval, the progression of the ''front'' of tritiated progesterone was parallel in endometrial and myometrial tissues, which spoke against the possibility of transcervical aspiration and subsequent absorption from the endometrial cavity. Furthermore, this latter possibility was formally excluded by Bulletti et al. when they repeated the ex vivo studies after obliterating the cervical canal with latex (25).

The nature of the mechanism responsible for the direct vagina-to-uterus transport still remains hypothetical. Putative candidates include passive diffusion, transport through the lymphatic system, or a facilitated diffusion with vein-to-artery passage and countercurrent transport. The latter possibility, albeit complex, let alone implausible at first glance, is a common mode of exchange of substances or heat in a variety of physiological systems. Countercurrent exchange can take place between fluids running in opposite directions and separated by a relatively large exchange surface. This situation exists when arteries and veins have an extensive course in close contact. That countercurrent exchanges take place between vagino-uterine veins and arteries was suggested by an earlier work of Cicinelli et al. (23). These authors demonstrated

higher (approximately two times) uterine artery concentration of progesterone than peripheral arterial values after vaginal administration of progesterone (23). In support of this concept are earlier works that alluded to the possibility of countercurrent diffusion phenomena also taking place between the ovaries and uterus (26–28).

Further work with the ex vivo model is ongoing to determine the extent of vascular involvement implicated in the direct vagina-to-uterus transport. However, it remains that our efforts to administer progesterone vaginally have unveiled a previously unknown local vagina-to-uterus transport system. Speculations on the physiological role of this newly discovered local "portal" system includes a putative involvement in a local transport of prostaglandins (PG) contained in sperm. Following this concept, PG reaching the uterus by this local portal system would activate uterine and tubal contractility and thus facilitate sperm transport without raising PG level in the peripheral circulation. This local transport system is dependent on vaginal and uterine anatomy, but not the nature of the molecule administered. Hence, direct vagina-to-uterus transport could be put into play for any molecule that needs to be administered vaginally. The drug to be administered, however, must remain in the vagina for a prolonged time either through repeated administrations (three times daily for progesterone) or by using some form of sustained-delivery apparatus, as with the bioadhesive vaginal gel. Most interesting clinically is the administration of drugs that exert their effects on the uterus.

IV. VAGINAL PROGESTERONE IN INFERTILITY: OPTIMAL RECEPTIVITY WITHOUT THE PAINFUL IM INJECTIONS

Infertility treatments require that only the native ovarian hormone, progesterone, be used for fear that synthetic compounds could be embryotoxic or lead to the masculinization of a female fetus. Hence, in the early days of IVF, clinicians only had the possibility of administrating progesterone by IM injections, using a peanut–sesame oils preparation.

The need for luteal support became essential with the onset of donor egg IVF. Here the challenge is to prime endometrial receptivity in women whose own ovarian function failed (or was suppressed in case of incipient ovarian failure) with the sole help of exogenous hormone. The first need is for estrogenization. Early investigators in this field used oral estrogen, E_2-valerate (8,9), or micronized E_2 (29). Later, similar efficacy was achieved with transdermal delivery systems (5,30). Yet, despite a more physiological profile of estrogen levels relative to the physiological E_2/E_1 ratio, no benefit could be seen on endometrial morphology by nonoral administration of E_2 (31).

The second need was to replace progesterone normally produced by the corpus luteum. The first investigators used IM progesterone for priming endometrial receptivity (8,9,29). Yet, similar or possibly improved efficacy at reproducing the endometrial changes of the luteal phase has been shown with vaginal progesterone given in the form of suppositories (50 mg t.i.d.) (5) or capsules (200 mg t.i.d.). Bourgain et al. reported that vaginal administration of 200 mg of progesterone t.i.d. induced an in-phase endometrium in most women whose ovaries were inactive (4). This endometrial efficacy was achieved despite lower than normal plasma progesterone levels (6). More importantly, when pregnancy rates were compared, this team of investigators found similar results with a trend toward a lower incidence of early embryo losses in women receiving vaginal progesterone (32). In a different study, it was documented that endometrial effects induced by vaginal progesterone in previously estrogenized women (physiological levels of E_2) were independent of the actual levels of E_2 during the progesterone treatment phase (7). Hence, it was through a logical extension of their experience that these teams adopted vaginal progesterone administration for luteal support in regular IVF (33).

More recently, the advent of a vaginal progesterone gel with sustained-release characteristics has ultimately permitted a reduction of the number of vaginal administrations to once-a-day. In oocyte donation, Gibbons et al. (15) first reported that two daily applications of the 8% gel (90 mg) resulted in pregnancy rates similar to those achieved with IM progesterone. Subsequently, however, these authors extended their trial and observed that once-daily administration was sufficient, with similar clinical (ongoing) pregnancy rates in the vaginal and IM groups of 48% (38%) and 52% (41%), respectively (16). Hence, once-a-day administration having been tested in the worst-case scenario (no endogenous progesterone), it was logically concluded that once-daily administration of the 8% gel is the best means of luteal support in all forms of infertility treatments (34). We know from the dose-ranging study mentioned earlier (20) that this is still far more than the minimum required for an in-phase endometrium and thus provides a reasonable safety margin. This is most reassuring if one application is forgotten, for example.

V. VAGINAL PROGESTERONE IN HRT: A CARDIOVASCULAR FRIENDLY OPTION WITHOUT SIDE EFFECTS

There is now overwhelming evidence that synthetic progestins prevent the risk of endometrial hyperplasia. But contrasting with those reassuring findings, there are also mounting suspicions that these molecules may interfere with some of the beneficial effects of estrogen treatments. This possible antagonism is particularly

concerning the beneficial cardiovascular effects of E_2. Moreover, synthetic progestins are also notorious for their side effects, particularly psychological ones. These effects have their share of responsibility in the poor long-term compliance that characterizes HRT. Progesterone, however, is uniquely different from the synthetic progestins in all these issues.

On the variety of cardiovascular endpoints studied to date, a dualistic situation most often prevails that casts the beneficial effects of E_2 and the antagonistic actions of progestins. Depending on the model studied, synthetic progestins antagonize part of all E_2 effects. Hence, on all these parameters, the beneficial effects recognized with E_2 treatments are opposed to some variable degree by the synthetic progestins most commonly used in HRT. In contrast, however, natural progesterone has been found cardiovascular neutral (see Chap. 16).

The first arena in which the conflicting effects of E_2 and progestins have been identified are blood lipids. These endpoints, originally believed to be the primary mechanism of action for the beneficial effects of E_2 on the cardiovascular system, are considered today as only one of the many mediators of E_2 effects (35–38). It remains consequential, however, that exogenous estrogen, particularly when taken orally, increases high-density lipoprotein (HDL) cholesterol (particularly the HDL_2 subfraction) and decreases LDL cholesterol levels (35–38). As stated earlier, synthetic progestins partially oppose these changes (35–38). Despite the amplitude of the net effects being variable and dependent on the nature of the synthetic progestin used, its concentration, and the degree of estrogenization (35–37), it remains an undebated observation that synthetic progestins at least partly antagonize the action of estrogens on lipids.

The deleterious effects of progestins on lipids are not shared by progesterone. In the Postmenopausal Estrogen/Progestin Intervention (PEPI) Trial, natural progesterone taken orally (200 mg/day) induced no important alterations of the lipid profile, when compared with women taking estrogens only (39). HDL cholesterol increased by 5.6 and 4.1 mg/dL in women who received conjugated equine estrogen (CEE) (0.625 mg/day) alone or in conjunction with progesterone, respectively. In contrast, the increase in HDL cholesterol was much more limited, ranging from 1.2 to 1.6 mg/dL, in women who received medroxyprogesterone acetate (MPA). The clinical consequences of progestin effects on blood lipids are unknown and probably less significant than originally thought. Moreover, speculations on lipid actions of synthetic progestins and progesterone made by analyzing lipid effects in isolation are probably futile and clinically irrelevant. On the contrary, we know today that effects on lipids must be analyzed in the context of all the other changes induced by any given treatment. In the case of progestins, direct vascular effects of hormones on vessels are probably overwhelmingly more important.

The renowned monkey model developed by Clarkson for studying the cardiovascular effects of hormones bears a lot of clinical relevance because to this

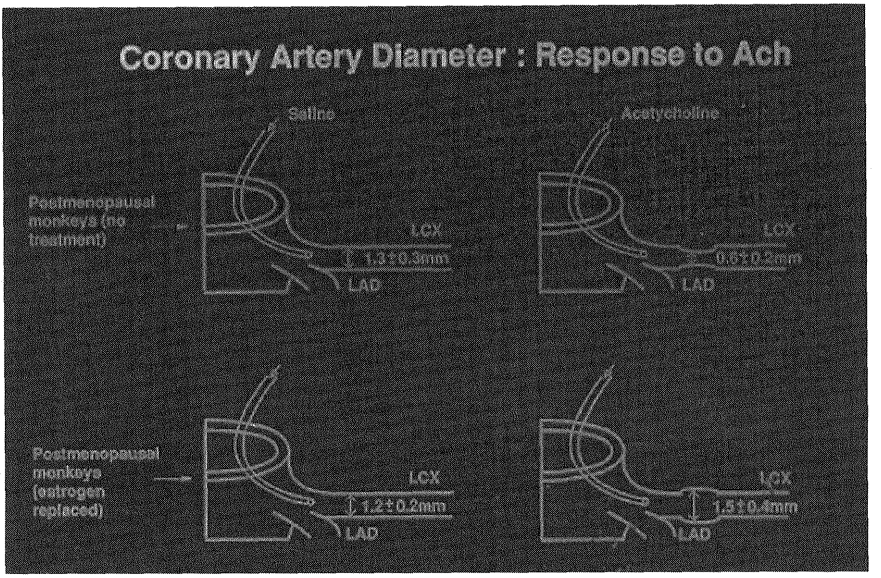

Figure 4 Effects of disease and hormones on vascular reactivity. Acetylcholine (ACh) induces a vasodilation of healthy coronary arteries, but vasoconstriction of atherosclerotic estrogen-deficient vessels. Short-term CEE treatment restores a vasodilative response, but this is blunted by 60% if MPA is concomitantly administered with CEE. On the contrary, addition of progesterone does not hamper the estrogenic effects on vascular reactivity to ACh. (From Ref. 46, with permission.)

date its findings have never been contradicted in human studies (40). Findings of Clarkson's team have all concurred to unveil similar, but yet not related, antagonisms between the effects of estrogens and synthetic progestins as just described for lipids. Contrasting with synthetic progestins, however, progesterone appeared neutral. Female monkeys fed a highly atherogenic diet developed less coronary atherosclerosis, when exposed to E_2 of either endogenous (41) or exogenous (41,42) source, than did untreated castrated animals. Remarkably, this beneficial effect of E_2 treatment was not diminished by concomitant administration of progesterone (42). Medroxyprogesterone acetate (MPA) however, completely opposed the inhibitory effects of CEEs on coronary artery atherosclerosis (43).

Studies on the effects of sex hormones on vascular reactivity have also revealed a similar opposition between E_2 and synthetic progestins (Fig. 4). Coronary arteries of castrated cynomolgus monkeys fed an atherogenic diet for more than 2 years acquired a constrictive response when exposed to acetylcholine (ACh). This represents a pathological response characteristic of atherosclerotic

estrogen-deficient coronary vessels. The arterial response returned to normal (vasodilation) when monkeys received short-term treatment with CEE (44,45). Yet, when MPA was added to the CEE regimen, the amplitude of the dilation induced by ACh was reduced by approximately 60% (46). A different group of investigators observed similar findings in a somewhat similar monkey model (47). With an established coronary vasospasm model, these latter authors observed that progesterone (physiological levels) in combination with E_2 protected against vasospasm, whereas the combination of MPA (low therapeutic range) and E_2 did not (47). Extending the studies of vascular reactivity to humans, Sullivan and his team observed that synthetic progestins, but not estrogens, enhanced the undesirable vasoconstrictor responsiveness (48).

Similar differences between the effects of estrogen, estrogen plus progestin, or estrogen plus progesterone have been observed on the uterine artery model taken as an amplified reflector of vascular effects of ovarian hormones. In early studies, exogenous E_2 given at physiological (49) or HRT levels (50) induced prompt and profound vasodilative effects in women of reproductive (49) or menopausal age (50). The vasodilation induced by E_2, however, was at least partly antagonized by synthetic progestins (51), but not vaginal progesterone (49).

Finally and most importantly for the clinician, similar antagonisms between synthetic progestins on estrogen effects have been recently reported in human trials. A wealth of observational studies have concurred to show that postmenopausal estrogen therapy significantly reduced the risk of death from cardiovascular disease (52). One epidemiological study failed to show that such protection was hampered when progestins (mainly MPA) were combined with estrogens (53). But in apparent contradiction with the optimistic finding of case–control epidemiological studies, a recent prospective, blinded and randomized trial reached different conclusions. The Heart and Estrogen/Progestin Replacement Study (HERS) compared the effects of a 4-year treatment of menopausal women with preexisting CHD with either placebo or CEE (0.625 mg/day) and MPA (2.5 mg/day, constantly). The results showed no cardioprotective effects of the combination treatment CEE–MPA (54). Knowing the beneficial effects of estrogens on cardiovascular ischemia in humans (52), and how MPA can interfere with cardiovascular effects of estrogens, we are led to believe that the null results of the HERS study (54) reflect the antagonistic effects of MPA on estrogenic action. Consistant with this hypothesis are the findings of Adams et al. (42,43). Even more important are the results of a prospective human trial made by Rosano et al. (55). These authors observed that cyclic treatment with MPA, but not vaginal progesterone gel, interfered with the anti-ischemic effects of E_2 already reported previously (56). Moreover puzzling, because unexpected, these authors observed that vaginal progesterone provided further anti-ischemic effects as compared with the E_2-only phase of treatment. This suggested that

Table 3 Cardiovascular Effects of Progestins: Comparison Between Progesterone and a Synthetic Progestin, MPA

	Estrogen only	Estrogen/MPA[a]	Estrogen–Progesterone
Lipids	HDL cholesterol ↑ HDL_2 LDL cholesterol ↓ (39)	Reverse changes induced by E_2 or no increment Larger or similar decrease (35, 37, 39)	No impairment of E effects on HDL No impairment of E effects on LDL (35, 39)
Vasodilative effects	Uterine artery: Rapid and profound dilation (49, 50)	Less dilation that with E_2 alone (51)	Dilation unaltered (49)
Coronary atherosclerosis the ''Clarkson model''	Prevents atherosclerosis development seen in untreated castrated females Endogenous E_2 (41) Exogenous E_2 in castrated animals (42)	MPA completely antagonizes beneficial effects of exogenous estrogens (43)	Progesterone does not interfere with beneficial effects of estrogens (42) Pregnancies provide better protection against coronary atherosclerosis than the menstrual cycle (64).
Vasoreactivity	In monkeys: Estrogen restores vasodilative response of atherosclerotic coronary arteries to acetylcholine (44, 45) In humans: In the presence of estrogen acetylcholine triggers vasodilative response to acetylcholine (48)	In monkeys: MPA blunts the vasodilative response to acetylcholine in estrogenized animals (46) In humans: In the presence of MPA acetylcholine triggers a vasoconstrictive response to acetylcholine (48)	In monkeys: Progesterone does not affect the vasodilative response to acetylcholine in estrogenized animals (44, 45).
Clinical studies	Epidemiological: Beneficial effects, RR ≈ 0.5 (52, 65)	Epidemiological: No difference from estrogen alone (53) Prospective: (II prevention) HERS study (54) No effect of CEE (0.625 mg + MPA (2.5 mg) (54) In symptomatic ischemic women MPA antagonized effects of E_2 (55)	Epidemiological: Cardioprotective effects linked to ovarian function (estrogen and progesterone). Prospective: In symptomatic ischemic women, progesterone improved effects of E_2 alone (55).

[a]MPA is the most extensively studied existing synthetic progestin. Effects of other products may differ.

progesterone might have cardiovascular effects on its own. Consistent with the possibility that progesterone might have cardioprotective effects of its own is the observation made by Clarkson's team that pregnancy offers improved cardioprotection as compared with the menstrual cycle levels of E_2 (40). The distinctive interactions of synthetic progestins and progesterone with the cardioprotective effects of estrogens are summarized in Table 3.

Progesterone also differs from progestins by the characteristically low incidence of side effects encountered. Progestins, on the contrary, are notoriously responsible for a wide variety of side effects, including negative mood changes (57), other CNS symptoms, and water retention. It is currently hypothesized that the difference in CNS effects (including effects on mood) between progesterone and synthetic progestins lies in divergent nongenomic properties of these molecules. In one study, it has been demonstrated that the reduced metabolite of MPA equivalent to the neuroactive steroid 3α-OH-5α-pregnane-20-one (allopregnanolone) failed to bind the steroid site of the $GABA_A$ receptor complex (58). Hence, this provides an explanation for clinical observation that MPA failed to exert the anxiolytic–sedative effects of progesterone (57,59).

VI. A SUSTAINED-RELEASE GEL OPENS THE DOOR TO LONG-TERM TREATMENTS: VAGINAL PROGESTERONE IN CYCLIC AND CONSTANT COMBINED OPTIONS FOR HRT

Although vaginal progesterone has been used in infertility for more than 12 years (4–6), the need for multiple daily administrations has greatly limited its potential for long-term use, such as in HRT. The recent development of a sustained-release gel, Crinone 4%, has opened new perspectives for prolonged treatment in HRT.

In previous work, the vaginal gel of progesterone 4 or 8% concentration has triggered full predecidual transformation of the endometrium, with only single daily (16) or even every 2-day administration (20). In women suffering from secondary amenorrhea, every second-day administration of the vaginal gel of progesterone 4 and 8% resulted in secretory transformation of the endometrium in 100 and 96%, respectively (60). The bleeding patterns achieved with these two dosing regimens were no different among themselves and were similar to those seen with two doses of the synthetic progestin, MPA (61) (Fig. 5).

In a separate trial, we studied the effects of every-day administration of the vaginal gel 4% for 10 days a month. As shown in Table 4, this treatment induced true withdrawal bleeding (i.e., bleeding occurring only after discontinuation of the progestin) in more than 90% of women (62). These data indicate that every-day administration should be the preferred regimen when optimal control of the bleeding pattern is sought. Because the number of vaginal applications

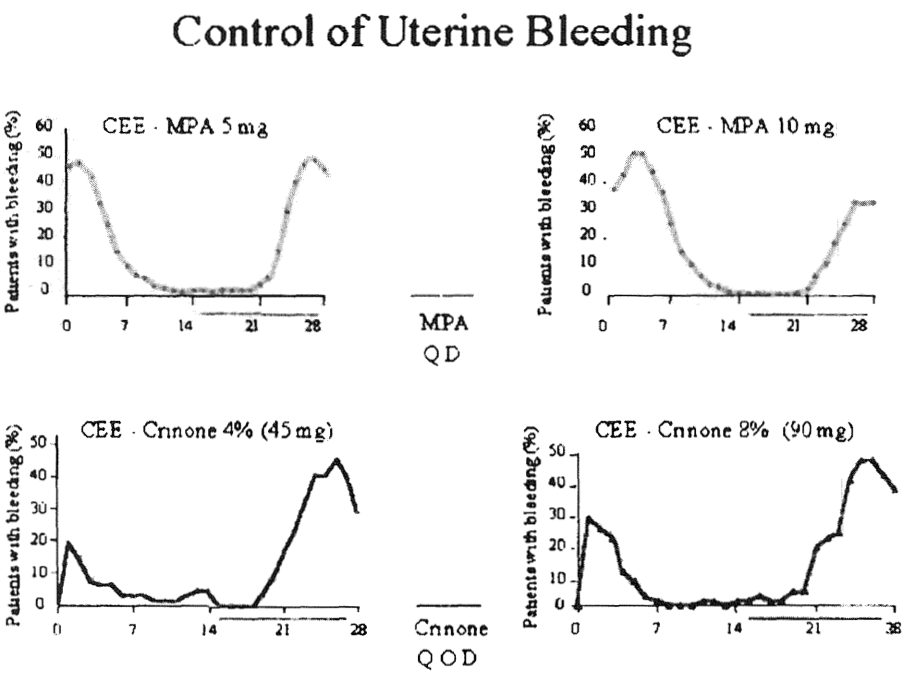

Figure 5 Bleeding patterns with MPA and vaginal progesterone. Bleeding patterns when two doses of MPA (5 and 10 mg) and vaginal gel of progesterone (4 and 8%) are administered in conjunction with conjugated equine estrogens (CEE 0.625 mg/day continuously). The bleeding patterns were similar with these two treatments, and in both cases were not affected by increasing the dose of progestin used.

of the gel is limited to once daily, this approach retains its appeal for long-term treatments (HRT) particularly for (a) women whose past experience indicates that they are intolerant to synthetic progestins; (b) women whose medical condition precludes ingesting hormones orally, notably those with coronary heart disease (CHD) (54); or (c) women who for personal reasons would rather avoid the oral intake of hormones. Our experience indicates that despite its apparent complexity, women are likely to show remarkable compliance if they participate in selecting the vaginal progesterone option for their HRT. This probably results from two distinct phenomena: (a) the notoriously low incidence of side effects with this physiological approach. This practical advantage is readily recognized by HRT users, particularly if they had a prior experience of side effects while using synthetic progestins; (b) the second basis for treatment compliance is linked to women being more likely to remain compliant with a therapeutic

Table 4 Vaginal Progesterone Gel in HRT

	Mean age	Type of bleeding		*n*	%
Group I	50 ± 1.5	Expected	Withdrawal only	63/69	91.3
		Abnormal	Breakthrough or other abnormal bleeding	6/69	8.7
Group II	58 ± 5.3	Expected	Amenorrhea (no bleeding)	54/67	80.6
		Acceptable	Isolated spotting—mild bleeding	9/67	13.4
		Unacceptable	Heavy bleeding—repeated spotting	4/67	6

The vaginal progesterone gel was used either in cyclic (group I) or combined (group II) association with estrogens as part of HRT. When used cyclically (10 days/month), the majority of women experienced only true withdrawal bleeding (bleeding occurring after the last dose of treatment). The constant combined regimen allowed 80% of women to remain amenorrheic throughout their treatment period.

option they participated to select. On the contrary, regimens that may be felt imposed are less likely to gain long-term compliance, even if their ease of use appears relatively superior.

Another approach for using the vaginal progesterone gel in menopause takes a maximum advantage of the sustained-release properties of the gel Crinone 4%. Because of the clinical appeal for constant combined or "no-bleed" regimens, we made an attempt to use the vaginal gel of progesterone in constant association with estrogen replacement therapy. Prefactory to this approach was the prerequisite to limit the number of vaginal administrations to a frequency judged reasonable and acceptable by women who begin HRT. Evidently, constant daily administration of a vaginal product could not be considered. Hence, following our quest for a practical constant combined regimen with vaginal progesterone, we concluded that twice weekly administration should be the best practical compromise. On the one hand, the sustained-release properties of the polycarbophil base gel cannot reasonably be extended more than 3.5 days because of the turnover time of the vaginal epithelium. On the other hand, it would not have seem practical to impose more frequent administrations of a vaginal product for a prolonged treatment, even if it were highly successful at maintaining a no-bleed situation. Preliminary work with 20 women who used this twice weekly, constant combined regimen showed very positive results. The vast majority of women stayed and remained amenorrheic throughout the treatment period (63). Encouraged by these early results, the regimen has been adopted in our gynecological practice. Results available from 67 women receiving this treatment for at least 6 months appear in Table 4. As can be seen, the vast

majority of women (> 80%) remained completely amenorrheic throughout their treatment period. These results are far superior than any published data with constant, combined regimens using oral hormones. In oral treatments, amenorrhea is a state usually reached only after months of spotting (61), and irregular bleeding is experienced in most participants. In our study, the endometrial thickness was measured with ultrasound. In women who received the vaginal progesterone gel twice weekly in conjunction with estrogen therapy, mean endometrial thickness was 3.9 ± 1.2 mm. An endometrial biopsy was performed each time the thickness was greater than 5 mm or bleeding occurred. None of these cases showed endometrial hyperplasia.

In conclusion, vaginal progesterone, the best nonoral option for short-term replacement in infertility, has also its place in long-term treatments. Because of the sustained-release properties of a new vaginal gel, it is now also possible to consider the vaginal progesterone option for HRT. It can be speculated that this physiological option will appeal to women who cannot or do not want to use synthetic progestins because of medical contraindications or subjective side effects.

VII. CONCLUSION

Progestins completely protect from the risk of estrogen-induced endometrial hyperplasia or cancer. Yet, because of side effects and interferences with the beneficial effects of E_2, there are circumstances when physiological replacement with natural progesterone administered vaginally is preferred. Today there is a multitude of practical options available for delivering progestins. At one extreme, the simplest option consists in taking a synthetic progestin orally. At the other end of the spectrum, the natural hormone progesterone can be administered vaginally, which represents the physiological option. Because of direct access to the uterus, vaginal progesterone using a sustained-release gel only minimally raises peripheral progesterone levels. Within the range of acceptable options are other compromise solutions. These include (a) oral progesterone, which however results in an unnatural elevation of progesterone metabolites with depressive CNS effects; or (b) nonoral synthetic progestins, such as NETA, through transdermal therapeutic systems. Although viable, this latter option carries the side effects of NETA, which are molecule-dependent.

Sadly, we must also realize that other non–FDA-supervised options are commonly proposed and even compared with treatments of proven efficacy. These include transdermal progesterone preparations that have been proved ineffective at raising plasma progesterone levels and do not prevent development of hyperplasia. Therefore, it is crucial that our discussion on the use of progestins

in gynecology differentiates the acceptable options, each with their particular advantages (e.g., ''simple'' or ''natural'') from other remedies (such as progesterone creams) that have no proved efficacy.

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REFERENCES

1. JA Simon, DE Robinson, MC Andrews, et al. The absorption of oral micronized progesterone: the effect of food, dose proportionality, and comparison with intramuscular progesterone. Fertil Steril 60:26–33, 1993.
2. K Nahoul, L Dehennin, R Scholler. Radioimmunoassay of plasma progesterone after oral administration of micronized progesterone. J Steroid Biochem 26:241–249, 1987.
3. K Nahoul, L Dehennin, M Jondet, M Roger. Profiles of plasma estrogens, progesterone and their metabolites after oral or vaginal administration of estradiol or progesterone. Maturitas 16:185–202, 1993.
4. C Bourgain, P Devroey, L Van Waesberghe, J Smitz, AC Van Steirteghem. Effects of natural progesterone on the morphology of the endometrium in patients with primary ovarian failure. Hum Reprod 5:537–543, 1990.
5. CL Schmidt, D de Ziegler, CL Gagliardi, et al. Transfer of cryopreserved-thawed embryos: the natural cycle versus controlled preparation of the endometrium with gonadotropin-releasing hormone agonist and exogenous estradiol and progesterone (GEEP). Fertil Steril 49:609–616, 1988.
6. P Devroey, G Palermo, C Bourgain, L Van Waesberghe, J Smitz, AC Van Steirteghem. Progesterone administration in patients with absent ovaries. Int J Fertil 34:188–193, 1989.
7. D de Ziegler, C Bergeron, C Cornel, et al. Effects of luteal estradiol on the secretory transformation of human endometrium and plasma gonadotropins. J CIin Endocrinol Metab 74:322–331, 1992.
8. P Lutjen, A Traunson, J Leeton, J Findlay, C Wood, P Renow. The establishment and maintenance of pregnancy using in vitro fertilization and embryo donation in a patient with primary ovarian failure. Nature 307:174–175, 1984.
9. D Navot, N Laufer, J Kopolovic, et al. Artificially induced endometrial cycles and establishment of pregnancies in the absence of ovaries. N Engl J Med 314:806–811, 1986.
10. D de Ziegler. Hormonal control of endometrial receptivity. Hum Reprod 10:4–7, 1995.

11. K Steingold, P Stumpf, D Kreiner, HC Liu, D Navot, Z Rosenwaks. Estradiol and progesterone replacement regimens for the induction of endometrial receptivity. Fertil Steril 52:756–760, 1989.
12. R Frydman, H Letur-Könirsch, D de Ziegler, D Bydlowski, A Raoul-Duval, J Selva. A protocol for satisfying the ethical issues raised by oocyte donation: the free, anonymous, and fertile donors. Fertil Steril 53:666–672, 1990.
13. S Soliman, S Daya, J Collins, EG Hughes. The role of luteal phase support in infertility treatment: a meta-analysis of randomized trials. Fertil Steril 61:1068–1076, 1994.
14. C Bourgain, J Smitz, M Camus, et al. Human endometrial maturation is markedly improved after luteal supplementation of gonadotrophin stimulated cycles. Hum Reprod 9:32–40, 1994.
15. WE Gibbons, JP Toner, P Hamacher, P Kolm. Experience with a novel vaginal progesterone preparation in a donor oocyte program. Fertil Steril 69:96–101, 1998.
16. JP Toner, WE Gibbons. Crinone 8% used once a day for replacement in donor egg recipients: a status report [abstr]. Presented at the ASRM, San Francisco, CA, 1998.
17. E Cicinelli, F Savino, I Cagnazzo, P Scorcia. Comparative study of progesterone plasma levels after nasal spray and intramuscular administration of natural progesterone in menopausal women. Gynecol Obstet Invest 35:172–174, 1993.
18. RJ Chetkowski, DR Meldrum, KA Steingold, et al. Biologic effects of transdermal estradiol. N Engl J Med 314:1615–1620, 1986.
19. A Cooper, C Spencer, MI Whitehead, D Ross, GJR Barnard, WP Collins. Systemic absorption of progesterone from Progest cream in postmenopausal women. Lancet 351:1255–1256, 1998.
20. R Fanchin, D de Ziegler, C Bergeron, C Righini, C Torrisi, R Frydman. Transvaginal administration of progesterone: dose–response data support a first uterine pass effect. Obstet Gynecol 90:396–401, 1997.
21. RW Noyes, AT Hertig, J Rock. Dating the endometrial biopsy. Fertil Steril 1:3–25, 1950.
22. RA Miles, RJ Paulson, RA Lobo, MF Press, L Dahmoush, MV Sauer. Pharmacokinetics and endometrial tissue levels of progesterone after administration by intramuscular and vaginal routes: a comparative study. Fertil Steril 62:485–490, 1994.
23. E Cicinelli, M Cignarelli, S Sabatelli, et al. Plasma concentrations of progesterone are higher in the uterine artery than in the radial artery after vaginal administration of micronized progesterone in an oil-based solution to postmenopausal women. Fertil Steril 69:471–473, 1998.
24. C Bulletti, D de Ziegler, C Flamigni, et al. Targeted drug delivery in gynaecology: the first uterine pass effect. Hum Reprod 12:1073–1079, 1997.
25. D de Ziegler, E Cicinelli, C Bulletti. Vaginal progesterone gel: evidence of direct vagina-to-uterus transport. Wyeth-Ayerst Laboratories sponsored Symposium: Vaginal Progesterone in Assisted Fertility: New Data on Implantation and Pregnancy Rates. ASRM, San Francisco, CA, 1998.

26. A Bendz, N Einer-Jensen, O Lundgren, PO Janson. Exchange of krypton-85 between the blood vessels of the human uterine adnexa. J Reprod Fertil 57:137–142, 1979.
27. A Bendz. The anatomical basis for a possible counter current exchange mechanism in the human adnex. Prostaglandins 13:355–362, 1977.
28. N Einer-Jensen. Local transfer of ^{133}xenon from the uterine horn to the ipsilateral ovary in the mouse, hamster and guinea-pig. J Reprod Fertil 40:479–482, 1974.
29. Z Rosenwaks. Donor eggs: their application in modern reproductive technologies. Fertil Steril 47:895–909, 1987.
30. K Droesch, D Navot, R Scott, D Kreiner, HC Liu, Z Rozenwaks. Transdermal estrogen replacement in ovarian failure for ovum donation. Fertil Steril 50:931–934, 1988.
31. KA Steingold, DW Matt, D de Ziegler, JE Sealey, M Fratkin, S Reznikov. Comparison of transdermal to oral estradiol administration on hormonal and hepatic parameters in women with premature ovarian failure. J Clin Endocrinol Metab 73:275–280, 1991.
32. G Pados, M Camus, A Van Steirteghem, M Bonduelle, P Devroey. The evolution and outcome of pregnancies from oocyte donation. Hum Reprod 9:538–942, 1994.
33. J Smitz, P Devroey, B Faguer, C Bourgain, M Camus, AC Van Steirteghem. A prospective randomized comparison of intramuscular or intravaginal natural progesterone as a luteal phase and early pregnancy supplement. Hum Reprod 7:168–175, 1992.
34. D de Ziegler, C Bulletti, B de Moustier. Endometrial preparation. In: MV Sauer, ed. Principles of Oocyte and Embryo Donation. New York: Springer-Verlag, 1998, pp 65–76.
35. RA Lobo. Effects of hormonal replacement on lipids and lipoproteins in postmenopausal women. J Clin Endocrinol Metab 73:925–930, 1991.
36. VT Miller, RA Muesing, JC LaRosa, DB Stoy, SE Fowler, RJ Stillman. Quantitative and qualitative changes in lipids, lipoproteins, apolipoprotein A-I, and sex hormone-binding globulin due to two doses of conjugated equine estrogen with and without a progestin. Obstet Gynecol 83:173–179, 1994.
37. JH Pickar, I Thorneycroft, M Whitehead. Effects of hormone replacement therapy on the endometrium and lipid parameters: a review of randomized clinical trials, 1985 to 1995. Am J Obstet Gynecol 178:1087–1099, 1998.
38. MA Espeland, SM Marcovina, V Miller, et al. Effect of postmenopausal hormone therapy on lipoprotein(a) concentration. Circulation 97:979–986, 1998.
39. The Writing Group for the PEPI Trial. Effects of estrogen or estrogen/progestin regimens on heart disease risk factors in postmenopausal women. The Postmenopausal Estrogen/Progestin Interventions (PEPI) Trial. JAMA 273:199-208, 1995.
40. TB Clarkson, MS Anthony, KP Klein. Hormone replacement therapy and coronary artery atherosclerosis: the monkey model. Br J Obstet Gynaecol 103:53-58, 1996.
41. MR Adams, JR Kaplan, TB Clarkson, DR Koritnik. Ovariectomy, social status, and atherosclerosis in cynomolgus monkeys. Arterioslerosis 5:192–200, 1985.

42. MR Adams, JR Kaplan, SB Manuck, et al. Inhibition of coronary artery atherosclerosis by 17-beta estradiol in ovariectomized monkeys: lack of an effect of added progesterone. Arteriosclerosis 10:1051–1057, 1990.
43. MR Adams, TC Register, DL Golden, JD Wagner, JK Williams. Medroxyprogesterone acetate antagonizes inhibitory effects of conjugated equine estrogens on coronary artery atherosclerosis. Arterioscerosis Thromb Vasc Biol 17:217–221, 1997.
44. JK Williams, MR Adams, HS Klopfenstein. Oestrogen modulates responses of atherosclerotic coronary arteries. Circulation 81:1680–1687, 1990.
45. JK Williams, MR Adams, DM Herrington, TB Clarkson. Effects of short-term oestrogen treatment on vascular responses of coronary arteries. J Am Coll Cardiol 20:452–457, 1992.
46. JK Williams, EK Honore, SA Washburn, TB Clarkson. Effects of hormone replacement therapy on reactivity of atherosclerotic arteries in cynomolgus monkeys. J Am Coll Cardiol 24:1757–1761, 1994.
47. K Miyagawa, J Rösch, F Stanczyk, K Hermsmeyer. Medroxyprogesterone interferes with ovarian steroid protection against coronary vasospasm. Nature Med 3:324–327, 1997.
48. JM Sullivan, BA Shala, LA Miller, JL Lerner, JD McBrayer. Progestins enhances vasoconstrictor responses in postmenopausal women receiving estrogen replacement therapy. North Am Menopause Society 2:193–199, 1995.
49. D de Ziegler, R Bessis, R Frydman. Vascular resistance of uterine arteries: physiological effects of estradiol and progesterone. Fertil Steril 55:775–779, 1991.
50. T Bourne, TC Hillard, MI Whitehead, D Crook, S Campbell. Oestrogens, arterial status, and postmenopausal women. Lancet 335:1470–1471, 1990.
51. TC Hillard, NC Siddle, MI Whitehead, DI Fraser, J Pryse-Davies. Continuous combined conjugated equine estrogen–progestogen therapy: effects of medroxyprogesterone acetate and norethindrone acetate on bleeding patterns and endometrial histologic diagnosis. Am J Obstet Gynecol 167:1–7, 1992.
52. MJ Stampfer, GA Colditz. Estrogen replacement therapy and coronary heart disease: a quantitative assessment of the epidemiologic evidence. Prev Med 20:47–63, 1991.
53. F Grodstein, MJ Stampfer, JE Manson, et al. Postmenopausal estrogen and progestin use and the risk of cardiovascular disease. N Engl J Med 335:453–461, 1996.
54. S Hulley, D Grady, T Bush, et al. Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. Heart and Estrogen/Progestin Replacement Study (HERS) Research Group. JAMA 280:605–613, 1998.
55. GMC Rosano, PM Sarrel, SL Chierchia, et al. Medroxyprogesterone acetate (MPA) but not natural progesterone (P) reverses the effect of estradiol 17β (E_2) upon exercise induced myocardial ischemia. A double-blind cross-over study [abstr]. Proceedings of the 8th International Congress on the Menopause, Sydney, Australia, November 3–7, 1996, p 82.

56. GMC Rosano, PM Sarrel, PA Poole-Wilson, P Collins. Beneficial effect of oestrogen on exercise-induced myocardial ischaemia in women with coronary artery disease. Lancet 342:133–136, 1993.
57. N Panay, J Studd. Progestogen intolerance and compliance with hormone replacement therapy in menopausal women. Hum Reprod Update 3:159–171, 1997.
58. JW McAuley, PD Kroboth, DD Stiff, IJ Reynolds. Modulation of (^{3}H) flunitrazepam binding by natural and synthetic progestational agents. Pharmacol Biochem Behav 45:77–83, 1993.
59. T Bäckström. Effects of natural and synthetic gestagens on mood and brain excitability. In: G. Berg, M. Hammar, eds. The Modern Management of the Menopause. A Perspective for the 21st Century. New York: Parthenon Publishing, 1994, pp 295–299.
60. MP Warren, BMK Biller, MM Shangold. A new clinical option for hormone replacement therapy in women with secondary amenorrhea: effects of cyclic administration of progesterone from the sustained release vaginal gel Crinone (4% and 8%) on endometrial morphologic features and withdrawal bleeding. Am J Obstet Gynecol 180:42–48, 1999.
61. DF Archer, JH Pickar, F Bottiglioni. Bleeding patterns in postmenopausal women taking continuous combined or sequential regimens of conjugated estrogens with medroxyprogesterone acetate. Obstet Gynecol 83:686–692, 1994.
62. D De Ziegler, R Ferriani, LAM Moraes, C Bulletti. Vaginal progesterone in menopause: Experience with a new sustained and controlled release gel of progesterone Crinone® 4% in cyclical and constant combined regimens. Proceeding of the conference Progesterone: A natural, life-supporting hormone. Montegridolfo, Italy, July 12–14, 1998. (In press) 1999.
63. F Al-Azzawi, D de Ziegler. Vaginal progesterone gel-based continuous combined HRT as an amenorrheic regimen [abstr]. Presented at IV European Congress on Menopause, Vienna, Austria, October 1997.
64. MR Adams, JR Kaplan, TB Clarkson. Pregnancy-associated inhibition of coronary artery atherosclerosis in monkeys. Arteriosclerosis 7:378–384, 1987.
65. MJ Stampfer, GA Colditz, WC Willet, JE Manson, B Rosner, FE Speizer, CH Hennekens. Postmenopausal estrogen therapy and cardiovascular disease. Ten-year follow-up from the nurses' health study. N Engl J Med 325:756–762, 1991.
66. D de Ziegler. Transvaginal administration of progesterone targets delivery to the uterus while minimizing systemic levels through a first uterine pass effect [abstr]. IFFS, Sydney, Australia, 1996.

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Pharmacology of Progestins: 17α-Hydroxyprogesterone Derivatives and Progestins of the First and Second Generation

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I. INTRODUCTION

In the combined estrogen–progestin oral contraceptives (OC), one type of estrogen prevails, that is, 17α-ethinyl estradiol (EE). This substance was first synthesized and tested by Inhoffen and Hohlweg in 1938 (i.e., 60 years ago) (1). Because EE and its methyl ether (mestranol) exhibited potent oral activity and were not protected by patents, there was little incentive to search for other oral estrogens for contraceptive purposes. Thus, for estrogens, synthetic efforts cannot be described as extensive. On the other hand, the first contraceptive study conducted by Pincus et al. (2) about 40 years ago, prompted the synthesis of numerous progestins, about a dozen of which have been clinically used in established contraceptives. In addition, at least three compounds are in various stages of preclinical and clinical development. Besides these compounds, certain modified progestins have been used in areas other than contraception (e.g., spironolactone as an antiandrogen).

II. DEFINITION

According to the classic definition, *progestins* transform the estrogen-primed endometrium into a secretory one and are able to support the development and maintenance of pregnancy. The advent of molecular biology has defined *progestins* as compounds that bind with progesterone receptors within the target cells. However, binding with progesterone receptors (PRs) does not preclude the progestin molecule from binding with other receptors or expressing effects other than progestogenic. For example, norethindrone, under certain circumstances, can stimulate the proliferation of the atrophic endometrium and cyproterone acetate is a recognized antiandrogen both in men and women (3–5).

III. STRUCTURE AND CLASSIFICATION

Contraceptive progestins are derived from the three tetracyclic structures, pregnane, estrane, and gonane (Fig. 1).

In characterizing the steroid molecule, the four rings of the tetracyclic structure are identified as A, B, C, and D. The numbering system of the steroid

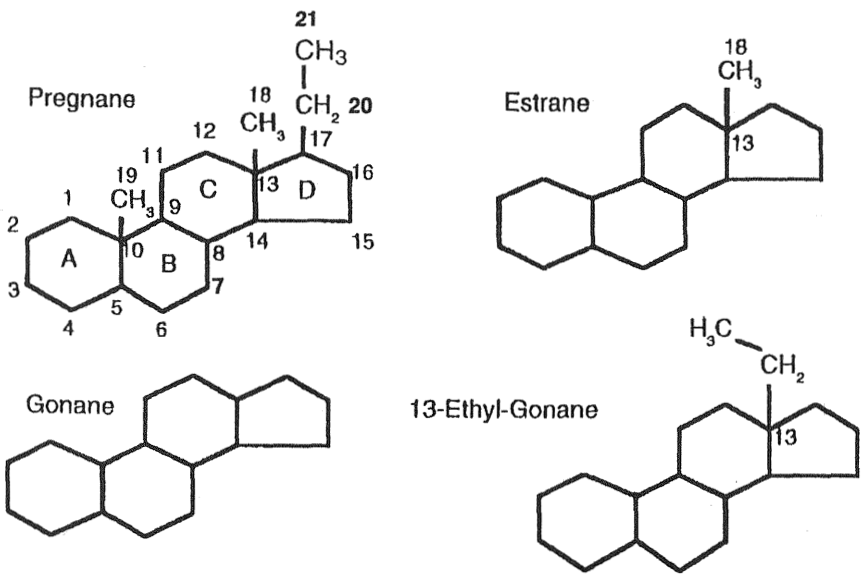


Figure 1 Skeleton steroid structures from which contraceptive progestins are derived (details in text).

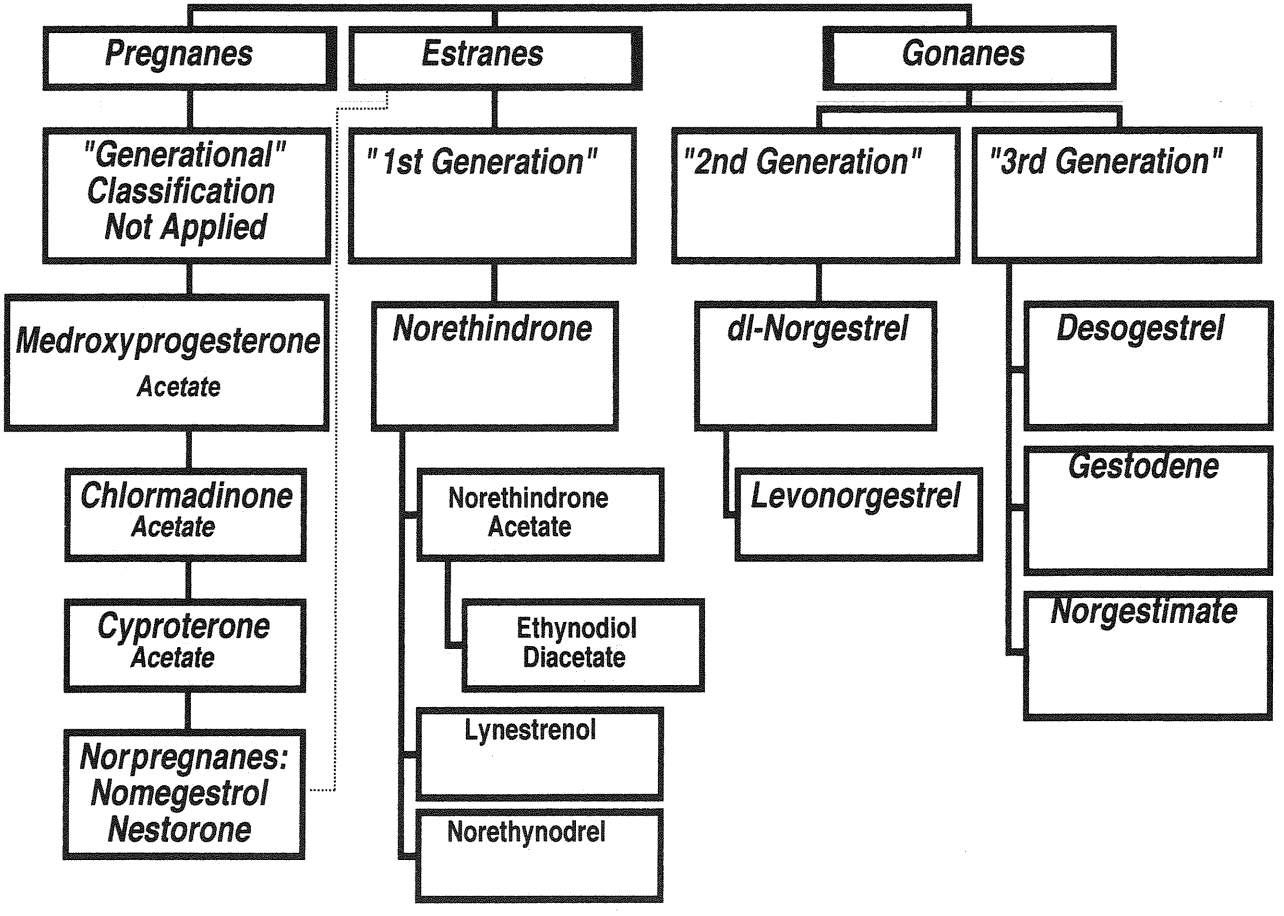


Figure 2 Classification of contraceptive progestins. Norpregnanes are a cross between pregnanes and estranes.

molecule designates the 17 carbons that form the steroid skeleton as C-1 through C-17, and the two angular methyl radicals are numbered C-18 and C-19. In the pregnanes, the two carbons of the side chain that is attached to C-17 are specified as C-20 and C-21. Progesterone is a typical example of the pregnane family.

The estrane structure lacks the C-19 angular methyl radical between rings A and B. Removal of a radical from a certain position in the molecule is abbreviated in chemical shorthand as NOR (i.e., no radical). Thus, the 19-norsteroids belong to the estrane series. As will be stressed later, removal of the C-19 methyl radical substantially increases the progestational activity of these norsteroids.

The gonane structure lacks both the C-18 and the C-19 angular methyl radicals. However, gonane progestins bear an ethyl group between rings C and D at C-13, and this chemical modification further enhances their progestational activity.

From these definitions, we have classified the contraceptive progestins into four groups as summarized in Fig. 2.

It has become currently fashionable to call the estrane progestins the "first-generation" OC, whereas the gonane progestins are divided into the "second-" and "third"-generation OC. It is difficult to understand why this generational classification omitted to encompass pregnanes, although these compounds entered the contraceptive field at its inception and continue to play an important role in the effort to curb the human population growth.

In this chapter, we will discuss various pharmacologic aspects of pregnanes, estranes, and gonanes of the second generation, related to contraception. Gonanes of the third generation are discussed in a subsequent chapter. It is indispensable, however, to bring up certain comparative features of progestins of the third generation in our review. The description of each type of progestin will begin with a brief discussion of the structure–function relation of the individual molecules, followed by pharmacokinetics and metabolic disposition. A separate section is devoted to the pharmacodynamic aspects of the discussed progestins.

IV. PREGNANES

A. Structure and Function

Pregnanes that are derived from the progesterone molecule reveal how seemingly minor structural modifications of the steroid molecule can have far-reaching consequences for biological activity (Fig. 3) (6). For progestogenic activity, the C-17 position is of key importance. Indeed, chemical manipulations at this site profoundly change the function of the molecule. Thus, progesterone loses its

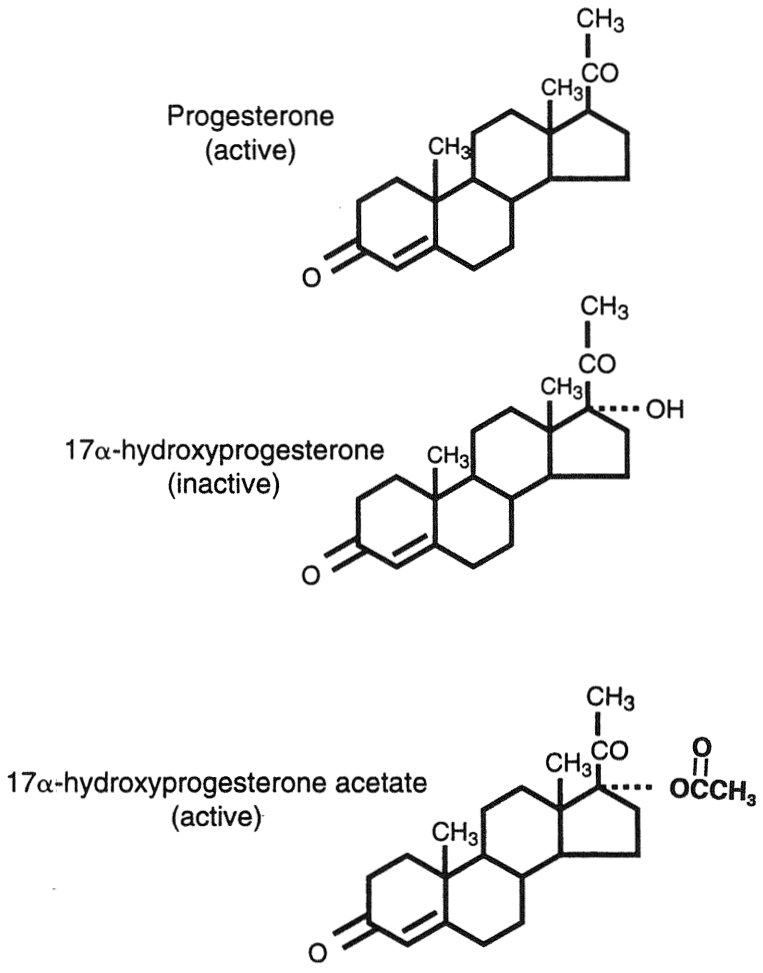


Figure 3 Pregnane progestins derived by manipulation of the progesterone molecule. For progestogenic activity, the C-17 position is of key importance. Progesterone loses its biological activity with the introduction of the hydroxyl group in position α to C-17. Acetylation of the C-17 hydroxyl restores progestogenic activity of the molecule. 17α-Hydroxyprogesterone acetate has been the starting point for the synthesis of other potent progestins (see Fig. 4). (From Ref. 6.)

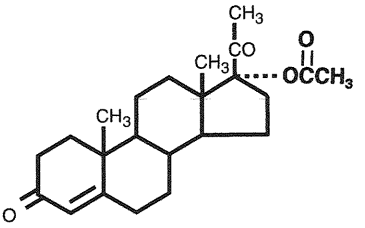
biological activity with the introduction of an α-hydroxyl group at C-17. Esterification of this group not only restores the progestational activity, but also renders this compound orally active. The resulting substance, 17α-hydroxyprogesterone acetate, became the starting point for the synthesis of other potent progestins. Further synthetic work showed the importance of the C-6 position in the B ring for oral progestational activity. The 6-methyl analogue, medroxyprogesterone acetate (MPA; Provera, Pharmacia–Upjohn Company) is used as a tablet for various gynecological indications. The injectable depot preparation is an effective 3-month contraceptive. Another C-6 methyl analogue, megestrol acetate (Megace), differs from MPA by an additional double bond between C-6 and C-7. This compound is used for management of breast, endometrial, and prostatic carcinomas.

Halogenation of C-6 led to compounds that are among the most potent progestins synthesized in the acetoxyprogesterone series; namely, the acetates of chlormadinone and cyproterone (Fig. 4). Both compounds are used in combination OC in several countries outside the United States. They are also highly effective antiandrogens. Recently, a chlormadinone acetate-containing combination OC has been successfully applied in women with acne (7). Cyproterone acetate has been established in the therapy of benign prostatic hypertrophy (BPH), prostatic carcinoma, and precocious puberty.

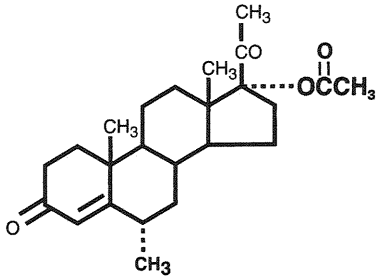
B. Certain Toxicological Aspects of Pregnanes

Medroxyprogesterone acetate, chlormadinone, and cyproterone acetates have been proscribed for contraceptive use in the United States for a number of years because preclinical toxicology had shown an accelerated occurrence of benign and malignant breast nodules in beagle dogs—a breed that shows a spontaneously high incidence of breast tumors, including carcinoma. Extensive clinical studies conducted by the World Health Organization (WHO) have lifted the cloud of potential carcinogenicity hanging over MPA, with the result that this compound is currently being used as an injectable contraceptive in the United States (8).

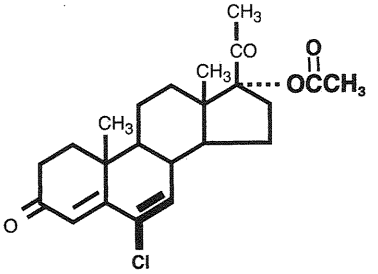
The acceleration of the development of breast nodules in beagle dogs was not observed in toxicological studies with various estranes and gonanes. The differences in carcinogenic potential among the various types of steroids have not been well studied in animals and remain unexplained. Also, long-term toxicological studies in monkeys have not shown formation of breast nodules. In monkeys, spontaneous breast carcinoma is unknown and, therefore, the induction of hormone-associated breast pathology would have been particularly important.



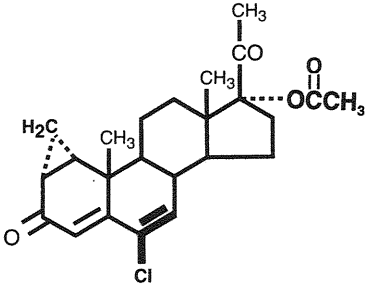
17a-HYDROXYPROGESTERONE
ACETATE
(weak progestogen)



MEDROXYPROGESTERONE
ACETATE
(progestogen)
orally active



CHLORMADINONE
ACETATE
(progestogen, antiandrogen)
orally active



CYPROTERONE
ACETATE
(progestogen, strong
antiandrogen)
orally active

Figure 4 C-6 is an important center of progestational activity. Introduction of methyl radical to C-6 results in medroxyprogesterone acetate—a potent progestin. Halogenation of C-6 results in compounds with high progestational and antiandrogenic activities. (From Ref. 6.)

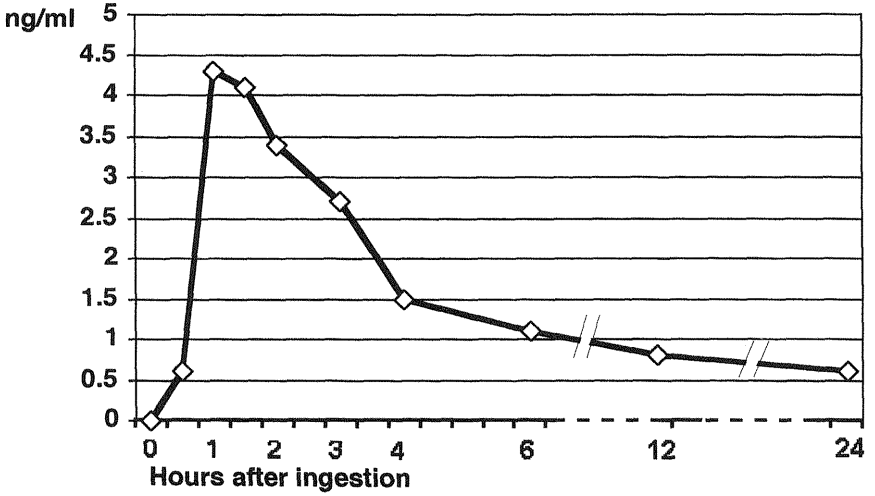


Figure 5 Serum concentrations of radioimmunoassay-labeled medroxyprogesterone acetate (MPA) before and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h after ingestion of 10 mg MPA. Three healthy women were examined. Reproduced here are representative data of one subject. (Modified from Ref. 9.)

C. Pharmacokinetics and Metabolism

The pharmacokinetics and metabolism of the pregnane progestins are illustrated with MPA. After an oral dose of 10 mg, maximum circulating concentration of 3–5 ng/mL is achieved 1–4 h after dosing. A gradual decline follows, and at 24 h after dosing, the circulating concentration ebbs to 0.5 ng/mL (Fig. 5) (9). The half-life of MPA is about 24 h. In the circulation, MPA is mostly bound nonspecifically to albumin.

MPA is metabolized by reduction of the A ring and hydroxylation, mainly at C-6 with subsequent glucuronidation. The acetate group remains intact (10). After injection of 150 mg of depot-MPA, MPA rapidly appears in the circulation. During the 20–40 days after injection, blood levels are relatively high, fluctuating between 1.5 and 3 ng/mL. At the end of 3 months after the injection the blood levels of MPA are about 0.5–1.0 ng/mL. Thereafter, the MPA levels decline to 0.2 ng/mL at the end of the sixth month, and during the seventh month after injection, MPA is undetectable in the circulation (Fig. 6) (11). The relatively high circulating levels of MPA during the first 3 months after injection of depot-MPA are paralleled by high protection against pregnancy, and repeated 3-month injections are recommended.

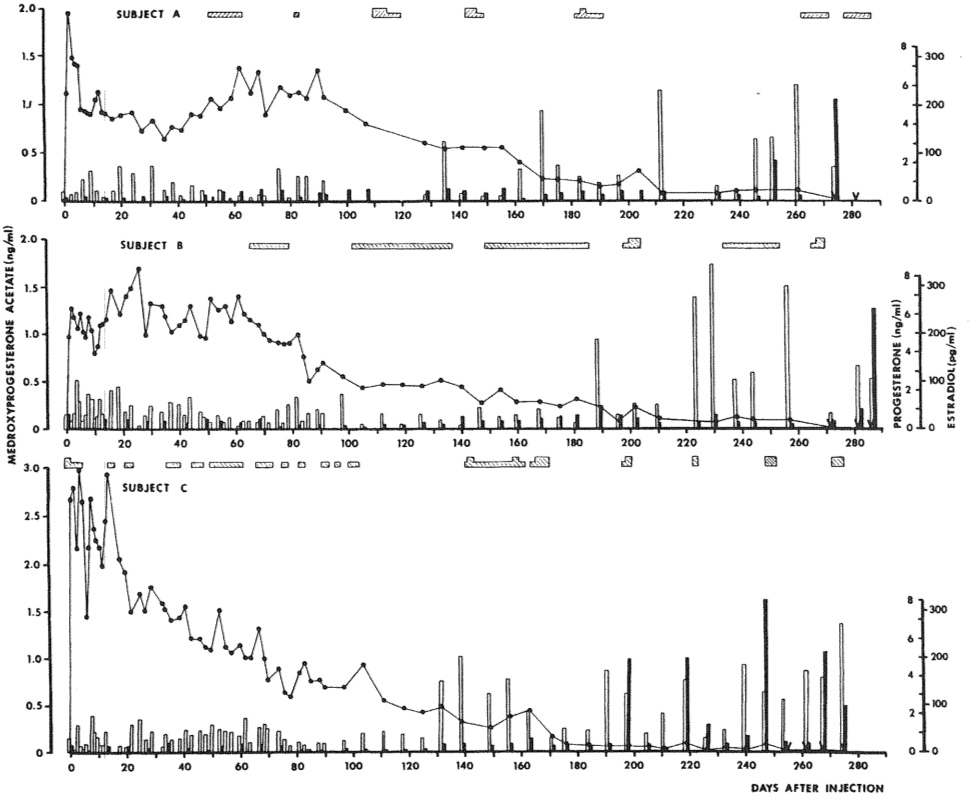


Figure 6 Serum medroxyprogesterone acetate (MPA, dots), estradiol (open bars), and progesterone (solid bars) concentrations in three women (subjects A, B, and C) following intramuscular injection of 150 mg depo-MPA. Hatched horizontal bars of full and half thickness indicate uterine bleeding and spotting, respectively. Undetectable levels of MPA are indicated by **v**. (From Ref. 11.)

V. ESTRANES: THE FIRST-GENERATION PROGESTINS

A. Structure and Function

The estrane progestins are derivatives of the testosterone molecule. Possibly, the most important estrane is 17α-ethinyl-19-nortestosterone, known under the generic names of norethindrone and, mostly in Europe, as norethisterone; it is abbreviated as NET. Figure 7 shows NET and its four derivatives which have been used in various combined oral contraceptives. These derivatives differ from the parent molecule by minor structural modifications, and they have to be

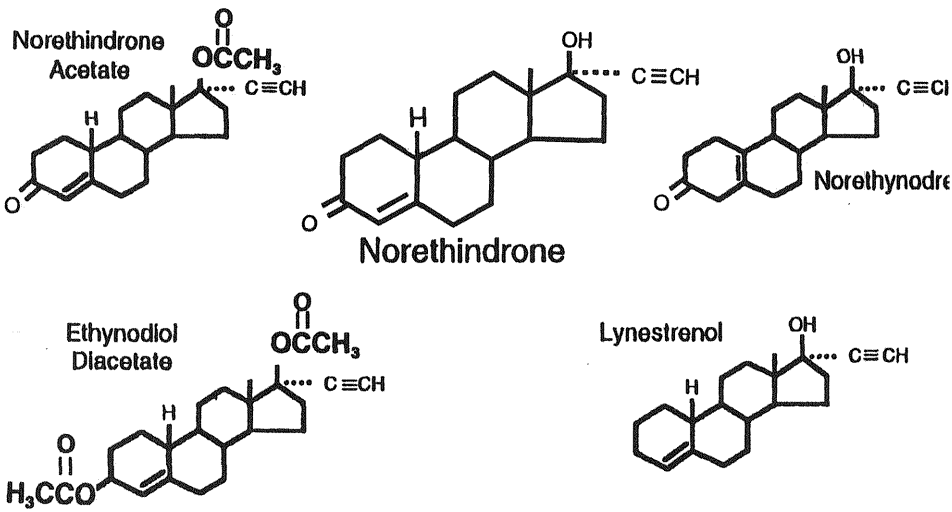


Figure 7 Norethindrone (NET) and its four derivatives. To become biologically active, these derivatives have to be metabolically converted to NET. (From Ref. 12).

metabolically converted to NET before they become biologically active (12). In this sense, they can be considered as prodrugs. Norethynodrel differs from NET by the position of the double bond in the A ring: in norethynodrel the double-bond is between C-5 and C-10, whereas in NET it is between C-4 and C-5. This modification affords norethynodrel some degree of estrogenicity. Norethynodrel was the progestin component of the first combined OC; however, today it is rarely used. Lynestrenol is characterized by the absence of an oxygen function at the C-3. NET-acetate bears an acetoxy group at C-17. Ethinodiol diacetate bears acetoxy groups on both C-3 and C-17.

The development of norsteroids is an example of biochemical ingenuity and innovation (Fig. 8). Henzl (13) and Edwards and Henzl (14) have given a detailed account of the development of contraceptive steroids.

The cornerstone in the quest for highly potent orally active progestins was the recognition that removal of C-19 methyl radical profoundly changes the biological properties of both the progesterone and testosterone molecules. For progesterone, removal of C-19 methyl group resulted in 19-norprogesterone, the first compound found to be more potent than progesterone itself. However, this compound was only parenterally active. Removal of the C-19 methyl radical from testosterone resulted in the loss of androgenicity of the parent molecule. Earlier, it had been found that attachment of the ethinyl group in the α-position ($\cdots C{\equiv}CH$) at C-17 afforded the molecule oral activity. Combination

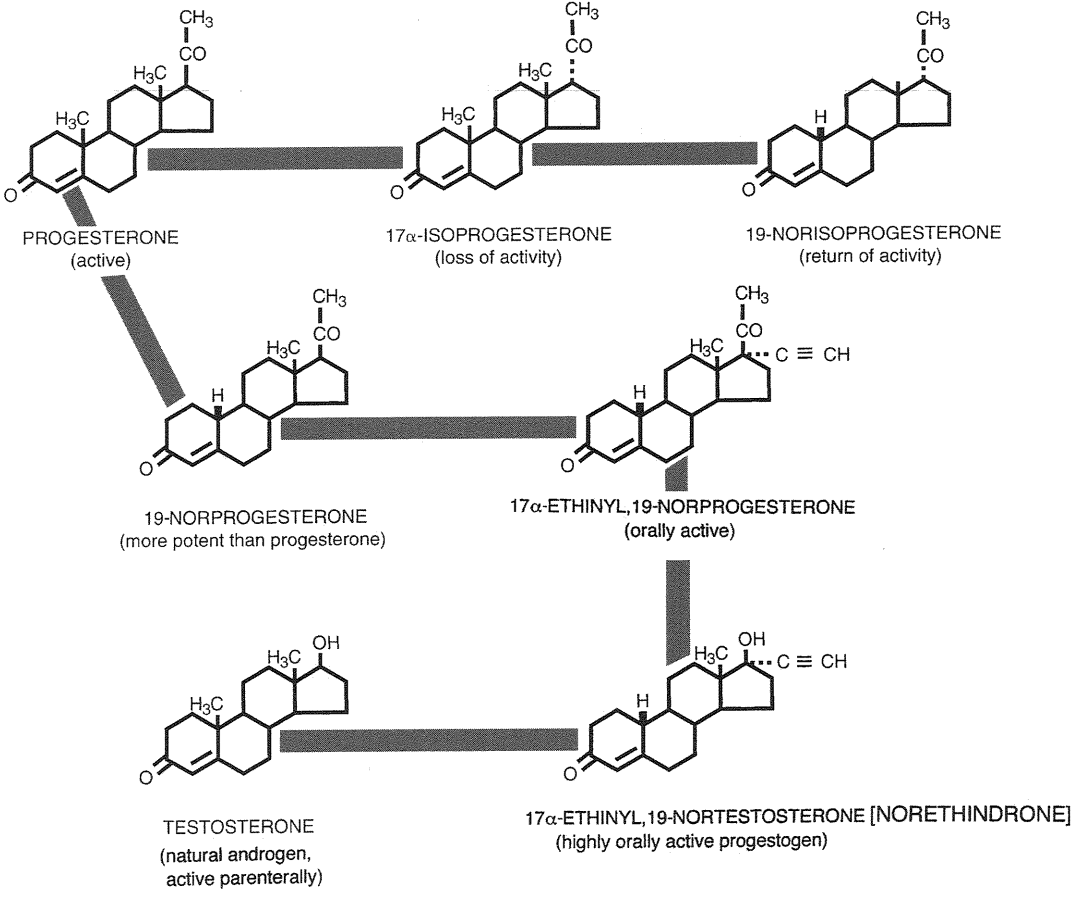


Figure 8 Development of norethindrone (for details see text). (From Ref. 6.)

of both described modifications (i.e., removal of the C-19 group and attachment of ethinyl group to C-17) resulted in norethindrone. This compound exhibited high progestational potency when administered orally and was the first oral progestogen more potent than progesterone given parenterally. The discovery of NET, accomplished by Djerassi et al. in 1951 (see details in Refs. 13 and 14), enabled the development of modern oral contraceptives. The synthetic process leading to NET, coupled with the earlier discovery that intermediate products for steroid synthesis could be obtained from roots of certain plants, principally of the genus *Dioscorea*, enabled the large-scale production of OC that made their wide distribution financially feasible.

Because NET is derived from testosterone, and "testosterone" is part of the chemical name of the compound, it is sometimes assumed that NET has androgenic properties. In fact, in the doses and combinations used in current clinical practice, NET does not exhibit any discernible clinical androgenicity. Interestingly, NET containing OC has been used in combination with a GnRH agonist for the management of hirsutism in women (15).

B. Pharmacokinetics and Metabolism

The four derivatives of NET have to be metabolically converted to NET to exercise their biological action. This conversion happens rapidly, with only NET being detected in the circulation within 30 min after oral administration of any of the derivatives (12). Compared with an intravenous dose, the bioavailability of NET given orally averages 60–70%, indicating a first-pass effect (12).

The single-dose pharmacokinetics of NET fits the two-compartmental model, with the $t_{1/2\alpha}$ of the rapid phase averaging 0.6 h and the slower $t_{1/2\beta}$ phase averaging 8.4 h (16). The pharmacokinetic parameters of NET are summarized in Table 1.

Table 1 Pharmacokinetic Parameters of Norethindrone (NET) in 20 Normal Adult Women After a Single Oral Dose of NET (1 mg) plus Ethinyl Estradiol (0.12 mg)

Parameter	Mean value ± standard deviation
Maximum plasma concentration (Cp_{max})	15.7 ± 6.19 ng/mL
Time to maximum plasma concentration (t_{max}) of NET	1.17 ± 0.65 h
Total area under plasma concentration vs. time curve (AUC)	84.5 ± 27.6 ng × h
Plasma half-life ($t_{1/2}$)	8.05 ± 1.92 h

Source: Ref. 16.

About 36% of the circulating NET is bound to serum hormone-binding globulin (SHBG), about 60% is bound to albumin, and the remainder circulates as free NET.

NET is mainly metabolized to glucuronide, but about 10–25% is metabolized to sulfate (12). The major plasma metabolite is the product of reduction of the A ring; namely, the 3β-hydroxy-5α-tetrahydro-derivative. This metabolite exhibits high affinity for the estrogen receptors, whereas NET by itself and other metabolites of NET do not (17,18). The estrogenic effects of NET have been demonstrated earlier; however, the explanation of the estrogenic activity of NET has been controversial (3). Besides the possibilities outlined in the foregoing, metabolic conversion of NET to EE has also been suggested as a reason for the estrogenic effect of NET. In perimenopausal women, the transfer constant in blood for the conversion of NET to EE was established at about 2%. Although small, this amount was considered sufficient to produce an estrogenic response (19).

VI. GONANE PROGESTINS OF THE SECOND GENERATION

A. Structure and Function

The gonane progestins are divided into two classes called the second and third generations. Compounds of the second generation include *dl*-norgestrel (NG) and levonorgestrel (LNG) (Fig. 9).

A brief discussion of the terms *optical isomerism* and *absolute configuration* is required to understand the differences in the stereochemical and biological properties of NG and LNG. Optical isomers are distinguished by their ability to rotate a plane of polarized light (sodium source) in solution. Thus, an isomer

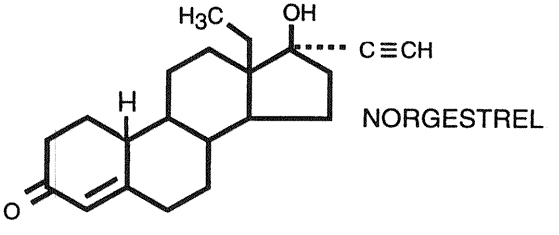


Figure 9 Levonorgestrel (LNG) is the *l* (−) enantiomer. Norgestrel (NG) is a racemic *d*/*l* (+/−) mixture composed of the depicted structure and its mirror image. Only the levorotatory enantiomer is biologically active.

that rotates the plane of polarized light in a clockwise direction is referred to as the dextrarotatory (*d*) or (+) enantiomer. Similarly, an optical isomer that rotates the plane of polarized light in the opposite direction is referred to as the levorotatory (*l*) or (−) enantiomer. An equal mixture of optical isomers is identified by the expression (+/−) and is often referred to as a *d*/*l* mixture (20).

Steroids prepared by total synthesis consist of a 1:1 mixture of *d* and *l* enantiomers. Norgestrel was the first progestin to be prepared by total synthesis and marketed as a *d*/*l* mixture. There are several disadvantages associated with the marketing of pharmaceutical products as *d*/*l* mixtures, such as the cost of synthesis and formulation. These disadvantages stem from the fact that the biological activity of a *d*/*l* mixture actually resides in only one of the optical isomers. In the case of NG, this prompted the development of synthetic methods that produced only the biologically active *levo* (−) enantiomer. As expected, the latter enantiomer, named levonorgestrel (LNG) exhibited twice the potency of NG in comparative bioassays. Thus, LNG was rapidly developed and soon replaced all NG-containing OC preparations.

The term absolute configuration refers to the actual arrangement of a molecule in space (21). There are various procedures available for determining the absolute configuration of a molecule, including X-ray crystallography, optical rotatory dispersion, and several chemical methods. Application of these procedures to several of the natural steroids revealed that progesterone has the absolute configuration represented by the structure depicted in Fig. 3 and not its mirror image. In fact, all steroids from plant and mammalian sources, and compounds derived from them, have the same absolute configuration (21–23).

Optical rotatory dispersion studies (24) and, subsequently, an X-ray crystallographic analysis (25) of LNG indicated that the biologically active enantiomer has the structure depicted in Fig. 9. This LNG has the same absolute configuration as the natural steroids, such as progesterone as well as clinically useful progestins described in this chapter. The name D-norgestrel has been employed to describe this circumstance. The use of the letters D and L originated from an early convention that was created to permit the unambiguous assignment of the absolute configuration to molecules bearing a single asymmetric carbon atom, such as glyceraldehyde and lactic acid (21). In an investigation of the structure of cholesterol, it was demonstrated that the hydroxyl-bearing carbon C-3 has the same absolute configuration as glyceraldehyde. Therefore, according to the convention, cholesterol would be categorized as D-cholesterol. Because the structure of LNG was correlated with that of cholesterol by X-ray studies, this enantiomer was called D-norgestrel (25). There is no relation between the sign of the optical rotation of an asymmetric substance [*d* or (+); *l* or (−)] and its absolute configuration, designated either D or L (see foregoing for details).

In recent years, the International Union of Pure and Applied Chemistry has adopted a more rigorous convention to define the absolute configuration of asymmetric molecules (26). However, the old convention is still used for the amino acids and sometimes for LNG.

The successful synthesis of LNG enabled the development of an OC with an extremely low progestin content. In one monophasic combination pill, 150 μg LNG is used with 30 μg EE. In the triphasic 21-day combination, a dose as low as 50 μg is given for the first 5 days, followed by 75 μg daily for 6 days, and 150 μg daily for the last 12 days. The progestin-only method employs a daily dose of 30 μg in a continuous, uninterrupted treatment regimen. For emergency contraception (interception), 750 μg LNG are recommended. In describing the pharmacokinetics of LNG, we will concentrate on these three doses.

B. Pharmacokinetics and Metabolism

The pharmacokinetics of the two optical isomers of *dl*-norgestrel differs substantially. Notably, the half-life of both the α- and the β-phase of the inactive dextrarotatory enantiomer is longer than that of LNG. Thus, in evaluating the pharmacokinetic data of NG, we need to consider whether LNG or the racemic mixture was assayed.

Fotherby has most comprehensively reviewed the pharmacokinetics of LNG, and his survey is the main source for the following description of the pharmacokinetics of LNG (27,28).

The pharmacokinetics of LNG displays certain unique features (Table 2). The compound's $t_{1/2}$ is about 14 h, with a range of 8–25 h. Only progestins of the third generation have similarly long $t_{1/2}$. Most importantly, the time required for the circulating levels of LNG to decline by 50% is about 15 h, whereas for NET, it is about 7 h. The difference in the elimination time is one of the reasons why contraceptive doses of NET must be higher than those of LNG. Concentrations of circulating LNG also vary widely within subjects and between individual subjects. That concomitant administration of estrogens is associated with a marked increase of the circulating concentrations of LNG is one of its important characteristics (28) (Fig. 10).

Another interesting feature of NG and LNG is their relation to SHBG. LNG, at a dose of 150 μg/day without an estrogen, decreases plasma concentrations of SHBG by 50%. However, SHBG is strongly affected by the estrogen component of the combined OC. EE given alone will increase plasma SHBG two- to threefold from baseline. However, it is important to realize that NG and LNG are effective antiestrogenic agents, which suppress estrogen-induced formation of SHBG. Monophasic combination treatment with 300 μg NG and 30 μg EE has been associated with only a minor (26%) and not statistically significant SHGB elevation from baseline (29). Results in users of triphasic LNG

Table 2 Pharmacokinetic Parameters of Levonorgestrel (LNG) given orally in Three Contraceptive Regimens: (a) Low-Dose Continuous Contraception (30 μg/day); (b) Monophasic Combination of 150 μg LNG with 30 μg Ethinyl Estradiol (EE) per day; (c) Emergency Contraception (750 μg)

	Plasma concentration of LNG (ng/ml) after oral dose of		
	30 μg	150 μg + 30 μg EE	750 μg
Parameter	2 studies $n = 8$	6 studies $n = 54$	3 studies $n = 26$
Time to maximum plasma concentration (t_{max}; h)	N/D		
Range of means across studies		1.0–2.4[b]	1.9[c]
Range of means of individual patients		0.2–3.1[b]	1.0–2.7[c]
Mean after achieving steady state		1.0 ± 0.2	
Maximum plasma concentration (Cp_{max}; ng/mL)	N/D		
Range of means across studies		3.2–3.8	9.0–11.2
Range of means of individual patients		1.5–6.8	8.1–18.4
Mean after achieving steady state		6.8 ± 2.1	7.1 ± 2.8
Total area under plasma concentration vs. time curve (AUC; ng × h)			
Range of means across studies	6.7[a]	20.2–37.8	116.0–124.0[d]
Range of means of individual patients	3.5–11.1	14.0–120.0	41.0–177.0[d]
Mean after achieving steady state		115.0 ± 52.0	118.0 ± 50.0
Terminal plasma elimination half-life ($T_{1/2\beta}$; hs)			
Range of means across studies	13.7–14.8	8.0–25.0	8.9–14.5
Range of means of individual patients	7.4–23.0	8.0–35.0	1.9–18.5
Mean (±SD) after achieving steady state		23.5 ± 8.6	12.0 ± 4.8

[a]Data of three women.
[b]Data of 42 women.
[c]Data of 10 women.
[d]Data of 16 women.
Legend: n, total number of women in studies; N/D, not determined.
Source: Ref. 27.

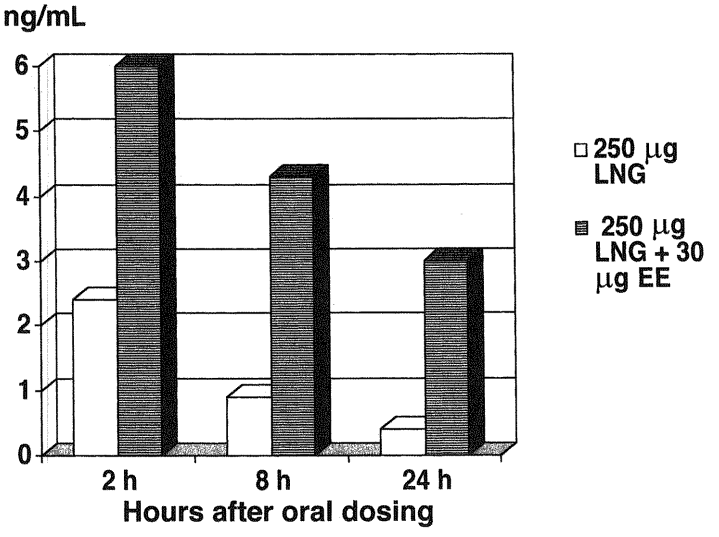


Figure 10 Mean serum levonorgestrel (LNG) concentrations in women receiving LNG alone (open columns) or with ethinyl estradiol (EE) (hatched columns). Concomitantly administered EE substantially elevates blood concentrations of LNG. (From Ref. 28.)

combination are inconsistent. After six treatment cycles, some studies reported elevations up to 70% from baseline (30–32), whereas at least one investigator detected a 6% decrease in SHBG, also after 6 months of treatment (33). The changes in SHBG concentrations should be viewed in the context that the use of combination oral contraception with cyproterone acetate and EE is associated with a fivefold increase of SHBG (see also Fig. 12).

LNG binds more strongly to SHBG than other progestins. This interaction does not explain the wide variability of blood levels of LNG after oral dosing or that circulating concentrations of LNG are elevated in the presence of estrogens.

Table 2 gives the elemental pharmacokinetic parameters for LNG. Few data are available for the 30-μg dose because only a total of eight women were examined in two studies. The $t_{1/2\beta}$ ranged in individual women from 7 to 23 h, with an average from 13 to 15 h.

More data are available for the contraceptive combination of 150 μg LNG + 30 μg EE. In six studies, 54 women were studied. There was a wide variability of values between individual subjects and among individual studies. Maximum serum levels ranged from 1.5 to 6.8 μg/L and were attained within 1–2 h after oral dosing. The mean $t_{1/2}$ ranged from 8 to 25 h. Maximum serum LNG levels after the single "interceptive" dose of 750 μg were three to four times higher, and the area under the concentration–time curve (AUC) was three to five times

greater than after ingestion of the combined dose of 150 μg with 30 μg of EE. These high LNG levels were necessary for an effective emergency contraception.

Pharmacokinetics of progestins released from silastic implants, vaginal rings, and intrauterine devices are discussed in other chapters of this book.

LNG is metabolized by reduction of the A ring, and conjugation to glucuronic and sulfuric acid, and further by oxidation at C-2 and C-16. About 20–67% of the administered radioactive dose is excreted in urine, and 21–34% is excreted in the feces. No metabolite with estrogenic activity has been identified (12).

VII. NORPREGNANES AND OTHER MODIFIED PROGESTINS

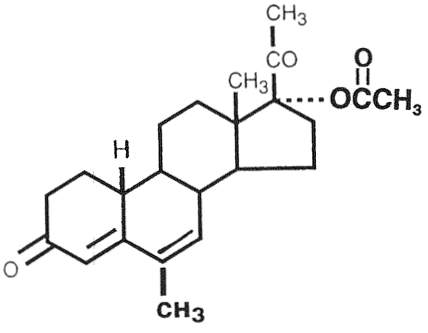
A. 19-Norpregnanes

The 19-norpregnanes are a cross between the pregnanes and estranes. These compounds are derived from progesterone, but lack the C-19 methyl radical (Fig. 11). Nomegestrol, an important member of this series, is currently undergoing extensive clinical investigation as a contraceptive implant. It has been intensively studied for its ability to inhibit uterine contractions (34,35).

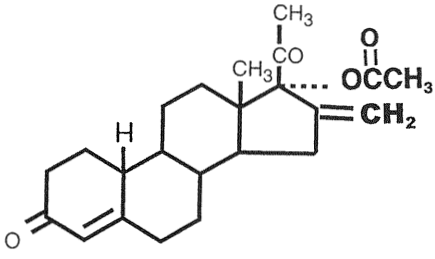
Nestorone (NES) is another 19-norpregnane of the 17α-acetoxy-progesterone series, which bears a methylene group on C-16. Thus, the compound is 16-methylene-17α-acetoxy,19-norpregn-4-ene-3,20-dione. In receptor assays NES showed progestational effects equal to or better than LNG, without estrogenic, androgenic, or anabolic activities (36). However, NES binds to glucocorticoid receptors. NES has low oral, but high parenteral progestational activity. Therefore, The Population Council is studying NES as a contraceptive in subdermal implants, vaginal rings, and transdermal formulations. The compound is suitable for nursing mothers because of its low oral action (see also Chap. 7).

B. Other Modified Progestins

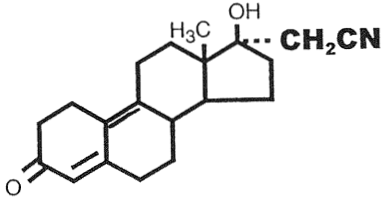
Dienogest is an interesting estrane. In this compound, the cyanomethyl group ($\cdots CH_2$ CN) has replaced the C-17 ethinyl group ($\cdots C{\equiv}CH$). There is an extra double bond between C-10 and C-11. Dienogest is 100% orally available. The terminal half-life of dienogest varies from 8 to 10 h, which is slightly longer than $t_{1/2}$ of NET, but it is shorter than that of LNG. It is claimed that the compound is without any androgenic activity and, supposedly, it affects glucocorticoids to a lesser degree than mifepristone (RU 486). The compound suppresses endometrial growth and was tested in the management of endometriosis. Preliminary studies in women with mild endometriosis have shown some beneficial effects. The



Nomegestrol



Nestorone



Dienogest

Figure 11 Chemical structure of two norprogestins: nomegestrol and nestorone. Dienogest is a modified norethindrone in which cyanomethyl has replaced the 17α-ethinyl group.

antigonadotropic activity of dienogest is relatively low, and 2 mg has to be used in combination with 30 μg EE in a 21-day–cyclic regimen (37) (see also Chap. 8).

VIII. PHARMACODYNAMIC ASPECTS OF PROGESTINS

A. Progestational Activity

The progestational activity of steroids can be established in several ways. The relative binding affinity (RBA) of the tested compound to progestin receptors is the most advanced method. Receptors can be cloned, and computerized receptor-binding assays can test hundreds of compounds. Receptors are obtained from various experimental animals, sometimes hormonally influenced in vivo, or from

Table 3 Relative Binding Affinities of Progestins to Human Endometrial Progesterone Receptors

Reference standard	Binding affinity (%)	Reference standard	Binding affinity (%)
Progesterone[a]	100	R5020[a]	100
Levonorgestrel	95	3-Ketodesogestrel	130
Norethindrone	85	Medroxyprogesterone acetate	115
Medroxyprogesterone acetate	78	Levonorgestrel	90
Megestrol acetate	70	Cyproterone acetate	90
Norgestrel	49	Progesterone	40
Norethynodrel	5	Desogestrel	1
Ethynodiol diacetate	5	Norgestimate	<0.1

[a]Originally, progesterone was used as the reference standard in the test. Later, it was replaced with a synthetic progestin promegeston (R 5020).
Source: Ref. 10.

human endometrium. Originally, tritiated progesterone was used as the reference standard. In recent years, it has been replaced by tritiated promegestone (R 5020), a highly potent synthetic progestin. RBA is expressed by the concentration of the tested steroid that competitively inhibits 50% of the reference standard (IC_{50}) (10). The method is highly effective and today's chemists do not need to wait for results of lengthy animal experimentation to learn about the biological activity of compounds they have synthesized. Table 3 gives the relative progestogenic potencies expressed as RBA for the most important contraceptive progestins. The high activity of NET in the receptor assay contrasts with the extremely low activity of NET derivatives. This indicates that NET derivatives acquire their biological activity only after in vivo metabolic conversion to NET.

Receptor-binding assays superceded the Clauberg test and its various modifications. This test evaluated secretory changes in the estrogen-primed uterus of immature rabbits.

In women, two tests have been used to determine the progestational activity of various compounds. The first one measures the delay of menses produced by the tested compound, given daily with 50 μg ethinyl estradiol, starting in the late luteal phase of the menstrual cycle (10,38,39). The dose required to delay menses for 20 days in 50% of the tested women (ED_{50}) is established. Comparative potencies of the most important progestins are given in Table 4.

The second test establishes the dose that transforms estrogen-primed endometrium into a secretory one. The endometrial response is graded from "no ef-

Table 4 Delay of Menses Test: Relative Potencies of First and Second Generation Contraceptive Progestogens, and Medroxyprogesterone Acetate[a]

Progestin	Relative potency[b]
Medroxyprogesterone acetate	0.2
Norethindrone acetate	0.5
Ethynodiol diacetate	0.5
Norethindrone	1.0
Norgestrel	3.0
Levonorgestrel	6.0

[a]Individual progestogens are given with 50 μg ethinyl estradiol, starting in the late luteal phase of the menstrual cycle. The hormones are given orally. The dose required to delay menses for 20 days in 50% of the tested women (ED_{50}) is established.
[b]Potency of norethindrone is assumed to be 1.
Sources: Ref. 10, 38, 39.

Table 5 Amount of Progestin Achieving Full Secretory Transformation of Estrogen-Primed Human Endometrium

Progestin	Daily dose (mg)	Number of days of administration
Oral administration		
Progesterone	2000	10–14
Medroxyprogesterone acetate	10	10–14
Norethindrone	5–10	10–14
Chlormadinone acetate	2–5	10–14
Parenteral administration		
Progesterone in oil	20	10
Norethindrone enanthate	100–200	1
Medroxyprogesterone acetate–depot	150	1

fect,'' with proliferative endometrium unaffected by the test compound, to ''good progestational effect,'' with fully developed late secretory phase endometrium. Table 5 gives comparative results of this test with various progestins.

B. Androgenic and Antiandrogenic Activity

The androgen receptor-binding test estimates the androgenicity of a compound by its ability to displace tritiated testosterone from the androgen receptor. The

tritiated synthetic androgen metribolone (R 1881) is now used instead of testosterone. Animal tests are conducted on immature or castrated rats, the endpoint being the weight increase of the prostatic glands.

In tests determining androgenicity by RBA of various molecules, the displacing potency of testosterone is 100%. The displacing potencies of LNG, NET, and MPA relative to testosterone are 42, 23, and 23%, respectively. Measured by prostatic growth, the potency of LNG is 15% (testosterone = 100) and that of NET is only 1.6%. In this biological test, MPA and chlormadinone acetate are inactive (10).

In humans, androgenicity is difficult to test clinically. It has been suggested that measurements of SHBG can reflect an overall balance between androgenic and estrogenic properties of a drug, specifically of a combined OC, as SHBG is raised by estrogen and reduced by androgen. Figure 12 shows the increase in circulating levels of SHBG during six contraceptive cycles with four different combination OC regimens. The lowest levels of SHBG were associated with the LNG monophasic regimen, whereas treatment with cyproterone acetate was associated with the highest SHBG concentrations (31,32).

C. Clinically Important Antiandrogenic Effects of OC

Contraceptive progestins exercise their antiandrogenic effect by two mechanisms. The first one is attributed to competitive binding of the compounds that are not androgenic per se to the androgen receptor. Of the pregnanes, chlormadinone and cyproterone acetates are the main representatives of contraceptive progestins, with high antiandrogenic potency. These compounds have high affinity for the androgen receptor, but lack the ability to activate it. Consequently, the receptor–antiandrogen complex fails to interact with the acceptor site of the gene, and the gene is not activated. Another antiandrogen is spironolactone, which is a modified pregnane, although it is not a contraceptive.

The second mechanism associated with the antiandrogenic effects of progestins is nongenomic and is estrogen mediated. The estrogen component of combined OC stimulates globulin synthesis, including SHBG. This protein, in turn, binds free testosterone and in this way reduces the circulating levels of this active androgen and its availability to target tissues. Generally, progestins counteract the formation of estrogen-induced SHBG. However, the degree of interference with the SHBG-stimulating effects of estrogens depends on the biological potency and characteristics of each individual progestin.

Levonorgestrel best illustrates the interplay of progestogenic, androgenic, and estrogenic properties of a combined OC. LNG is a highly active progestin. However, relative to other progestins, it also has increased androgenic activity. In women who are using combined oral contraception, the increase in circulating SHBG is not sufficient to bind free testosterone to a clinically meaningful extent.

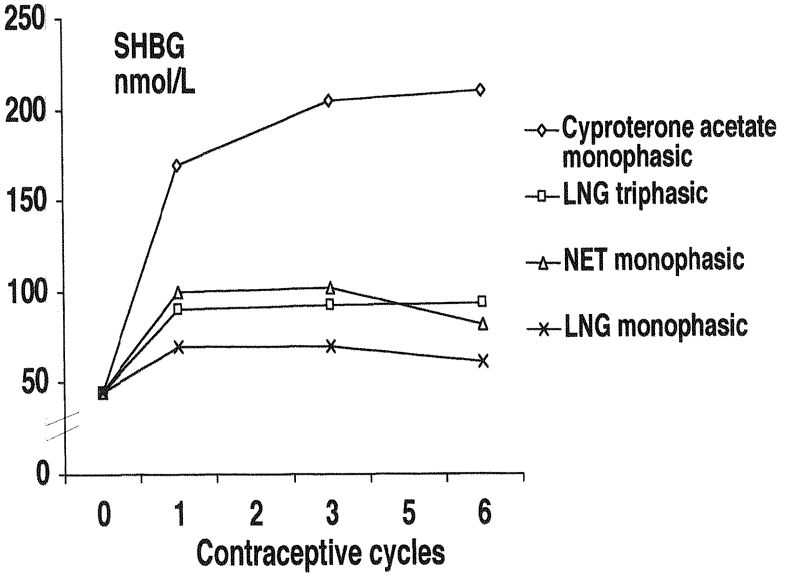


Figure 12 Increase in circulating levels of sex hormone-binding globulin (SHBG) during six contraceptive cycles with four different contraceptive regimens. Presented are the adjusted mean values (corrected for differences in pretreatment values). The following contraceptive regimens were used:

Progestin and type of regimen	Daily dose of progestin (μg)	Daily dose of ethinyl estradiol (EE) (μg)
Cyproterone acetate (CPA); monophasic (21 days)	2000	35
Levonorgestrel (LNG); triphasic (6–5–10 days)	50–75–125	30–40–30
Norethindrone (NET); monophasic (21 days)	1000	35
Levonorgestrel (LNG); monophasic (21 days)	150	30

(From Ref. 31.)

However, androgenic signs, such as acne, hirsutism, and male-type alopecia are recognized adverse events in a few women receiving pure LNG in contraceptive implants.

In contrast to LNG, norgestimate, a progestin of the third generation, does not interfere with the estrogen-stimulated formation of SHBG. Women taking a norgestimate/EE OC have a fivefold increase in circulating levels of SHBG, with about a 50% reduction of free testosterone concentrations in plasma. Clinically,

these changes translate into a 50% reduction of total acne lesions during the use of the norgestimate OC for 6 months (40). The antiandrogenic action of OC has been recently reviewed (41).

D. Lipid Metabolism

Important differences exist among progestins in their effect on lipid metabolism, particularly on the cardioprotective lipoproteins. Estrogens increase total cholesterol, but they also increase the high-density lipoprotein fraction (HDL) and decrease the low-density lipoprotein fraction (LDL) of cholesterol. Progestins exert an antagonistic effect on these positive actions of estrogens by various mechanisms, including an increase in the activity of hepatic lipase which degrades HDL. However, there are quantitative differences among individual progestins, and the net metabolic effect depends on an intricate interplay between the two components of the combined contraceptives, the type and dose of the progestin, and the treatment regimen, whether it is monophasic, biphasic, or triphasic (42). In general, the higher the androgenic properties of a progestin, the more pronounced are the negative effects on cardioprotective lipoproteins.

The effects of MPA on lipid metabolism have been studied mainly in women receiving hormone replacement therapy (HRT). A 21-day treatment regimen of estradiol valerate, 2 mg/day, combined for the last 10 days with MPA, 10 mg/day, has shown a slight increase in HDL and a small, but significant, reduction of LDL, over the 6-month treatment period (43). In general, when HRT is given by transdermal delivery systems, the hormonal effects on lipid metabolism are less pronounced than after oral administration. Once-a-month injections of MPA combined with an estrogen, given as a contraceptive over a 1-year period, were associated with only small changes in the lipid profile (44).

Figure 13 compares the effects of three generations of monophasic combinations on the lipoprotein profile. Of the first-generation progestins, norethindrone (combined with EE) significantly elevates HDL and significantly reduces LDL. This effect can be considered as beneficial. Of the second-generation progestins, levonorgestrel (combined with EE) increases LDL only slightly, but significantly reduces HDL—an undesirable effect. Norgestimate, a third-generation progestin (combined with EE), significantly elevates HDL, with only slight and nonsignificant elevation of LDL. This indicates an overall beneficial profile of norgestimate (42,45).

E. Carbohydrate Metabolism

Both estrogens and progestins are associated with the development of insulin resistance. Of the pregnanes, 150 mg of MPA alone, given by intramuscular, 3-month–depot injections, causes deterioration of glucose tolerance or hyperin-

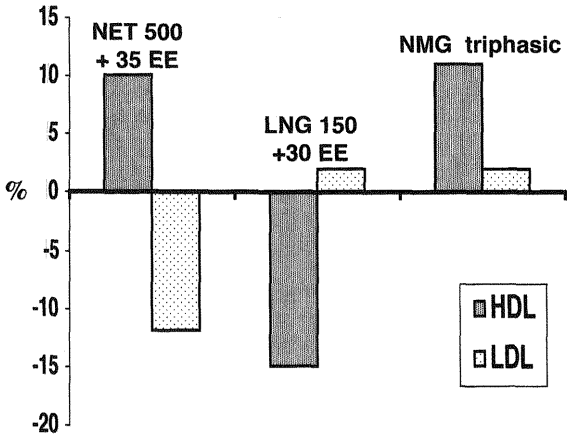


Figure 13 Percentage change from baseline in HDL and LDL in users of three generations of oral contraception. Contraceptive codes: NET 500 + 35 EE: combined norethindrone (NET) 500 μg and ethinyl estradiol (EE) 35 μg/day, monophasic; LNG 150 + 30 EE: combined levonorgestrel (LNG) 150 μg and EE 35 μg/day, monophasic; NMG triphasic: EE 35 μg/day for 21 days, combined with norgestimate (NMG) 180 μg/day for the first 7 days, followed by 215 μg/day for 7 days and 250 μg/day for the last 7 days. (From Refs. 42 and 45.)

sulinemia or both (46,47). Of the other pregnane progestins, data are available on the low-dose (0.5 mg daily) continuous contraceptive regimens with chlormadinone acetate and megestrol acetate. Most reports indicate that the two steroids have no or minor effects on carbohydrate metabolism.

Figure 14 compares three generations of OC relative to two split products of proinsulin, C peptide, and insulin. C peptide is a better indicator of pancreatic function than insulin itself. In general, increased insulin levels indicate resistance to insulin. Norethindrone in combination with EE has some influence on carbohydrate metabolism, but the elevations of C peptide and insulin are modest, just passing the statistical significance of 0.05. On the other hand, levonorgestrel combinations with EE have a highly significant effect in elevating both C peptide and insulin. The third-generation progestin desogestrel, combined with EE, has no significant effect on C peptide, although it significantly elevates insulin (42,46).

F. Progestins and Coronary Circulation

Interventional cardiologists have demonstrated that, after an acute infusion of estrogen into dogs, the cross section of the coronary arteries expands by about

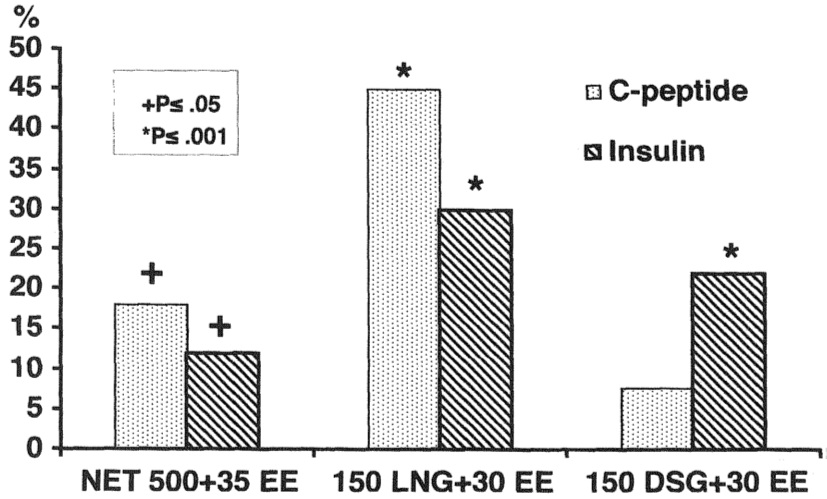


Figure 14 C peptide and insulin in response to oral glucose tolerance test: difference in incremental area (%) between oral contraceptive users and nonusers. Contraceptive codes: NET 500 + 35 EE: combined norethindrone (NET) 500 μg and ethinyl estradiol (EE) 35 μg/day, monophasic; LNG 150 + 30 EE: combined levonorgestrel (LNG) 150 μg and EE 35 μg/day, monophasic; 150 DSG + 30 EE: combined desogestrel (DSG) 150 μg/day and EE 30 μg/day, monophasic. (From Refs. 42 and 46.)

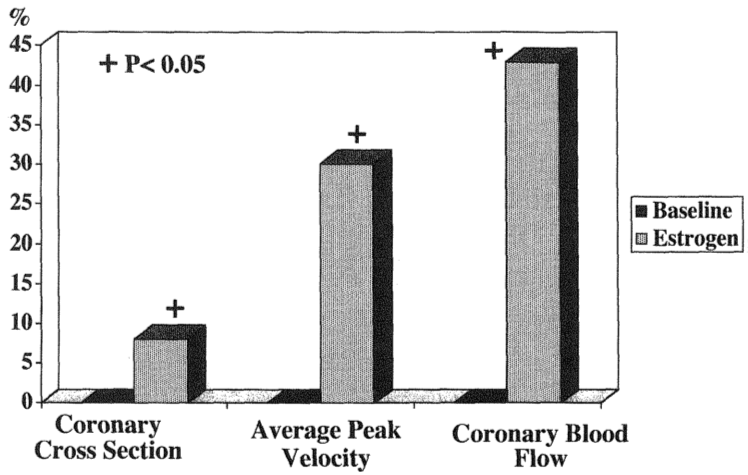


Figure 15 Estrogen-induced coronary vasodilation in dogs. A solution of 17β-estradiol hemisuccinate was infused directly into the coronary circulation at concentrations increasing from 10^{-10} to 10^{-6} mol/L. (From Ref. 48.)

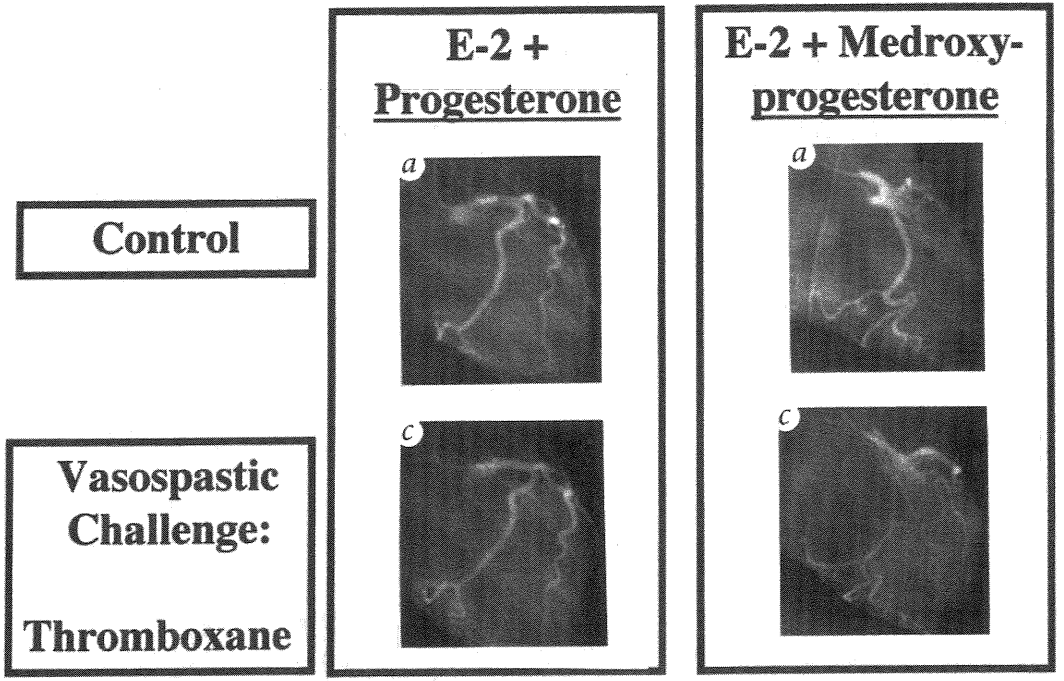


Figure 16 Medroxyprogesterone acetate (MPA) interferes with ovarian steroid protection against coronary vasospasm in monkeys: Twelve rhesus monkeys, at least 3 months after ovariectomy, received silastic implants with 200 mg of estradiol (E_2). Two weeks later, silastic implants containing either 400 mg progesterone or 400 mg MPA were implanted. Six weeks after the start of the experiment (i.e., when the monkeys had been 4 weeks under the influence of both E_2 and the two respective progestogens) intracoronary catheters were introduced and standard angiograms and hemodynamic measurements were obtained. Both treatment groups were then challenged by an injection of thromboxane, a potent vasospastic agent. Thromboxane had virtually no vasospastic effect in animals that received E_2 and progesterone. Monkeys treated with MPA responded with massive vasospasm. (From Ref. 49.)

10%. This translates into a 32% increase in blood flow velocity and over a 40% increase in the volume of blood passing through the coronaries (Fig. 15); this effect is nongenomic (48).

To determine whether progestins counteract the described beneficial effects of estrogens on the coronary circulation, a long-term experiment was conducted (49). Ovariectomized monkeys received silastic implants containing estradiol; 2 weeks thereafter, they received silastic implants containing either progesterone or MPA. Six weeks after estradiol-only capsules were implanted, the coronaries of the monkeys were catheterized and standard angiograms and hemodynamic measurements were obtained. Both treatment groups were then challenged by an injection of thromboxane, a potent vasospastic agent. Thromboxane had virtually no vasospastic effect in animals that received estradiol and progesterone. However, monkeys treated with MPA responded with massive vasospasm (Fig. 16). In other words, MPA counteracted the vasodilatory effect of estradiol (see also, Chap. 16).

It would be interesting to determine whether MPA shares its coronary vasoconstrictive effects with other synthetic progestins. There is some indication that nomegestrol does not counteract the vasodilating effect of estradiol (50).

IX. NOTE ON CONTRACEPTIVE PROGESTINS AND VENOUS THROMBOEMBOLISM

In the preceding section we have discussed pharmacodynamic differences between the three generations of oral contraceptives. Previously, we have evaluated the clinical relevance of toxicological studies showing development of breast nodules in connection with pregnanes, but not with estranes and gonanes. This section touches on some differences in the adverse event profile among third-generation progestins. Specifically, the section discusses the alleged differences between individual third-generation progestins relative to venous thromboembolism (VTE).

It has been suggested that the third-generation OC desogestrel and gestodene, but not norgestimate, double the risk of nonfatal VTE when compared with the first- and second-generation OC containing norethindrone or its derivatives, and levonorgestrel (51–54). These results were surprising, because we have associated the thrombotic phenomena with the estrogen content of OC. The data were immediately questioned and the relation of the various progestins to VTE became controversial (55–57). Indeed, studies showing an excess of VTE in gestodene and desogestrel users over users of norgestimate containing OC were much disputed.

In VTE, do first- and second-generation progestins differ from those of the third-generation? Are there indeed toxicological differences among the third-generation progestins? These are important questions that can be resolved only

by extensive basic research, carefully designed clinical studies, and epidemiological surveillance.

X. CONCLUSION AND MESSAGE

Each of the clinically effective progestins is a unique chemical entity. In this chapter we have stressed repeatedly that seemingly minor structural modifications can profoundly affect the biological function of the steroid molecule. Although large amounts of information have been accumulated on the relation between the chemical structure and physiological response of steroids, less in-depth data were generated addressing the relation between the structure and toxicological effects of individual steroid molecules. These are challenges to come. Indeed, numerous scientific and practical problems must be solved to continue improving the safety of the highly effective method of hormonal contraception.

REFERENCES

1. HH Inhoffen, W Hohlweg. Neue per os wirksamme weibliche Keimdruesenhormonderivate: 17-Aethinyl-oestradiol and Pregnen-in-on-3-ol-17. Naturwissenschaften 26:96, 1938.
2. G Pincus. The Control of Human Fertility. New York: Academic Press, 1956.
3. MR Henzl, J Jirasek, J Horsky, J Presl. Proliferative effect of 17-alpha-ethinyl, 19-nortestosterone. Arch Gynaekol 199:335–339, 1963.
4. F Neumann. The antiandrogen cyproterone acetate: discovery, chemistry, basic pharmacology, clinical use and tool in basic research. Exp Clin Endocrinol 102: 1–32, 1994.
5. F Kuttenn, C Rigaud, F Wright, P Mauvais-Jarvis. Treatment of hirsutism by oral cyproterone acetate and percutaneous estradiol. J Clin Endocrinol Metab 51:1107–1111, 1980.
6. MR Henzl. Synthetic sex steroids. In: EY Adashi, JA Rock, Z Rosenwaks, eds. Reproductive Endocrinology, Surgery, and Technology. Philadelphia: Lippincott–Raven, 1996, pp 585–604.
7. HP Zahradnik, J Goldberg, JO Andreas. Efficacy and safety of the new antiandrogenic oral contraceptive Belara. Contraception 57:103–110, 1998.
8. WHO Collaborative Study on Neoplasia and Steroid Contraceptives. Breast cancer and depot-medroxyprogesterone acetate: a multinational study. Lancet 338: 833–838, 1991.
9. M Hiroi, FZ Stanczyk, U Goebelsmann, PF Brenner, ME Lumkin, DR Mishell, Jr. Radioimmunoassay of serum medroxyprogesterone acetate (Provera) in women following oral and intravaginal administration. Steroids 26:373–386, 1975.
10. FZ Stanczyk. Structure–function relationships, potency, and pharmacokinetics of progestogens. In: RA Lobo, ed. Treatment of the Postmenopausal Woman: Basic and Clinical Aspects. New York: Raven Press, 1994, pp 69–89.

11. A Ortiz, M Hiroi, FZ Stanczyk, U Goebelsmann, DR Mishell, Jr. Serum medroxyprogesterone acetate (MPA) concentrations and ovarian function following intramuscular injection of depot-MPA. J Clin Endocrinol Metab 44:32–38, 1977.
12. JW Goldzieher. Pharmacology of contraceptive steroids. In: D Shoupe, FP Haseltine, eds. Contraception. New York: Springer–Verlag, 1993, pp 17–24.
13. MR Henzl. Evolution of steroids and their contraceptive and therapeutic use. In: D Shoupe, FP Haseltine, eds. Contraception. New York: Springer–Verlag, 1993, pp 1–16.
14. JA Edwards, MR Henzl. Chemical synthesis. In: IS Fraser, RPS Jansen, R Lobo, M Whitehead, eds. Estrogens and Progestogens in Clinical Practice. Philadelphia: WB Saunders, 1999, pp 55–70.
15. JS Heiner, GA Greendale, AK Kawakami, PS Lapolt, M Fisher, D Young, HL Judd. Comparison of a gonadotropin-releasing hormone agonist and a low dose oral contraceptive given alone or together in the treatment of hirsutism. J Clin Endocrinol Metab 80:3412–3418, 1996.
16. FZ Stanczyk, EJ Mrozsczak, MR Henzl, T Ling, R Runkel, I Miyakawa, U Goebelsmann. Plasma levels and pharmacokinetics of norethindrone and ethinylestradiol administered in solution and as tablet to women. Contraception 28: 241–251, 1983.
17. MV Oropeza, MG Campos, AE Lemus, G Garcia, G Perez-Palacios, H Ponce-Monter. Estrogenic actions of norethisterone and its A-ring reduced metabolites. Induction of *in vitro* uterine sensitivity to serotonin. Arch Med Res 25:307–310, 1994.
18. J Garza-Flores, M Menjivar, M Cardenas, M Reynoso, GA Garcia, G Perez-Palacios. Further studies on the antigonadotropic mechanism of action of norethisterone. J Steroid Biochem 38:89–93, 1991.
19. MJ Reed, MS Ross, LC Lai, MW Ghilchik, VH James. In vivo conversion of NET to ethynylestradiol in perimenopausal women. J Steroid Biochem Mol Biol 37:301–303, 1990.
20. DJ Cram, GS Hammond. Organic Chemistry. New York: McGraw-Hill, 1959, pp 129–145.
21. EL Eliel. Chemistry of Carbon Compounds. New York: McGraw-Hill, 1959, pp 85–115.
22. FZ Stanczyk, S Roy. Metabolism of levonorgestrel, norethindrone, and structurally related contraceptive steroids. Contraception 42:67–96, 1990.
23. LF Fieser, M Fieser. Steroids. New York: Reinhold, 1990, pp 330–336.
24. H Smith, GA Hughes, GA Douglas, et al. Totally synthetic hormones. Part 2. 13*b*-alkylgona-1,2,3,5(10)-triene, 13*b*-alkylgon-4-en-3-ones and related compounds. J Chem Soc 1963:4472–4492, 1963.
25. NJ DeAngelis, TH Doyne, RL Grob. Crystal and molecular structure of *d*-norgestrel, a progestational steroid. Acta Crystallogr 31B:2040–2043, 1975.
26. Revised tentative rules for nomenclature of steroids. IUPAC Commission on the Nomenclature of Organic Chemistry and IUPAC-IUB Commission on Biochemical Nomenclature. Steroids 13:277–310, 1969.
27. K Fotherby. Levonorgestrel. Clinical pharmacokinetics. Clin Pharmacokinet 28: 203–215, 1995.

28. K Fotherby. Potency and pharmacokinetics of gestagens. Contraception 41:533–550, 1990.
29. A Chapdelaine, J-L Desmarais. Clinical evidence of the minimal androgenic activity of norgestimate. Int J Fertil 34:347–352, 1989.
30. A Janaud, J Rouffy, D Upmalis, M-P Dain. A comparison study of lipid and androgen metabolism with triphasic oral contraceptive formulations containing norgestimate or levonorgestrel. Acta Obstet Gynecol Scand Suppl 156:33–38, 1992.
31. N Van der Vange, MA Blankenstein, HJ Kloosterboer, AA Haspels, HH Thijssen. Effects of seven low-dose combined oral contraceptives on sex hormone binding globulin, corticosteroid binding globulin, total and free testosterone. Contraception 41:345–352, 1990.
32. PD Darney. The androgenicity of progestins. Am J Med 98(Suppl IA):104S–110S, 1995.
33. RS London, A Chapdelaine, D Upmalis, W Olson, J Smith. Comparative contraceptive efficacy and mechanism of action of the norgestimate-containing triphasic oral contraceptive. Acta Obstet Gynecol Scand Suppl 156:9–14, 1992.
34. EM Coutinho, JC de Souza, C Athayde, IC Barbosa, F Alvarez, V Brache, ZP Gu, EE Emuveyan, AO Adekunle, L Devoto, MM Shaaban, HT Salem, B Affandi, OM de Acosta, J Mati, OA Lapido. Multicenter clinical trial on the efficacy and acceptability of a single contraceptive implant of nomegestrol acetate, Uniplant. Contraception 53:121–125, 1996.
35. B Carbonne, D Carbol, M-S Clerget, G Germain. Effect of nomegestrol acetate on spontaneous and sulprostone-induced uterine contractions in pregnant cynomolgus monkeys monitored by telemetry. Am J Obstet Gynecol 178:150–155, 1998.
36. N Kumar, Y-Y Tsong, SS Koide, K Sundaram. Nestorone progestin, a highly selective 19-nor-progesterone derivative lacking androgenic and estrogenic activity. In press.
37. M Oettel, H Breitbarth, T Graeser, D Huebler, G Kaufmann, Y Katsuki, V Patchev, Y Shibutani, L Sobek, H Zimmermann. The pharmacological profile of dienogest. Proceedings of the 5th Congress of the European Society of Contraception. 17–20 June 1998, Prague. Eur J Contracep Reprod Health Care 3(suppl 1):76, 1998.
38. MR Henzl. Contraceptive hormones and their clinical use. In: SSC Yen, RB Jaffe, eds. Reproductive Endocrinology 3rd ed. Philadelphia: WB Saunders, 1991.
39. W Dorflinger. Relative potency of progestins used in oral contraceptives. Contraception 31:557–570, 1985.
40. GP Redmond, WH Olson, JS Lippman, ME Kafrissen, TM Jones, JL Jorizzo. Norgestimate and ethinyl estradiol in the treatment of acne vulgaris: a randomized placebo-controlled trial. Obstet Gynecol 89:615–622, 1997.
41. MR Henzl, JA Edwards. Anti-androgen action of hormonal steroids: basis for management of disorders of the pilosebaceous unit (PSU) in young women. Proceedings of the 5th Congress of the European Society of Contraception. 17–20 June 1998, Prague. Eur J Contraception Reprod Health Care 3(suppl 1):130, 1998.
42. IF Godsland, D Crook, R Simpson, T Proudler, C Felton, B Lees, V Anyaoku, M Devenport, V Wynn. The effects of different formulations of oral contraceptive

agents on lipid and carbohydrate metabolism. N Engl J Med 323:1375–1381, 1990.
43. FP Chen, N Lee, CH Wang, WJ Cheng, YK Soong. Effects of hormone replacement therapy on cardiovascular risk factors in postmenopausal women. Fertil Steril 69:267–273, 1998.
44. United Nations Development Programme. Comparative study of the effects of two once-a-month injectable steroidal contraceptives (Mesigyna and Cyclofem) on lipid and lipoprotein metabolism. Contraception 56:193–207, 1997.
45. RT Burkman, Jr, ME Kafrissen, W Olson, J Osterman. Lipid and carbohydrate effects of a new triphasic oral contraceptive containing norgestimate. Acta Obstet Gynecol Scand Suppl 156:5–8, 1992.
46. IF Godsland. The influence of female sex steroids on glucose metabolism and insulin action. J Intern Med 240(suppl 738):1–60, 1996.
47. W Spellacy. Oral contraceptives effect on glucose metabolism. In: D Shoupe, FP Haseltine, eds. Contraception. New York: Springer-Verlag, 1993, pp 25–33.
48. K Sudhir, TM Chou, WL Mullen, D Hausmann, P Collins, PG Yock, K Chatterjee. Mechanism of estrogen-induced vasodilation: in vivo studies in canine coronary conductance and resistance arteries. J Am Coll Cardiol 26:807–814, 1995.
49. K Miyagawa, J Rosch, F Stanczyk, K Hermsmeyer. Medroxyprogesterone interferes with ovarian steroid protection against coronary vasospasm. Nature Med 3:324–327, 1997.
50. JK Williams, MR Adams. Estrogens, progestins and coronary artery reactivity. Nature Med 3:273–274, 1997.
51. World Health Organization Collaborative Study of Cardiovascular Disease and Steroid Hormone Contraception. Venous thromboembolic disease and combined oral contraceptives: results of international multicentre case–control study. Lancet 346:1575–1582, 1995.
52. World Health Organization Collaborative Study of Cardiovascular Disease and Steroid Hormone Contraception. Effect of different progestagens in low oestrogen oral contraceptives on venous thromboembolic disease. Lancet 346:1582–1588, 1995.
53. H Jick, SS Jick, V Gurewich, MW Meyers, C Vasilakis. Risk of idiopathic cardiovascular death and nonfatal venous thromboembolism in women using oral contraceptives with differing progestagen components. Lancet 346:1589–1593, 1995.
54. MR Henzl. Safety of modern oral contraceptives. Lancet 347:257–258, 1996.
55. RDT Farmer, RA Lawrenson, CR Thompson, JG Kennedy, IR Hambleton. Population-based study of risk of venous thromboembolism associated with various oral contraceptives. Lancet 349:83–88, 1997.
56. JD Douketis, JS Ginsberg, A Holbrook, M Crowther, EK Duku, RF Burrows. A reevaluation of the risk for venous thromboembolism with the use of oral contraceptives and hormone replacement therapy. Arch Intern Med 157:1522–1530, 1997.
57. WO Spitzer. The 1995 pill scare revisited: anatomy of a non-epidemic. Hum Reprod 12:2347–2357, 1997.

6

Clinical Pharmacology of Third-Generation Gestogens

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I. INTRODUCTION

The pharmacokinetics and pharmacodynamics of synthetic gestogens have been comprehensively reviewed (1,2), and additional reviews have appeared (3–6). This chapter will deal only with three gestogens derived from levonorgestrel (LNG; 13β-ethyl-17α-ethinyl-17β-hydroxygen-4en-3-one): desogestrel (DSG; 11-methylene-3-deoxy-LNG); gestodene (GSD; Δ15-LNG); and norgestimate (NGM; 17β-acetoxy-LNG-3-oxime). These three gestogens have been described as "third-generation" gestogens, a term that should not be used because (a) it has never been properly defined and, therefore, leads to confusion; (b) it is sometimes used in reference to the gestogens themselves and sometimes in reference to the combined oral contraceptives (COC) containing them; (c) several COC containing various doses of the gestogen in combination with various doses of ethinyl estradiol (EE) are in use and under development, and not all may be classified as third-generation formulations; (d) it could imply that there is some similarity in the pharmacology and clinical effects of the three formulations which, as this chapter will show, is not true; (e) the classification of NGM is anomalous because its conversion in vivo to LNG, a "second-generation" gestogen, may account for most, if not all, of its biological activity; (f) it has been used to refer to the date of introduction of the COC, but there is a large difference in these dates; COC containing DSG were first marketed in 1981, those with GSD in 1987, and those with NGM after a preliminary introduction in 1986, were not mainly marketed until 1992.

Levonorgestrel is a 13β-ethyl gonane steroid (in contrast with norethisterone which is a 13β-methyl gonane). The major aspect that DSG, GSD, and NGM have in common is their chemical structure; they can all be considered to be derived from LNG, and generically, they can be considered as derivatives of LNG (DLNG).

This chapter will deal mainly with the clinical pharmacology of the DLNG and COC containing them. Animal pharmacology and in vitro studies will be mentioned only briefly where they are relevant to the human pharmacology.

II. PHARMACOKINETICS

A. Absorption and Bioavailability

The gestogens are readily absorbed after oral administration. In most subjects peak serum concentrations (C_{max}) occurs between 1 and 2 h, although in a few, C_{max} may occur from 2 to 4 h and there appears to be no difference among the three gestogens. However, there are important differences in their bioavailability (7).

1. DSG

Desogestrel is a prodrug and, after ingestion by humans, it is rapidly and almost quantitatively converted in the liver in a two-stage process (hydroxylation at C-3 followed by oxidation of the C-3-hydroxy group to the C-3-ketone) to 3-keto-desogestrel, the biologically active metabolite. After oral administration DSG was detected in blood in only low concentration during the first 3 h (1). Serum concentrations of 3-keto DSG are similar after giving DSG or 3-keto DSG in the same dose. Three studies of bioavailability, as measured by the area under the serum concentration–time curve (AUC), gave values of 76$\pm$22%, 81$\pm$27%, and 62 $\pm$ 7% with a mean overall of 73% (7).

2. GSD

Gestodene is not a produg and is absorbed without modification so that its bioavailability is almost 100%. Four studies gave values of 88$\pm$18%, 99$\pm$11%, 111 $\pm$ 18%, and 87 $\pm$ 19%, with an overall mean bioavailability of 96% (7).

3. NGM

Norgestimate is a prodrug, and determination of its bioavailability is difficult owing to its rapid metabolism after ingestion. NGM itself can be detected in blood for less than 6 h after oral administration, and it is present in only very low

concentrations. Hydrolysis of the 17β-acetyl group occurs rapidly, some in the intestinal tract and during absorption, resulting in LNG-3-oxime, which appears in the circulation in measurable amounts (4). This is surprising because oximes are also readily hydrolyzed in vivo, to some extent in the gastrointestinal tract. Considerable hydrolysis of LNG-oxime does occur, for LNG itself is a major metabolite in blood and accounts for 20–25% of the dose (8). Because of this rapid and extensive metabolism of NGM, methods other than AUC are required to determine its overall bioavailability.

From the values given for DSG and GSD it will be noted that there is a large variation in the values both within any study and between studies. Part of this variability is due to intersubject variability and part to the different methods employed in the studies. The bioavailability of sex steroids and their measurement are discussed in a recent review (7).

B. Serum Concentrations

Most of the information available relates to the gestogen administered with EE. Values quoted are means calculated from published reports. Values are given for single-dose oral administration, when steady-state levels will not have been achieved, and also for steady-state values (i.e., values obtained usually at or near the end of a treatment cycle) after 3 weeks of daily oral dosing. Steady-state concentrations will be higher and more relevant to the pharmacological activity of the gestogen than single-dose values. The time to achieve steady-state conditions will be longer than that calculated on pharmacokinetic considerations (about 4 days) because the estrogen in the COC will increase serum sex hormone-binding globulin (SHBG) concentration to which the gestogens show variable degrees of binding. SHBG concentrations do not reach plateau levels until about day 10 of the treatment cycle, and serum gestogen concentrations will continue to increase until this time. (Note: these are better described as 'pseudo'–steady-state conditions because the COC is administered only once per day and serum concentrations will continue to show daily increases and decreases.)

1. DSG

After a single dose of 150 μg DSG with 30 μg EE, serum DSG concentrations increase rapidly to reach C_{max} of 1.5 ng/mL, falling rapidly to 0.5 ng/mL at 4 h, and then more slowly to 0.3 ng/mL at 8 h and 0.1 ng/mL at 24 h (1). Under steady-state conditions C_{max} values would be 4 ng/mL falling to 2 ng/mL at 4 h, 1.7 ng/mL at 8 h, and 0.8 ng/mL at 24 h (2). The ratio of steady-state to single-dose concentrations is about 2.7 at C_{max} and about 8.0 at 24 h.

2. GSD

After a single dose of 75 μg GSD with 30 μg EE, serum GSD concentrations reach C_{max} of 3.7 ng/mL, slowly decreasing to 2.2 ng/mL at 4 h, 1.1 ng/mL at 8 h, and 0.6 ng/mL at 24 h (4). Under steady-state conditions C_{max} is 10 ng/mL, decreasing to 8.8 ng/mL at 4 h, 7.0 ng/mL at 8 h, and 5 ng/mL at 24 h. The ratio of steady-state to single-dose concentrations is about 2.7 at C_{max} and about 8.3 at 24 h.

3. NGM

Little information is available for this gestogen. With doses not used in contraception (360 μg NGM + 70 μg of EE), both after single-dose and under steady-state conditions, NGM disappeared rapidly from serum, and none was detected after 5 h (4). LNG-3-oxime (17-deacetyl NGM) reached a peak at 1 h of about 4 ng/mL and was still readily detectable (concentration about 0.8 ng/mL) at 36 h. With a contraceptive dose (250 μg NGM + 35 μg EE) in 12 women (8), LNG was a major metabolite, and it was calculated that about 22 ± 6% of the NGM dose was converted to LNG.

C. Serum Binding

Only small amounts of the gestogen are present in serum in an unbound state, and it is this fraction that appears to be responsible for the biological activity. Most of the gestogen in serum binds to albumin and SHBG. The binding to albumin is loose, and the gestogen can readily dissociate to maintain the level of unbound steroid. The binding to SHBG is much tighter; hence, dissociation occurs less readily. Measuring the distribution of the gestogen between the unbound and the two protein-bound fractions can be difficult because the method of estimation may itself disturb the equilibrium. Furthermore, the equilibrium between bound and unbound in vivo is constantly changing, partly as a result of changes in the serum gestogen concentration throughout the day and the treatment cycle, and partly as a result of changes in the serum concentration of SHBG throughout the treatment cycle. Administration of EE alone in a daily dose of 30 μg will increase serum SHBG concentrations two- to threefold by day 10 of treatment, decreasing during the pill-free interval (1). Although some gestogens in COC will antagonize this estrogenic effect, it is not inhibited by DSG or GSD. It has been claimed that the amount of DSG bound to SHBG almost doubles from 37 to 69% as a result of the increase in serum SHBG concentrations during the treatment cycle (9). This large increase in SHBG binding is at the expense of albumin binding (decreased from 63 to 36%) and also leads to a decrease

in the unbound fraction from 2.1 to 1%. For GSD, which has a higher-binding affinity for SHBG, under similar conditions, the SHBG bound increases from 48 to 76%, with decreases from 48 to 24% and from 2 to 0.6% for albumin-bound and unbound, respectively. The characteristics of binding to SHBG differs between DSG and GSD. The binding of GSD is tighter; thus, it dissociates more slowly from SHBG than does DSG. Further details on the binding of DSG and GSD will be found in Kuhnz et al. (10,11). Little is known about the binding of NGM. One major metabolite LNG-oxime, does not bind to SHBG, but LNG itself binds strongly. After a single dose of LNG + EE about 50% of LNG binds to SHBG, 47.5% to albumin, and 2.5% is unbound (1). Because little increase, if any, in serum SHBG concentrations occurs with this formulation, there is little change in these values under steady-state conditions. Reported values for the proportions of the gestogens in the bound and unbound states vary (a) among studies owing to the differing methodologies used; (b) with the time of sampling in the treatment cycle; and (c) owing to the large intersubject variability in the pharmacokinetics of the gestogens.

D. Metabolism

Little is known about the metabolism of gestogens. Although DSG and GSD can be considered to be derivatives of LNG, these are not converted to LNG in vivo, in contrast with NGM for which the modifications of the LNG molecule are those that can be readily hydrolyzed.

1. DSG

Between 40 and 50% of the dose is excreted in urine, and 30–35% in feces (4). Although the main urinary metabolites are conjugated (glucuronides 25–60%, sulfates 20–40%), surprisingly large amounts (12–28%) are voided in an unconjugated form (4). Metabolism mainly appears to follow the usual initial stages for most steroids—reduction of the 4-en-3-one structure of ring A to the 3,5-tetrahydrosteroids, followed by substantial hydroxylation.

2. GSD

Although GSD differs from LNG by only the double bond at C15–C16, it is not converted to LNG in vivo. About 50% of the dose is excreted in urine (25% as glucuronides, 40% as sulfates, and 25% unconjugated) and about 33% in feces (4). The major pathway of reduction of 4-en-3-one structure to the tetrahydrosteroids is followed, and substantial hydroxylation occurs at C-1, C-6, and C-11.

3. NGM

As indicated earlier, NGM is rapidly metabolized and is present in blood only in very small amounts and for only a few hours after ingestion. Three metabolites, LNG-17-acetate, LNG-3 oxime, and LNG, may appear in the circulation, but the acetate is rapidly hydrolyzed. About 40% of the dose is excreted in urine (57% as conjugates, 12% unconjugated) and 16–49% in feces. No NGM or LNG is found in urine (4). The urinary metabolites are similar to those of LNG, tetrahydrosteroids, and hydroxylated steroids (at C-2α and C-16β) and even trihydroxysteroids. The pattern of urinary metabolites reinforces the idea that LNG is the major metabolite of NGM. Some esters are hydrolyzed in the gastrointestinal tract; therefore, seems likely that some conversion of NGM to LNG-3-oxime occurs during absorption. In addition, esterases are widely distributed in body tissues. Hydrolysis of oximes can also occur in the gastrointestinal tract, and they are usually readily hydrolyzed in tissues (12).

Both endogenous and exogenous steroids are metabolized in vivo to a great extent by the hepatic cytochrome system. Under in vitro conditions, ethinyl steroids can inhibit the enzyme system, and GSD was a more potent inhibitor than other gestogens tested (6). Considering the artificial conditions of the in vitro studies, the findings are unlikely to have any relevance to the in vivo situation, and no information is available concerning such an effect in vivo.

E. Pharmacokinetic Parameters

Available data for pharmacokinetic parameters for the three gestogens are summarized in Table 1. It must be emphasized that these values

1. Relate to the gestogen administered with EE in contraceptive doses. Values for the gestogen administered alone are likely to be very different owing to an interaction between the estrogen and gestogen.
2. Are compiled from published reports and, where possible, are consensus values. For some parameters (e.g., bioavailability and serum concentrations) sufficient studies have been performed to determine a consensus value, whereas for others (e.g., volume of distribution and clearance) often only data from a single study is available.
3. Will vary according to whether they apply to single dose or steady-state conditions.
4. Will vary widely among the different studies owing to the differing methods that have been used in their derivation
5. Will show a very wide variation (more than fourfold) between subjects and for some parameters, information is available to show that there is an almost equally wide within-subject variability.

Table 1 Pharmacokinetic Parameters for Gestogens Values Relate Mainly to Gestogen Administered with 30–35 μg EE

			NGM (250 μg)[a]	
	DSG (150 μg)[a]	GSD (75 μg)[a]	LNG oxime	LNG
Serum concentration (ng/mL)				
Peak	1.5	3.7	4.0	—
24 h	0.1	0.6	1.0	—
Absorption time (h)	1.7	1.3	—	—
Volume of distribution (L)	130	32	—	110
Clearance (L/h)	8	3	—	6
Elimination half-life (h)	12	11	16	14
Bioavailability (%)	73	96	22[b]	95
SHBG binding affinity (% DHT)	5	17	0	13
Serum binding (%)				
Albumin	65	24	—	50
SHBG	31.5	75.5	0	48
Unbound	3.5	0.5	—	2

[a]Daily dose.
[b]Value relates to amount of LNG resulting from NGM administration.
Source: Ref. 1.

Little information is available on the correlation of the parameters with subject characteristics. The parameters are likely to be affected by many factors that may influence the absorption, binding, and metabolism of the gestogens; most of these effects have been inadequately studied (13). Further details of the pharmacokinetics of gestogens and how they might relate to pharmacological activity were summarized by Fotherby (1).

F. Pharmacokinetics: Conclusions

Because of the large number of factors, excluding pharmaceutical ones, that may operate between oral administration of a drug and its appearance in the systemic circulation (e.g., metabolism in the gastrointestinal wall, variations in absorption, metabolism in intestinal tract and on first passage through the liver [first-pass effect], binding in blood, rate of metabolism and elimination), it has long been recognized in pharmacology that dose is not a satisfactory basis for comparison of the biological activity of different drugs and that blood concentrations might be a more satisfactory parameter. Two of the gestogens

(DSG and GSD) considered in this chapter, to some extent, appear to substantiate this. In combination with 30 μg EE, the daily dose of DSG (150 μg) required for an antifertility effect is twice that of GSD (75 μg) and the daily dose of the gestogen administered alone for ovulation inhibition is 60 μg for DSG and 40 μg GSD (i.e., relative to dose, GSD is 1.5–2 times as potent as DSG). However, serum concentrations of GSD are much higher than those of DSG. For 75 μg GSD + 30 μg EE and 150 μg DSG + 30 μg EE, serum concentration ratios of GSD/DSG after single doses are 2.5:1 at C_{max} and 5:1 at 24 h, and under steady-state conditions, these ratios are even higher 3:1 at C_{max} and 8:1 at 24 h, even though the dose of GSD is only half that of DSG. These aspects are considered in greater detail by Fotherby (1). Although definite conclusions cannot be made from the limited amount of information available for some of the parameters shown in Table 1, it seems likely that the main determinant of the large difference in the serum concentrations of GSD and DSG is the avid binding of GSD to SHBG. This restricts GSD to mainly the intravascular compartment and leads to a low volume of distribution to tissues and also a low clearance compared with DSG, which binds with a much lower affinity.

There is little difference between GSD and DSG in the elimination half-life (t_{el}). This is partly because t_{el} is a derived parameter and depends on volume of distribution and clearance. A compound with a low volume of distribution and a low clearance may have a value for t_{el} similar to that of one with a high volume of distribution and high clearance. Caution has to be used in the interpretation of t_{el} values for t_{el} varies widely and is very dependent on the procedures used in its estimation. In a few studies, t_{el} is derived from volume of distribution and clearance as determined by computer analysis after intravenous administration of the gestogen, whereas most studies determine t_{el} graphically from serum concentrations after oral administration.

Difficulties in the determination of the distribution of the gestogens among the various serum fractions mean that the values shown in Table 1 are only approximations of the in vivo situation. Minor variations in the amount bound are likely to have a major effect on the very small unbound fraction, especially for drugs that are highly bound and have a low volume of distribution. The increase in SHBG during a cycle of COC use as a result of the estrogen component leads to a decrease in the unbound fraction from 2.1 to 1% for DSG (a decrease of about 50%) and a larger decrease about 70% for GSD from 2 to 0.5% (10). Despite the difference between GSD and DSG for the percentage in the unbound fraction, it seems possible that the concentrations of unbound GSD and DSG in serum and, hence, presumably in extracellular fluid and in contact with tissue cells, may be similar. If this is so, it suggests that the biological response to the gestogen will be determined by the extent to which the gestogen interacts will the cell receptors.

As is evident from Table 1, little information is available for the pharmacokinetics of NGM; therefore, comparison with DSG and GSD is not possible. Table 1 includes information for LNG, which is the major metabolite of NGM, and for which a large amount of information is available (14).

It is apparent from this summary that although structural differences between the gestogens derived from LNG are minor, they do lead to marked differences in their pharmacokinetics. As the next section will show, although there are differences among the gestogens in their pharmacodynamic effects, these differences cannot yet be correlated with the pharmacokinetic differences.

III. PHARMACODYNAMICS

Unlike many drugs, the dose regimen and doses used in COC are not based on pharmacokinetic characteristics. The dose regimen of 1 pill/day is based on user convenience. The doses used are decided by clinical studies to determine the optimum dose of estrogen and gestogen, in combination, that will provide efficient contraception by inhibiting ovulation and a satisfactory pattern of regular menstruation.

A. Ovulation Inhibition

The modern low-dose COC provide satisfactory inhibition of pituitary gonadotropin secretion to prevent follicular growth and ovum maturation in most treatment cycles (15,16). Some ovarian activity with initial follicular development occurs in many women during the interval between cycles of treatment (the pill-free interval), and this is quickly inhibited when commencing the next cycle of treatment. However, evidence such as serum hormone measurements and ultrasonography of the ovary suggests that in occasional cycles, follicular development may occur, which in rare instances, may lead to ovulation and the possibility of pregnancy. Follicular development is less likely to occur when pill-taking starts on day 1 of the initial cycle, as is now more usual, than on day 5. Missing pills during the treatment cycle, or more importantly at the beginning or end of the cycle so that the pill-free interval is lengthened, or an interaction between the COC and other drugs taken concomitantly, may also increase the likelihood of follicular development and ovulation. The gestogen component of the COC appears to be responsible mainly for ovarian suppression, and this action is reinforced by the estrogen. Two lines of evidence from human pharmacology support this conclusion.

First, several clinical trials have been performed and at least one product marketed with COC in which the estrogen dose has been reduced to 20 μg, without a reduction in the gestogen dose. These formulations still inhibit ovu-

lation, and their effectiveness is no less than that of the corresponding COC containing 30 μg of EE (2).

Second, studies have been performed to determine the minimum daily dose of gestogen per treatment cycle administered alone to inhibit ovulation. Values reported (17) in noncomparative studies are GSD, 40 μg/day, DSG, 60 μg/day; and NGM, 200–250 μg/day. Thus, in this assay, GSD is more potent than DSG, and both are considerably more potent than NGM. If, as the pharmacokinetic studies suggest, NGM is active by conversion to LNG, the dose of NGM alone would be metabolized to 50–60 μg LNG, and this is the daily dose of LNG required to inhibit ovulation. However, these ovulation inhibition studies have included only a few subjects during few treatment cycles and the "endpoint" is often indistinct. Administration of the gestogen alone may lead to (a) complete inhibition of ovarian activity, with no follicular development and with no increase in estradiol and progesterone secretion; or (b) varying degrees of follicular development and estradiol secretion, but without formation of a corpus luteum or secretion of progesterone.

There are also marked variations among subjects and possibly between different cycles in the same subject. Accordingly, the values are to be seen as an approximate indicator of the activity of the gestogen. They do show, however, that the daily dose required to inhibit ovulation in this assay, in which the drug is given daily, is only about half of the daily dose in the COC for which the drug is given 3 weeks out of 4, for GSD and DSG, but approximately equal to the daily dose for NGM.

B. Lipid Metabolism

About 200 studies have been published on the effect of COC on serum concentrations of the major lipids; thus, although there is enough information on this aspect, insufficient information is available on the details of these changes and the mechanisms responsible. Serum lipid changes are determined by the relative and absolute dose of estrogen and gestogen in the COC. Estrogens increase serum concentrations of cholesterol, triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) and decrease low-density lipoprotein cholesterol (LDL-C). These changes can be counteracted by gestogens, depending on their dose and structure (2). Many of the studies with COC have been comparative ones. For COC containing DSG or GSD (30 μg EE with either 150 μg DSG or 75 μg GSD or GSD triphasic) there is no change in serum cholesterol or LDL-C concentrations, but significant increases in TG and HDL-C which tend to be more pronounced with DSG than with GSD (2). Much less information is available for other lipids; the HDL apoprotein (apo-A1) increases and the LDL apoprotein (apo-B) decreases. Although two HDL subfractions (HDL_2 and HDL_3) can be measured by a relatively simple precipitation method, ultracentrifugation gives

more accurate and reproductible results. In addition, ultracentifugation enables other subclasses to be estimated (e.g., at least seven subclasses of LDL are now recognized), and there is some evidence to suggest that the various subclasses differ in their effect on atherosclerosis or are associated with different degrees of risk of developing cardiovascular disease (CVD). Both VLDL and HDL can also be separated into a number of subclasses.

Any changes that occur in lipid metabolism on starting a COC regimen are usually manifest within the first 3 months and usually do not progress with continued use, and they return to pretreatment levels on discontinuing the COC (2).

Little information is available for COC containing NGM. Preliminary studies indicate no change in serum total cholesterol and LDL-C concentrations, but in contrast with DSG and GSD, HDL-C is increased without any increase in TG (2). Triglycerides do increase, however, with the triphasic formulation of NGM (2).

Numerous other factors affect lipid metabolism in addition to estrogens and gestogens, and changes in serum lipid concentrations will be a result of these factors. It also must be emphasized that most of the studies of the effect of estrogens and gestogens on serum lipids have been performed with subjects in the fasting state; therefore, they may not be directly relevant to the normal situation. It has been suggested that changes in serum lipids, particularly TG, postprandially may be more important for the risk of developing CVD, but little information is available on the effect of estrogen and gestogens on postprandial lipid levels (13).

Another serum lipoprotein [lipoprotein (a), Lp(a)], similar to LDL, but with an extra protein that is structurally similar to plasminogen, has been claimed to be an indicator of the risk of developing CVD (6). However, increased levels of Lp(a) appear to be important only when LDL is high, and because serum levels of Lp(a) are mainly genetically determined, the effects of estrogen or gestogen are minimal.

C. Carbohydrate Metabolism

Despite numerous investigations during the almost 40 years COC have been used, the mechanisms by which they may affect carbohydrate metabolism is still unclear. Initially, any effects observed were ascribed to the estrogen component. However, the relative lack of effect of estrogen administered alone in doses similar to those of the COC shifted the emphasis to the gestogen. Most recent studies suggest that any effects observed result from a synergistic action of the estrogen and gestogen (18).

All the modern low-dose COC have what appear to be only minor effects. Mostly, there is no change in fasting serum levels of glucose and insulin. During an oral glucose tolerance test (OGTT), minor increases may occur in

serum glucose and, particularly, in serum insulin responses. Direct comparative studies showed no differences between DSG and GSD, although one study (18) suggested that GSD COC may affect insulin levels more than DSG. There is much less information for NGM, but it also does not seem to have any effect. Changes are more apparent when an intravenous glucose tolerance test (IVGTT) is used. In a large study involving 296 COC users (19), computer modeling of the glucose and insulin responses to an IVGTT showed differences among various COC, with a reduction in insulin sensitivity. However, the insulin changes and differences among the COC were minor, and it was concluded that the changes were primarily due to the estrogen, with the gestogen having a slight modifying action. The validity of this approach needs substantiating, and the conclusions require confirmation. It must also be emphasised that the OGTT results may not be entirely relevant to the normal in vivo situation. Subjects also vary in their pancreatic reserve and, although for most subjects, COC use will have little effect on carbohydrate metabolism, for a few it may be a sufficient challenge to produce an impaired insulin response.

With short-term COC use the effects are minor and, although some studies suggest the effects become more marked with long-term COC use, this is not supported by other studies or by clinical findings (19). It is apparent that even with long-term COC use, any changes in carbohydrate metabolism are unlikely to be clinically significant, with no increase in incidence of diabetes, diabetic symptoms, or impaired glucose tolerance (4).

Few studies have investigated the changes on COC use in other hormonal factors (e.g., growth hormone, glucagon, or adrenocortical hormones) involved in regulation of carbohydrate metabolism. No study has found changes in the serum concentrations of glycosylated proteins, even though these are sensitive to consistent increases in blood glucose levels. For a more detailed account of COC and carbohydrate metabolism see the review by Hirsch and Goldzieher (20).

D. Hematological Factors

Two somewhat recent reviews of the effect of COC use on hematological factors have been published (21,22). From the large numbers of publications on this topic, three conclusions are regularly repeated: (a) any changes that occur are mainly due to the estrogen; (b) the estrogen effect may be modified by gestogens; and (c) any changes in coagulation are matched by changes in fibrinolysis; therefore, there is no change in the hemostatic balance. It is also often claimed that the newer COC have a smaller effect. However, the experimental basis for these conclusions is often suspect. There is great variability in the results from various studies, and the changes are often minor and of doubtful significance. In many investigations, particularly the earlier ones, the methodology has been inadequate. These factors make the interpretation of the results questionable.

A comprehensive review that adopts a different approach to an analysis of the results of the effect of COC on hematological factors has been published by Kluft and Lansink (23). Because of the large inter- and intrasubject variability in the levels of the hemostatic variables, they compared the mean intersubject coefficient of variation of published studies for control data (women not using COC) with that of women using COC and calculated the percentage change (COC use versus control). By applying this approach to COC containing DSG or GSD, 15 hematological factors showed significant changes. Of these, 6 can be excluded owing to a small number of data points. For the remaining 9, six (plasminogen, prothrombin, and factors VIIc, VIII, X, and XII) showed significant increases and three (protein S, t-plasminogen activator [t-Pa]), and plasminogen activator inhibitor [PAI] showed significant decreases. Application of a similar approach for results from women using COC containing LNG showed little difference from the foregoing results for DSG and GSD. It must be stressed that most of the studies included in these analyses did not involve a direct comparison of LNG-containing COC with DSG and GSD-containing COC.

The changes with DSG-containing COC were similar to those containing GSD, and this finding receives confirmation from the few direct comparative studies that have been performed and in which no significant differences were apparent.

Although this approach is interesting, it is not without its drawbacks. Whereas for many coagulation factors, large changes in concentration or activity (> 50%) are necessary before any clinical consequences result, the range of normal values for almost all of the hematological factors is wide, such that for any individual subject, a change from a level pre-COC use near the low end of the normal range to a level near the high end of the normal range while using COC could still be highly significant, although the levels do not fall outside the normal range.

Little information is available for NGM COC. Recent direct comparisons of COC containing NGM or GSD suggest no differences in either procoagulatory changes or fibrinolysis (24,25).

E. Serum Proteins and Androgens

An increase in synthesis and secretion of several hepatic proteins, mainly globulins, occurs with oral administration of estrogen, and this is particularly marked with ethinyl estradiol. Many of these proteins act as a transport mechanism for other molecules, particularly ones with a low aqueous solubility (e.g., vitamins, minerals, and hormones). Most information is available for SHBG (sex hormone-binding globulin) owing to its central role in the biological activity of sex steroids. Less information is available for cortisol-binding globulin and ceruloplasmin (copper-carrying protein), and even less for thyroxin-binding glob-

ulin, transferrin (iron-carrying protein), and retinol (vitamin A)-binding protein. The magnitude of the estrogen-induced stimulation varies with the dose of EE administered and with the protein being measured (e.g., EE 30 μg daily for 14 days will increase serum SHBG concentrations by 177% and ceruloplasmin concentrations by only 69%) (26). The estrogen-induced increase in some proteins may be inhibited by gestogens, depending on their type and dose; thus, administration of 150 μg DSG or 75 μg GSD with 30 μg EE will not inhibit the increase in serum SHBG induced by EE, whereas 150 μg LNG will inhibit most (> 95%) of the increase (26). The estrogen-induced increase in ceruloplasmin is not inhibited by any of the three gestogens under similar conditions. Administration of COC containing EE 30 μg + DSG 150 μg, or EE 30 μg + GSD 75 μg, for a treatment cycle will increase serum SHBG concentration by 100–300% (11); less information is available for EE 35 μg + NGM 250 μg, and reported increases are smaller (100–200%) (2). With both DSG and GSD COC, the increases for ceruloplasmin are 65–140%, and for cortisol-binding globulin 100–200% (26). Although both cortisol-binding and thyroid-binding globulins are increased with COC use, there is no change in cortisol or thyroid hormone activity because only the bound hormones increase, without any increase in the unbound biologically active fraction. Although EE does not bind to SHBG, the gestogens do to varying extents (see Table 1). As SHBG is the main carrier for testosterone, it has been suggested that the binding of the gestogen to SHBG will displace some of the bound testosterone, leading to an increased serum concentration of unbound testosterone, the biologically active moiety. There is a large amount of information for EE + DSG, less information for EE + GSD, and only sporadic reports for EE + NGM, to show that this does not occur and that concentrations of both total and unbound testosterone in serum decrease (2). The reason for this decrease seems to be a general decrease in secretion of C-19 steroids related to the androgens by inhibition of the tropic hormone that stimulates the synthesis and secretion of these steroids from the ovary or the adrenal cortex (2).

IV. CONCLUDING REMARKS

During the past 37 years, the metabolic effects of COC have been widely, but somewhat superficially, investigated. In the early years, the methods used were limited in scope and often did not meet the reliability criteria of accuracy, specificity, sensitivity, and reproducibility. Developments in methodology have improved the validity of the results and widened the scope of investigations. Also, during this time, the doses of estrogen and gestogen in the COC have been markedly reduced, and new gestogens have been developed. These new formulations need to be assessed clinically and pharmacologically and compared with

existing products. The investigations summarized in this chapter indicate that the currently used low-dose COC produce only minor changes in most metabolic parameters. It might be argued that even minor changes with long-term use of the COC might have a significant clinical effect, but there is no evidence to substantiate this belief, and any metabolic changes that do occur do not usually appear to progress with continued use of the COC.

The main areas of metabolism studied—lipid metabolism, carbohydrate metabolism, and hematological factors—are those that are relevant to the development of cardiovascular disease. This is partly because cardiovascular disease was the first major adverse side effect ascribed to COC use. Although effects of the new COC on carbohydrate metabolism are minor, they may affect the insulin response to a glucose tolerance test, indicating glucose intolerance and hyperinsulinemia. There is an increased risk of cardiovascular disease in diabetes, and this has been ascribed by some to hyperinsulinemia. Hyperinsulinemia is a risk factor for CVD and is associated with an increase in blood pressure, increased TG concentrations, with a decrease in serum HDL-C, and changes in some hematological factors (27).

The concern that this effect on carbohydrate metabolism may increase the risk of CVD is not supported by the findings that there is no increase in the incidence of diabetes in the long-term user and no increase in serum levels of glycosylated hemoglobin (20). These changes in carbohydrate metabolism, therefore, may not be important, even if they should occur under normal in vivo conditions.

The recognition of serum lipids as important indicators of CVD risk directed attention to the changes induce by COC. With the passage of time, COC-induced changes in serum lipid levels have become less important, although it is still widely thought that COC that have what appear to be desirable effects (e.g., increasing HDL-C and decreasing LDL-C and VLDL-C) are to be preferred. These views may be modified again when more detailed information about COC-induced changes in lipid metabolism becomes available. Use of COC containing DSG and GSD, and to a lesser extent NGM, is associated with increases in serum TG concentrations. The role of TG as a risk factor for CVD is still controversial and the effect of COC on postprandial hyperlipidemia and especially TG has been little studied. Elevation of serum TG levels may increase blood levels of some coagulation factors, especially factor VII, which may be important in causing thrombosis (28). In addition, other hematological factors may be altered in hyperlipidemia. Estrogens have widespread effects on lipid metabolism (29), most of which are beneficial. There is insufficient information to decide how many of these and to what extent they are changed by concurrent administration of the various gestogens.

Changes in the blood levels of hematological factors may be a poor indicator of thrombosis risk, and local events in the vessel wall and subendo-

thelial space, such as LDL oxidation, or release of vasoactive peptides and prostaglandins, may be much more important. It is of interest that four of the five hemostatic factors identified in the analysis by Kluft and Lansink (23) as showing significant increases in women using low-dose estrogen COC containing DSG or GSD (27), are the same as those identified by a similar approach by Beller and Ebert (21) in women using COC containing EE 50 μg or more and different progestogens.

Almost all the parameters measured in the pharmacodynamic studies show a wide interindividual variation. This necessitates using a satisfactory method of analyzing the results obtained in the clinical trials. The statistical method used in most of the investigations has been inadequate, involving mainly comparing the mean and variance of one group of subjects with that of another group. Owing to the wide intersubject variability, variances are usually large so that any changes caused by COC administration have to be very large to be significant. Most take the view that changes that occur without the values lying outside the normal range are of no significance, a conclusion that may be invalid. Clinical significance may not always correlate with statistical significance. In most studies, there are usually a few subjects, who show an abnormal response in one or more of the parameters being measured, the ''outliers,'' with the possibility that these subjects may show an exaggerated clinical response.

During the past few years the term ''selectivity'' has been widely used relative to COCs containing DSG, GSD, and NGM. These COCs were considered to be safer, with a lower incidence of serious side effects, than COCs containing other gestogens. However, the term selectivity has never been adequately defined. It usually invokes the androgenicity of gestogens. However, it is my belief that, none of the gestogens used in COC are androgenic in humans (30,31) and the definition of selectivity is, therefore, invalid.

Although CVD is still considered to be the major serious side effect of COC use, the relative risk of CVD for COC users in epidemiological studies has decreased during the past 30 years to very low levels. This is due to various factors (e.g., better prescribing habits with discouragement of subjects with CVD risk factors from using COC, improvement in epidemiological analyses to eliminate bias and confounding factors, and reduction in the doses of estrogen and gestogen in the COC). The risk for developing CVD with COC in women younger than the age of 35–40 years and who do not smoke, is now so low, particularly for arterial disease, but less so for venous disease, that it could be argued that it is due to an idiosyncracy of the affected women to the COC and, therefore, it is unlikely that it would show any correlation or relation to metabolic changes. For venous disease, metabolic changes appear less important than for arterial disease because the mechanisms involved in the two clinical conditions are different. It seems that about half of the cases of venous thrombosis occurring on COC use are due to these subjects having an inherited thrombophilia, often due to a gene mutation (32).

From this review, two points regarding COCs that contain NGM are evident:

1. There is little information on its pharmacodynamic effects so that it cannot be considered in the same way as DSG and GSD COCs.
2. There is still controversy over the extent to which it is metabolized in vivo to LNG and, if so, whether the LNG produced is sufficient to account for all the pharmacological activity of NGM. A decisive clinical trial comparing use of 250 μg NGM + 35 μg EE with 50–60 μg LNG + 35 μg EE is necessary.

The major question still outstanding is the extent to which third-generation COCs containing DSG and GSD are an improvement over, or preferable to, other low-estrogen–dose COCs. The efficacy and acceptability relative to menstrual bleeding patterns of the earlier COCs is such that it is difficult to improve on them. The pharmacological activity of the COC reviewed does not indicate any marked improvement of third-generation COCs over second-generation ones. The major difference is in lipid metabolism, the third-generation COC increasing serum HDL-C (which might be beneficial) and serum TG (which may not be beneficial), whereas a second-generation COC that contains LNG produces little change in either HDL-C or TG (2).

Controversy still exists over the extent of serious clinical side effects. It was hoped that DSG and GSD COCs would further reduce the low incidence of arterial disease in COC users: this may be a forlorn hope if the occurrence in women younger than 40 years, who do not smoke, is due to a drug idiosyncrasy or a rare genetic mutation. For venous thrombosis, evidence would suggest that the incidence is higher with DSG and GSD COC use than with LNG COC. Both of these aspects are still very controversial (33), and it may be some considerable time before they are resolved.

REFERENCES

1. K Fotherby. Potency and pharmacokinetics of gestogens. Contraception 41:533–550, 1990.
2. K Fotherby, ADS Caldwell. New progestogens in oral contraception. Contraception 49:1–32, 1994.
3. K Fotherby. Pharmacokinetics of gestagens: some problems. Am J Obstet Gynecol 163:323–328, 1990.
4. K Fotherby. Pharmacokinetics and metabolism of progestins in humans. In: JW Goldzieher, K Fotherby, eds. Pharmacology of Contraceptive Steroids. New-York: Raven Press, 1994, pp 99–126.
5. JR Newton. Classification and comparison of oral contraceptives. Hum Reprod Update 1:231–263, 1995.

6. H Kuhl. Comparative pharmacology of newer progestogens. Drugs 51:188–215, 1996.
7. K Fotherby. Bioavailability of orally administered sex steroids. Contraception 54:59–69, 1996.
8. W Kuhnz, H Blode, M Mahler. Systemic bioavailability of levonorgestrel after single oral administration of a norgestimate-containing oral contraceptive. Contraception 49:255–263, 1994.
9. GL Hammond, WP Boichinfuso, M Orava, CL Smith, A Van den Ende, A Van Enk. Serum distribution of two contraceptive progestins. Contraception 50:301–318, 1994.
10. W Kuhnz, M Pfeffer, G Al-Acoub. Protein binding of gestodene and desogestrel. J Steroid Biochem 35:313–318, 1990.
11. W Kuhnz, B Schutt, B Wolosczak. Influence of changes in serum SHBG on the protein binding of contraceptive steroids. J Steroid Biochem 48:573–580, 1994.
12. FS Khan, K Fotherby. In vivo metabolism of norethisterone-3-oxime. J Steroid Biochem 9:229–232, 1978.
13. K Fotherby. A cortical evaluation of the pharmacokinetics of contraceptive steroids. In: R Snow, P Hall, eds. Steroid Contraceptives and Women Response. New York: Plenum Press, 1994, pp 125–132.
14. K Fotherby. Levonorgestrel, clinical pharmacokinetics. Clin Pharmacokinet 28: 203–215, 1995.
15. G Benagiano, FM Primiero, C Bastianelli, G Abbadonte, F Grotanelli, G Tunnera. Ovarian activity during oral contraception. Adv Contracept 6:245–251, 1990.
16. C Fitzgerald, W Feichtinger, J Spona, M Elstein, F Ludicki, U Muller, C William. Comparison of the effects of two oral contraceptives on ovulation inhibition. Adv Contracept 10:5–18, 1994.
17. R Barentsen. Progestogens: pharmacological characteristics. Eur Menopause J 3:266–271, 1996.
18. D Crook, I Godsland, M Worthinghton, C Felton, A Proudler, J Stevenson. A comparative metabolic study of two low-dose oral contraceptives. Am J Obstet Gynecol 169:1183–1189, 1993.
19. I Godsland, C Walton, C Falton, A Proudler, A Patel, V Wynn. Insulin resistance, secretion and metabolism in oral contraceptive users. J Clin Endocrinol 74:64–70, 1992.
20. KE Hirsch, JW Goldzieher. Carbohydrate metabolism. In: JW Goldzieher, K Fotherby, eds. Pharmacology of Contraceptive Steroids. New York: Raven Press, 1994, pp 345–356.
21. FK Beller. Coagulation, thrombosis and contraceptive steroids. In: JW Goldzieher, K Fotherby, eds. Pharmacology of Contraceptive Steroids. New York: Raven Press, 1994, pp 309–333.
22. H Kuhl. Effects of progestogens on haemostasis. Maturitas 24:1–19, 1996.
23. C Kluft, M Lansink. Effect of oral contraceptives on haemostasis variables. Thromb Haemost 78:315–326, 1997.
24. P Quehenberger, U Lohner, S Kapiotis, S Handler, B Schneider, J Huber, W Speiser. Increased levels of factor VII and decreased PrS during use of oral contraceptives. Thromb Haemostat 76:729–734, 1996.

25. P Quehenberger, U Lohner, S Kapiotis, S Handler, B Schneider, J Huber, W Speiser. Effects of third generation oral contraceptives on fibrinolytic parameters. Fibrinolysis and Proteolysis 11:97–101, 1997.
26. K Fotherby. Interactions of contraceptive steroids with steroid-binding proteins and the clinical implications. Ann NY Acad Sci 538:313–324, 1988.
27. K Fotherby. Metabolic interrelationships, cardiovascular disease and sex steroids. Contraception 57:183–187, 1998.
28. GJ Miller. Hyperlipidaemia and hypercoagulability. Prog Lipid Res 32:61–69, 1993.
29. RH Knopp. Multiple beneficial effects of estrogen on lipid metabolism. J Clin Endocrinol 82:3952–3954, 1997.
30. K Fotherby. The selectivity of gestogens. Gynecol Endocrinol 10(suppl 2):59–63, 1996.
31. K Fotherby. The myth of the androgenicity of levonorgestrel. In: AT Teichmann, A Corbin, eds. Levonorgestrel. Stuttgart: Georg Thieme, 1998 pp 25–30.
32. T Koster, FR Rosendaal, H Deronde, E Briet, JP Vandenbroucke, RM Bertina. Venous thrombosis due to poor anticoagulant response to activated protein C. Lancet 342:1503–1506, 1993.
33. World Health Organization. Cardiovascular disease and steroid hormone contraception. WHO Technical Series 877. Geneva, Switzerland, 1998.

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Pharmacology of New Progestogens: The 19-Norprogesterone Derivatives

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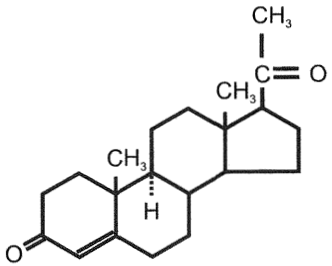
I. INTRODUCTION

Several new progestins have been introduced in the last decade. Although the so-called third-generation progestins derived from 19-nortestosterone, were essentially developed for contraceptive use (see Chaps. 6 and 9), the19-norprogesterone molecules have been mainly studied for progesterone-replacement therapy. In contrast with some 19-nortestosterone–derived progestins, the 19-norprogesterone–derived compounds are devoid of androgenic properties.

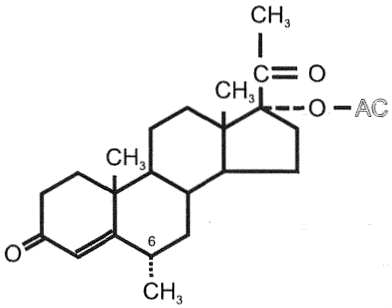
The group of 19-norprogesterone derivatives include promegestone and its derivative trimegestone, dimegestone, nomegestrol acetate, and nestorone. The latter being ineffective orally was developed for use in controlled-released delivery systems contrary to the former, which are developed for oral administration.

II. STRUCTURE AND METABOLISM

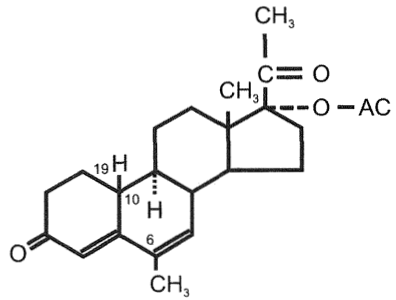
The structure of the 19-norprogesterone molecules is shown in Fig. 1 and Table 1. 19-Norprogesterone is inactive when administered orally. However, the



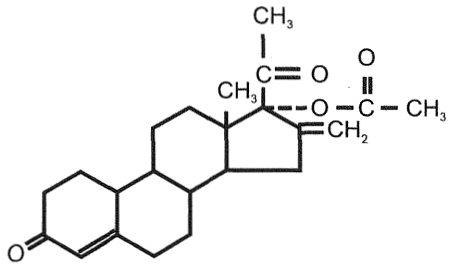
progesterone



medroxyprogesterone acetate



nomegestrol acetate



nestorone

Figure 1 Structure of 19-norprogesterone molecules.

modification of its chemical structure with the addition of a double bond at C6–7, a methyl group at C-6, and an acetoxy function at the C-17 position, results in nomegestrol acetate, an orally active compound (1).

Although Nestorone is very similar in structure to nomegestrol acetate, the double bond at C6–7 and the methyl group at C-6 are missing. This difference may account for the absence of oral activity of the Nestorone molecule.

Table 1 Structural Formulas of Some 19-Norprogesterone Molecules

Nomegestrol acetate	17α-Acetoxy-6α-methyl-19-norpregna-4,6-diene-3, 20-dione (TX 066)
Nestorone	16-Methylene-17α-acetoxy-19-norpregn-4-ene-3,20-dione (ST 1435)
TX 525	1,2α-Methylene,6α-Methyl,17α-acetoxy,19-norprogesterone

The 19-norprogesterone molecules are metabolized mainly through hydroxylations. Dimegestone and promegestone are essentially hydroxylated at C-21. In the latter compound, the hydroxylation results in its increased activity (2). Nomegestrol acetate undergoes mono- and dihydroxylations (3).

III. PHARMACOLOGICAL PROPERTIES

A. Promegestone

Promegestone is one of the most-studied molecules known under the code name R 5020. It binds specifically to the progesterone receptor, with a relative binding affinity, compared with 100 for progesterone, of 220 after 2 h of incubation and 535 after 24 h (2).

It showed no binding affinity to the estrogen, androgen, mineralocorticoid, or glucocorticoid receptors. The compound has strong antiestrogenic potency both in vitro and in vivo. It decreases the uterotropic activity of estrogens and also decreases human breast cancer cell proliferation in in vitro models (4). Promegestone does inhibit luteinizing hormone (LH) secretion in animal models (5) and, when given at higher doses to normal-cycling women, leads to anovulation.

The pharmacokinetics of the molecule indicate a rapid absorption and a short half-life. Owing to these properties, the molecule has been used as a tool in biochemical studies, but has not been developed for clinical use except for limited indications, such as treatment of anovulatory and irregular cycles.

Through C-21 hydroxylation, other metabolites with enhanced progestational potency have been identified and are under development (6).

B. Trimegestone

Trimegestone is one of the active metabolites of promegestone and is also a potent progestin devoid of any androgenic effect (7).

This 19-norpregnane derivative is being developed for postmenopausal therapy. Ross et al. (7) studying the effects of three doses of trimegestone on the endometrium of estrogen-treated postmenopausal women showed a secretory transformation with the lowest dose of 0.1 mg/day.

Pharmacokinetic studies have indicated a t_{max} of 0.5 h. and a C_{max} of 25.0 ± 3.2 ng/mL, after a single oral dose administration of 1 mg, and of 46.0 ± 3.9 ng/mL after ingestion of 2 mg. The half-life ($t_{\frac{1}{2}}$) was on average 13.8 h, and the product is designed for a once-daily administration (8).

In animal models, the compound potentiated the bone-sparing effect of estradiol, which makes it very suitable for hormonal replacement therapy (HRT) (9).

C. Nomegestrol Acetate

Among the progesterone derivatives, nomegestrol acetate is one of the most potent progestins and exerts a strong effect on the endometrium (1,3,10).

This agent, when given orally, is four times more potent than medroxyprogesterone acetate (MPA) by the McPhail test and is about half as potent as MPA in the inhibition of estradiol-induced uterotropic action (1). Its binding affinity to the progesterone receptor is 2.5 times higher than that of progesterone (11) and also higher than that of MPA and chlormadinone acetate (CLA) (12).

Nomegestrol acetate does not stimulate the growth of the rat ventral prostate and is able to counteract the testosterone effect on this organ by 62% (13). Its antiandrogenic effect is higher than that of CLA, but lower than that of cyproterone acetate (3,13).

The compound does not bind to the estrogen receptor (2), the aldosterone receptor, or the glucocorticoid receptor (14,15). Also, it does not induce sodium retention or exert antidiuretic activity (15). Nomegestrol acetate does not exert any estrogenic effect in animals or in cell cultures of endometrial Ishikawa cell lines and MCF7 or T47D breast cancer cell lines (16,17).

Although the antigonadotropic activity of the compound is weak in animal models, it inhibits ovulation effectively in humans (18). Also the administration of the progestin to postmenopausal women receiving estradiol treatment, resulted in a decrease in the frequency of pulsatile LH secretion, similar to that observed with norethisterone acetate (NETA) (19). The authors showed that this antigonadotropic effect is induced through the progesterone receptor. The antiovulatory effect of nomegestrol acetate occurs with a dose of 1.25 mg/day when given orally (18), and at much lower doses when given continuously, by subdermal implants (20).

The pharmacokinetic profile of the progestin when given orally shows a C_{max} reached within 2–4 h and a prolonged elimination half-life of 35–50 h, which is much longer than most progestins, such as MPA (24 h) or NETA (20 h) (19,21). Compared with other 19-norprogesterone molecules, such as promegestone, nomegestrol exhibits a much longer half-life. These properties led to the clinical use of nomegestrol acetate for the treatment of several conditions. It induces a full endometrial secretory transformation with oral doses as low as 0.5 mg/day when given to post-menopausal women receiving estrogen therapy by implants (10).

Because of the lack of androgenic activity of the molecule, this agent does not modify glucose and insulin response in oral glucose tolerance tests (22) and does not induce any changes in the lipid profile (23). Moreover, although some progestins may oppose the vasodilator action of estrogens (see also Chaps. 4 and 16), nomegestrol acetate does not modify the coronary dilator response to therapy in atherosclerotic female rhesus monkeys (24). It also does not oppose the estrogen-dependent vasodilator response in the human uterine artery (25).

D. TX525

Another new synthetic 19-norprogesterone derivative sharing structural characteristics of nomegestrol acetate also showed potent pseudogestagenic activity. It is four to nine times as potent as MPA, but only half as active as cyproterone acetate. The molecule has no androgenic or estrogenic activities and appears to be a potent antiovulatory agent in rodents (26,27).

E. Nestorone

Nestorone (NES), 16-methylene-17α-acetoxy-19-norpregnene-3,20,-dione (previously called ST 1435) is a progestin that has several features that make it ideal for use both for contraception and hormone replacement therapy (HRT). NES has low oral activity, but is very potent parenterally when given in sustained-release formulations (28,29). The pharmacological profile of NES has been compared with that of levonorgestrel (LNG) and 3-keto-desogestrel, two 19-nortestosterone–based progestins that are widely used for contraception, in in vitro and in vivo bioassays (30). 3-Keto-desogestrel showed the highest-binding affinity to rat uterine progesterone receptors (PR) followed by NES, levonorgestrel (LNG), and progesterone (Table 2). The binding affinity of NES to androgen receptors (AR) prepared from rat prostate, was negligible. However, both levonorgestrel and 3-keto-desogestrel showed significant binding to AR (30). None of the progestins bound to estrogen receptors (30). The progestational activity of NES in the Clauberg, pregnancy maintenance, and ovulation inhibition tests was higher than that of levonorgestrel. The in vivo androgenic activity of these progestins in castrated immature male rats correlated well with their binding affinity to AR (30). Both levonorgestrel and 3-keto-desogestrel showed significant androgenic activity. In contrast, NES showed no androgenic activity

Table 2 Relative Binding Affinity (RBA) and Biopotency of NES

Activity	P	NES	LNG	DSG	T
RBA (PR)	100	490	450	980	ND
Progestational (ED_{50}, μg)[a]	100	1.0	10	ND	ND
RBA (AR)	ND	0.2	73	39	100
Androgenic (ED_{50}, μg)[b]	ND	NA	140	110	45

P, progesterone; NES, nestorone; LNG, levonorgestrel; DSG, desogestrel; T, testosterone; PR, progesterone receptors; AR, androgen receptors; ED_{50}, effective dose 50%; ND, not determined; NA, no activity.

[a]McPhail index.

[b]Prostate stimulation in castrated rats.

Source: Ref. 30.

(see Table 2). NES does not bind to sex hormone-binding globulin (SHBG) (31). This accounts for its short half-life and rapid clearance, compared with other synthetic progestins (32). Both levonorgestrel and 3-keto-desogestrel bind strongly to SHBG. NES showed no uterotropic or antiuterotropic activity in ovariectomized rats (30). However, it blocked estrogen-induced vaginal cornification (30). NES showed significant binding to glucocorticoid receptors (30). However, it showed no glucocorticoid activity in in vivo models. Because of its excellent pharmacological profile and lack of oral activity, NES is being formulated into various parenteral dosage forms (33).

Subdermal implants designed to last 2 years and releasing about 80–100 μg/day of NES, have proved to be highly effective for contraception (34). No ovulation occurred when the mean serum NES concentrations were higher than 40 pg/mL. Cervical mucus showed a decrease in quantity and thickening, leading to decreased permeability in sperm penetration tests. There were frequent irregular bleeding patterns, a phenomenon common to progestin-only contraceptive methods.

Contraceptive vaginal rings are flexible rings made of silicone elastomer measuring approximately 58 mm in diameter. The rings contain steroid cores of different lengths to meet dose requirements. Rings delivering steroids by the vaginal route have several advantages over other methods of contraception. Unlike oral contraceptives they do not require daily administration or attention. In contrast to injectables, implants and intrauterine devices (IUDs), they are under user control and can be terminated at will (see also Chap. 12).

Rings delivering NES alone at doses of 50, 75, or 100 μg have been investigated for continuous use and were shown to inhibit ovulation consistently (T Jackanicz, The Population Council, personal communication). Combination rings containing NES and ethynyl estradiol are being investigated for 3-weeks in and 1-week out cyclic use (29,35).

Bioavailability studies have shown that NES is absorbed through the skin readily. Hence, various transdermal formulations of NES are being investigated (36,37). By adjusting the dose of NES and estrogen, both gels and patches have the potential of being developed for contraception and other indications.

In summary, initial studies in rodents and primates have demonstrated that NES has little progestational activity when given orally. However, when administered subcutaneously or intramuscularly, the molecule was 10–1400 more potent than progesterone applied by the same route (28). Therefore, this agent is being essentially developed for administration through sustained-release drug delivery systems (28,29).

Several studies are ongoing in which NES is administered either through implants or vaginal rings or transdermally, exploring its potential use for contraception and for the treatment of postmenopausal women. The lack of androgenic or estrogenic side effects makes NES highly attractive in the aforementioned indications.

IV. CONCLUSION

As has also been stressed in Chap. 5, minor structural modifications can profoundly affect the biological activity of a progestational steroid. In this chapter, the 19-norprogesterone molecules have been shown to be very potent compounds able to exert antiestrogenic and antigonadotropic activities at levels comparable with those observed with 19-nortestosterone derivatives. The main characteristics of these progesterone derivatives relate to their lack of androgenic activity. This may account for a better safety profile that makes this class of compounds highly attractive for future developments both for HRT and for contraception.

REFERENCES

1. J Paris, R Thevenot, P Bonnet, M Granero. The pharmacological profile of TX 066 (17α-acetoxy-6-methyl-19 nor-4,6-pregnadiene-3,20-dione), a new oral progestative. Arzneimittelforschung Drug Res 33:710–715, 1983.
2. JP Raynaud, T Ojasoo, T Vaché. Hormones naturelles et artificielles. Aspects pharmacologiques. In: P Mauvais-Jarvis, R Sitruk-Ware, eds. Médecine de la Reproduction. 2nd ed. Paris: Flammarion Médecine/Sciences, 1986, pp 521–537.
3. H Kuhl. Comparative pharmacology of newer progestogens. Drugs 51:188–215, 1996.
4. F Vignon, S Bardon, D Chalbos, H Rochefort. Anti-estrogenic effect of R5020, a synthetic progestin in human breast cells in culture. J Clin Endocrinol Metab 56:1124–1130, 1983.
5. F Labrie, L Ferland, L Lagace. High inhibitory activity of R 5020, a pure progestin at the hypothalamoadenohypophyseal level on gonadotropin secretion. Fertil Steril 28:1104–1112, 1977.
6. JP Raynaud, T Ojasoo. The relevance of structure–affinity relationships in the study of steroid hormone action. In: H Eriksson, JA Gustafsson, eds. Steroid Hormone Receptors: Structure and Function. Amsterdam: Elsevier, 1983, pp 141–170.
7. D Ross, V Godfree, A Cooper, J Pryse-Davies, MI Whitehead. Endometrial effects of three doses of trimegestone, a new orally active progestogen, on the postmenopausal endometrium. Maturitas 28:83–88, 1997.
8. E Sultan, C Logeais, M Mouren, B Chinal, P Molinier, B Lenfant. Single and multiple-dose pharmacokinetics of trimegestone in postmenopausal women. VIth World Conference on Clinical Pharmacology and Therapeutics. August 4–9, 1996 [abstr 262].
9. L Lepescheux, C Chadelat, C Couillard, J Secchi, D Philibert. Trimegestone potentiates the curative effects of estradiol on bone mass in ovariectomized rats while preventing uterotrophy [abstr]. J Bone Min Res 10(suppl. 1):M 403, 1995.
10. DI Fraser, ML Padwick, MI Whitehead, J White, TA Ryder, J Pryse-Davies. The effets of the addition of nomegestrol acetate to post menopausal oestrogen therapy. Maturitas 11:21–34, 1989.

11. J Botella, J Duc, R Delansorne, J Paris, B Lahlou. Structure–activity and structure–affinity relationships of 19-nor-progesterone derivatives in rat uterus. J Endocr Invest 13:905–910, 1990.
12. I Duc, J Botella, R Delansorne, J Paris. Interaction of (^{3}H) nomegestrol acetate with cytosolic progesterone receptors from the rat uterus. Steroids 56:325–328, 1991.
13. I Duc, J Botella, P Bonnet, F Fraboul, R Delansorne, J Paris. Antiandrogenic properties of nomegestrol acetate. Drug Res 45:70–74, 1995.
14. J Botella, J Porthe-Nibelle, J Paris, B Lahlou. Interaction of new 19-norprogesterone derivatives with progestagen, mineralocorticoid and glucocorticoid cytosolic receptors. J Pharmacol (Paris) 17:699-706, 1986.
15. J Paris, J Botella, P Fournau, P Bonnet, R Thevenot. Extinction of mineralocorticoid effects in 19-norprogesterone derivatives: structure–activity relationships. J Pharmacol Exp Ther 243:288–291, 1987.
16. J Botella, E Duranti, V Viader, I Duc, R Delansorne, J Paris. Lack of estrogenic potential of progesterone or 19-norprogesterone derived progestins as opposed to testosterone or 19-nortestosterone derivatives on endometrial Ishikawa cells. J Steroid Biochem Molec Biol 55:77–84, 1995.
17. WH Catherino, C Jordan. Nomegestrol acetate, a clinically useful 19-norprogesterone derivative which lacks estrogenic activity. J Steroid Biochem Mol Biol 55:239–246, 1995.
18. B Bazin, R Thevenot, C Bursaux, J Paris. Effect of nomegestrol acetate, a new 19-norprogesterone derivative, on pituitary-ovarian function in women. Br J Obstet Gynaecol 94:1199–1204, 1987.
19. B Couzinet, J Young, S Brailly, P Chanson, JL Thomas, G Schaison. The antigonadotropic activity of progestins (19-nortestosterone and 19-norprogesterone derivatives) is not mediated through the androgen receptor. J Clin Endocrinol Metab 81:4218–4223, 1996.
20. K Barnhart, L Devoto, R Pommer, T Pettermann, J Robinovic, E Coutinho. Neuroendocrine mechanism of anovulation in users of contraceptive subdermal implant of nomegestrol acetate (Uniplant). Fertil Steril 67:250–255, 1997.
21. E Ezan, H Benech, R Bucourt. Enzyme immunoassay for nomegestrol acetate in human plasma. J Steroid Biochem Mol Biol 46:507–514, 1993.
22. P Dorangeon, JL Thomas, H Choisy, M Lumbroso, MC Hazard. Effects of nomegestrol acetate on carbohydrate metabolism. Diabetes Metab 19:441–445, 1993.
23. A Basdevant, C Pelissier, J Conard, H Degrelle, TT Guyenne, JL Thomas. Effect of nomegestrol acetate (5 mg/d) on hormonal, metabolic and hemostatic parameters in premenopausal women. Contraception 44:599–605, 1991.
24. JK William, J Mark Cline, EK Honoré, R Delansorne, J Paris. Coadministration of nomegestrol acetate does not diminish the beneficial effects of estradiol on coronary artery dilator responses in nonhuman primates (*Macaca fascicularis*). Am J Obstet Gynecol 179:1288–1294, 1998.
25. D de Ziegler, M Zartarian, MC Micheletti, CG Linh, E Shaerer. Cyclical administration of nomegestrol acetate does not alter the vasodilatation exerted by estradiol on uterine artery. Contracept Fertil Sex 22:767–770, 1994.

26. R Delansorne, J Botella-Shields, I Duc, E Duranti, S Guesdon, S Galliani, J Paris. Steroid receptor profile of TX 525, a new synthetic progestin. Xth International Congress on Hormonal Steroids, Quebec City, June 17–21, 1998, abstr 107.
27. R Delansorne, P Bonnet, F Puccio, MN Chardin, A De Giovanni, V Duranti, S Gribaudo, D Guiraud, M Llanas, J Paris. In vivo reproductive endocrinological profile of TX 525, a new orally active synthetic progestin. Xth International Congress on Hormonal Steroids, Quebec City, June 17–21, 1998, abstr 108.
28. E Coutinho. Clinical experience with implant contraception. Contraception 18: 411–427, 1978.
29. F Alvarez-Sanchez, V Brache, T Jackanicz, A Faundes. Evaluation of four different contraceptive vaginal rings: steroid serum levels, luteal activity, bleeding control and lipid profiles. Contraception 46:387–398, 1992.
30. N Kumar, Y-Y Tsong, SS Koide, K Sundaram. Nestorone progestin: a highly selective 19-nor-progesterone derivative lacking androgenic and estrogenic activities. (Unpublished.)
31. PLA Lähteenmäki, S Diaz, P Miranda, H Croxatto, P Lähteenmäki. Milk and plasma concentrations of the progestin of ST-1435 in women treated parenterally with ST-1435. Contraception 42:555–562, 1990.
32. G Noé, A Salvatierra, O Heikinheimo, X Maturana, HB Croxatto. Pharmacokinetics and bioavailability of ST-1435 administered by different routes. Contraception 48:548–556, 1993.
33. O Heikinheimo, G Noé, M Haukkamaa, P Lähteenmäki. The progestin ST-1435 rapid metabolism in man. Contraception 50:275–289, 1994.
34. S Diaz, V Schiappacasse, M Pavez, A Zepeda, AJ Moo-Young, A Brandeis, P Lähteenmäki, HB Croxatto. Clinical trial with Nestorone subdermal contraceptive implants. Contraception 51:33–38, 1995.
35. M Laurikka-Routti, M Haukkamaa, O Heikinheimo. A contraceptive vaginal ring releasing ethinyl estradiol and the progestin ST-1435: bleeding control, serum steroid concentration, serum lipids and serum chemistry. Contraception 42:111–120, 1990.
36. M Haukkamaa, M Laurikka-Routti, O Heikinheimo. Transdermal absorption of the progestin ST-1435: therapeutic serum steroid concentrations and high excretion of the steroid in saliva. Contraception 44:269–276, 1991.
37. HB Croxatto, A Zepeda. Transdermal contraceptive systems, In: R Sitruk-Ware, CW Bardin, eds. Contraception. Newer Pharmacological Agents, Devices, and Delivery Systems. New York: Marcel Dekker, 1992, pp 101–115.
38. A Robbins, CW Bardin. Nestorone progestin. This ideal progestin for use in controlled release delivery systems. In: C Bulletti, D De Ziegler, S Guller, M Levitz, eds. The Uterus—Endometrium and Myometrium. Ann NY Acad Sci. 828:38–46, 1997.

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8

Hybrid Progestins: The Example of Dienogest

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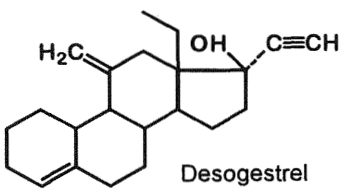
I. INTRODUCTION

The history of the progestins began with progesterone, and now again, general interest is focused on this natural hormone produced by the corpus luteum. There are several progesterone formulations on the market, including orally, intravaginally, and (as gel) transdermally active ones. However, at least the oral formulations have a big disadvantage: progesterone shows a very strong liver first-pass effect, and the 5α-reduced metabolites possess side effects on the central nervous system (CNS) (1,2).

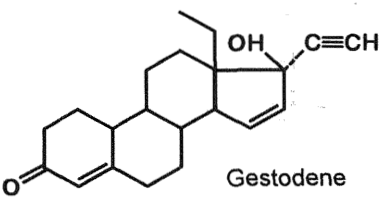
Thus, it seemed reasonable to develop progestins that were highly effective when used orally, and that were at least partly similar to progesterone in their pharmacodynamic profile (Table 1). Examples of such progestins include the norpregnanes promegestone (R 5020), nomegestrol (acetate), and trimegestone, and the androstane derivative drospirenone (3). It now became interesting to know if it was also possible to find substances resembling progesterone in certain pharmacodynamic features among the modern 19-nortestosterone derivatives. Because the structural feature that 19-norprogestins have in common (i.e., the toxicologically not inconspicuous 17α-ethinyl group) (4,5), was absent in the so-called C_{21} progestins suggested searching for norprogestins without the 17α-ethinyl group. The first compound in this group, dienogest (17α-cyanomethyl-17β-hydroxy-4,9-estradien-3-one; code STS 557), is now on the market in Germany (Fig. 1). Dienogest was selected from a group of potential progestins characterized by a methyl group substituted in position 17α and was first synthesized and pharmacologically characterized under the leadership of Ponsold

Table 1 Progestin and Oral Contraception

Aim	Means	Problem
Oral contraception	Progestin alone	Amenorrhea
	⇓	
Regular bleeding	Combined estrogen–progestin pills	Large doses
	⇓	
Stable cycles, low doses	Modern 19-norprogestins	Residual androgenicity
	⇓	
Combination of desirable effects of 19-nor- and C21-progestins	New hybrid progestins	?



Desogestrel



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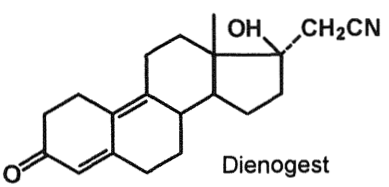
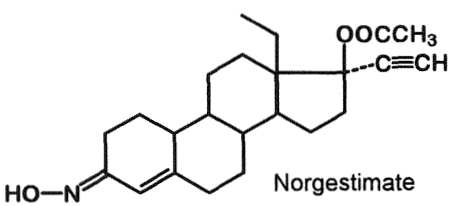
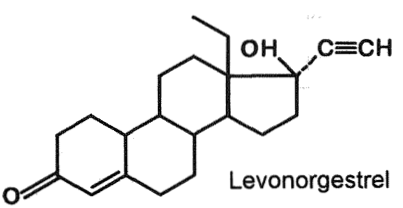


Dienogest



Norgestimate



Levonorgestrel

Figure 1 Chemical structure of dienogest in comparison with other 19-norprogestins. Note the cyanomethyl group at position 17α, as well as the double bond between C-9 and C-10 in ring B of the molecule.

(6). This novel type of substitution appeared to be favorable, because it enabled directed changes in the physicochemical properties of the steroids chosen. By variation of the substituent in the 17α-methyl group, hydrophobicity, polarity, and the size of the substituent were changed and, therefore, it became possible to optimize absorption, distribution, and metabolism (6).

The aim of this chapter is to present selected and, so far, relatively unknown, more or less recent findings of the pharmacoendocrine dynamic profile, postcoital fertility inhibition in females, fertility inhibition in males, and antiproliferative action of dienogest. Also presented here are investigations into the microbial transformation of the steroid. These findings have already been published elsewhere individually, but they have not yet been analyzed and discussed in connection with each other.

II. PHARMACOENDOCRINE PROFILE OF DIENOGEST

Current overviews are given in Oettel et al. (7–10) and Foster and Wilde (11). Dienogest exhibits moderate binding affinity for the human and rabbit uterine progesterone receptor (approximately 10% compared with progesterone). This may be due to a relatively fast dissociation of the dienogest–receptor complex (12,13). Nevertheless, the 17α-cyanomethyl group is tolerated preferably by the progesterone receptor and contributes to the high selectivity of receptor binding. This is indicated by negligible binding affinities for the estrogen, glucocorticoid, and, especially, mineralocorticoid receptors. Dienogest exhibits low competitive binding to the androgen receptor, which is well in keeping with the preclinical and clinical findings of its lack of androgenic effects.

The ED_{50} values for the expression of alkaline phosphatase in T47D cells well agree with the relatively moderate binding affinity of dienogest to the progesterone receptor (14). In contrast with this, dienogest showed a high progestational (transformatory) activity in the Clauberg/McPhail assay, using immature rabbits. After oral administration dienogest was five times as active as levonorgestrel and ten times as active as medroxyprogesterone acetate (MPA) (8,15). Additionally, unlike MPA, dydrogesterone, and NETA, dienogest maintained pregnancy in ovariectomized mice and rats (9,15).

As Vij et al. (16) found in the progesterone-treated rat uterus, dienogest enhanced the concentration of cytosolic as well as of nuclear progesterone receptors. However, in the deciduoma tissue, the number of nuclear receptors was reduced by this progestin. This may have been an antiprogestational effect, as emphasized by Kitchlu and Mehrota (17) observing that dienogest inhibits the progesterone-dependent deciduoma formation in rats. The progestational effect of dienogest on the rabbit uterus was only partially antagonized by the antiprogestin–antiglucocorticoid mifepristone (RU 486), whereas in this assay

the progestational activities of MPA and progesterone were fully abolished by the antiprogestin (15).

Surprisingly, unlike progesterone, dienogest does not bind at all to cortisol-binding globulin (CBG), nor does it show any binding to sex hormone-binding globulin (SHBG), whereas other 19-norprogestins do (13). The 4,9-diene structural element seems to contribute to this property by analogy with this element in promegestone, another progestin that does not bind to CBG (18). Consequently, a displacement of the binding of endogenous steroids to SHBG (e.g., testosterone or 5α-dihydrotestosterone) or CBG (e.g., cortisol or progesterone) by dienogest is impossible. This suggests that the endocrine profile will not be affected by displacement of endogenous steroids from specific transport proteins.

We determined the free fraction of dienogest in the plasma of female rats, rabbits, beagle dogs, and female volunteers using the method of centrifugal ultrafiltration with centrifree micropartition systems (19). The reference compound was levonorgestrel. Surprisingly, dienogest had a considerably higher fraction of free, non–protein-bound compound in plasma, up to 10% of the total concentration (the unbound fractions of the other progestins ranging between 1 and 3%). This extremely large fraction of biologically active steroid obviously contributes to the strong progestational effect of dienogest, including strong tissue penetration in vivo. The residual 90% of the compound was bound nonspecifically to albumin. Similarly, the concentrations of dienogest in saliva are also very high (6.0 $\pm$ 1.0% of the plasma total dienogest) (19).

The contradiction between only moderate progesterone receptor binding and very strong progestational activity in the endometrium can be explained by the high levels of free (i.e., biologically active) dienogest, the influence of the conjugated 9(10)-double bond inhibiting some metabolization pathways, and the specific pharmacokinetic parameters of this progestin (20).

The endocrine–pharmacological profile of dienogest, very logically, derives from its receptor specificity (Table 2). Apart from its very strong progestational activity on the endometrium, estrogenic, antiestrogenic, and androgenic activities are completely absent. Antigonadotropic actions (e.g., inhibition of the secretion of follicle-stimulating hormone [FSH] and luteinizing hormone [LH]) are weak. In cyclic women a reliable antiovulatory effect is achieved by oral administration of 1 mg dienogest per day predominantly by peripheral mechanisms (e.g., inhibition of the preovulatory, ovarian 17β-estradiol peak). This prevents the positive feedback on the pituitary, and the ovulatory peaks of FSH and LH fail to occur (23). We compared the ovulation inhibition dose (mg/day) as a mark for the antigonadotropic action with the so-called endometrium transformation dose (mg/over 14 days) in the known Kaufmann assay (24) using estrogen-primed postmenopausal women (determination of the total progestin dose over 14 days that leads to full secretory transformation of the endometrium). Estimating the quotient between ovulation inhibition dose (central activity) and the transforma-

Table 2 Endocrinopharmalogical Profiles of Several Progestins in Laboratory Animals

Progestin	Progestational activity: Endometrial glandular proliferation in immature rabbits	Estrogenic effect: Vaginal smear or uterine growth assays in rodents	Antiestrogenic effect: Vaginal smear or uterine growth assays in rodents	Androgenic effect: Hershberger assay in immature male rats	Antiandrogenic effect: Hershberger assay in immature male rats	Inhibition of gonadotropin secretion: Gonadal growth or gonadotropin estimations in immature rats
Progesterone	+	−	+	−	−	−
Chlormadinone acetate	++	−	+	−	+	+
Cyproterone acetate	++	−	(+)	−	++	+
Norethisterone acetate	++	+	+	+	−	++
Levonorgestrel	+++	−	++	+	−	+++
Desogestrel	+++	−	+	−	−	+++
Gestodene	+++	−	+	+	−	+++
Dienogest	+++	−	−	−	+/++	−

−, no/scarce effect; (+), weak effect; +, distinct effect; ++, strong effect; +++, very strong effect.
Sources: Refs. 8,9,15,21,25.

Table 3 Pharmacoendocrinological Assays in Which Dienogest Shows Significant Antiandrogenic Properties

Assay	Ref.
Inhibition of androgen biosynthesis in human ovarian slices in vitro	27
Androgen receptor competition on human genital skin fibroblasts	28 cited in 10
Prostatic cancer cells LNCaP in vitro	29 cited in 8
Hershberger assay in immature male rats	9 15
Influence on vocal fold elasticity in female beagle dogs	30 cited in 10

tion dose (peripheral activity) we found the following ranking: norethindrone acetate, levonorgestrel, gestodene: 1; desogestrel and norgestimate: 3; cyproterone acetate and chlormadinone acetate: 5–7; and finally dienogest: 17. We can see that dienogest shows the highest ratio (i.e., there is a pronounced dissociation with favorable effects of dienogest on the uterus). Dienogest comes close to naturally occurring progesterone. One might also speculate on a uterine first-pass effect of dienogest after oral ingestion (8,25). Despite its low antigonadotropic activity, dienogest is perfectly qualified for combination with natural estrogens for oral contraception (replacement of ethinyl estradiol) (26).

The antiandrogenic activities of dienogest deserve particular notice. As far as we know, dienogest is the only 19-nortestosterone derivative to have definite and clinically relevant antiandrogenic activities. Table 3 lists the most important preclinical assays in which dienogest shows a statistically significant antiandrogenic effect (for references, see Table 3).

III. POSTCOITAL FERTILITY INHIBITION

In rats, dienogest arrested the division of early embryos resting in the tube. At the same time, an acceleration of tubal egg transport was observed (31). The acceleration of tubal egg transport, the morphological changes in the endometrium shown by scanning electron microscopy, and the effects on the outcome of blastocyst transfer, all investigated in rats, suggest a peripheral mechanism of action when dienogest is given postcoitally. Postcoital fertility-inhibiting effects were found in mice, rats, rabbits, and baboons (32,33). The significant fertility inhibition in baboons was seen when dienogest, 0.4 mg/animal was given orally immediately after the mating period (33). However, when the mating period was extended to 42 h after single dosage of dienogest to baboons, the conception rate was not reduced, indicating that dienogest possesses no precoital fertility-inhibiting activities (34).

These animal studies were encouraging enough to initiate corresponding studies in women. The administration of 0.5 or 2.0 mg dienogest during the LH peak neither prevented ovulation nor interfered with corpus luteum function (35). Fifty-eight fertile female volunteers between 20 and 45 years of age were enrolled in a clinical trial to evaluate the efficacy and tolerance of dienogest as a postcoital contraceptive. An oral dose of 2 mg dienogest was administered immediately after each coitus. The 58 women reported 872 intercourses during 302 cycles. The frequency of ingestion was on average three times per cycle. Pregnancy occurred in 14 women. The observed pregnancy rate referring to all intercourses was 1.6%. The incidence of expected pregnancies in relation to the coital exposures was 4.0%. As a result, the risk of pregnancy was reduced 2.5 times by dienogest. This corresponds to a high Pearl index of 55.6 or a reduction to 60% in the number of pregnancies to be expected if no dienogest was used after unprotected intercourse. Bleeding irregularities occured in 18.9% of all cycles (36). On the other hand, the U.S. Food and Drug Administration (FDA) has approved an emergency contraceptive containing 0.5 mg levonorgestrel and 0.1 mg ethinyl estradiol to be administered twice at an interval of 12 h within 72 h after unprotected intercourse, resulting in a 75% reduction in the number of pregnancies (37). Apart from the additional ethinyl estradiol, the chosen levonorgestrel total dose is about ten times higher than necessary for daily dosage in oral contraceptives. For dienogest, that could mean a total dosage of 20 mg instead of 2 mg postcoitum. The aforementioned clinical studies, with only 2 mg dienogest once, the aim of which was to establish optimal doses or regimens (e.g., two higher dosages 12 h apart) were not continued. From our personal point of view, it is rather unlikely to realize an acceptable emergency contraception with dienogest.

IV. MALE FERTILITY INHIBITION

As early as 1980 in male hybrid mouse-mating tests over 35 days (38), dienogest showed potent antifertility activities in comparison with levonorgestrel and chlormadinone acetate. A certain dissociation was seen between fertility inhibition and mating rate. All changes were reversible. In male rats, the reduction of testicular growth, testicular DNA content, and plane of tubular cross-section, after dienogest treatment over 14 days was less than that for levonorgestrel. The LH-suppressive effect of dienogest was about 15 times lower than that of levonorgestrel. These male rat findings were confirmed by Warikoo and Das (39). Treatment of the male rats with 1.0 mg dienogest subcutaneously for 3 weeks reduced the fertilizing ability and motility of spermatozoa. The initial motility of spermatozoa, recovered from the terminal segment of epididymis, and the glycerylphosphorylcholine content of the epididymis were also reduced, indi-

cating an impairment of the epididymal function. Twenty-eight percent of the seminiferous tubules in testis were partly or completely devoid of germinal cells. On the other hand and in contrast with the known progestin effects, the treatment had no effect on the weight of the accessory male genital organs. Acid phosphatase activity in the ventral prostate was significantly reduced. The oral treatment with dienogest 5 mg/rat over 60 days led to spermatogenic arrest and loss of libido (40). The subcutaneous dosage of dienogest 10 mg/kg for 4 weeks induced atrophy of the seminiferous tubules in adult male rats, with a reduction in tubule diameter and in the number of round spermatids at stage VII. Elongated spermatids were not detected. Leydig cells were atrophied from the second week of administration, with a concomitant decrease in blood levels of testosterone. The blood levels of FSH and LH were decreased from the third week of treatment. Dienogest induced sterility in 50% of rats after 2 weeks of treatment, but after 4 weeks, none of the treated males mated. Normal fertility and normal levels of testosterone and FSH were restored after 6 weeks, and of LH after 4 weeks of withdrawal of treatment (41).

To ascertain the effects on the reproductive and endocrine functions in male rabbits, dienogest was given orally daily over 8 weeks. In doses of 10 and 20 mg/animal, fertility inhibition was accompanied by a decrease of spermatogenesis and sexual activity. At the chosen dose of 5 mg/day, dienogest caused a decrease of sperm motility, semen fructose content, and sterility of the bucks. On the other hand, libido, semen volume, sperm number, sialic acid content in semen, serum LH, and testosterone levels remained unaffected. Concomitant injection of testosterone did not reverse the suppressive effect of dienogest on male fertility. The motility of human sperm was lowered markedly by dienogest in vitro. The human sperm penetration and pronuclear formation were significantly reduced in the in vitro fertilization assay with zona-free hamster eggs. The question arises from these findings whether there is a "dosage window" for dienogest, in which fertility is safely inhibited, but libido remains unaffected. The development of male contraceptives without concomitant androgen supplementation, therefore, might very well be worth investigating (42,43).

In the bonnet monkey (*Macaca radiata*), daily intramuscular treatment with 10 mg/animal for 12 weeks resulted in a significant decline in the count, motility, acrosin, and hyaluronidase activities, and the fertilizing ability (zona-free hamster egg penetration assay) of spermatozoa by the sixth week of initiation of the administration. The circulating levels of dienogest were low after 1 week and increased from the second week of treatment, when the serum level of testosterone was significantly reduced. Complete recovery was observed by the 11th week after withdrawal of treatment (44,45). Unfortunately, the studies on male bonnet monkeys provided no information on the aforementioned dosage window. Therefore, Sharma and Das (46), concomitantly with dienogest 12 mg/monkey daily for 15 weeks IM, applied the depot androgen, testosterone

bucyclate 40 mg IM as a single dose. The sperm count was reduced from the 10th week, with near azoospermia ensuing in the 13th week, which continued until the 12th posttreatment week. The motility was low from the 8th week of treatment to the 6th week after withdrawal of treatment. The fertilizing ability of spermatozoa was abolished from the 8th week. The serum level of testosterone was naturally maintained within the normal range during the treatment period. The authors conclude that dienogest in combination with testosterone bucyclate may have the potential to be used as a contraceptive for men (46,47). Currently, a clinical trial is under way with male volunteers to examine the nature and the site of testicular or posttesticular antifertility action of dienogest in men.

V. ANTIPROLIFERATIVE ACTION

What should be pointed out first is that dienogest acts favorably on endometriosis if used at a low dosage (48). This is in contrast with other progestins that are used in much higher dosages for endometriosis treatment (e.g., as the progestational component in oral contraceptives or for hormone replacement) (49). In rats and rabbits with surgical induction of endometriosis, we found a pronounced beneficial effect of dienogest. With various time intervals between surgical autotransplantation of the endometrial explants, ovariectomy, steroid treatment, and autopsy, dienogest was effective for the prevention and therapy of experimental endometriosis (50). In the model of renal subcapsular autotransplantation of endometrial tissue in rats, dienogest diminished the volume of endometriosis implants to the same extent as did danazol, which has also been a ''gold standard'' in clinical trials on endometriosis (51). Mifepristone (RU 486) reduced the suppressive action of dienogest on endometrial cells only somewhat, but not totally. On the basis of these findings, the inhibitory action of dienogest on cell proliferation seems to be dependent on its progestational effect (52).

The effects of dienogest on the intracellular-signaling systems, with and without RU 486, were also assessed by Katsuki et al. (51). The compound ameliorated the endometrial implant-induced alterations of the immune system (i.e., increased the natural killer activity of peritoneal fluid and splenic cells, decreased the number of peritoneal fluid cells, and decreased interleukin-1β production by peritoneal macrophages). As opposed to this, danazol had none of these specific effects. The inhibition of protein kinase A activity and the decreased cyclic-AMP content, which are also effects of dienogest and danazol, were due to the progestational activity. Unlike danazol, dienogest also inhibited the protein kinase C activity of rat endometrial cells in the presence of RU 486. This finding substantiates the view that dienogest also induces inhibitory actions by mechanisms other than those due to its progestational properties (52). Importantly, in contrast to gonadotropin-releasing hormone (GnRH) analogues,

dienogest shows bone protective effects (9,51). This is not normal for all progestins, because, for example, medroxyprogesterone acetate use is associated with a significant reduction in bone density (53). Also, dienogest does not affect lipid metabolism in rats (54) or in humans (55). Dienogest is now being tested in various clinical studies for its clinical effect on endometriosis (56,57).

Another aspect of the antiproliferative action of dienogest refers to specific antitumor activities of the progestin. An association between breast tumor development and reproductive hormones has been suspected since Beatson, in 1896, discovered the beneficial effect of ovariectomy in some patients with metastatic breast cancer. Nowadays, it is well known that estrogens promote the growth of breast tumor cells. On the other hand, the involvement of progestins, alone or in combination, is controversial (58).

At first, we were surprised to find that in T47D cells given dienogest—despite its very strong progestational activities—the expression of alkaline phosphatase was less than that of progesterone (14). More comprehensive studies were presented by Katsuki et al. (15). At oral doses of 0.01–1 mg/kg per day in ovariectomized mice, dienogest significantly suppressed the 17β-estradiol benzoate (E_2)-dependent tumor growth of transplanted HEC-88nu cells, which were unresponsive to known progestins, such as medroxyprogesterone acetate (MPA; 100 mg/kg per day, administered orally) and norethindrone (NET; 100 mg/kg per day administered orally). HEC-88nu cells expressed estrogen receptor (ERα), but not progesterone receptors (PR). The suppressive effect of dienogest on tumor growth was not diminished in the presence of excess MPA. Additionally, Ishikawa cells and MCF-7 cells expressing both ERα and PR were used. Dienogest also suppressed the E_2-dependent tumor growth of both Ishikawa and MCF-7 cells, both of which responded to MPA. However, the minimum effective dose of dienogest (0.01–1 mg/kg per day) was much lower than that of MPA (100 mg/kg per day). In contrast, dienogest did not suppress the E_2-induced increase in uterine weight, indicating no antiestrogenic activities, whereas MPA and NET suppressed it significantly. The question about the mode of action must remain open for the time being. The fact that dienogest suppresses E_2-dependent tumors without itself having antiestrogenic effects, may be due to hitherto unknown antiestrogenic metabolites or an independent mode of action still unexplained.

It remains for more extensive clinical studies to also demonstrate the antiproliferative effect of dienogest in humans.

The antiproliferative action of dienogest is also useful for HRT in postmenopausal women. A continuous combined regimen of 2 mg estradiol valerate plus different doses of dienogest (0.5, 1.0, 2.0, 3.0, or 4.0 mg) has been tested in 120 postmenopausal women over 6 months. Endometrial histology at the end of the treatment period and bleeding patterns were evaluated. The histomor-

phological pattern of the endometrium showed a clear dose dependence. The percentage of atrophic endometrium was directly correlated with the increase of the dienogest dose in the preparation used (e.g., between 55 and 60% atrophic specimens after 6 months treatment with 3 or 4 mg/day of dienogest), whereas proliferative specimens were more often seen in preparations with lower doses of dienogest (0.5 and 1.0 mg). No case of hyperplastic endometrium was found in this study (25).

VI. THE MICROBIOLOGY OF DIENOGEST

The broad variety of microbial reactions opens possibilities to investigate the potential metabolic pathways as well as the behavior of drugs in the environment in a relatively uncomplicated way. It can be concluded from microbial transformation experiments using *Rhodosporidium toruloides* and *Mycobacterium smegmatis* (59), *Rhodosporidium rubrum*, *Rhodotorula glutinis*, and *Clostridium paraputrificum* (20,60); that is aerobes, anaerobes, and fungi, that the chemical structure of dienogest makes both biotransformation and degradation in the environment much easier. None of the microbial metabolites of dienogest show a higher endocrinological activity than the mother substance (61).

An unusual result was published by Klinger et al. (62). Using two different oral contraceptives (dienogest plus ethinyl estradiol [EE], or desogestrel plus EE), they studied the influence of hormonal contraceptives on microbial flora of gingival sulcus in cyclic women. The occurrence of the periodontopathogenic microorganism *Prevotella intermedia* was significantly higher in plaque samples in the desogestrel–EE group than in the dienogest–EE group.

VII. CONCLUSION

A new segment between pharmacodynamic profiles of modern 19-norprogestins and conventional derivatives of natural progesterone has been created by progestins without the 17α-ethinyl group, pioneered by dienogest.

The following properties of dienogest derive from the norprogestins:

Strong progestational activity on the endometrium
Short half-life
High oral bioavailability
Perfect cycle control if combined with estrogens (e.g., for oral contraception)
Low liver impact
Normal toxicological or genotoxicological patterns

Properties that derive from the progesterone derivatives include

Excellent tolerability
Antiandrogenic action
Relatively low antigonadotropic activity
Mainly peripheric mode of action
Antiproliferative activities
Low penetration through the skin
Dosage in the milligram range

Original characteristics of dienogest include the following:

Receptor selectivity
No binding to serum transport proteins
Extremely high level of unbound, free drug in the blood serum
No accumulation after daily intake
Postcoital inhibitory effects with weak antigonadotropic effects
Low-dose antiendometriotic effects
In liver and vessel wall, no cancellation of estrogen effects
To a very large extent, neutrality for hepatic metabolism of xenobiotics
Very easy microbial transformation

Only a few, selected features have been discussed in the present chapter. However, also presented here are relatively unknown, more or less recent findings on the postcoital fertility inhibition in female animals and in women, on fertility inhibition in male animals, and the in vitro as well as in vivo antiproliferative action. The interesting special investigations into the microbial transformation are pilot studies for similar investigations using other steroids.

Such hybrid progestins (promegestone, trimegestone, nomegestrol, drospirenone) may be increasingly used clinically in the future. Dienogest paves the way for this new and very interesting group.

REFERENCES

1. FS Wu, HM Yu, JJ Tsai. Mechanism underlying potentiation by progesterone of the kainate-induced current in cultured neurons. Brain Res 779:354–358, 1998.
2. R Maggi, A Poletti, LA Casulari, F Pimpinelli, F Piva, MR Zanisi, L Martini. Effects and metabolism of steroid hormones in human neuroblastoma cells. Steroids 63:257–262, 1998.
3. U Fuhrmann, R Krattenmacher, EP Slater, KH Fritzemeier. The novel progestin drospirorenone and its natural counterpart progesterone: biochemical profile and antiandrogenic potential. Contraception 54:243–251, 1996.
4. FP Guengerich. Mechanism based inactivation of human liver microsomal cytochrome P450IIIA4 by gestodene. Chem Res Toxicol 3:363–365, 1990.

5. R Boecker, B Kleingeist. Der Einfluβ von Dienogest auf das humane Cytochrom P-450 Enzymsystem in vitro. In: AT Teichmann, ed. Dienogest-Präklinik und Klinik eines neuen Gestagens. Berlin: Walter de Gruyter, 1995, pp 141–147.
6. M Huebner, K Ponsold, M Oettel, R Freund. Eine neue Klasse hochwirksamer Gestagene: 17$\alpha$$CH_2$X-substituierte Gona-4,9(10)-diene. Arzneimittelforschung Drug Res 20:401–404, 1980.
7. M Oettel, S Bervoas-Martin, W Elger, S Golbs, G Hobe, G Kaufmann, M Mathieu, C Moore, C Puri, P Ritter, G Reddersen, R Schön, G Strauch, H Zimmermann. A 19-norprogestin without 17α-ethinyl group I: dienogest from a pharmacokinetic point of view. Drugs Today 31:499–516, 1995.
8. M Oettel, W Carol, W Elger, G Kaufmann, C Moore, W Römer, G Klinger, B Schneider, J Schröder, L Sobek, F Walter, H Zimmermann. A 19-norprogestin without 17α-ethinyl group II: dienogest from a pharmacodynamic point of view. Drugs Today 31:517–536, 1995.
9. M Oettel, W Elger, M Ernst, R Freund, A Kurischko, B Schneider, W Stölzner. Experimentelle Endokrinpharmakologie von Dienogest. In: AT Teichmann, ed. Dienogest—Präklinik und Klinik eines neuen Gestagens. 2nd ed. Berlin: Walter de Gruyter. 1995, pp 11-21.
10. M Oettel, H Breitbarth, W Elger, T Gräser, D Hübler, G Kaufmann, C Moore, V Patchev, W Römer, J Schröder, L Sobek, H Zimmermann. The pharmacological profile of dienogest. Eur J Contracept Reprod Health Care 3:1–12, 1998.
11. RH Foster, MI Wilde. Dienogest. Drugs 56:825–833, 1998.
12. U Verma, KR Laumas. Screening of anti-progestins using in vitro human uterine progesterone receptor assay system. J Steroid Biochem 14:733–740, 1981.
13. M Juchem, M Schaffrath, K Pollow, G Hoffmann, G Kaufmann. Dienogest: Bindungsstudien an verschiedenen Rezeptor-und Serumproteinen. In: AT Teichmann, ed. Dienogest—Präklinik und Klinik eines neuen Gestagens. 2nd ed. Berlin: Walter de Gruyter, 1995, pp 141–147.
14. L Sobek, D Di Lorenzo, M Oettel, G Kaufmann. Normal and stable transfected cancer cell lines: tools for a screening of progestogenic, antiprogestogenic and antiglucocorticoid substances. Methods Findings Exp Clin Pharmacol 16:545–551, 1994.
15. Y Katsuki, S Sasagawa, Y Takano, Y Shibutani, D Aoki, Y Udagawa, S Nozawa. Animal studies on the endocrinological profile of dienogest, a novel synthetic steroid. Drugs Expt Clin Res 23:45–62, 1997.
16. U Vij, K Murugesan, JC Kalita, A Farooq. Interaction of antiprogestins with progesterone receptors in rat uterus. J Steroid Biochem 32:279–282, 1989.
17. S Kitchlu, PK Mehrota. Relative potentiality of some contragestational agents on trauma induced deciduoma formation in immature rats. Indian J Exp Biol 35:589–593, 1997.
18. D Philibert, T Ojasoo, J-P Ranaud. Properties of the cytoplasmic progestin-binding protein in the rabbit uterus. Endocrinology 101:1850–1861, 1977.
19. G Hobe, G Klinger. Determination of free nonprotein-bound dienogest in plasma and of salivary dienogest in female volunteers taking Certostat for oral contraception. 1998, (submitted).

20. K Schubert, G Schumann, G Kaufmann. Influence of a 9-double bond on stereospecific microbial 4,5-reductions. J Steroid Biochem 18:75–80, 1983.
21. RA Edgren. Issues in animal pharmacology. In: JW Goldzieher, ed. Pharmacology of the Contraceptive Steroids. New York: Raven Press, 1994.
22. H-D Taubert, H Kuhl. Kontrazeption mit Hormonen. Ein Leitfaden für die Praxis. 2nd ed. Stuttgart: Georg Thieme Verlag, 1995, p 76.
23. M Oettel, W Carol, T Gräser, G Klinger, U Mellinger, C Moore, AE Schindler, UH Winkler. Der Einfluß einer Ethinylestradiol–Dienogest-Kombination auf die Serum-Androgen-Konzentrationen. Zentralbl Gynäkol 119:597–606, 1997.
24. W Böhm, E Hempel, G Carlsohn. Zur Problematik der Transformationsdosenbestimmung von Gestagenen. Vortrag; III. Jenaer Symposium zur hormonalen Kontrazeption. Jena, Germany. April 10–11, 1984.
25. T Gräser, M Oettel. Organ targeting with the oral progestin dienogest. Drugs Today 32(suppl H):43–55, 1996.
26. H Hoffmann, C Moore, H Zimmermann, W Elger, S Schwarz, T Gräser, M Oettel. Approaches to the replacement of ethinylestradiol by natural 17β-estradiol in combined oral contraceptives. Exp Toxicol Pathol 50:458–464, 1998.
27. P Schürenkämper, K Lisse. Effects of the new progestagen STS 557 on the biosynthesis of steroids in tissue slices of the human ovary. Exp Clin Endocrinol 86:185–189, 1985.
28. HU Schweikert, D Schnabel. Trial to investigate the antiandrogenic efficacy of Valette in comparison to placebo by evaluation of the number of androgen receptors in the interscapular region of female acne patients before and after treatment. Interim Report; 1998, (submitted).
29. L Sobek. Antiandrogene Effekte von Dienogest und Cyproteronacetat in LNCaP–Zellkulturen. Mitteilung an die Jenapharm GmbH, May 1995.
30. R Dettmann, B Kramp. Chronische Toxizitätsprüfung der Substanz STS 557 am Beagle-Hund—Supplementary Report. University Rostock.
31. M Oettel, A Kurischko, J Strecke, M Koch. Embryotoxic effects of post-coital fertility inhibiting steroids. Arch Toxicol Suppl 4:248–251, 1980.
32. J Strecke, W Stölzner, H Freund, A Kurischko, M Koch, W Römer, R Stracke, M Oettel. Interceptive activities of STS 557 in rabbits, mice and rats. Exp Clin Endocrinol 81:122–136, 1983.
33. A Komor, NP Goncharov, K Schubert, M Oettel. STS 557 as an interceptive in baboons. Exp Clin Endocrinol 81:217–221, 1983.
34. VD Castracane, JW Goldzieher. Evaluation of STS 557 as a postcoital contraceptive in the baboon. Contraception 23:335–339, 1981.
35. G Köhler, G Göretzlehner, K Rudolf, M Rüting, J Meissner, S Kunkel, K Schollberg. The effect of a single midcycle administration of 0.5 or 2.0 mg dienogest (17α-cyanomethyl-17β-hydroxyestra-4,9-dien-3-one) on pituitary and ovarian function—investigation for the use as a postcoital contraceptive. Exp Clin Endocrinol 81:299–301, 1984.
36. G Köhler, E Canzler, S Lembke, I Amon, HJ Ahrendt. Postkoitale Kontrazeption mit Dienogest. Zentbl Gynäkol 109:1296–1302, 1987.
37. SL Nightingale. From the Food and Drug Administration. JAMA 280:1472, 1998.

38. M Oettel, H Tkocz, O Löw, G Hesse, A Kurischko. Effects of levonorgestrel and STS 557 on testis in rodents. Endokrinologie 76:13–22, 1980.
39. PK Warikoo, RP Das. Fertilizing ability of spermatozoa of rats treated with STS 557. Arch Androl 11:157–159, 1983.
40. A Srivastava, JP Maikhuri, BS Satty. Evaluation of STS-557 as an oral contraceptive in male rat. Contraception 36:253–272, 1987.
41. J Chaudhary, RK Sharma, SS Majumdar, DPS Bhatte, MM Misro, RP Das. Effect of STS-557 (17α-cyanomethyl 17β-hydroxy-estra-4,9(10)-dien-3-one) on blood hormone levels, the testis, accessory sex organs and fertility of rats. Int J Androl 13:398–407, 1990.
42. H Freund, G Hesse, M Oettel. Effect of STS 557 on semen composition, fertility and sexual behaviour of male rabbits. Contraception 21:641–650, 1980.
43. M Oettel, H Freund, G Hesse, MH Raj, K Dietz, KH Chemnitius, W Stölzner. Inhibition of male fertility by STS 557. Exp Clin Endocrinol 81:137–145, 1983.
44. SS Majumdar, RK Sharma, RP Das. Effects of STS 557 on semen and semen functions in bonnet monkey (*Macaca radiata*)—a preliminary report. Contraception 37:631–641, 1988.
45. SS Majumdar, J Chaudhary, RK Sharma, RP Das. Contraceptive potentiality of STS-557—a feasibility study in male bonnet monkey (*Macaca radiata*). Contraception 41:641–653, 1990.
46. RK Sharma, RP Das. Effects of STS-557 and 20 AET-1 on sperm functions and serum level of testosterone in bonnet monkey (*Macaca radiata*). Contraception 45:483–491, 1992.
47. RK Sharma, RP Das. Effect of fertility regulating agents on motility and zona-free hamster egg penetration by spermatozoa of bonnet monkey. Ind J Expt Biol 30:977–981, 1992.
48. G Köhler. Die Therapie der Endometriose mit Dienogest: eine Renaissance der Gestagene. In: AT Teichmann, ed. Dienogest—Präklinik und Klinik eines neuen Gestagens. 2nd ed. Berlin: Walter de Gruyter, 1995, pp 243–251.
49. G Köhler, G Göretzlehner, I Amon. Endometriosetherapie mit Dienogest. Zentbl Gynäkol 109:795–801, 1987.
50. M Oettel, T Bocker, T Güttner, M Horn, W Stölzner. Studie zur Entwicklung eines geeigneten Modells der Endometriosis externa bei Kaninchen und Ratten und die Effekte von Dienogest und Danazol. Fertilität 8:131–135, 1992.
51. Y Katsuki, Y Takano, Y Futamura, Y Shibutani, D Aoki, Y Udagawa, S Nozawa. Effects of dienogest, a synthetic steroid, on experimental endometriosis in rats. Eur J Endocrinol 138:216–226, 1998.
52. A Kauppila. Reappraisal of progestins in endometriosis therapy. Eur Endocrinol 138:134–136, 1998.
53. T Cundy, J Cornish, H Roberts, H Elder, IR Reid. Spinal bone density in women using depot medroxyprogesterone contraception. Obstet Gynecol 92:569–573, 1998.
54. Y Katsuki, Y Shibutani, D Aoki, S Nozawa. Dienogest, a novel synthetic steroid, overcomes hormone-dependent cancer in a different manner than progestins. Cancer 79:169–176, 1997.

55. G Köhler, G Göretzlehner, K Brachmann. Lipid metabolism during treatment of endometriosis with the progestin dienogest. Acta Obst Gynecol Scand 68:633–635, 1989.
56. C Moore, M Oettel. The endocrine pharmacological profile of the progestin dienogest (DNG) for treatment of endometriosis. VI World Congress on Endometriosis. Québec City, Canada, June 30–July 4, 1998. Poster 340.
57. A Müller, B Christensen, A Henkel, C Moore, AE Schindeler. Treatment of endometriosis with the novel progestin dienogest. VI World Congress on Endometriosis. Québec City, Canada, June 30–July 4, 1998, Poster 341.
58. WGEJ Schoonen, JWH Joosten, HJ Klosterboer. Effects of two classes of progestagens, pregnane and 19-nortestosterone derivatives, on cell growth of human breast tumor cells: I. MCF-7 cell lines. J Steroid Biochem Mol Biol 55:423–437, 1995.
59. K Schubert, G Hobe, G Kaufmann, G Schumann, K Wehrberger, C Hörhold. Studies on biotransformation of STS 557. Exp Clin Endocrinol 81:168–174, 1983.
60. C Hörhold-Schubert, G Hobe, G Kaufmann, G Schumann. Zur Biotransformation von Dienogest durch Mikroorganismen. In: AT Teichmann, ed. Dienogest—Präklinik und Klinik eines neuen Gestagens. 2nd ed. Berlin: Walter de Gruyter, 1995, pp 51–58.
61. M Oettel, G Kaufmann, A Kurischko. Das endokrinologische Profil von Metaboliten des Gestagens Dienogest. Pharmazie 48:541–545, 1993.
62. G Klinger, S Eick, G Klinger, W Pfister, T Gräser, C Moore, M Oettel. Influence of hormonal contraceptives on microbial flora of gingival sulcus. Contraception 57:381–384, 1998.

9

Progestogens in Contraception: Third-Generation Pills

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I. INTRODUCTION

Since the beginning of the 1980s, three major new progestogens have been introduced in the field of oral contraception, essentially in combined oral contraceptives (COCs) that also contain ethinyl estradiol in a low daily dose ($\leq$ 35 μg). These progestogens are desogestrel (DSG), gestodene (GSD), and norgestimate (NGM). All three belong to the 13-ethyl gonane group of steroids and, as such, are derived from the parent ethyl gonane levonorgestrel (LNG). Chemically speaking, they differ from earlier progestogens, such as norethynodrel, lynestrenol, and norethindrone, that are 13-methyl gonanes (or estranes). However, all these compounds belong to the 19-nortestosterone group.

The recent classification of DSG, GSD, and NGM as "third-generation progestins" is a rather misleading, historical, classification because on the one side the more prominent so-called second-generation representative LNG is also a 13-ethyl gonane and on the other side NGM, which is a prodrug, is probably acting essentially through conversion to its main metabolite LNG (1) and could as well be classified as a second-generation progestogen. These third-generation progestins were designed and developed to obtain potent progestogens capable of exerting adequate contraceptive effects, such as those shown by LNG, but at the expense of much less clinical and metabolic androgenic-type effects that are clearly displayed by LNG and believed to be involved in an adverse effect on the cardiovascular system.

After a brief review of the more salient points resulting from the pharmacodynamic and metabolic comparison of third-generation progestogens with

Table 1 Composition of Currently Available COCs Containing Third-Generation Progestogens

Progestogen	Type of Combination	Composition
Desogestrel	Monophasic 20 μg	DSG + EE 21 × 150/20 μg/day
	Monophasic 30 μg	DSG + EE 21 × 150/30 μg/day
	Biphasic	DSG + EE 7 × 25/40 + 15 × 125/30 μg/day
	Sequential	EE 7 × 50 μg/day, + DSG + EE 15 × 125/50 μg/day
Gestodene	Monophasic 20 μg	GSD + EE 21 × 75/20 μg/day
	Monophasic 30 μg	GSD + EE 21 × 75/30 μg/day
	Triphasic	GSD + EE 6 × 50/30 + 5 × 70/40 + 10 × 100/30 μg/day
Norgestimate	Monophasic	NGM + EE 21 × 250/35 μg/day
	Triphasic	NGM + EE 7 × 180/35 + 7 × 215/35 + 7 × 250/35 μg/day

Abbreviations: DSG, desogestrel; GSD, gestodene; NGM, norgestimate; EE, ethinyl estradiol.

LNG, the major question as to which new COC containing one of these progestogens may be considered or not as an improvement over second-generation low-dose oral contraceptives (OCs) will be envisaged through a review of their comparative clinical and safety aspects.

II. CLASSIFICATION OF COCS CONTAINING THIRD-GENERATION PROGESTOGENS

A range of preparations containing low doses of both ethinyl estradiol (EE) and new generation progestogens are available either in monophasic or multiphasic combinations to meet potentially optimal acceptability and safety, with preserved effectiveness, in women presenting with somewhat different clinical features (Table 1).

III. MAIN PROPERTIES OF THIRD-GENERATION PROGESTOGENS VERSUS LEVONORGESTREL

A. Desogestrel

Desogestrel's structural formula is depicted in Fig. 1. It is a prodrug nearly quantitatively and rapidly converted to 3-keto-desogestrel when taken orally. 3-keto-DSG, also named etonogestrel, is a potent progestogen, with only mild androgenic properties and with an ensuing high selectivity index (progestogen/androgen potency quotient) (2). This index, however, is of limited clinical significance, as it has been developed from in vitro receptor binding affinities and, therefore, is difficult to extrapolate as a marker of biological activity (1). Its progestagenic activity is similar to that of LNG, and results in powerful inhibition of gonadotropin levels, ovulation, and cervical mucus, with a low endometrial secretory transformation dose, whether given alone or combined with small concentrations of EE (20 or 30 μg/day) (Tables 2 and 3). The substantial increase in serum concentrations of sex hormone-binding globulin (SHBG) during use of low-dose COCs containing DSG (3.5 times basal pretreatment levels; see Table 3) is another interesting marker of the low androgenic–antiestrogenic properties of these OCs.

B. Gestodene

Gestodene is the most potent, directly active progestogen currently available (see Fig. 1), twice as potent as LNG, which means that its antigonadotropic, antiovulatory, and other contraceptive effects, including the endometrium transformation

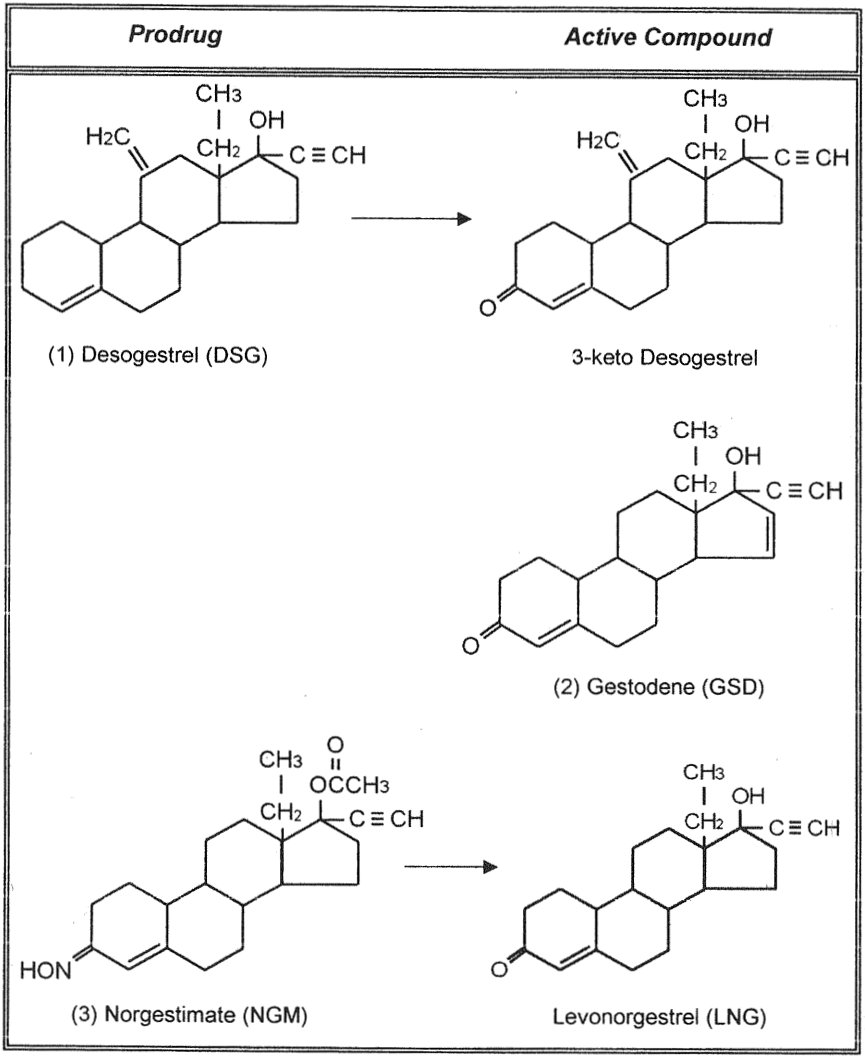

Figure 1 Third-generation progestogens.

Table 2 Relative Binding Affinity of Various Progestogens to Cytosolic Steroid Receptors and Serum Binding Proteins

Progestogen	PR	AR	ER	GR	MR	SHBG	CBG
Progesterone	50	0	0	10	100	0	36
Desogestrel	1	0	0	0	0	0	0
3-Keto-desogestrel	150 (260)[a]	20	0	14	0	15	0
Gestodene	90 (350)[a]	100	0	27	350	40	0
Norgestimate	5	0	0	1	0	0	0
Levonorgestrel	150 (135)[a]	45	0	2	70	50	0

Abbreviations: PR, (rabbit uterus) progesterone receptor (promegestone = 100 and [a]Org 2058 = 100); AR, (rat prostate) androgen receptor (metribolone = 100); ER, (mouse uterus) estrogen receptor (estradiol = 100); GR, (rat thymus) glucocorticoid receptor (dexamethasone = 100); MR, (rat kidney) mineralocorticoid receptor (aldosterone = 100); SHBG, sex hormone-binding globulin (dihydrotestosterone = 100); CBG, corticosteroid-binding globulin (cortisol = 100).
Source: Ref. 1.

dose, are obtained with half the concentrations necessary for LNG (see Tables 2 and 3). Moreover, the ratio between the progestogenic and androgenic effects of GSD is distinctly higher than that of LNG, and the increase in SHBG induced by COCs containing GSD is only slightly less marked (three times over basal levels) than with preparations containing DSG. These data collectively indicate a low androgenic potential of these OCs.

C. Norgestimate

Norgestimate is a prodrug (see Fig. 1) with extensive and rapid metabolism after oral intake. Its binding affinity to the progesterone receptor is low, and very low to the androgen, estrogen, glucocorticoid, and mineralocorticoid receptors. LNG itself is a major metabolite of NGM, accounting for 20–25% of the ingested dose. When 250 μg of NGM are taken orally, an ensuing average serum LNG level of 0.5 ng/mL is obtained, which may explain (although it is still somewhat controversial), the antiovulatory and antigonadotropic properties, as well as the inhibitory effect on cervical mucus, and the endometrium transformation obtained with combined preparations containing 250 μg of NGM. This corresponds roughly to the effect of a daily oral dose of 50–60 μg of LNG (1,4). Accordingly, the androgenic activity of OCs containing NGM is low, and the SHBG increase is similar to that observed during use of COCs containing DSG or GSD (see Tables 2 and 3).

Table 3 Selected Pharmacokinetic and Pharmacodynamic Properties of Second- and Third-Generation Progestogens

	Bioavailability (oral route)	SHBG binding of progestin + EE[a]	Selectivity index (progest:androg potency)[c]	Increase in SHBG from baseline using corresponding COCs (Prog + EE)	Minimum oral daily dose inhibiting ovulation in women	Endometrial secretory transformation dose
3-Keto-desogestrel	75%	69%	40	×3.5	60 μg/day	2–3 mg/cycle
Gestodene	100%	76%	26	×3.0	40 μg	2 mg
Norgestimate	NA[b]	NA	NA	×3.3	200–250 μg	5–10 mg
Levonorgestrel	95%	50%	8.8	×1.75	60 μg	4–6 mg

[a]EE, Ethinyl estradiol.
[b]NA, not available; norgestimate thought to be active essentially through its major metabolite levonorgestrel.
[c]Ratio of relative binding affinity (RBA) of progestogens to progesterone receptors in rabbit uterus: RBA to androgen receptors in rat prostate.
Source: Refs. 1–3.

IV. CONTRACEPTIVE EFFICACY

As well delineated by Trussell et al. (5), current studies of contraceptive effectiveness are still hampered by methodological flaws that reduce reliability and comparability of studies related to OC efficacy. More specifically, the life-table analysis (actuarial method for calculating the failure rate as a function of time) should be preferred to the Pearl Index (PI) method, and large, comparable study populations should be randomly assigned to the new OC product under study and older agents, allowing differential failure rates for subgroups to be calculated. Moreover, strict criteria for observation of subjects under study, using the ''intention-to-treat'' analysis, as recommended by Good Clinical Practice (GCP) guidelines should be carefully followed (6).

More than 40 studies have been devoted to the evaluation of contraceptive efficacy of low-dose OCs containing third-generation progestogens versus low-dose and standard-dose OCs containing second-generation progestogens, such as LNG or norethindrone (NET). Reviews of these studies (6,7) report a uniformly low pregnancy rate for the new formulations (zero to 1.1 pregnancy per 100 woman-years [WY] of exposure) and an efficacy similar to that of older products. By recalculating with the 13-cycle PI formula, the contraceptive efficacy of new formulation low-dose OCs, Newton interestingly shows that with a more accurate methodology, taking GCP guidelines into account, the overall PI value is close to 1:100 WY, whereas studies performed before implementation of these guidelines reported PI values from 0.05 to 0.27 (6), with both lower method and user failure rates. In some studies, the triphasic preparations, hampered either by a lower dosage of steroids during the first OC phase or by the increased complexity of multiphasic drug intake, display—although not convincingly—a slightly higher failure rate (8,9). However, a mean overall PI of low-dose new COCs close to 1 is considerably better than the failure rate of 3:100 WY reported for the general population of OC users in the United States (5).

Moreover, when the extent of ovarian activity during the use of COCs is appreciated by sequential ultrasound measurement of follicular activity and assessment of serum concentrations of ovarian hormones and gonadotropins, it appears that low-dose preparations containing new-generation progestogens induce an adequate suppression of ovulation if not always of follicular or ovarian secretory activity (10,11). Even with incorrect use of the new COCs (missed tablets, extension of pill-free interval, vomiting), although less inhibition of ovarian activity or even escape ovulation are recorded (12,13), occurrence of pregnancy is usually prevented, implying that other contraceptive mechanisms such as cervical mucus hostility and endometrial alteration are effective (14). Comparison of low-dose OCs containing progestogens of newer versus older generation products yield either similar results in terms of suppression of ovarian activity (15) or a better inhibition with new OCs, and this also holds true for new-generation triphasics (16).

Even new preparations containing only 20 μg of ethinyl estradiol with DSG or GSD induce an equally good suppression of ovarian function (17), although this does not seem to be true with the older preparation containing 20 μg EE + 1 mg norethindrone. It must be remembered that in the new preparations containing DSG and GSD, the progestin concentration used is approximately twice the dose necessary for ovulation inhibition. By contrast the dose of NGM contained in OCs (250 μg) is only slightly higher than the ovulation-inhibiting dose (200 μg) and, accordingly, for preserving a high contraceptive effectiveness, the 35 μg dose of EE contained in these OCs should probably not be reduced (1).

V. CYCLE CONTROL

Ideal cycle control during COC use could be defined by the occurrence of a regular withdrawal bleeding in addition to no unscheduled bleeding (i.e., breakthrough bleeding plus spotting) during tablet taking (18). The best possible cycle control is important to obtain, as unscheduled vaginal bleeding and amenorrhea are a leading cause of low acceptability and compliance and, hence, a high discontinuation rate in OC users (7,19,20). More than 40 extensive cycle control studies have now been published concerning new low-dose OCs—either comparative or noncomparative versus older-generation OCs. Most of these studies, however, are hampered by the use of nonstandardized protocol design, and varying definitions for bleeding characteristics, leading to a wide variation of results that render evaluation and comparison more difficult. Therefore, it has been proposed (6) that, in addition to a "classic cycle control analysis," which is usually performed, an analysis of total bleeding experiences of women during a reference period (of 90 days) should be performed according to a protocol proposed by the WHO (21).

Notably, women using COCs usually have a better cycle control than women not using OCs, making it understandable that spontaneous irregular cycles and unscheduled bleeding are a noncontraceptive indication for OC use (18,21).

There are numerous, often very large studies of cycle-control data in women using OC preparations containing the new progestogens DSG, GSD, and NGM combined with low doses of EE (35, 30, or 20 μg). However, another weakness of many of these studies is that they are noncomparative or not performed against a sufficiently near comparator.

A large, exemplative, open trial of the monophasic preparation containing DSG 150 μg + EE 30 μg was reported in 11,605 women over 69,630 cycles, with a rate of unscheduled bleeding (breakthrough plus spotting) of about 7% at cycle 3, decreasing to 3% at cycle 6. A low rate of amenorrhea (1–2%), and a low dropout rate of 13%, with only 2% of women discontinuing treatment

because of irregular bleeding, were also reported (22). Two studies of the low-dose formulation DSG 150 μg + EE 20 μg, conducted in a total of 13,015 cycles in 530 women, yielded similar rates of irregular bleedings: 11% at 3 months and 9% at 6 months (23,24). This rate of unscheduled bleeding seems somewhat higher than with the preparation containing 30 μg of EE instead of 20 μg, and this difference was significant in one comparative study (25). However another trial comparing these particular combinations showed that cycle control and tolerance were similar with both formulations tested (26).

A large trial of the monophasic preparation containing GSD 75 μg + EE 30 μg was reported in 1095 women over 14,281 cycles, with cycle-control data very near those reported earlier for the combination containing DSG 150 μg + EE 30 μg. A low incidence of amenorrhea (0.5% of all cycles) was observed in this study. Similar results were observed with the corresponding triphasic preparation containing GSD + EE (i.e., irregular bleeding rates of 9–16% at cycle 1 and 6–9% at cycle 6) (27). The monophasic preparation containing 30 μg of EE was comparatively tested more recently against the combination containing GSD 75 μg + EE 20 μg, showing low and comparable levels of unscheduled bleeding with both preparations in relation to all cycles, with a rate of 14% for the preparation containing 20 μg of EE and 8.8% for the 30 μg preparation; in both cases, low discontinuation rates of 9.8 and 7.2% were recorded, respectively (28).

More limited but corresponding data were reported with the use of the preparation containing NGM 250 μg + EE 35 μg, or with its triphasic counterpart. Data from a large controlled comparison of the monophasic preparation with another combination containing a second-generation progestogen (norgestrel; NG) showed that in women who took the NGM monophasic preparation over 9000 cycles (29), an unscheduled bleeding rate of 12% at cycle 1 rapidly declined to less than 6% after cycle 4. Failure to experience withdrawal bleeding was a very rare event, about 1% during cycles 1–6 and 0.4% during cycles 13–24. Very similar data were reported with the triphasic NGM formulation (30).

Some comparative studies with new-generation OCs reported somewhat contradictory results: comparison of monophasic preparations containing DSG, GSD, and NGM showed that the overall incidence of irregular bleeding was slightly, but statistically significantly lower, with the GSD preparation versus the other new OCs (31,32), whereas other studies could not show any statistically significant difference between preparations when using the classic cycle analysis (33–35). Overall, comparative studies of new-generation preparations indicate a similar favorable cycle control for all these products.

When reviewing cycle control data from studies comparing preparations containing new-generation progestogens with older products (7), such as those containing levonorgestrel or norethindrone, similar results are obtained in terms of low rate of irregular bleedings. New preparations achieve this excellent result

usually with dosages, particularly of EE, that are often much lower than in older products. Moreover, decreasing rates of bleeding over time, and particularly low rates of amenorrhea with new OCs result in higher continuation rates compared with older preparations, but differences are usually not significant (7).

VI. OVERALL ACCEPTABILITY OF NEW-GENERATION OCS

Overall acceptability of an OC is related to parameters of cycle control and also minor side effects, such as weight gain, mastalgia, acne, and other signs of androgenization, and is best reflected by long-time continuation rates, a measure of compliance to any OC use. COCs containing new progestogens have been repeatedly reported (6,7) to exert a good cycle control and are expected to generate fewer side effects owing to their higher progestogenic to androgenic activity ratio (i.e., ''selectivity'') when compared with older COCs. Indeed, most of the studies reporting data on cycle control, mentioned in the foregoing, also showed excellent overall acceptability and particularly low annual discontinuation rates (usually less than 15%) when new OCs are used. When compared with each other, OCs containing third-generation progestogens showed similar results in terms of overall acceptability. When comparing them with low-dose monophasic or triphasic preparations containing the older progestogen LNG, significant differences in acceptability could not be demonstrated clearly between these preparations (36,37).

A. Effects on Body Weight

Although there have been reports concerning weight increases in women treated by high-dose progestogens for endometriosis, and in tall girls prepubertally treated by high-dose estrogens, and in spite of frequent statements that the use of previous generations high-dose COCs may be associated with weight gain (38), well-controlled studies of early high-dose formulations, such as the study of Goldzieher et al. (39) could not substantiate this assumption. No evidence of any weight-increasing effect of standard and low-dose older preparations could be firmly demonstrated (40), in spite of the well-known, although frequently restricted, effect of contraceptive steroids used in COCs on water and electrolyte, lipid, carbohydrate, and nitrogen metabolism. When reviewing data from either carefully controlled studies of body weight changes or body fat distribution (41), it essentially appears that over periods of OC use varying from 6 months to 3 years, most women (70–80%) show only slight weight fluctuations (less than 2 kg), whereas a few experience a more sustained increase or decrease in body weight, maybe in predisposed individuals, with

an unclear causal relation to OC use. Moreover, body fat distribution remains unchanged.

Large-scale efficacy and safety trials with new low-dose OCs containing DSG, GSD, and NGM in monophasic or multiphasic regimens, combined with small doses of EE (20–35 μg) show the same pattern of only small variations of body weight and an attending low discontinuation rate for that reason (6). One of these typical large studies reports tolerability of the low-dose monophasic EE 20 μg + GSD 75 μg during 3 years of use in 670 young women for 19,095 evaluable cycles: 55% of women had maintained a body weight near baseline after 36 cycles of OC use, whereas a body weight increase of 2 kg was reported for 29%, and a loss of 2 kg occurred in 17% of these women; only 6 women discontinued OC use for alleged weight increase (42). In a particularly well-controlled study, Reubinoff et al. (43) observed after 6 months of use of monophasic EE 30 μg + GSD 75 μg a marginal weight gain of more than 0.5 kg in 30% of OC users as well as in 30% of the nonusers in the control group; simultaneously, 20% of the users and of the nonusers had lost more than 0.5 kg body weight. The users who gained weight had a statistically significantly higher weight (+ 1.13 kg) and body fat (+ 1.8%) than the control nonusers. This effect of OCs on body weight was judged as very discrete, and no abdominal fat accumulation—a marker of increased risk of cardiovascular disease—could be demonstrated.

Overall, no significant differences in body weight changes could be recorded in different comparative studies bearing on new-generation low-dose OCs (6).

B. Androgenicity of New OCs: Effect on Acne and Hirsutism

Appreciation of the androgenic potential of progestogens in clinical studies is difficult, and the so-called selectivity index (see Table 3)—a theoretical approach to this potential—is not helpful. Clinical assessment of acne and hirsutism (either improvement or worsening) in users of COCs or their progestogen component may be useful, but this assessment is usually far from quantitative. Serum levels of sex hormone-binding globulin (SHBG) could be considered as an interesting, although imperfect, marker of "estrogenicity" or "androgenicity" of OCs, as EE stimulates liver synthesis of this transport protein, and androgens and 19-nortestosterone-derived progestogens have an opposite effect. The ensuing SHBG level is, accordingly, the result of both opposing steroid actions when COC use is analyzed (see Table 3). Moreover, as SHBG is avidly binding testosterone, any increase in this binding protein is attended by a decrease in free, biologically active blood testosterone, with an ensuing decrease in androgenization potential, although this mechanism is certainly not the only one related to COC effect on acne and hirsutism (44–47).

When older OC preparations were used, it was clearly demonstrated that estrogen-dominant OCs may improve preexisting acne, whereas progestogen-dominant OCs—and more specifically those containing NG or LNG—may exacerbate or even trigger acne or seborrhea and hirsutism (48,49). Additionally, they exert a potentially deleterious effect on lipid and carbohydrate metabolism, in plausible association with the high androgenicity of NG.

If new-generation progestogens, such as DSG (50), are, when used alone, able to moderately decrease SHBG concentrations in relation to their mild androgenic action, this effect is completely blunted in low-dose COCs owing to the presence of EE (see Table 3). In several open efficacy and acceptability studies bearing on the use of almost all low-dose COCs containing third-generation progestogens (review in Refs. 6,7), a subjective improvement of preexisting acne was noted in as frequently as 25–95% of the women treated, although these studies were not specifically designed to demonstrate efficacy on acne. By contrast, it is common clinical experience to observe triggering or worsening of acne in women using COCs containing first- or second-generation progestogens, even in low-dose monophasic preparations. However, when these progestogen concentrations are further decreased and associated with adequate levels of EE, such as in the triphasic combination of EE + LNG, the androgenic effect on the skin is much less obvious. Comparative studies of GSD and LNG triphasic formulations yielded similar results in terms of clinical assessment of acne, increase in SHBG concentrations, and decrease in circulating androgens (51). Similarly, DSG-containing OCs improve preexisting acne significantly more than do LNG-containing OCs (52). Even when compared with OC preparations containing the antiandrogen cyproterone acetate and EE, DSG-containing OCs improve acne similarly or nearly as well in the treated subjects (53–55). We recently assessed, as a primary outcome measure, the effect of a triphasic COC that contained GSD on preexisting mild to severe facial acne in a group of 33 young women. Acne was carefully monitored during 13 cycles of treatment by strict clinical evaluation, sebum production, and superficial follicular biopsy. Global evaluation of the patients' acne status revealed a statistically significant improvement in 95% of the women (56). OC preparations containing NGM seem to yield favorable results, similar to those obtained with DSG and GSD when effects on acne and related endocrine parameters are considered (57). The overall beneficial effect on the severity of acne of different COCs that contain new-generation progestogens is probably in relation not only to a favorable effect on SHBG, androgen, and free testosterone levels, but also to a very low intrinsic androgenic activity.

Provided prior adequate investigation and etiological assessment are performed in *hirsute women*, COCs have been proposed for therapy, and used extensively (44,46,47,49). As far as OCs containing new progestogens are concerned, Bilotta and Farilli (22), in a large open efficacy and tolerability trial, used DSG + EE for more that six treatment cycles in 11,605 women. Acne disappeared or improved in over 80% of 1021 women with this preexisting lesion.

Nearly 500 women in the same study experienced an improvement or disappearance of hirsutism in almost 50% of cases, whereas it newly occurred in only 2% of these women. In more specifically controlled studies, COCs containing low doses of DSG displayed a similar effectiveness when hirsutism was evaluated by different methods (58,59) in different populations of OC users, including women presenting with polycystic ovarian disease. Interestingly, a study comparing the use of EE + DSG and EE + cyproterone acetate for 2 years in hirsute women showed a significant improvement that was similar for both preparations (60). A comparative study bearing on two low-dose OCs containing DSG or GSD also showed a similar and significant decrease of hirsutism in both treatment groups (61).

VII. EFFECTS ON LIPID AND CARBOHYDRATE METABOLISM AND HEMOSTASIS

The metabolic effects of OCs have been described in detail in other chapters of this book. Accordingly, only the main features of the metabolic action of OCs are summarized in this section.

A. Lipoprotein Metabolism

As far as lipoprotein metabolism is concerned, there is a general agreement that changes in plasma lipid profile clearly reflect the balance of estrogen and progestogen dose and type in OCs. Older-generation OCs were able to decrease circulating levels of HDL cholesterol (particularly, HDL_2-chol) and to increase LDL cholesterol and specifically its small dense fractions, while only modestly increasing triglyceride (TG) serum levels (62). By contrast, newer low-dose OCs containing less androgenic progestogens (DSG, GSD, NGM), which are slightly more estrogen-dominant preparations than older-generation OCs, do increase large VLDL and triglyceride circulating levels in the users, as well as hepatic uptake of LDL and intermediate-density lipoprotein (IDL). However, they do not change—or eventually slightly increase—HDL cholesterol or the cholesterol and apolipoprotein content of its subfractions, HDL_2 and HDL_3, potentially indicating a preserved reverse cholesterol transport (63,64). The particularly small changes in lipid and lipoprotein levels observed during use of third-generation low-dose OCs do not seem biochemically or clinically related to an increased risk of atherogenic cardiovascular disease (64–68).

B. Carbohydrate Metabolism

Relative to carbohydrate metabolism, use of all generation OCs has been associated with reduced glucose tolerance and increased resistance to insulin. These

alterations were obvious in users of first- and second-generation high-dose OCs, particularly those containing LNG (69). High-dose OC users with predisposing risk factors developed impaired glucose tolerance more frequently than nonusers (69,70). Considerably fewer changes in glucose tolerance and insulin sensitivity have been observed with low-dose older- and newer-generation OCs, and a recent large prospective study was unable to find any appreciable increase in the risk of non–insulin-dependent diabetes mellitus either among current users of OCs or among past users (71). However, when studying a large population of young healthy white women, it was demonstrated that women's use of OCs was one of the four most important determinants of insulin sensitivity (72). This is in good agreement with the large study of Godsland and colleagues showing a significantly decreased insulin sensitivity index in users of low-dose OCs of any generation (73). However, with third-generation low-dose OC use, glucose tolerance is only marginally decreased by 10–20% versus pretreatment baseline, and pancreatic beta-cell function seems only discretely altered as C peptide concentrations, representing pancreatic insulin secretion, are essentially unchanged. Only small changes in insulin response to an oral glucose challenge are reported when low-dose COCs containing DSG or GSD are used (74). The mechanism underlying these limited alterations of carbohydrate metabolism is mostly unknown, and these changes cannot be clearly attributed to the estrogen or the progestogen component of OCs. Although it was previously postulated that insulin resistance was induced by the progestogen component, a hypothesis formulated by Godsland and co-workers suggests that the estrogen component of OCs is primarily responsible for any insulin resistance, and it is eventually combined with progestogen-associated changes in insulin half-life (68,74). Considering the important cardiovascular effect of chronic hyperinsulinemia and insulin resistance, well described by Reaven (75) as a multifaceted metabolic syndrome, it is essential to appreciate whether OC use can contribute to an increased cardiovascular risk by altering parameters of insulin sensitivity. However, when analyzing the limited influence of current OCs on these parameters and considering, as Godsland and co-workers do (76), that the insulin resistance seen in OC users differs on many points from that associated with arterial disease, it is far from obvious that current low-dose OCs may have a deleterious effect on the cardiovascular system through their effects on carbohydrate metabolism.

VIII. HEMOSTASIS

Pharmacodynamic studies, particularly related to the use of COCs containing high-dose or standard-dose of EE combined with first- or second-generation progestogens, showed adverse effects of lipid and carbohydrate metabolism and

raised concern about increased arteriosclerosis (67,70). However, epidemiological findings showed increased risks of myocardial infarction, stroke, and venous thromboembolism in current, but not past, users of any type and generation of COCs, suggesting a thrombotic effect of combined OCs, rather than an atherosclerotic mechanism (77).

Early reports already drew attention to the effects of COCs on the hemostatic system, and established that high doses of estrogens increased risk for thrombotic cardiovascular disease (78).

The increase in thrombotic disease seems mainly related to the estrogenic component of OCs (79), with good evidence that CVD risk is decreased when estrogen content is decreased from 100 to 50 μg, but less obviously so when further decreased to 30 or 20 μg of EE (77,80). Because a modulating role of progestogens was also suspected (80), new lower-dose compounds, such as (*levo*)norgestrel were first introduced, and followed by less androgenic and more metabolically neutral steroids, such as the third-generation DSG, GSD, and NGM, in a general effort to lower the effects of new OCs on hemostasis variables, lipid and carbohydrate metabolism (7).

Very recent epidemiological studies seem to indicate that myocardial infarction (81) and cerebral thrombosis (82) are less prevalent in women using low-dose third-generation OCs than other high- or low-dose OCs of first and second generation, in spite of the observation of a potential increase in venous thromboembolism in third- versus second-generation OC users (83). Therefore, it is of paramount importance to analyze the effect of all these OCs and their separate components on hemostatic variables to uncover biological plausibility supporting, at least partly, the epidemiological data recently reported.

Many reports have documented the effects of various OCs on hemostatic variables (84,85). However, the drastic reduction in estrogen and progestogen content of OCs, the change in type of progestogens, the earlier age of the women taking OCs, and for a longer period of time, the better selection of candidates for OC use (lower risk population) or, by contrast, preferential prescribing of low-dose new-generation OCs (supposed to be safer) to a population with a higher risk of CVD, and the more precise diagnosis of CVD, do effectively profoundly alter data concerning OCs and their users, making it mandatory to rely on recent well-conducted epidemiological studies that accurately reflect these changes. The challenge is then to relate the observed changes in vascular risk to hemostatic changes induced by new-generation OCs and to compare them with older OC preparations.

The remaining problem is that there is no established way to assess thrombogenicity of one particular OC preparation, and that there is no definite causal relation between particular hemostatic changes induced by OCs and specific cardiovascular risk (85–87).

A. Effects of COCs on Plasma Concentrations of Components of the Coagulation and Fibrinolysis System

1. Coagulation Factors

In an extensive review, Kluft and Lansink (85) studied the changes in hemostatic variables, expressing the results, for the sake of clarity, as percentages of the interindividual variation coefficient of each variable, in women using low doses of EE (30–35 μg) associated with either second- or third-generation progestogens. Results encompassing the study of 37 hemostasis parameters were tabulated. In about 15 variables, the change induced by COCs was of a magnitude close to the standard deviation of the population range, reflecting a significant shift of the distribution. Many procoagulant factors were increased by all COCs formulations used, either in terms of concentration, activity, or both. OC use has been shown to increase fibrinogen, prothrombin, factor VIII (F VIII), F XII, and F VII levels, and to significantly reduce activated partial thromboplastin time (APTT), among other parameters (85). These changes are observed as well with high-dose OCs as with low-dose preparations of any generation, and are more pronounced with OC preparations containing more than 35 μg of EE. Reducing EE from 30 to 20 μg in third-generation preparations, containing the same dose of progestogens, is not accompanied by differential changes in coagulation variables in women using these OCs (88). As far as progestogens are concerned, the first-generation progestogen norethindrone, may have some estrogen-like effect on hemostasis, probably in relation to some of its metabolic products that exert estrogenic activity (89). Low-dose second-generation OCs induce small changes in coagulation factors (90) and, when comparing these results with the effects of third-generation OCs, it seems that they are very close to each other, but with some exceptions: vitamin K-dependent F VII, and to a smaller extent F X and prothrombin, seem to increase somewhat more during use of third-generation OCs containing gestodene and even more so with desogestrel than during use of OCs containing LNG (85). If an increase in coagulation variables augments the coagulation potential without necessarily leading to an increased vascular risk, a recent observation has shown that a prothrombin gene mutation that increases plasma prothrombin levels by 30% in heterozygous carriers versus noncarriers, is an established genetic risk factor for venous thrombosis, including cerebral vein thrombosis (91). This risk is further strongly raised in women presenting with the prothrombin gene mutation and using OCs.

2. Coagulation Inhibitors

Antithrombin III (AT III), protein C (PC), and protein S (PS) play a central inhibitory role in the prothrombin to thrombin activation step, which is of

Table 4 Prevalence of the Deficiency of Coagulation Inhibitors Antithrombin III, Protein C, Protein S, and Resistance to Activated Protein C in the Asymptomatic Population, in Thrombosis Patients, and in Thrombosis Patients with a Positive Family History

	Prevalence (%)		
	Population	Thrombosis patients	Thrombosis patients with a positive family history
Antithrombin III deficiency	0.1	1.2	4.2
Protein C deficiency	0.3	3.6	4.9
Protein S deficiency	0.1	2.4	5.1
Activated protein C resistance	5.7	28.0	46.0
Total	6.2	35.2	60.2

Source: Ref. 94.

paramount importance in the coagulation process. Decreases in plasma activities of these coagulation inhibitors can precipitate venous thrombosis. Indeed, it is now well delineated that an increased risk of venous thrombosis (i.e., essentially deep vein thrombosis and pulmonary embolism, or cerebral vein thrombosis) can occur in predisposed individuals (thrombophilia). Aside from acquired risk factors (such as obesity, autoimmune disease, or others), inherited abnormalities of the hemostatic system may explain one-third of all venous thromboembolism (VTE). Deficiencies in AT III, PC, and PS have been already well described. More recently, resistance to activated protein C (APCR), characterized by the inability of activated PC to prolong the clotting time in an APTT reaction, was described (92). This APC resistance is essentially, but not only, caused by a mutation of the substrate of APC, coagulation factor Va (factor V Leiden mutation) (93) which, therefore, cannot be neutralized by APC and leads to increased thrombin generation. Table 4 reproduced from Winkler (94) summarizes data concerning prevalence in Western Europe of deficiencies in coagulation inhibitors and in APCR in the asymptomatic population, in thrombosis patients, and in thrombosis patients with a positive family history—which underlines the importance of inherited disturbances in thrombophilia. It is important to consider that the thrombophilic trait may create a latent prothrombotic state that may become thrombogenic in the presence of precipitating risk factors, such as pregnancy, postpartum, surgery, immobilization, and OC use. In the Leiden Thrombophilia Study (95) the risk of VTE in the heterozygous carriers of the factor V Leiden mutation is already increased 7-fold, and there is a 28-fold higher risk for Leiden mutation-positive OC-users in comparison with women

not genetically predisposed and not taking OCs. In that setting, it is important to consider that COCs reduce plasma concentration and activity of AT III and PS by about 10–20% versus baseline and do not interfere, or increase slightly PC concentrations (85,87). Many studies clearly show that AT III levels and activity are inhibited by OCs containing high doses of EE ($\geq$ 50 μg) and that second- and third-generation OCs containing 35 μg EE or less have much less or no effect on AT III levels and activity (88,96). Whereas total protein S antigen concentrations are frequently reported to be decreased with use of any type of OC (97), there is some controversy over free PS and PS activity that can remain unchanged or even decreased slightly in some studies using low-dose third-generation OCs (88). PC levels and activity are usually increased with any type of OCs (87). APC sensitivity is slightly reduced (by about 10%) during use of either second- or third-generation OCs (85,98). The mechanism of this reduction is not well understood; however, as small variations of F V, F VIII, and PS (which are known to be caused by OCs) may overcome the anticoagulant effect of APC, this could be a partial explanation of this OC-induced APCR of unknown clinical significance (98). A new in vitro assay measuring the effect of APC on thrombin generation in the plasma of women using OCs showed more thrombin generation during use of third-generation OCs than during second-generation OC use. This APCR-like response during use of third-generation OCs was interpreted by the authors (99), to be associated with an increased risk factor for VTE. However, these results are at variance with the classic APCR measurements, which do not provide differential results in users of second- versus third-generation OCs. Moreover, no VTE patients have been tested using this method, and additional data are necessary for better interpretation of these results (87).

3. *Fibrinolytic Activity*

There is a rather large consensus in numerous studies related to the assessment of fibrinolysis variables during OC use, indicating a significant increase in plasminogen concentration and activity, and α_2-antiplasmin antigen (88,90,100), a decrease in histidine-rich glycoprotein (101), a decrease in tissue plasminogen activator (t-PA), and an even more important decrease in plasminogen activator inhibitor 1 (PAI-1) concentration and activity (85,87,88,101). Altogether these results point to an increased fibrinolytic potential during OC use, whatever the type and dose of the preparation used.

4. *Coagulation and Fibrinolysis Activation Markers*

These markers are generated in the proteolytic process of the coagulatory (thrombin-generating) or the fibrinolytic (plasmin-generating) cascade. Coagulation activation markers are prothrombin fragment 1+2 (F 1+2), reflecting throm-

bin generation rate, thrombin–antithrombin (TAT) complexes, and fibrin peptide A (FPA), whereas the corresponding markers of fibrinolysis are plasmin–antiplasmin (PAP) complexes, reflecting plasmin generation rate, and D-dimer fibrin degradation products, another measure of plasmin activity on polymerized fibrin.

FPA levels are increased during use of second- and third-generation OCs, but eventually slightly less with a preparation containing 20 μg EE (85); F 1+2 and TAT are increased in the same way during use of OCs containing 35, 30, or 20 μg EE, irrespective of the progestogen contained in the combined preparation.

Fibrinolysis activation during OC use has been observed with all combined OCs tested, with significant increases in PAP complexes and D-dimers.

These markers of hemostatic activity clearly indicate that OCs increase the basal rate of thrombin and plasmin generation and the comprehensive review of Kluft and Lansink (85) as well as other reports show that this activation is largely dependent on the presence of EE in the OCs used and almost unchanged when EE dosages are reduced from 35 to 20 μg. Additionally, no differential effect of the progestogen component of OCs has been described (102–104). It has been frequently hypothesized that increased coagulation potential and activity was balanced by increased fibrinolysis potential and activation. However, changes observed during OC use in coagulation and fibrinolysis variables do not correlate (105) and cannot be systematically considered as part of a balanced process. Indeed, some women become "high hemostatic responders" when exposed to OCs (106). Moreover, women presenting with inherited or acquired prothrombotic abnormalities may be facing the risk of vascular, mainly venous, occlusion with notable coagulation–fibrinolysis imbalance (107).

IX. RISK OF CARDIOVASCULAR DISEASE AND NEWER ORAL CONTRACEPTIVES

The association between OC use and cardiovascular disease, including venous thromboembolism (VTE), myocardial infarction (MI), stroke, and hypertension has been a major safety issue ever since the 1960s, inasmuch as OCs are more than ever extensively used by young and generally healthy women for longer and longer time periods. Early concern about cardiovascular safety first led to reduction of estrogens to the 50-μg and then considerably sub–50-μg level and, secondly, to reduction of progestogen dosage and introduction of new progestogens with a more neutral metabolic profile. They culminate at the time being in a number of third-generation combined OCs (see Table 1), thought to have a better cardiovascular risk profile than older first- and second-generation OCs, this notion not only being supported by medical literature, but also forcefully put forward by the pharmaceutical industry, which invested many resources in

the development of new progestins. Several recent epidemiological studies have been devoted to analysis of the cardiovascular effect of the sub-50 second- and third-generation OCs (< 50 μg EE), some of them reaching a size sufficient to more objectively ascertain whether preparations containing new progestogens were indeed safer than older combinations.

A. Venous Thromboembolism

The surprise came suddenly at the end of 1995 and beginning of 1996, when four studies reported, through somewhat different epidemiological approaches, that current use of low-dose COCs was not only still associated with a significantly increased risk of VTE—which was already known—but also that products containing DSG or GSD were apparently associated with a 1.5–3.1 greater risk of VTE than OCs containing second-generation progestogens (mainly LNG) (95,108–110). COCs containing NGM were not taken in consideration because of its too recent introduction into the market, accordingly reducing the number of cases available for study. In later studies or reanalyses, subjects using NGM were grouped with LNG users, as it is usually considered that NGM is potentially acting mainly by its major metabolite, LNG. The most important results from these initial studies are summarized in Table 5. To evaluate these results, it is important to consider that current prevalence of idiopathic VTE in OC nonusers is fewer than 1:10,000 woman-years (WY) and that the incidence of VTE in first- and second-generation OC users was 8:10,000 to 9:10,000 WY in the RCGP 1978 report (111), but fell to 4.1:10,000 WY in mainly low-dose second-generation OC users in the report of Gertsman et al. (112). Data available from two of the four 1995–96 studies reported in Table 5 indicate an incidence of VTE of 1.0:10,000 and 1.6:10,000 WY in second-generation OC users (WHO and Boston Collaborative Drug Surveillance Programme [BCDSP] studies, respectively) and 2.1:10,000 and 2.9:10,000 WY in third-generation OC users in the same studies. Accordingly, the absolute risk of VTE in third-generation OC users recently described by no means exceed the prevailing occurrence rates of VTE described in 1991 by Gertsman et al. in users of second-generation low-dose OCs (112). Additionally, it is important to remember that the incidence of VTE in pregnancy and postpartum is about 6:10,000 WY, well higher than values currently reported for absolute risk of VTE in OC users. Finally, in terms of public health interest, VTE case–fatality is 1–2% (i.e., much lower than for other cardiovascular accidents, such as myocardial infarction and stroke, for which case–fatality is about 10–20% and higher) (113,114). Accordingly, excess mortality attributable to VTE in OC users has been calculated to be 1:million to 1.5:million WY (115). The observed 1.5–3.1 increase in relative risk of VTE in current users of third-generation OCs versus second-generation OCs reported in the 1995–1996 studies is altogether surprising and unexpected, although this

Table 5 Main Results of Initial Venous Thromboembolism Studies

	WHO (all regions) (108)	BCDSP nonfatal VTE (110)	Transnational study (109)	Leiden Thrombophilia study (95)
Type of study[a]	Case–control adjusted ORs	Nested case–control analysis	Case–control adjusted ORs	Case–control adjusted RR
Adjusted OC use vs. no use	4.1 (3.2–5.2)	—	4.0 (3.1–5.3)	—
DSG vs. second-generation[b] OCs	2.4 (1.3–4.6)	2.2 (1.1–4.4)	1.5 (1.1–2.2)	2.5 (1.2–5.2)
GSD vs. second-generation OCs	3.1 (1.6–4.6)	2.1 (1.0–4.4)	1.5 (1.0–2.2)	—

VTE, venous thromboembolism; ORs, odds ratios; RR, relative risk; numbers in parens, 95% confidence intervals; adjusted, adjusted for different confounding variables.

[a]OCs, oral contraceptives; DSG, desogestrel; GSD, gestodene; LNG, levonorgestrel.

[b]"Second generation OCs," levonorgestrel-containing OCs for WHO and Boston Collaborative Drug Surveillance Programme (BCDSP) studies, all second-generation OCs for Transnational study, and second- + first-generation OCs for Leiden study.

increase, translated in terms of absolute risk of morbidity and mortality, is very limited. Important questions have been raised concerning potential bias and confounding factors in these epidemiological studies and subsequent studies (see following section) are not yielding the same results, also pointing to some potential limitations and bias in the initial studies. Additionally, no clear and plausible biological mechanism can currently explain the discrepancies in relative risk of VTE observed between second- and third-generation OCs.

1. *Limitations, Bias, and Confounding Factors in the "Initial" 1995–1996 Studies and Confrontation with Newer Studies*

A comprehensive discussion of these important epidemiological aspects has been provided in reviews by Spitzer (116) and Walker (117). However, their conclusions are divergent, as, for the former author discrepancies of results for risk of VTE between second and third-generation OCs are "more likely to be explained by bias inherent in observational research than by a causal relationship," whereas for the latter author, the "most plausible explanation of the available data is that combined OCs containing DSG and GSD carry a very small risk of VTE which exceeds the even smaller risk carried by products containing LNG, the position of NGM being uncertain" (116,117).

a. Consistency or Inconsistency of Studies

The odds ratios (ORs) for VTE in the four initial studies are consistent, but the rate-ratios [ratio of the relative risks (RR)] of third- versus second-generation OCs are small even when statistically significant. However, consistent findings going in the same direction may suggest the presence of the same systematic errors in all studies. In fact, two subsequent studies by Farmer's group investigated the risk of VTE during use of second- versus third-generation COCs, using the British Mediplus database of patient record (118) or the German Mediplus database (119). In the U.K. study, the rate-ratio of VTE in users of third-generation relative to second-generation OCs after adjustment for age, was 1.68 (95% CI 1.04–2.75), at the limit of statistical significance. Farmer stressed that residual confounding by age could have hampered initial studies. Indeed, age distribution of second- and third-generation products was not similar and might reflect prescribing bias (118). However, reanalyses of WHO and BCDSP studies do not confirm this potential distortion. In the German study, no significant differences relative to VTE risk between users of second- and third-generation OCs were found (OR 0.77 [95% CI 0.38-1.57]), with no significant age difference between users and control subjects (119). Another important and heretofore unexplained element of inconsistency lies in the observation not only in the WHO, BCDSP, and Transnational studies, but also in the UK Mediplus study that, among users of third-generation OCs, the risk of VTE was higher

in users of 20 μg than of 30 μg EE combined with the same dose of DSG (150 μg). These results are paradoxical, as the progestogen concentration was constant; a decrease in the EE dosage should reasonably not increase the risk for VTE in a biologically plausible manner. Therefore, unsuspected confounding factors have to be postulated (recent introduction to the market, prescribing bias, and such).

b. First Use, Duration of Use, and Time Trends

Vessey and Doll (120) reported, as early as 1969, that the risk for VTE during OC use is higher in the first year than at a later period (120). *First-time use* is more likely to occur with newly introduced third-generation products (new-starter effect). Reanalyses of the Transnational study by Suissa (121) also showed that for first-time users of OCs, no significant difference was found for risk of VTE between second- and third-generation OC users. A decrease in relative risk of VTE is recorded with increased *duration of use*, as well demonstrated in a large recent Danish case–control study (114) with sufficient power to ensure the possibility of correction for duration of use. As described in the initial studies, current users of OCs with second- and third-generation progestogens had an OR for VTE of 1.8 (1.1–2.9) and 3.2 (2.2–4.4), respectively, when duration of use was not taken into account. After adjustment for duration of use, however, no significant differences were found between users of OCs containing different types of progestogens. Moreover, this study distinctly showed that the risk for VTE among current users of (all) OCs was primarily influenced by duration of use, with significantly decreasing ORs over time: less than 1 year: 5.1 (3.1–8.5); 1–5 years: 2.5 (1.6–4.1); and more than 5 years 2.1 (1.5–3.1). As control for duration of use in the initial studies was not always adequate, particularly in Leiden and BCDSP studies (95,110), it implies that, at least in the latter studies, risk estimates of third- versus second-generation OCs could have been overestimated. Additional analysis of Transnational study database by Lewis et al. (122) showed important changing trends in OC use as a function of time: the highest risk for VTE was not confined to particular OC formulations, but correlated very significantly with recency of market introduction of OCs. A reasonable explanation may be found in *preferential prescribing* by physicians of third-generation low- and very low-dose COCs, because of their perceived improved safety profile. Accordingly, a new user will receive the more recent OC, with an attending higher risk for VTE, and women with cardiovascular risk factors will be switched from older second-generation OCs to a newer third-generation product, potentially ''safer'' for them. Numerous physician surveys across Europe clearly indicate this prescribing trend (123,124) that leads, in epidemiological studies, to an unexpected bias owing to *attrition of susceptibles (healthy user effect)*. A striking illustration of prescribing bias is provided by a recent study of Lidegaard (125), aiming at assessing potential preferential

prescribing of OCs according to different thrombotic risk factors: women with familial thrombotic predisposition were four times more likely to be prescribed third- versus second-generation OCs, compared with women without such a disposition. It was concluded that, for thrombotic diseases, for which familial predisposition and duration of use of OCs play a role for the pill-associated risk, these differences in prescribing may introduce biases in observational, nonrandomized studies, unless confounder control is conducted (125). The unexplained observation that risk for VTE is higher with use of the recently introduced very low-dose OC, containing 20 μg EE and 150 μg DSG may be partly related to prescribing bias, such as just described.

2. *Biological Plausibility*

If one refers to the numerous literature devoted to the effects of OCs and, more specifically of low-dose and very low-dose second- and third-generation OCs on hemostasis (see foregoing), there seems to be a reasonable agreement that all combined OCs tested may constitute a precipitating risk factor for VTE (87), essentially relative to their content in EE. The increase in some coagulation factors and fibrinolysis variables and the decrease in some coagulation inhibitors seem particularly related to EE action on the hepatocyte, with possibly restricted effect of progestogens, but the general mechanism of action of OCs is largely unknown (85). The only potential discrepancy may be observed between low-dose OCs containing desogestrel versus other progestogens relative to the effects on levels of F VIIc, which seem somewhat more increased during use of the former progestogen (85). However, this increase is probably not associated with the occurrence of VTE (126). An unverified hypothesis could be that third-generation COCs are more "estrogenic" than second-generation COCs containing the same amount of EE, because of a less androgenic–antiestrogenic balance of the new OCs, owing to their content of less androgenic progestogens. A direct effect of this estrogen dominance is exemplified by the increase in serum levels of SHBG during use of third- versus second-generation OCs. However, the SHBG increase recorded under the influence of 20 μg EE + 150 μg DSG is slightly less than with the same combined preparation containing 30 μg. In that prospect, the paradoxical increased VTE risk, which has been described in many studies, reported in the foregoing with the 20- versus the 30-μg preparation, seems rather biologically implausible. The study of Rosing and colleagues (99) assessed, with the use of a personal in vitro method, the effects of APC on thrombin generation in the plasma, showing a significantly decreased APC sensitivity in the plasma samples from women using third-generation versus second-generation OCs. They described these results as a type of "acquired APC resistance," as observed in heterozygous female carriers of factor V Leiden mutation. However, in the same study, measurement of APC resistance by a validated method did not

show any differential result for users of different OCs. Validation of Rosing's method, which is in progress, and repetition of this study with an appropriate (and not a cross-sectional) design are mandatory before adequate assessment of additional observations can be conducted. Anyway, if, to say the least, poor biological plausibility exists to explain a probably very restricted increased risk of VTE with use of third- versus second-generation OCs, this can, however, not be considered a crucial flaw. As recently stressed by Walker (117), "in drug research, it is almost tautological that serious adverse effects will not have a well understood physiological basis when they are first observed."

B. Acute Myocardial Infarction

The WHO, the BCDSP, and the Transnational epidemiological studies cited earlier (108–110), were designed not only for appreciation of risk for VTE, but also mainly for evaluation of risk for acute myocardial infarction (AMI) and stroke. Earlier epidemiological studies showed that current use of OCs was associated with a two- to fourfold increase in risk of AMI, which may be further increased by smoking or clinical risk factors, such as hypertension (127). However, changes in the formulations of OCs, prescription to low-risk and to younger women are probably associated with a lower risk for AMI, which is now lower than twofold when compared with no use (128). Only a few studies have directly assessed the risk of AMI in users of low EE-containing OCs by progestogen type: the WHO, the BCDSP, and the Transnational studies cited earlier, as well as the specific study by Sidney et al. (129) were specifically designed for this purpose. From these studies, it is difficult to draw a definite answer for any differential effect of OCs containing third-generation progestogens on the risk of AMI. The BCDSP study is hampered by a very low number of cases, and by a comparison of risk for AMI in current and noncurrent users, which may be misleading (128). The Transnational study (81) reported an overall OR for AMI of 2.3 (1.4–4.1) during use of any low-dose OC; for second-generation OCs, the OR for AMI was 3.0 (1.5–5.6) and for third-generation OCs 0.8 (0.3–2.3). Seven cases and 49 controls were exposed to third-generation OCs. The rate-ratio for third- versus second-generation OCs was 0.3 (0.1–0.9), showing a significantly lower risk for AMI in case of third-generation versus second-generation OC use, with no increase in risk between third-generation OC use and non-OC users. However, the study of Sidney et al. (29) reported a relative risk of less than 1.0 for low-estrogen OCs containing levonorgestrel. In the WHO case–control study (130), the overall OR for AMI in current users of any type of OC was 5.01 (2.54–9.90) in Europe; however, this risk estimate reflected coexistence of other risk factors for AMI. The study had unhappily insufficient power to examine whether progestogen dose and type had any effect on AMI risk. It will be difficult to establish clearly whether progestogen type or dosage

are effectively modifying the effect of current use of OCs for MI because of the low prevalence of that disease in young women. However, it is important to acknowledge the fact that the risk of MI during use of third-generation OCs is probably very low, because the case–fatality rate for that disease is elevated (113).

C. Stroke

Both the incidence of stroke in young women and the relative risk of stroke in OC users has been falling for some years (131). For OC use, the risk of stroke decreased owing to younger age at use of OCs, avoidance of risk factors such as hypertension, and use of lower–steroid-dose preparations. The risk of thromboembolic stroke decreases with decreasing doses of EE in OCs (82). Two recent American epidemiological studies (132,133) concluded that the use of low-dose OCs was not associated with an increased risk of stroke. The large case–control WHO and Transnational studies (134,135) revealed no different rate-ratios of second- and third-generation OCs for thrombotic stroke. By contrast, the recently reported Danish cerebral thrombosis and oral contraceptive case–control study (82) concluded not only that use of OCs with 50 μg of EE (but not lower dosages) and OCs with second-generation progestogens increased the risk of thromboembolic stroke significantly, whereas OCs containing third-generation progestogens did not. In the Danish study, both levonorgestrel- and norgestimate-containing OCs constituted the second-generation group. Consequently, these contrasting results ask again for additional, clarifying data concerning the potential differential effect of second-generation progestogens. In that prospect, Schwartz et al. (132) already noted that a higher risk of hemorrhagic stroke was associated with current use of OCs containing the second-generation progestogen levonorgestrel.

X. SUMMARY AND CONCLUDING REMARKS

In the last 18 years or so three third-generation progestogens—DSG, GSD, and NGM—have been increasingly used in COCs. These progestogens were designed to have a higher progestogenic to androgenic activity ratio than older second- and first-generation progestogens (e.g., LNG and norethindrone) and, accordingly, to exert a lower metabolic influence, potentially leading to fewer side effects and fewer serious sequelae, particularly for the cardiovascular system.

Available literature clearly shows that whether in monophasic or in multiphasic combinations with particularly small dosages of EE, the resulting third-generation COCs demonstrate a high level of contraceptive effectiveness, certainly comparable with the efficacy of higher-dose older generation OCs, even in

the case of suboptimal compliance with OC intake. In terms of cycle control, all new products, including the 20-μg preparations, appear to be roughly equivalent to each other, and there seems to be a nonsignificant trend to a lower frequency of spotting, breakthrough bleeding, absence of withdrawal bleeding, and accordingly, a lower discontinuation rate for these reasons when third-generation OCs are compared with older-generation products.

Overall acceptability is also based on low rates of body weight alteration and androgenic side effects. Here again, use of low-dose COCs containing third-generation progestogens tend to better improve acne and hirsutism than do second-generation OCs, probably because of their slight estrogen dominance and low intrinsic androgenic potential.

Lipid and lipoprotein metabolism is essentially unaltered in healthy users of third-generation OCs, except for a slight increase in triglyceride levels. This rather neutral effect is significantly better than the one recorded during use of older-generation OCs. Although a mild degree of glucose intolerance and insulin resistance is still observable during use of new OCs, it seems generally less marked than with older OCs, and of limited clinical significance. Overall, the metabolic effect of low-dose OCs does not seem to induce any increased atherogenic risk in the users.

Collectively, available studies indicate that use of low-dose OCs that contain third-generation progestogens is attended by high level contraceptive effectiveness and cycle control, with excellent overall acceptability and low discontinuation rate. However, accurate comparison between different OC preparations in large randomized trials, extending on sufficient periods of time, is still needed.

All studied combined OCs seem to increase, at least slightly, molecular markers reflecting in vivo activation of coagulation and fibrinolysis, increase some procoagulant and profibrinolytic variables, and alter coagulation inhibitors. It is felt that in individuals with acquired or inherited increased thrombotic risk, OC intake may serve as a precipitating factor for arterial and, even more so, for venous thrombotic accidents. Most of the changes of hemostatic variables recorded in OC users are related to the dose of EE and accordingly less frequently observed with new-generation OCs, which also contain the lowest concentrations of EE. Higher levels of factor VII are observed with third- versus second-generation COCs, and it has been postulated—with a still unvalidated procedure—that third-generation OCs may increase in vitro thrombin generation, the clinical significance of which is difficult to evaluate.

As far as serious vascular sequelae are concerned, epidemiological studies are hampered by low frequency of cardiovascular accidents in young women using or not using OCs, which renders interpretation of these studies difficult. If we take into consideration established risk factors, such as hypertension and smoking, recent studies, which are still controversial and based on too few cases, tend to show that risk of AMI and stroke seems to be lower in users of low-dose third- versus second-generation OCs. This is a very important observation in

view of the high case–fatality rate associated with these diseases and necessitates further evaluation. By contrast, large epidemiological studies have reported an unexpected twofold increased risk of VTE in users of third- versus second-generation OCs. This observation is paradoxical because third-generation OCs essentially contain low levels of EE—which should normally be accompanied by a reduced risk for VTE—and because third-generation progestogens are not known to be associated with a biologically plausible increased thrombotic risk. Bias and confounding factors that hamper these studies may indeed account for some, but probably not all, of the discrepancy observed. Newer studies with more-focused adjustment for risk factors and anticipated biases have yielded very similar results for risk of VTE when using either second- or third-generation OCs. It should be stressed, however, that the small increase in risk of VTE shown in the initial studies during use of third-generation OCs was not likely to have any significant public health effect in view of both the low incidence of VTE that was recorded, and the low case–fatality rate of this disease. Accordingly, the warning issued by the UK Committee on Safety of Medicines (CSM) after review of the initial VTE studies, to switch when possible from third- to second-generation OCs triggered an inappropriate pill scare in many countries, which led to panic interruption of contraception and a dramatic increase in subsequent abortion rates (136,137).*

To further evaluate third-generation OCs, and according to a judicious request of the Committee for Proprietary Medicinal Products (CPMP) of the European Medicines Evaluation Agency (EMEA), two large specific studies have been designed and are in progress. The first one is a randomized, double-blind study comparing COCs containing third-generation progestogens with preparations containing LNG, for illustrating differences in the profiles of common side effects. The second one is a randomized, open-label, parallel group, comparative study of seven monophasic OCs containing either a second- or a third-generation progestogen, to investigate the discrepancy between alleged differences in risk of VTE between second- and third-generation OCs and the knowledge on the effects of OCs on hemostasis parameters (138). We could not agree more.

Finally, beyond critical analysis of the effects of third-generation OCs, the quest for ever safer oral contraceptives is still open: lower dosages of available steroids, combined according to different regimens, new progestins (derived, for example, from spironolactone or 19-norprogesterone), and estradiol in place of EE, are currently being extensively tested.

**Note:* In April 1999, the UK Medicines Commission issued the new warning that, provided women are fully informed of the very small risks for VTE when using oral contraception, the type of pill to be used (i.e., either second or third generation) is for the woman, together with her doctor or other family planning professionals, to decide in light of her individual medical history—A U-turn in advice on the use of third generation OCs when compared to earlier warnings.

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REFERENCES

1. H Kuhl. Comparative pharmacology of newer progestogens. Drugs 51:188–215, 1996.
2. HJ Kloosterboer, CA Vonk-Noordegraaf, EW Turpijn. Selectivity in progesterone and androgen receptor binding of progestagens used in oral contraceptives. Contraception 38:325–332, 1988.
3. N van der Vange, MA Blankenstein, HJ Kloosterboer, AA Haspels, JHH Thijssen. Effects of seven low-dose combined oral contraceptives on sex hormone binding globulin, corticosteroid binding globulin, total and free testosterone. Contraception 41:345–354, 1990.
4. K Fotherby. Pharmacokinetics and metabolism of progestins in humans. In JW Goldzieher, K Fotherby, eds. Pharmacology of Contraceptive Steroids. New York: Raven Press, 1994, pp 99–126.
5. J Trussell, RA Hatcher, W Cates Jr, FH Stewart, K Kost. A guide to interpreting contraceptive efficacy studies. Obstet Gynecol 76:558–567, 1990.
6. JR Newton. Classification and comparison of oral contraceptives containing new generation progestogens. Hum Reprod Update 1:231–263, 1995.
7. L Speroff, A De Cherney. The Advisory Board for the New Progestins. Evaluation of a new generation of oral contraceptives. Obstet Gynecol 81:1034–1047, 1993.
8. E Ketting. The relative reliability of oral contraceptives: findings of an epidemiological study. Contraception 37:342–348, 1988.
9. J Guillebaud. Your Questions Answered. Contraception. Singapore: Churchill Livingstone, 1993.
10. CJCM Hamilton, HJ Hoogland. Longitudinal ultrasonographic study of the ovarian suppressive activity of a low-dose triphasic oral contraceptive during correct and incorrect pill intake. Am J Obstet Gynecol 161:1159–1162, 1989.
11. G Shaw, S Killick, M Elstein. Assessment of ovarian activity in a gestodene containing triphasic oral contraceptive. Br J Fam Plann 18:76–78, 1992.
12. B Hedon, P Cristol, A Plauchut, AM Vallon, F Desachampts, ML Taillant. Ovarian consequences of the transient interruption of combined oral contraceptives. Int J Fertil 37:270–276, 1992.
13. BM Landgren, G Csemiczky. The effect on follicular growth and luteal function of "missing the pill." Contraception 43:149–159, 1991.
14. SR Killick, K Bancroft, S Oelbaum, J Morris, M Elstein. Extending the duration of the pill-free interval during combined oral contraception. Adv Contracept 6:33–40, 1990.

15. G Benagiano, FM Primiero, C Bastianelli, G Abbondante, F Grottanelli, G Tunnera. Ovarian activity during oral contraception. Adv Contracept 6:245, 1990.
16. N van der Vange. Ovarian activity during low dose oral contraceptives. In: G Chamberlain, ed. Contemporary Obstetrics and Gynaecology. London: Butterworths, 1988, pp 315–326.
17. C Fitzgerald, W Feichtinger, J Spona, M Elstein, F Lüdicke, U Müller, C Williams. A comparison of the effects of two monophasic low dose oral contraceptives on the inhibition of ovulation. Adv Contracept 10:5–18, 1994.
18. GE Robinson. Low-dose combined oral contraceptives. Br J Obstet Gynaecol 101:1036–1041, 1994.
19. R Chez. Clinical aspects of three new progestogens: desogestrel, gestodene, and norgestimate. Am J Obstet Gynecol 160:1296–300, 1989.
20. MJ Rosenberg, SC Long. Oral contraceptives and cycle control: a critical review of the literature. Adv Contracept 8(suppl 1):35–45, 1992.
21. EM Belsey, The Task Force on Long-Acting Systemic Agents for Fertility Regulation. Menstrual bleeding patterns in untreated women and with long-acting methods of contraception. Adv Contracept 7:257–270, 1991.
22. P Bilotta, S Favilli. Clinical evaluation of a monophasic ethinylestradiol/desogestrel-containing oral contraceptive. Arzneimittelforschung 38:932–934, 1988.
23. HE van Kets. Belgian trial of an oral contraceptive containing 0.150 mg desogestrel and 0.020 mg ethinylestradiol. Acta Obstet Gynecol Scand Suppl 144:13–17, 1987.
24. J Nevinny-Stickel. German trial of an oral contraceptive containing 0.150 mg desogestrel plus 0.020 mg ethinylestradiol. Acta Obstet Gynecol Scand Suppl 144:19–21, 1987.
25. M Akerlund, A Rode, J Westergaard. Comparative profiles of reliability, cycle control and side effects of two oral contraceptive formulations containing 150 μg desogestrel and either 30 μg or 20 μg ethinyl oestradiol. Br J Obstet Gynaecol 100:832–838, 1993.
26. R Tuimala, M Saranen, U Alapiessa. A clinical comparison in Finland of two oral contraceptives containing 0.150 mg desogestrel in combination with 0.020 mg or 0.030 mg ethinylestradiol. Acta Obstet Gynecol Scand Suppl 144:7–12, 1987.
27. S Skouby. Oral contraception with a triphasic combination of gestodene and ethinyl estradiol: results of a multicenter clinical study. Int J Fertil 32:45–48, 1987.
28. J Endrikat, U Müller, B Düsterberg. A twelve-month comparative clinical investigation of two low-dose oral contraceptives containing 20 μg ethinylestradiol/75 μg gestodene and 30 μg ethinylestradiol/75 μg gestodene with respect to efficacy, cycle control and tolerance. Contraception 55:131–137, 1997.
29. SL Corson. Efficacy and clinical profile of a new oral contraceptive containing norgestimate: U.S. clinical trials. Acta Obstet Gynecol Scand Suppl 152:25–31, 1990.
30. KM Andolsek. Cycle control with triphasic norgestimate and ethinyl estradiol, a new oral contraceptive agent. Acta Obstet Gynecol Scand 71:22–26, 1992.

31. K Brill, C Müller, J Schnitzker, M Albring. The influence of different modern low-dose oral contraceptives on intermenstrual bleeding. Adv Contracept 7(suppl 2):51–61, 1991.
32. RJE Kirkman, JH Pedersen, P Fioretti, HE Roberts. Clinical comparison of two low-dose oral contraceptives, Minulet and Mercilon, in women over 30 years of age. Contraception 49:33–46, 1994.
33. G Benagiano. Comparison of two monophasic oral contraceptives: gestodene/ethinyl estradiol versus desogestrel/ethinyl estradiol. Int J Fertil 34:31–39, 1989.
34. P Affinito, M Monterubbianesi, M Primizia, V Regine, C Di Carlo, MJ Farace, G Petrillo, C Nappi. Efficacy cycle control and side effects of two monophasic combination oral contraceptives: gestodene/ethinyl estradiol and norgestimate/ethinyl estradiol. Gynecol Endocrinol 7:259–266, 1993.
35. S Koetsawang, C Charoenvisal, L Banharnsupawat, S Singhakovin, O Kaewsuk, S Punnahitanont. Multicentre trial of two monophasic oral contraceptives containing 30 mcg ethinylestradiol and either desogestrel or gestodene in Thai women. Contraception 49:387–398, 1994.
36. G Cullberg, G Samsioe, RF Andersen, P Bredesgaard, NB Andersen, H Ernerot, E Fanoee, P Fylling, PE Haach-Sorensen, P Klottrup, JH Pedersen, T Sandager. Two oral contraceptives, efficacy, serum proteins, and lipid metabolism. Contraception 26:229–243, 1982.
37. NB Loudon, RJE Kirkman, JA Dewsbury. A double-blind comparison of the efficacy and acceptability of Femodene and Microgynon-30. Eur J Obstet Gynecol Reprod Biol 34:257–266, 1990.
38. UB Lindberg, N Crona, G Silfverstolpe, P Björntop, M Rebuffé-Scrive. Regional adipose tissue metabolism in postmenopausal women after treatment with exogenous sex steroids. Horm Metab Res 22:345–351, 1990.
39. JW Goldzieher, LE Moses, E Averkin. A placebo-controlled double-blind crossover investigation of the side-effects attributed to oral contraceptives. Fertil Steril 22:609–623, 1971.
40. S Carpenter, LS Neinstein. Weight gain in adolescent and young adult oral contraceptive users. J Adolesc Health Care 7:342–344, 1986.
41. RE Litchfield, KK Grunewald. Oral contraceptives and fat patterning in young adult women. Hum Biol 60:793–800, 1988.
42. B Düsterberg, H Ellman, U Müller, E Rowe, B Mühe. A three-year clinical investigation into efficacy, cycle control and tolerability of a new low-dose monophasic oral contraceptive containing gestodene. Gynecol Endocrinol 10:33–39, 1996.
43. BE Reubinoff, A Gurbstein, D Meirow, E Berry, JG Schenker, A Brzezinski. Effects of low-dose estrogen oral contraceptives on weight, body composition, and fat distribution in young women. Fertil Steril 63:516–521, 1995.
44. UJ Gaspard. Role of antiandrogens in the treatment of acne in hirsutism and virilism. In: VB Mahesh, RB Greenblatt, eds. Hirsutism and Virilism—Pathogenesis, Diagnosis and Management. Boston: John Wright-PSG, 1983, pp 369–394.
45. UJ Gaspard, MA Romus, D Gillain, J Duvivier, E Demey-Ponsart, P Franchimont. Plasma hormone levels in women receiving new oral contraceptives containing ethinyl-estradiol plus levonorgestrel or desogestrel. Contraception 27:577–590, 1983.

46. T Rabe, K Grunwald, K Feldmann, B Runnebeaum. Treatment of hyperandrogenism in women. Gynecol Endocrinol 10(suppl 3):1–44, 1996.
47. SM Slayden, R Azziz. The risk of androgen excess in acne. In: K Azziz, JE Nestler, D Dewailly, eds. Androgens Excess Disorders in Women. Philadelphia: Lippincott-Raven 1997, pp 131–140.
48. VP Barranco, DD Jones. Effect of oral contraceptives on acne. South Med J 67:703–706, 1974.
49. R Azziz, F Gay. The treatment of hyperandrogenism with oral contraceptives. Semin Reprod Endocrinol 7:246–254, 1989.
50. G Cullberg, LA Mattsson. Androgenic, oestrogenic and antioestrogenic effects of desogestrel and lynestrenol alone: effects on serum proteins, sex hormones and vaginal cytology. In: RF Harrison, W Thompson, J Bonnar, eds. Trends in Oral Contraception. Hingham, MA: MTP Press, 1984, 17–24.
51. H Refn, A Kjaer, AM Lebech, B Borggaard, L Schierup. Clinical and hormonal effects of two contraceptives: correlation to serum concentrations of levonorgestrel and gestodene. Contraception 41:259–269, 1990.
52. R Palatsi, E Hirvensalo, P Liukko. Serum total and unbound testosterone and sex hormone binding globulin (SHBG) in female acne patients treated with two different oral contraceptives. Acta Derm Venereol (Stockh) 64:517–523, 1984.
53. M Levrier, H Degrelle, Y Bestaux, M Bourry-Moreno, JP Brun, F Sailly. Efficacité sur l'acné des contraceptifs oraux. Rev Fr Gynecol Obstet 83:573–576, 1988.
54. R Erkkola, E Hirvonen, J Luikko, R Lumme, H Männikkö, S Aydinlik. Ovulation inhibitors containing cyproterone acetate or desogestrel in the treatment of hyperandrogenic symptoms. Acta Obstet Gynecol Scand 69:61–65, 1990.
55. THOM Dieben, L Vromans, A Theeuwes, HGT Coelingh Bennink. The effects of CTR-24, a biphasic oral contraceptive combination, compared to Diane-35 in women with acne. Contraception, 50:373–382, 1994.
56. GE Pierard, N Nikkels-Tassoudji, V Goffin, UJ Gaspard, P Slachmuylders, P Lacante. Acne improvement in young women using a low-dose triphasic oral contraceptive containing gestodene and ethinylestradiol (Tri-Minulet). Interim evaluation of an open non-controlled clinical study combined with objective biometrological methods. Gynecol Endocrinol 10(suppl 5):61–66, 1996.
57. RS London, A Chapdelaine, D Upmalis, W Olson, J Smith. Comparative contraceptive efficacy and mechanism of action of the norgestimate-containing triphasic oral contraceptive. Acta Obstet Gynecol Scand 71:9–14, 1992.
58. G Cullberg, L Hamberger, LA Mattsson, H Mobacken, G Samsioe. Effects of a low-dose desogestrel–ethinylestradiol combination on hirsutism, androgens and sex hormone binding globulin in women with a polycystic ovary syndrome. Acta Obstet Gynecol Scand 64:195–202, 1985b.
59. S Venturoli, E Porcu, L Gammi, R Fabbri, R Paradisi, C Flamigni, D Patrono, M Capelli, C Paoletti. The effects of a desogestrel and ethinylestradiol combination in normal and hyperandrogenic young girls: speculations on contraception in adolescence. Acta Eur Fertil 19:129–134, 1988.
60. A Porcile, E Gallardo. Long-term treatment of hirsutism: desogestrel compared with cyproterone acetate in oral contraceptives. Fertil Steril 55:877–881, 1991.

61. GA Sobbrio, A Granata, F D'Arrigo, D Arena, A Panacea, F Trimachi, D Granese, C Pulle. Treatment of hirsutism related to micropolycystic ovary syndrome (MPCO) with two low-dose oestrogen oral contraceptives: a comparative randomized evaluation. Acta Eur Fertil 21:139–141, 1990.
62. JC La Rosa. The varying effects of progestins on lipid levels and cardiovascular disease. Am J Obstet Gynecol 158:1621–1629, 1988.
63. C Harvengt, JP Desager, U Gaspard, M Lepot. Changes in lipoprotein composition in women receiving two low-dose oral contraceptives containing ethinyloestradiol and gonane progestins. Contraception 37:565–575, 1988.
64. A Chapdelaine, JL Desmarais, R Derman. Clinical evidence of the minimal androgenic activity of norgestimate. Int J Fertil 34:347–352, 1989.
65. H Kühl, W März, C Jung-Hoffmann, F Heidt, W Gross. Time-dependent alterations in lipid metabolism during treatment with low-dose oral contraceptives. Am J Obstet Gynecol 163:363–369, 1990.
66. MJ Tikkanen. Role of plasma lipoproteins in the pathogenesis of atherosclerotic disease, with special reference to sex hormone effects. Am J Obstet Gynecol 163:296–304, 1990.
67. UJ Gaspard. Clinical relevance of plasma lipid changes during use of new low-dose oral contraceptives. Adv Contracept 7(suppl 3):180–194, 1991.
68. D Crook, I Godsland. Safety evaluation of modern oral contraceptives: effects on lipoprotein and carbohydrate metabolism. Contraception 57:189–202, 1998.
69. RK Kalkhoff. Effects of oral contraceptive agents on carbohydrate metabolism. J Steroid Biochem 6:949–956, 1975.
70. UJ Gaspard, PJ Lefebvre. Clinical aspects of the relationship between oral contraceptives, abnormalities in carbohydrate metabolism, and the development of cardiovascular disease. Am J Obstet Gynecol 163:334–343, 1990.
71. L Chasan-Taber, WC Willett, MJ Stampfer, DJ Hunter, GA Colditz, D Spiegelmann, JE Manson. A prospective study of oral contraceptives and NIDDM among U.S. women. Diabetes Care 20:330–335, 1997.
72. JO Clausen, K Borch-Jonsen, H Ibsen, RN Bergman, P Hougaard, K Winther, O Pedersen. Insulin sensitivity index, acute insulin response and glucose effectiveness in a population-based sample of 380 young healthy Caucasians. J Clin Invest 98:1195–1209, 1996.
73. IF Godsland, C Walton, C Felton, AJ Proudler, A Patel, V Wynn. Insulin resistance, secretion and metabolism in users of oral contraceptives. J Clin Endocrinol Metab 74:64–70, 1992.
74. UJ Gaspard, F Demeyer, CB Jaminet, AJ Scheen, PJ Lefebvre. Influence of two low-dose oral contraceptives containing ethinylestradiol (20 μg) and desogestrel or gestodene on carbohydrate metabolism during 1 year of use. Gynecol Endocrinol 10(suppl 2):179–181, 1996.
75. GM Reaven. Pathophysiology of insulin resistance in human disease. Physiol Rev 75:473–486, 1995.
76. IF Godsland. Sex steroids and changes in the cardiovascular system: carbohydrate metabolism. Gynecol Endocrinol 10(suppl 2):59–64, 1996.
77. L Rosenberg, JR Palmer, MI Sands, D Grimes, U Bergman, J Daling, A Mills. Modern oral contraceptives and cardiovascular disease. Am J Obstet Gynecol 177:707–715, 1997.

78. WHW Inman, MP Vessey, B Westerholm, A Engelund. Thromboembolic disease and the steroidal content of oral contraceptives—a report to the Committee on Safety of Drugs. Br Med J 2:203–209, 1970.
79. LE Bottiger, G Boman, G Eklund, B Westerholm. Oral contraceptives and thromboembolic disease: effects of lowering oestrogen content. Lancet 1:1097–1101, 1980.
80. TW Meade, G Greenberg, SG Thompson. Progestogens and cardiovascular reactions associated with oral contraceptives and a comparison of the safety of 50- and 30 μg oestrogen preparations. Br Med J 280:1157–1161, 1980.
81. MA Lewis, LAJ Heinemann, WO Spitzer, KD Mac Rae, R Bruppacher. The use of oral contraceptives and the occurrence of acute myocardial infarction in young women. Contraception 56:129–140, 1997.
82. O Lidegaard, S Kreiner. Cerebral thrombosis and oral contraceptives. Contraception 57:303–314, 1998.
83. TMM Farley, J Collins, JJ Schlesselman. Hormonal Contraception and risk of cardiovascular disease—an international perspective. Contraception 57:211–230, 1998.
84. J Bonnar. Changes in coagulation and fibrinolysis with low dose oral contraceptives. Adv Contracept 7(suppl 3):285–291, 1991.
85. C Kluft, M Lansink. Effect of oral contraceptives on haemostasis variables. Thromb Haemostas 78:315–326, 1997.
86. EF Mammen. Oral contraceptives and blood coagulation: a critical review. Am J Obstet Gynecol 142:781–790, 1982.
87. UH Winkler. Blood coagulation and oral contraceptives—a critical review. Contraception 57:203–209, 1998.
88. JL David, F Demeyer, D Gillain, U Gaspard. Hemostasis global profile during monophasic oral contraception by norgestimate or desogestrel associated with low-dose ethinylestradiol (preliminary results). Gynecol Endocrinol 10(suppl 2): 137–139, 1996.
89. H Kuhl. Effects of progestogens on haemostasis. Maturitas 24:1–19, 1996.
90. M Notelovitz, CS Kitchens, FY Khan. Changes in coagulation and anticoagulation in women taking low-dose triphasic oral contraceptives: a controlled comparative 12-month clinical trial. Am J Obstet Gynecol 167:1255–1261, 1992.
91. I Montinelli, E Sacchi, G Landi, E Taioli, F Duca, PM Mannucci. High risk of cerebral-vein thrombosis in carriers of a prothrombin-gene mutation and in users of oral contraceptives. N Engl J Med 338:1793–1797, 1998.
92. B Dahlbäck. The protein C anticoagulant system: inherited defects as basis for venous thrombosis. Thromb Res 77:1–43, 1994.
93. RM Bertina, RPC Koeleman, T Koster, FR Rosendaal, RJ Dirven, H de Ronde, PA van der Velden, PH Reitsma. Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature 369:64–67, 1994.
94. UH Winkler. Activated protein C resistance and deficiencies of antithrombin III, protein C or protein S and the risk of thrombo-embolic disease in users of oral contraceptives. Eur J Contracept Reprod Health Care 3:65–74, 1998.
95. KWM Bloemenkamp, FR Rosendaal, FM Helmerhorst, HR Büller, JP Vandenbroucke. Enhancement by factor V Leiden mutation of risk of deep-vein throm-

bosis associated with oral contraceptives containing a third-generation progestagen. Lancet 346:1593–1596, 1995.

96. A Basdevant, J Conard, C Pelissier, TT Guyene, C Lapousterle, M Mayer, B Guy-Grand, H Degrelle. Hemostatic and metabolic effects of lowering the ethinyl-estradiol dose from 30 mcg to 20 mcg in oral contraceptives containing desogestrel. Contraception 48:193–204, 1993.
97. P Quehenberger, U Loner, S Kapiotis, S Handler, B Schneider, J Huber, W Speiser. Increased levels of activated factor VII and decreased plasma protein S activity and circulating thrombomodulin during use of oral contraceptives. Thromb Haemostas 76:729–734, 1996.
98. M Hellgren, PJ Svensson. Resistance to activated protein C as a basis for venous thromboembolism associated with pregnancy and oral contraceptives. Am J Obstet Gynecol 172:210–213, 1995.
99. J Rosing, G Tans, GAF Nicolaes, MCLGD Thomassen, R Van Oerle, PMEN Van der Ploeg, P Heijnen, K Hamulyak, HC Hemker. Oral contraceptives and venous thrombosis: different sensitivities to activated protein C in women using second- and third-generation oral contraceptives. Br J Haematol 97:233–238, 1997.
100. JL David, UJ Gaspard, D Gillain, R Raskinet, MR Lepot. Hemostasis profile in women using low-dose oral contraceptives. Am J Obstet Gynecol 163:420–423, 1990.
101. J Jespersen, KR Petersen, SO Skouby. Effects of newer oral contraceptives on the inhibition of coagulation and fibrinolysis in relation to dosage and type of steroid. Am J Obstet Gynecol 163:396–403, 1990.
102. GB Melis, F Fruzzetti, AM Paoletti, F Carmassi, P Fioretti. Fibrinopeptide A plasma levels during low-estrogen oral contraceptive treatment. Contraception 30:575–583, 1984.
103. L Daly, J Bonnar. Comparative studies of 30 μg ethinylestradiol combined with gestodene and desogestrel on blood coagulation, fibrinolysis and platelets. Am J Obstet Gynecol 163:430–437, 1990.
104. UH Winkler. Hemostasis in oral contraception: the role of progestogens. Gynecol Endocrinol 7:59–64, 1993.
105. JA Gevers-Leuven, C Kluft, RM Bertina, LW Hessel. Effects of two low-dose oral contraceptives on circulating components of the coagulation and fibrinolytic systems. J Lab Clin Med 109:631–636, 1987.
106. KWM Bloemenkamp, FR Rosendaal, FM Helmerhorst, T Koster, RM Bertina, JP Vandenbroucke. Hemostatic effects of oral contraceptives in women who developed deep-vein thrombosis while using oral contraceptives. Thromb Haemost 80:382–387, 1998.
107. JP Vandenbroucke, T Koster, E Briet, PH Reitsma, R Bertina, FR Rosendaal. Increased risk of venous thrombosis in oral contraceptive users who are carriers of factor V Leiden mutation. Lancet 344:1453–1457, 1994.
108. WHO Collaborative Study of Cardiovascular Disease and Steroid Hormone Contraception. Effect of different progestogens in low oestrogen oral contraceptives on venous thromboembolic disease. Lancet 346:1582–1588, 1995.

109. WO Spitzer, MA Lewis, LAJ Heinemann, M Thorogood, KD Mac Rae. Third generation oral contraceptives and risk of venous thromboembolic disorders: an international case–control study. Br Med J 312:83–88, 1996.
110. H Jick, SS Jick, V Gurewich, MW Myers, C Vasilakis. Risk of idiopathic cardiovascular death and nonfatal venous thromboembolism in women using oral contraceptives with differing progestogen components. Lancet 346:1589–1593, 1995.
111. Royal College of General Practitioners. Oral contraceptives, venous thrombosis and varicose veins: Royal College of General Practitioners' Oral Contraceptive Study. J R Coll Gen Pract 28:203–209, 1978.
112. BB Gerstman, JM Piper, DK Tomita, WJ Ferguson, BV Stadel, EE Lundin. Oral contraceptive estrogen dose and the risk of deep venous thromboembolic disease. Am J Epidemiol 133:32–37, 1991.
113. D Levy, TJ Thom. Death rates from coronary disease. Progress and a puzzling paradox. N Engl J Med 339:915–917, 1998.
114. O Lidegaard, B Edström, S Kreimer. Oral contraceptives and venous thromboembolism. Contraception 57:291–301, 1998.
115. N Weiss. Bias in studies of venous thromboembolism in relation to the use of new formulations of oral contraceptives [letter]. Contraception 55:189–190, 1997.
116. WO Spitzer. The 1995 pill scare revisited: anatomy of a nonepidemic. Hum Reprod 12:2347–2357, 1997.
117. AM Walker. Newer oral contraceptives and the risk of venous thromboembolism. Contraception 57:169–182, 1998.
118. RDT Farmer, RA Lawrenson, CR Thompson, JG Kennedy, IR Hambleton. Population-based study of risk of venous thromboembolism associated with various oral contraceptives. Lancet 349:83–88, 1997.
119. RDT Farmer, JC Todd, MA Lewis, KD Mac Rae, TJ Williams. The risks of venous thromboembolic disease among German women using oral contraceptives: a database study. Contraception 57:67–70, 1998.
120. MP Vessey, R Doll. Investigation of relation between use of oral contraceptives and thromboembolic disease. A further report. Br Med J 2:651–657, 1969.
121. S Suissa, L Blais, WO Spitzer, J Cusson, M Lewis, L Heinemann. First-time use of newer oral contraceptives and the risk of venous thromboembolism. Contraception 56:141–146, 1997.
122. MA Lewis, LAJ Heinemann, KD Mac Rae, R Bruppacher, WO Spitzer. The increased risk of venous thromboembolism and the use of third-generation progestogens: role of bias in observational research. Contraception 54:5–13, 1996.
123. HW Van Lunsen. Recent oral contraceptive use patterns in four European countries: evidence for selective prescribing of oral contraceptives containing third generation progestogens. Eur J Contracept Reprod Health Care 1:39–45, 1996.
124. C Jamin, J De Mouzon. Selective prescribing of third generation oral contraceptives (OCs). Contraception 54:55–56, 1996.
125. O Lidegaard. The influence of thrombotic risk factors when oral contraceptives are prescribed. A control-only study. Acta Obstet Gynecol Scand 76:252–260, 1997.

126. T Koster, FR Rosendaal. Factor VII and fibrinogen levels as risk factors for venous thrombosis. A case control study of plasma levels and DNA polymorphisms. Thromb Haemost 71:719–722, 1994.
127. M Thorogood. Oral contraceptives and myocardial infarction: new evidence leaves unanswered questions. Thromb Haemost 78:334–338, 1997.
128. D Petitti, S Sidney, CP Quesenberry. Oral contraceptive use and myocardial infarction. Contraception 57:143–155, 1998.
129. S Sidney, DB Petitti, CP Quesenberry Jr, AL Klatsky, MK Ziel, S Wolf. Myocardial infarction in users of low-dose oral contraceptives. Obstet Gynecol 88:939–944, 1996.
130. WHO Collaborative Study of Cardiovascular Disease and Steroid Hormone Contraception. Acute myocardial infarction and combined oral contraceptives: results of an international multicentre case–control study. Lancet 349:1202–1209, 1997.
131. M Thorogood. Stroke and steroid hormonal contraception. Contraception 57:157–167, 1998.
132. SM Schwartz, DS Siscovick, WT Longstreth Jr, BM Psaty, RK Beverly, TE Raghunathan, D Lin, TD Koepsell. Use of low-dose oral contraceptives and stroke in young women. Ann Intern Med 127:596–603, 1997.
133. DB Petitti, S Sidney, A Bernstein, S Wolf, C Quesenberry, HK Ziel. Stroke in users of low-dose oral contraceptives. N Engl J Med 335:8–15, 1996.
134. WHO Collaborative Study of Cardiovascular Disease and Steroid Hormone Contraception. Haemorrhagic stroke, overall stroke risk, and combined oral contraceptives: results of an international, multicentre, case-control study. Lancet 348:505-510, 1996.
135. LAJ Heinemann, MA Lewis, M Thorogood, WO Spitzer, I Guggenmoos-Holzmann, R Bruppacher, the Transnational Research Group on Oral Contraceptives and the Health of Young Women. Case–control study of oral contraceptives and risk of thromboembolic stroke: results from international study on oral contraceptives and health of young women. Br Med J 315:1502–1504, 1997.
136. JJ Ferguson, MGV Jenkins. General practitioners in England prescribed second generation pills instead [Letter to the Editor]. Br Med J 313:363, 1996.
137. OE Iversen, ST Nilsen. Abortions increased by nearly 8% in Norway [Letter to the Editor]. Br Med J 313:363–364, 1996.
138. JM Alexandre, K Strandberg. EMEA and third generation oral contraceptives [Letter to the Editor]. Lancet 350:290, 1997.

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10

Levonorgestrel-Releasing Contraceptive Implants

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I. INTRODUCTION

Research on contraceptive implants began with the demonstration that steroids could be released from capsules manufactured from the polymer Silastic. Various progestins were investigated, and by 1974 the six-capsule Silastic drug-delivery system, which was to be termed the Norplant contraceptive system, was perfected and made ready for clinical trials. By 1978 sufficient data were available from these clinical studies to indicate that the failure rate of Norplant implants was unexpectedly low: 0.6% after 2 years. In 1983, Leiras Pharmaceuticals of Turku, Finland, was licensed to manufacture Norplant, and Finland became the first country to give regulatory approval for the distribution of this new contraceptive. In 1984, the World Health Organization (WHO) made a technical evaluation of the Norplant method in response to a request by the United Nations Fund for Population Activities (UNFPA). WHO concluded that Norplant implants are an effective, reversible, long-term method of fertility regulation that has particular advantage for women who wish to have an extended period of contraceptive protection. After approval of Norplant implants by the U.S. Food and Drug Administration (FDA), Wyeth-Ayerst Laboratories began to distribute this implantable contraceptive in the United States as the Norplant system. More than 50 countries have now approved Norplant implants for distribution for use in women.

II. WHAT IS THE NORPLANT CONTRACEPTIVE SYSTEM?

Norplant implants are a method of providing a progestin-only contraceptive for women. They consist of six soft, flexible capsules of Silastic rubber that contain levonorgestrel. This hormone is also used in other contraceptives, such as the progestin-only minipill and in several widely used progestin–estrogen combination pills. The capsules are inserted in a superficial plane beneath the skin on the inside of the upper arm. It is recommended that Norplant implants be inserted at the time of menstruation. Once in place, levonorgestrel diffuses through the wall of the Silastic capsule into the bloodstream and provides effective protection against pregnancy during the first month and through 5 years of use (1).

Immediately after implantation, the dose of levonorgestrel provided by Norplant implants is about 85 μg/day. This declines to about 50 μg/day by 9 months, to about 35 μg/day by 18 months, and to about 30 μg/day over the remaining 5 years of use.

The blood levels of levonorgestrel that result as this progestin diffuses out of the Silastic capsules are lower than those usually observed in women using combined oral contraceptives containing 100–150 μg of levonorgestrel. As noted below, however, the greater effectiveness of the implants relates to the constancy of the blood levels, rather than the peaks and valleys that occur after the use of each pill. The concentrations of levonorgestrel in women using Norplant implants are constant, the serum concentrations in individual women show considerable variation, depending on individual clearance rates, body weight, and other possible factors. Nonetheless, levels in an individual remain relatively consistent: women with high levels tend to maintain such levels, and women with low levels tend to maintain low levels.

III. MECHANISM OF ACTION

The mechanism of action of Norplant implants is of interest in view of the high effectiveness that results from a low dose of levonorgestrel. Every-other-day blood samples from a randomly selected group of users show serum progesterone levels compatible with ovulation in some women (2). Furthermore, the percentage of women with elevated serum progesterone levels suggestive of ovulation increases with longer durations of Norplant implant use. After 3–5 years, approximately 50% of women using implants have progesterone levels consistent with ovulation. Thus, ovulation suppression cannot account entirely for this method's extremely high effectiveness (2).

Norplant implant users with regular menstrual periods are more likely to have elevated progesterone consistent with ovulation. In such women, the luteal phase progesterone levels are 50% lower than in ovulatory women who are not

taking hormonal contraceptives. In these regularly cycling Norplant users, the midcycle surges of luteinizing hormone are blunted even though cyclic estradiol secretion by the ovary continues (2). These results suggest that not all Norplant implant users who have elevated progesterone levels ovulate. Such individuals may secrete progesterone from a luteinized follicle or from an ''inadequate corpus luteum.'' Ultrasonic studies show that many women who have regular menses while using Norplant implants develop a follicle that does not rupture, but regresses over several weeks.

Examination of cervical mucus from Norplant implant users shows that most women have scanty mucus that is extremely viscous, and thus sperm penetration is impaired (3). These observations suggest that levonorgestrel increases the viscosity of cervical mucus, thus changing its structure. As a result, sperm cannot enter the uterus and fertilization cannot occur (3).

Norplant implants, therefore, mediate their effects on fertility by a variety of mechanisms, including suppression of ovulation, alteration of cervical mucus, and possible other effects. These actions of levonorgestrel are mediated on the cervix and on the pituitary–gonadal axis and are the principal mechanisms by which Norplant implants provide extremely effective contraception.

To ensure that the woman is not pregnant at the time of capsule placement and to ensure contraceptive effectiveness during the first cycle of use, it is advisable that insertion be done during the first 7 days of the menstrual cycle or immediately following an abortion. However, Norplant implants may be inserted at any time during the cycle provided pregnancy has been excluded and a nonhormonal contraceptive method is used for at least 7 days following insertion. Insertion is not recommended before 6 weeks postpartum in women who are breastfeeding.

IV. EFFECTIVENESS

In the original pivotal trials on Norplant implants, only 0.2% of all continuing users became pregnant in the first year (4). In the second year, the pregnancy rate was 0.5% for all users. During years 3–5, the pregnancy rates rose to slightly over 1% (Table 1). The cumulative rate was 3.9% at 5 years. After 2 years of use, differences in pregnancy rates were noted among women of differing weights. For example, in women weighing less than 50 kg, there were almost no pregnancies, even after 5 years of use, whereas in women weighing more than 70 kg, the yearly pregnancy rate rose to 2.5–5.1% in years 3 and 4. In the clinical trials from which these data were derived, Norplant implants were manufactured from two types of silicone rubber tubing. Approximately one-fourth of the women used Norplant implants manufactured from silicone rubber that was slightly more flexible (soft tubing) and that released slightly more

Table 1 Annual and 5-Year Cumulative Discontinuation Rates per 100 Users of Norplant Made Predominantly with Hard Tubing

	Year 1	Year 2	Year 3	Year 4	Year 5	Cumulative
Pregnancy	0.2	0.5	1.2	1.6	0.4	3.9
Bleeding problems	9.1	7.9	4.9	3.3	2.9	25.1
Medical problems (excluding bleeding irregularities)	6.0	5.6	4.1	4.0	5.1	22.4
Personal reasons	4.6	7.7	11.7	10.7	11.7	38.7
Continuation	81.0	77.4	79.2	76.7	77.6	29.5

Table 2 Annual and 5-Year Cumulative Discontinuation Rates per 100 Continuing Users of Norplant Implants Made with Soft Tubing

	Year 1	Year 2	Year 3	Year 4	Year 5	Cumulative
Pregnancy	0.0	0.0	0.3	0.0	1.0	1.3
Bleeding problems	5.1	4.9	4.2	2.0	2.6	17.5
Medical problems (excluding bleeding irregularities)	4.3	5.9	4.8	4.1	3.7	20.8
Planning pregnancy	0.9	2.9	6.4	8.8	6.4	23.2
Personal reasons	1.3	3.5	2.4	5.1	5.3	16.4
Continuation	88.3	83.7	82.8	80.0	80.0	39.3

levonorgestrel than the implants made from less flexible (hard tubing) that were used in the other three-fourths of the women. The study showed that women using implants manufactured from soft tubing had fewer pregnancies than those who used implants made with hard tubing.

Because most of the results in the pivotal trials were from women using implants made from hard tubing, the efficacy data used in the labeling reflect the results of hard tubing (see Table 1). A subsequent clinical trial confirmed that the effectiveness of Norplant implants made with soft tubing is much greater than that of implants made with hard tubing (5). In a study of 511 women, only 3 women became pregnant with a cumulative 5-year rate of 1.3:100 continuing users (Table 2). Women younger than 25 years of age had a cumulative pregnancy rate at 5 years of 2.8%, and women older than 25 years had a rate of 0.7% of continuing users. No women who weighed less than 79 kg at admission became pregnant; the three who became pregnant weighed 79, 82, and 100 kg. Implants

made with soft tubing are the contraceptive devices that are marketed throughout the world.

When implants were used for longer than 5 years, the yearly pregnancy rate rose to 2.5–3.0% for all users (4). The latter rates are comparable with those for oral contraceptives (OCs) and are higher than during the previous years. Therefore, Norplant implants are replaced at the end of 5 years. This schedule provides a 1-year margin for noncompliance in which the protection afforded is equivalent to that of OCs (4).

To place the effectiveness of Norplant implants in perspective, typical pregnancy rates in various contraceptive methods for the first year of use are as follows: spermicides, 21%; diaphragm, 18%; male condom, 12%; female condom, 21%; progestin-only pill, 7–9%; combined pill, 2–6%; Copper T 380A, 0.8%; Norplant, 0.09%; female sterilization, 0.4%; and male sterilization, 0.15% (6). The efficacy of most methods partly depends on the reliability of use. By contrast, the efficacy of Norplant implants, similar to that of intrauterine devices (IUDs) and sterilization, does not depend on patient compliance.

The effects of Norplant implants are completely reversible. Observations in women who terminated use because they desired to become pregnant suggested that fecundity is about the same as it was at the time of marriage. The life-table pregnancy rates were 24% at 1 month after removal, 90% at 1 year, and 95% at 2 years (4).

V. INSERTION AND REMOVAL

Insertion and removal are not difficult procedures, but instructions must be followed closely. It is strongly advised that all health care professionals who insert and remove Norplant implant capsules be instructed in the procedures before they attempt them. A proper insertion just under the skin will ensure that implants are not displaced from the site of insertion and will facilitate removals. Proper implant insertion and removal should result in minimal scarring. If the capsules are placed too deeply, they can be harder to remove. The original method of removal was developed by a group of physicians with extensive experience in Norplant use. As is true with many medical procedures, other physicians have had ideas about how removal could be performed. As a result, several modifications of the removal procedure have been described in the medical literature, including the pop-out method, the U-technique, and the hook–traction technique (7). Physicians who have used multiple procedures report that implants are relatively easy to remove with all procedures when they have been inserted correctly, and are difficult with any technique when they have been inserted improperly. There have been infrequent reports of the use of general anesthesia during the removal procedure; this is generally not required.

Before initiating the removal procedure, all Norplant capsules should be located by palpation. If all six capsules cannot be palpated, they may be localized by ultrasound (7 MHz), or x-ray, with a technique similar to compression mammography. If all capsules cannot be removed at the first attempt, removal should be attempted later when the site has healed. Bruising may occur at the implant site during insertion or removal. Other cutaneous reactions that have been reported include blistering, ulcerations, and sloughing. There have been reports of arm pain, numbness, and tingling following these procedures. In some women, hyperpigmentation occurs over the implantation site, but this is usually reversible following removal.

There have been reports of capsule displacement (i.e., movement), most of which involve minor changes in the positioning of the capsules. However, infrequent reports of significant displacement (a few to several inches) have been received. In some instances displacement is associated with improper insertion. For example, in one woman a Norplant implant that was inserted into a muscle migrated along the muscle to the end of the arm. Such displacement is sometimes associated with pain or discomfort. In the event that capsule movement occurs, the removal technique may need to be modified, such as additional incisions or visits.

Dunson et al. (7) reported the complications associated with removal of Norplant implants in 3416 users from 11 countries. More than three-fourths of all complications were due to implants that were broken by the health care provider doing the removal (1.7%), embedded implants below the subdermal plane (1.2%), and displaced implants (0.6%). Removal complications occurred in 4.6% of women in this study. Most complications resulted in longer removal times. The mean removal times were 11.5 min and 29.7 min for implants without and with complications, respectively. It was concluded that proper insertion by well-trained health care providers will reduce the number of difficult removals.

VI. CONTRAINDICATIONS

Who should not use Norplant implants? Until greater experience is available, clinical judgment and experience with other hormonal contraceptives suggest that Norplant implants should not be used in women with active thrombophlebitis or thromboembolic disorders, undiagnosed abnormal genital bleeding, known or suspected pregnancy, acute liver disease, benign or malignant liver tumors, known or suspected carcinoma of the breast, history of idiopathic intracranial hypertension, or hypersensitivity to levonorgestrel or any of the components of the Norplant implants.

VII. ADVERSE EFFECTS

A. Menstrual Problems

The major reason women with Norplant implants discontinue their use is because of menstrual problems similar to those associated with other progestin-only methods (1,4,5). Therefore, women must be warned of the probability of irregular bleeding or spotting. A typical woman is likely to have increases in the numbers of bleeding or spotting days per cycle. Menstrual diaries show that women who eventually terminate Norplant implant usage because of menstrual problems tend to have more bleeding days than do other users. Even though menometrorrhagia is the prime reason for terminating implant use, hemoglobin values are not usually lower in women who stop use because of menstrual problems. Furthermore, anemia is a rare side effect of the implants. These findings are consistent with the observations that menstrual flow volume decreases during implant use (8). Thus, women who keep menstrual diaries over a 5-year period document an overall decrease in bleeding events. The total number of bleeding days per year declines significantly from a mean of 54 days per year in year 1 to 44 in year 5 (8). The total number of spotting days per year also decreases significantly after the first year.

Disruption of the normal cycle during implant use produces long intervals without overt bleeding, although spotting often occurs in such intervals. Because delayed menses can be interpreted as pregnancy, a long interval of amenorrhea may be stressful for women who are not counseled in advance and who are not reassured that the Norplant implants are unlikely to fail. In fact, the typical woman who becomes pregnant while using Norplant implants has regular menstrual bleeding that is then interrupted by a period of amenorrhea, which signals the onset of pregnancy. Thus, women with oligomenorrhea or amenorrhea are unlikely to become pregnant and should be counseled accordingly.

B. Medical Problems

A significant number of women stop using the implants for medical reasons other than menstrual problems (see Table 1).

1. Delayed Follicular Atresia and Ovarian Cysts

Ovarian cysts develop in some women using Norplant implants. These cysts, some of which are 7–10 cm in diameter, may be detected by palpation at the time of routine pelvic examination, and regress over several weeks. Daily ultrasonographic studies performed in Norplant implant users who had regular menses show that the following sequence occurs in some cycles: a dominant follicle is

selected, ovulation does not occur, the follicle persists for up to 2 weeks past the time of expected ovulation, and involution gradually occurs (2). We, therefore, conclude that use of Norplant implants is associated with delayed follicular atresia in some women. Because the enlarged follicles may be indistinguishable from ovarian cysts of other causes to the casual examiner, women using Norplant implants and their health care providers should be advised that delayed atresia occurs and that invasive measures are not required. Rather, these women should be observed for 6 weeks or until regression occurs. If regression does not occur, the ovarian enlargement must be further evaluated. The incidence of ovarian cysts requiring surgery is no greater than that in women who do not use hormonal contraception.

2. *Infection or Pain*

Another medical reason for terminating Norplant implant use is infection at the site of insertion. This rare event (0.7% of users) is usually associated with inadequate asepsis and no longer occurs once health care providers gain experience. If infection occurs, suitable treatment is instituted; if infection persists, the implants should be removed. Pain or itching at the implant site occurs in 3.7% of users (4). These complaints are usually transient.

3. *Drug Interactions*

Certain drugs used to treat epilepsy increase the risk of pregnancy in implant users because they increase the rate of levonorgestrel metabolism. Evidence of drug interaction is most convincing with phenytoin or carbamazepine (9,10).

4. *Ectopic Pregnancy*

Because ovulation and subsequent pregnancy do occasionally occur, there is concern that users may be subject to a heightened risk of ectopic pregnancy, a factor of marked importance in users of progestin-only contraception. Ectopic pregnancies have occurred among users of Norplant capsules at an average rate of 1.3:1000 woman-years. The risk of ectopic pregnancy may increase, with duration of use, and possibly with increased body weight. Thus, implants must be removed after 5 years (1,4,5).

To put this ectopic pregnancy rate into perspective, the rate for all women in the United States in 1992 was calculated from the combined ectopic pregnancies treated in hospitals plus outpatient clinics. In that year 108,800 ectopic pregnancies were recorded in approximately 60 million women, a rate of 1.8:1000. The reason that Norplant implants might reduce ectopic pregnancy rate is because this contraceptive is so effective at protecting women against

all types of pregnancy. Nonetheless, a pregnancy in a Norplant implant user is more likely to be ectopic than in women not using contraception, as about 20% of Norplant users who become pregnant will have an ectopic pregnancy. Thus, physicians should be alert to the possibility of ectopic pregnancy in all Norplant users who have had regular menses followed by amenorrhea and who have a positive pregnancy test.

5. Foreign Body Carcinogenesis

Rarely, cancers occur at the site of foreign bodies or old scars. None has been reported in clinical trials of Norplant implants. In rodents, which are highly susceptible to such cancers, the incidence decreases with decreasing size of the foreign body. Because of the resistance of human beings to these cancers and because of the small size of the capsules, the risk to users of Norplant implants is judged to be minimal.

6. Idiopathic Intracranial Hypertension

Idiopathic intracranial hypertension (pseuduotumor cerebri) is a disorder of unknown etiology that is seen most commonly in obese women of reproductive age. Growth hormone treatment is also associated with the appearance of this disorder. There have been reports of idiopathic intracranial hypertension in Norplant implant users. Cardinal signs include visual disturbances and headaches. The headaches are often different from those previously experienced by the women for frequency patterns, severity, or duration. Of particular importance are those headaches that are unremitting, and in obese patients with these symptoms with recent weight gain. These women should be screened for papilledema, and, if present, the patient should be referred to a neurologist for further diagnosis and care. Norplant implants should be removed from patients experiencing this disorder.

7. Other Adverse Reactions

Approximately 50% of Norplant users with medical problems (see Tables 1 and 2) have complaints commonly associated with other steroid-containing contraceptives. Comparative clinical trials suggest that the following adverse reactions occurring during the first year are probably associated with use of Norplant implants: headache, nervousness, nausea, dizziness. adnexal enlargement, acne, mastalgia, weight gain, and hirsutism. In addition, the following adverse reactions have been reported with a frequency of 5% or more during the first year and possibly may be related to Norplant implant use: breast discharge, cervicitis, musculoskeletal pain, abdominal discomfort, leukorrhea, and vaginitis (4,5).

The pivotal studies used to define the adverse reactions to Norplant implants involved 2470 women. This exposure, even though quite large for a newly introduced method, is not sufficient to determine whether Norplant implants are associated with many of the rare, but serious, risks of combined OCs.

VIII. BENEFITS

A. Contraception

Pregnancy and abortion are leading causes of death in women younger than 35 in developing countries. To put this into perspective, women in the United States are 38 times more likely to die in an automobile accident than from pregnancy or abortion. Nonetheless, contraception can also reduce maternal mortality even in the United States. Child mortality can also be significantly reduced by preventing teenage pregnancy and by spacing of births. Thus, in addition to allowing women to have children when they want, contraception has a beneficial effect on both maternal and child health. One might argue that the more effective the contraceptive, the greater is this health effect. The Norplant implant system is one of the most effective contraceptives yet devised, ranking with sterilization and modern IUDs (such as the TCu 380A), for preventing pregnancy.

B. Convenience

When women are questioned on what they liked best about Norplant implants after 5 years of use, the majority respond "convenience." That is, after insertion, implants promoted long-term contraception that does not require daily intake, use with each intercourse, or frequent return to the clinic for new supplies.

C. Reduction of Adverse Reactions

Many women who choose to use Norplant implants do so owing to previously discontinued OCs because of annoying medical problems, such as nausea, lightheadedness, and weight gain. Many former users of OCs continued to use Norplant because they felt there were fewer adverse reactions.

D. Prevention of Anemia

The average blood loss during the first year of implant use was not increased, even though bleeding and spotting did increase. After the first year, menstrual blood loss was reduced, and hemoglobin levels rose (8).

IX. WHO MAY WISH TO USE NORPLANT IMPLANTS

1. Women who desire highly effective, low-dose hormonal contraception
2. Women who want long-term contraception after completing their family, but do not want sterilization
3. Women who wish to delay having children for long periods
4. Women who cannot use estrogen
5. Women who are unhappy with other forms of contraception

X. WHO MAY NOT WISH TO USE NORPLANT IMPLANTS

1. Women who are happy with their present method of contraception
2. Women who do not want to use a method that requires a visit to a health care provider to discontinue
3. Women who do not wish to or cannot pay the up-front cost of Norplant implants, even though they know that the overall cost over 5 years is lower
4. Women who do not wish to tolerate irregular menstrual bleeding, should it occur

XI. NORPLANT II

During the development of the Norplant implant system, clinicians involved in the clinical trials realized that an implant system with fewer than six capsules would likely be much easier to insert and remove (4). After many studies, implants were structurally modified from capsules to covered rods and lengthened so that two 4-cm covered rods (Norplant II) could release the same amount of levonorgestrel as six 3-cm capsules. Five-year clinical trials showed that the safety and efficacy of Norplant II implants were the same as the Norplant implant manufactured with soft tubing (11). These clinical trials also confirmed that Norplant II implants were easier to insert and remove. In 1995 an application was made to the FDA to market Norplant II implants in the United States. The Norplant II contraceptive was recently approved by the FDA.

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REFERENCES

1. Bardin CW. Long-acting steroidal contraception: an update. Int J Fertil 1989; 34 (suppl):88–95.
2. Alvarez-Sanchez F, Brache V, Tejada AS, Faundes A. Abnormal endocrine profile among women with confirmed or presumed ovulation during long-term Norplant use. Contraception 1986; 33:111–119.
3. Croxatto HB, Diaz S, Salvatierra AM, et al. Treatment with Norplant subdermal implants inhibits sperm penetration through cervical mucus in vitro. Contraception 1987; 36:192–201.
4. Sivin I, International experience with Norplant and Norplant 2 contraceptives. Stud Fam Plann 1988; 19:81–94.
5. Sivin J, Mishell DR Jr, Darney PH, Wan L , Christ M. Levonorgestrel capsule implants in the United States: a 5-year study. Obstet. Gynecol. 1998; 92:337–344.
6. Trussell J, Hatcher RA, Cates W Jr, et al. Contraceptive failure in the United States: an update. Stud Fam Plann 1990; 21:51–54.
7. Dunson TR, Amatya RN, Krueger SL. Complications and risk factors associated with the removal of Norplant implants. Obstet Gynecol 1995; 85:543–548.
8. Nilsson CG, Holma P. Menstrual blood loss with contraceptive subdermal levonorgestrel implants. Fertil Steril 1981; 35:304–306.
9. Haukkamaa M. Contraception by Norplant subdermal capsules is not reliable in epileptic patients on anticonvulsant treatment. Contraception 1986; 33:559–565.
10. Odlind V, Olsson SE. Enhanced metabolism of levonorgestrel during phenytoïn treatment in a woman with Norplant implants. Contraception 1986; 33:257–261.
11. Sivin I, Campodonico I, Kiriwat O, Holma P, Diaz S, Wan L, Biswas A, Viegas O, el din Abdalla K, Anant MP, Pavez M, Stern J. The performance of levonorgestrel rod and Norplant contraceptive implants: a 5-year randomized study. Hum Reprod 1998: 13:3371–3378.

11

Progestin-Releasing Intrauterine Devices

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I. DEVICES

There are two commercially available progestin-releasing intrauterine devices (IUDs), or intrauterine systems (IUSs), as these are called by the manufacturing companies. The first intrauterine progesterone-releasing contraceptive system on the market was Progestasert. It received regulatory approval in the United States in 1976 and has been on the market since that date. It is manufactured in the United States in Palo Alto, California by ALZA Corporation and is currently available in the United States, Canada, and France. The drug reservoir contains 38 mg of progesterone, with a release rate of 65 μg/day. According to the company (1), the system is indicated for 1 year of intrauterine contraception in parous women who are in a stable, mutually monogamous relationship, and who have no history of pelvic inflammatory disease. After 12 months, the woman must return to her clinic to have the system removed or replaced.

The intrauterine levonorgestrel-releasing system Mirena was approved in Finland in 1990. It is manufactured by Leiras OY, Turku, Finland, and it is currently available in Austria, Belgium, Denmark, Estonia, Finland, France, Germany, Greece, Israel, Italy, Luxembourg, Netherlands, Poland, Portugal, Singapore, Switzerland, and United Kingdom for contraceptive use. It is also available in most countries for treatment of menorrhagia and in some countries for menopausal use.

A steroid reservoir surrounds the vertical arm of a T-shaped carrier device. The levonorgestrel (LNG) is dispersed in polydimethylsiloxane and is covered

with a membrane of the same material, which controls the release rate. The reservoir contains 52 mg of LNG. The initial release is 20 μg/24 h. The release rate slowly decreases to about 15 μg/24 h at 5 years, and to about 12 μg/24 h at 7 years. The plasma levels of levonorgestrel are of 170 pg/mL at 1 year, 150 pg/mL at 2 years, and 140 pg/mL at 5 years (P. Lähteenmäki, personal communication).

The device's currently approved lifetime is 5 years; removal should occur in the sixth year. If the woman desires continued contraception by this method the device can be replaced with a new one at the same visit for removal of the old device.

There are current reviews on progestin-releasing IUDs for historic and developmental aspects (2) and for instructions on clinical use (3). Only the research on the LNG-releasing IUD has been reviewed (4), and a risk–benefit assessment of its use has been published (5,6). The interested reader is referred to these reports for additional information.

II. PROGESTASERT

The name Progestasert first appeared in the report of Pharris et al. in 1974 (7). The device in their study was T-shaped with a steroid reservoir. The release was controlled by ethylene vinyl acetate copolymer, and progesterone was dispersed in silicone fluid with barium sulfate to make it radiopaque. The lifetime of the device was 1 year.

The requirements for toxicological studies were minimal with Progestasert for two reasons. First, the intrauterine release of progesterone was in amounts that are only fractions of the daily release of progesterone in the uterine cavity by the placenta during pregnancy. Second was the short lifetime of the device. Therefore, the Progestasert system was quickly introduced to the market. After 3 years on the market, the effectiveness of the device was well evaluated in a well-designed double-blind study by Newton et al. (8). In this randomized study, 600 women used either an inert control device without progesterone or the active Progestasert for 1 year. The active Progestasert and the control device were visually identical so that neither the women nor the doctors could differentiate them at insertion.

A. The Contraceptive Effectiveness of Progestasert

The study by Newton et al. (8) demonstrated that the intrauterine release of progesterone significantly reduced the pregnancy rate compared with the control device. The cumulative net pregnancy rate at 1 year for parous women was 0.9:100 women. Finally, the hopes and the hypothesis of the development of this device had now, 1979, been proved to be correct. However, the hope that

the release of progesterone would decrease the expulsion rate was not verified. The clinical performance was evaluated in the second part of their study.

The package insert dated 12/89 indicates higher total pregnancy rates of 1.8 for parous and 2.5 for nulliparous per 100 women in 1 year of use. The total pregnancy rate includes ectopic pregnancies (0.5 for parous and 0.4 for nulliparous per 100 women) (1). Progestasert's lack of protection against ectopic pregnancy has been a main concern; in fact, it may even slightly increase the risk (9–11). Effectiveness no better than first-generation copper IUDs, and duration of action of only about 1 year are factors in Progestasert's limited use as a contraceptive method.

B. Endometrial Protection in Hormonal Replacement Therapy

There is no demonstration that the minute amount of progesterone released in the uterine cavity from Progestasert has any systemic effects. Studies do show, however, that during the use of Progestasert there is a significant reduction of menstrual blood loss (12). The reduction of blood loss is the result of the suppression of the endometrium. Two investigations have evaluated the use of intrauterine release of progesterone in hormonal replacement therapy (HRT) (13,14). The intrauterine administration of progesterone did not change the circulating progesterone concentrations (13,14); therefore, no systemic effects of progesterone were observed. The estrogen-induced rise in high-density lipoprotein (HDL) cholesterol is not suppressed (13,14), and the slight increase in serum estradiol concentration during the last months of the first year use (14) show that estradiol's effect on the synthesis of sex hormone-binding globulin (SHBG) is not affected. In addition, the effect on the lumbar spine mineral density during the first year was similar to that with estrogen-only replacement therapy (14).

The studies on endometrial morphology revealed a uniform progestin effect. Late secretory changes and pseudodecidualized stroma were observed, but without proliferation during the later part of treatment with progesterone (13,14). The decrease in the duration of spotting and bleeding corresponded with the endometrial morphology. The treatment was highly accepted, but the duration of the progesterone release from Progestasert is 18 months maximum.

III. MIRENA, LEVONORGESTREL-RELEASING INTRAUTERINE SYSTEM

A. Effectiveness and Mode of Contraceptive Action

The clinical trials have demonstrated that intrauterine release of levonorgestrel (LNG) is highly effective during long-term use. It is equally effective among

young and older women, with Pearl pregnancy rates from 0 to 0.2 over 7 years of use. It seems that the rare failures to prevent accidental pregnancies are associated with unnoticed expulsion or with problems with the insertion. The insertions have been performed during the first 7 days of a woman's cycle, immediately after abortion, or during lactation, with good results. The method is highly accepted by well-informed women, and the continuation rates have been about 80:100 women at 1 year (15) and up to 56:100 women at the end of 6 years of use (16).

In several trials there were no pregnancies. The duration of the trial was 3 years in India (17) and 4 years in the Netherlands (18). In the multinational study in Europe there were no pregnancies among 1138 women over 5 years in clinics in Denmark, Hungary, and Sweden. Faundes and associates reported no pregnancies during 7 years (19). The Pearl pregnancy rate of zero in these trials also indicates that there were no ectopic pregnancies. Sivin et al. (20) reported in the Population Council trial no ectopic pregnancies during 7 years with an experience of 3371 woman-years. Prevention of implantation does not explain this high rate of effectiveness because it does not account for the absence of ectopic pregnancies. The mode of the contraceptive action, therefore, is the prevention of fertilization. Croxatto and coworkers (21) speculate that there must be an extrauterine mechanism of action of the IUD in the prevention of fertilization. However, it is possible that copper-releasing IUDs do prevent fertilization with mechanisms that are different from those associated with the use of Mirena, the LNG-releasing IUS.

The intrauterine release of LNG has a strong local action (22) owing to the high tissue concentration of progestin in the endometrium (23). The endometrium becomes insensitive to the circulating estradiol as the high tissue concentration of progestin inhibits the synthesis of the estradiol receptor (24). As a result, a strong antiproliferative effect is seen. The proliferate and secretory endometrium produce many highly active compounds (e.g., prostaglandins, estrogen and estrogen–progesterone-induced growth factors, and other bioactive peptides). When the endometrium is suppressed, the production of many of these regulatory factors ceases. On the other hand, progestin stimulates the synthesis of proteins, such as insulin-like growth factor-binding protein in the stroma (25). It is more likely that some of these changes in the normal function of the endometrium are responsible for the prevention of fertilization.

The prevention of ectopic pregnancies is in sharp contrast with the results from trials with Progestasert and its bioequivalent LNG device used by WHO (26). This device initially released 2 μg LNG daily, but the release in vivo is unreported. The development of this approach was discontinued owing to the high number of accidental pregnancies and ectopic pregnancies observed. The endometrial suppression, as judged by bleeding control and the endometrial mor-

phology, is far less than that with Mirena. The studies on endometrial function during use of Progestasert and Mirena could demonstrate the difference in the function and could elucidate how Mirena prevents fertilization.

B. Bleeding Control, Menstrual Blood Loss and Amenorrhea

During the first 3 months following insertion, the endometrium is transformed into a thin, inactive epithelium that resembles that seen during lactational amenorrhea or during the postmenopausal years. The suppression of endometrium leads to a substantial reduction in duration and amount of menstrual bleeding. Many women, about 20%, have no bleeding at all at the end of the first year after insertion. With the Mirena IUS in place, the endometrium becomes nonproliferative and is insensitive to the estradiol produced by the ovaries. Therefore, this endometrial amenorrhea occurs in spite of completely normal ovarian function (16,27). Amenorrhea is not a side effect of the treatment, it is a sign that the system is in the proper fundal position. The correct fundal position is necessary to ensure the uniform domination of the endometrium by progestin. The correct position is also important for prevention of expulsion, for full noncontraceptive health benefits, and for uniform therapeutic effects. Amenorrhea is also highly accepted in well-informed women; in Sweden the cumulative gross removal rate at 5 years was only 1.7:100 women (27), and Tang reported high acceptance of amenorrhea in Chinese women who were treated for menorrhagia with Mirena (28). Understanding this medically induced local amenorrhea, or, better stated, absence of bleeding, is important in the counseling of potential users of Mirena. Women, providers, and medical personnel need to be informed that amenorrhea during use of Mirena is the goal of its use, rather than being a side effect. It is not a symptom of pregnancy, nor a disturbance of ovarian or pituitary function. Normal menstruation returns in 1 month after the removal of the device, even among women who have been amenorrheic for 7 years (27). Removals for the reason of amenorrhea are unnecessary in well-informed women, and most women find that they prefer the absence of menstrual bleeding.

Women with heavy menstruation or women who cannot use conventional IUDs because of menorrhagia can use Mirena with good results. Well-informed women tolerate the infrequent or irregular bleeding. The discontinuation for bleeding problems is significantly less at 1 year for users of Mirena than for users of copper IUDs (19).

No reduction in menstrual blood loss indicates that the device is not properly placed in the uterine cavity. Dislocation of the device in the myometrium or in the peritoneal cavity reduces its effectiveness on both bleeding control and pregnancy prevention (29,30).

C. Insertion of Mirena

The timing of insertion in menstruating women has been during the first 7 days of the cycle. Postabortal insertions have been done immediately after a first-trimester legal abortion (length of gestation less than 12 weeks). The experience with 5 years follow-up has been very satisfactory. The insertions during lactational amenorrhea have been performed during full lactation 6–8 weeks after delivery. In postmenopausal use, insertions were preceded by 1 month's use of estrogen replacement therapy to prime the cervical canal.

The correct fundal placement is important for suppression of the entire endometrium when Mirena is used for therapeutic purposes. Therefore, paracervical infiltration of local anesthesia and dilation of the cervical canal to 5 mm should be used liberally. Before the insertion, the cervical os is inspected for any sign of cervical infection. Any infection is treated before insertion of the device. Because the insertion technique is different from other IUDs, special emphasis should be given to training in the correct technique. The device is inside the insertion tube during passage through the cervical canal. When the tip of the tube has passed the inner os of the cervical canal, the side arms of the device are released by withdrawing the tube down to a mark on plunger. Then the device is gently lifted upward with the tube into the fundal position. The device should be completely released from the tube by pulling the tube down to the ring part of the plunger. First the plunger and then the tube is removed. With this technique, insertions are safe, and the need for using sound is minimized. Sounding is contraindicated in insertions during lactation.

D. Endometritis and PID

The most important factor in acquiring pelvic inflammatory disease (PID) is the predisposition to sexually transmitted diseases (STDs). Therefore, IUDs, including the progestin-releasing ones, are for women at a low risk for STDs. Special emphasis must be placed on the selection of the users of the method and on counseling. Many studies show a transiently increased risk of STD during the first months after insertion because of the contamination of the uterine cavity by pathogens during the insertion. Therefore, any suspected existing infection of the vagina or cervical canal must be diagnosed and treated before the insertion. Adherence to this policy has eliminated the higher risk of infection during the first months after the insertion (31,32). Furthermore, the infections observed were not related to the parity in a large randomized study with two different copper IUDs. The highest infection rate was in young women less than 25 years of age. The older women had a very low rate of infection during 5 years of follow-up (33). It was concluded that IUDs do not cause infection, but they do not protect against the STD infection progressing to PID. Furthermore, it was

concluded that the reason for infections among young women using IUDs was the sexual behavior of their partners or of the young women (31).

Careful studies evaluating Mirena were conducted in large modern gynecological hospitals with excellent facilities and round-the-clock accessibility to the women in the studies, and which provided follow-up and diagnosis. These studies show that Mirena gives significant protection against the progression of STD infection to PID (34). The women using the control device, a copper IUD, had a lower rate of PID than the average population because of selection, aseptic insertion, and counseling. Only after 3 years was the rate of PID in 3000 women using Mirena, found to be significantly lower than that in copper IUD users. The analysis after 5 years demonstrated a significant prevention of the progression of vaginal and cervical infections to PID and endometritis (32).

The pilot study with 5-years follow-up did show an even lower rate of PID with the use of Mirena than the low rate in the copper IUD users (35). The Population Council study (20), which was not designed to detect PID, reported the same PID rate in women using Mirena or copper IUDs. However, the clinics with good diagnostic facilities reported very low rates of infection among users of Mirena, rates lower than the clinical findings for users of copper IUDs (36). Similar to the Population Council study, the Indian field trial of different IUDs did not include modern PID diagnosis (17) in its design. The typical and strong local action of the progestin released from LNG IUSs on the cervical mucus and on the endometrium, and the reduction of menstrual blood loss (MBL), may contribute to the reduction in relative risk of PID and endometritis seen in sexually active young women. The reduction of the risk of PID is also seen in users of oral contraceptives (OCs), although the effects on cervical mucus and endometrium of OCs are less pronounced than those of LNG IUSs.

E. Therapeutic Use of Mirena

1. Sterilization

Mirena is a highly effective long-acting contraceptive method with a Pearl pregnancy rate 0.0–0.2 for 7 years (19,20). Therefore, Mirena can be used to replace tubal sterilization. A recent study reports that the cumulative probability of pregnancy 10 years after sterilization was 18.5:1000 procedures (37). Tubal sterilization does not rule out the possibility of ectopic pregnancy, even many years after the procedure. The 10-year cumulative probability of ectopic pregnancy for all methods of tubal sterilization combined was 7.3:1000 procedures (38). The same U.S. collaborative, multicenter cohort study has further reported the cumulative probability of hysterectomy after sterilization. The risk of hysterectomy was significantly higher if women reported at the time of sterilization a history of heavy menstrual flow, severe menstrual pain, or bleeding more than

7 days during menstrual cycles (39). For some of these women, hysterectomy later became necessary, as tubal ligation has no beneficial effect on heavy menstrual blood loss or dysmenorrhea; whereas Mirena is the method of choice for treatment of these conditions, in addition to its contraceptive effect. As a contraceptive method, Mirena is also reversible (40), contrary to sterilization. As an alternative to permanent sterilization, Mirena would be highly acceptable to those in their early reproductive years. Among American women using contraception, nearly half of those between the ages 35 and 44 have chosen tubal ligation as their method. In countries where Mirena is available, the continuation rate in the same age group is high: 87:100 women at 1 year (15).

2. *Iron Deficiency Anemia*

All studies in which menstrual blood loss (MBL) has been quantitatively measured demonstrate a significant reduction of MBL during the use of Mirena. This reduction is seen as early as 3 months after insertion and persists for the duration of use of the device. The reduction of MBL results in an improvement of blood hemoglobin. A prospective study with use of Mirena over 40 months in a population with a high incidence of anemia showed improved body iron stores and hemoglobin concentrations (41). Thus, Mirena is useful in the prevention and the treatment of iron deficiency anemia and the depletion of iron stores by heavy menstrual blood loss.

3. *Menorrhagia*

The analysis of the results of a large comparative study in Europe (15) showed that heavy menstrual blood loss was the reason given for discontinuation of use by ten times more women with copper IUDs than by women using Mirena as their contraceptive method. Scholten et al., 1989 (18) reported a significant reduction in MBL of 11 women, 7–12 months after insertion of Mirena. The mean reduction was from 117 to 17 mL, with significant increases in hemoglobin and serum ferritin levels ($p < 0.005$).

Andersson and Rybo (42) and Milsom et al. (43) studied the treatment of idiopathic menorrhagia with Mirena. They included women who each had MBL of over 80 mL/menses during two consecutive cycles and who had a normal or slightly enlarged uterus. In both studies the reduction of MBL was approximately 82% after 3 months and 96% after one year. Comparatively, the rate of reduction of MBL was 21% by flurbiprofen and 44% by tranexamic acid. Tang and Lo (28) reported that Mirena was the safest and most effective medical therapy for menorrhagia. They also studied the acceptability of the treatment in well-informed Chinese women. They emphasized the importance of preuse counseling for long-term compliance.

The noninvasive diagnosis of adenomyosis is possible by transvaginal ultrasonography and by nucleomagnetic resonance imaging (44). Fedele et al. (45) reported their experience with using Mirena in the treatment of adenomyosis-associated menorrhagia. They observed a significant reduction in MBL and a significant increase in hemoglobin, serum ferritin, and serum iron levels. Lipid metabolism and clotting variables remained unchanged. A study in Finland reported that 67% of menorrhagic women who were treated with Mirena while awaiting hysterectomy canceled their surgery because they were satisfied with treatment with Mirena (46).

These studies uniformly demonstrate that Mirena is an effective, long-term treatment for menorrhagia. This less expensive method could replace the invasive procedures for treating this disorder, such as endometrial ablation and hysterectomy, both of which require hospitalization. Furthermore, surgical treatments for menorrhagia result in loss of fertility and are not widely available in developing countries. Thus, the intrauterine levonorgestrel-releasing system is a simple, highly effective, fertility-sparing alternative to the invasive treatments of menorrhagia.

F. Intrauterine Levonorgestrel Release in Hormonal Replacement Therapy

The use of unopposed estrogen replacement therapy leads to increased risk of endometrial cancer (47–49). Therefore, a woman with her uterus still intact needs addition of a progestin as a second component in her hormonal replacement therapy (HRT). The duration of modern sequential progestin is 12–14 days per cycle (50). Depending on the dose and the quality of the progestin, effects can be observed that could counteract the beneficial effects of estrogen therapy. The progestin component can cause unwanted steroidal side effects (51), and it does cause withdrawal bleeding. Because of these side effects, the conventional use of progestin may lead to dissatisfaction with or discontinuation of the entire replacement therapy.

The progestin treatment in HRT can be focused on the target, the endometrium, by releasing progestin locally in the uterine cavity. The daily dose is lower than in the systemic sequential or continuous progestin administration, causing fewer side effects. The tissue concentration of progestin in the endometrium during the use of Mirena far exceeds that found with high systemic doses of levonorgestrel (23), explaining the strong suppression of the endometrium (22) and induction of the absence of the bleeding (52). The endometrium is suppressed because the high concentration of progestin inhibits the synthesis of the endometrial receptor (24). Therefore, the endometrium is insensitive to estradiol, and no proliferative activity is seen (53,54), despite the continuous estrogen replacement.

Women using the levonorgestrel-releasing system for contraception had less risk of PID and endometritis (32,34) compared with those using copper-releasing devices. The peri- and postmenopausal use of Mirena is not associated with PID or endometritis (52,53,56). The large long-term clinical trial for the safety and effectiveness of this contraceptive method shows a significant reduction in the occurrence of uterine fibroids in women who used this device over a period of 7 years (55). This could be of importance in long-term use of Mirena in HRT.

The reported trials on using LNG IUSs as the progestin component of HRT show uniform positive results. Estradiol was administrated continuously by oral route (52), by subdermal implants (56), or by transdermal delivery (53), and ultrasound was used to record the suppression of the endometrium. The endometrium was thin despite the continuous administration of estradiol. After an initial 3- to 4-month phase of occasional spotting, most women had no bleeding or spotting. During the last month of the 1-year treatment, 83–88% of the women had no bleeding or spotting (52,54). All studies reported the uniform finding of a rapid reduction of subjective complaints of symptoms that may have been related to estrogen deficiency. This shows that the intrauterine administration of levonorgestrel had no antagonistic effect on estrogen action outside the uterine cavity.

Endometrial biopsies revealed a pronounced progestin effect on the endometrium and stroma (54). Neither proliferation nor endometrial pathology were observed. All of these studies (52–54,56) reported a high acceptance and high continuation of the treatment. The study with 3 years of follow-up reported a continuation rate of 82:100 women at 3 years (54). One reason for the high continuation rate could be that most women prefer the absence of bleeding in HRT (57). Another factor contributing to the acceptance of this type of HRT could be the absence of systemic progestin-related side effects. Finally, it is a simplified treatment in that women need only remember to take the estrogen component. According to the investigators, discontinuations of the treatment because of pain and bleeding may have been related to the small size of the uterine cavity (53) of those women. Body weights and blood pressures did not change significantly during the study period, and no other significant changes were observed (52).

G. Problems, Side Effects, and Counseling

A common problem with levonorgestrel-releasing IUSs is a change in bleeding pattern, especially during the first months of use. Users should be informed that more frequent episodes of spotting in the early stage are to be expected, and that such spotting is typical of progestin treatment and is not harmful. The goal of treatment is the reduction of amount and duration of menstrual bleeding.

The first indication of this reduction is longer intervals between bleeding. MBL is progressively reduced until there is no bleeding at all. Absence of bleeding is a health benefit, rather than a sign of pregnancy or ovarian or pituitary dysfunction. Successful use of Mirena requires intensive counseling with the users, providers, and community health personnel. This is important to avoid unnecessary removals for amenorrhea.

Some side effects of Mirena reported in studies were considered to be related to the low daily release of levonorgestrel. These steroidal side effects were headache, depression, other mood changes, breast tenderness, acne, and other skin problems. The gross discontinuation rate in Europe for all these side effects combined was 2.7:100 women (15). In another study outside Europe (58), the discontinuation rate for these hormonal reasons was 0.7, not unlike that found in users of copper IUDs. Women should be informed about the effectiveness and the mode of action of this method, and reassured that fertility and normal menstrual function return immediately after removal of the IUS.

IV. RECENT DEVELOPMENTS

Young nulliparous women with small uterine size have few options among the contraceptive methods. These women typically have problems with conventional IUDs, and they are less than ideal users of Norplant. The methods requiring daily motivation have poor compliance and low continuation rates. Being both fertile and sexually active early, this population particularly needs a safe, effective, long-term contraceptive method specifically designed for this phase of reproductive life. Studies have shown that the current models of levonorgestrel-releasing intracervical devices (ICDs) (59) have many of the same benefits as Mirena, with a possible lifetime of 10 years. The acceptability study shows that this method is as well-accepted as Mirena (60). It is safe and effective and could be on the market in 6–10 years. The great challenge of the next century is providing the opportunity for young, urban women to become well-educated. Meeting this challenge requires removing the obstacles of unplanned pregnancy and childbearing that too often interfere with the goals and potential of our youth.

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REFERENCES

1. Söderström RM. Progestasert Intrauterine Progesterone Contraceptive system. In: Bardin CW, Mishell DR Jr, eds. Proceedings from Fourth International Conference on IUDs. Newton, MA: Butterworth-Heinemann, 1994; 319–327.
2. Luukkainen T, Progestin releasing intrauterine contraceptive device. In: Bardin CW, Mishell DR Jr., eds. Proceedings from Fourth International Conference on IUDs. Newton, MA: Butterworth-Heinemann, 1994;32–41.
3. Toivonen J, Luukkainen T. Progestin-releasing intrauterine devices. In: Bardin CW, ed. Current Therapy in Endocrinology and Metabolism. St. Louis, MO: Mosby, 1997; 281–285.
4. Luukkainen T, Toivonen J. Levonorgestrel-releasing IUD as method of contraception with therapeutic properties. Contraception 1995; 52:269–276.
5. Sturridge F, Guillebaud J. A risk-benefit assessment of the levonorgestrel-releasing intrauterine system. Drug Safety 1996; 15:430–440.
6. Sturridge F, Guillebaud J. Gynaecological aspects of the levonorgestrel-releasing intrauterine system. Br J Obstet Gynaecol 1997; 104:285–289.
7. Pharris BB, Erickson RE, Bashaw J, et al. Progestasert: a uterine therapeutic system for long-term contraception: I. Philosophy and clinical efficacy. Fertil Steril 1974; 25:915–921.
8. Newton J, Szoutagh F, Ledch P, Rowe P. A collaborative study of progesterone intrauterine device (Progestasert). Fertil Steril 1979; 19:575–589.
9. Zador G, Nilsson BA, Nilsson B, et al. Clinical experience with the uterine progesterone system (Progestasert). Contraception 1976; 13:559–569.
10. Rantakylä P, Ylöstalo P, Järvinen PA, Vierjoki A. Ectopic pregnancies and the use of intrauterine device and low progestogen contraception. Acta Obstet Gynecol Scand 1977; 56:61–62.
11. Snowden R. The Progestasert and ectopic pregnancy. Br Med J 1977; 2:1600–1601.
12. Rybo G, Bergqvist A. Comparison of menstrual blood loss with the Progestasert and Cu-T200: preliminary results. In: Symposium Proceedings Clinical Experience with Progesterone Uterine Therapeutic System. Acapulco, Oct. 15–16, 1976. Exerpta Medica 1978; pp 106–110.
13. Shoupe D, Meme D, Merzrow G, Lobo RA. Prevention of endometrial hyperplasia in postmenopausal women with intrauterine progesterone. N Engl J Med 1991; 325:1811–1812.
14. Archer DF, Viniegra-Sibal A, Hsiu J-G, et al. Endometrial histology, uterine bleeding and metabolic changes in postmenopausal women using a progesterone-releasing intrauterine device and oral conjugated estrogens for hormone replacement therapy. Menopause 1994; 1:109–116.
15. Luukkainen T, Allonen H, Haukkamaa M, et al. Effective contraception with levonorgestrel-releasing intrauterine device. 12 month report of an European multicenter study. Contraception 1987; 36:169–179.
16. Xiao B, Zeng T, Wu S, Sun H, Xiao N. Effect of levonorgestrel-releasing intrauterine device on hormonal profile and menstrual pattern after long-term use. Contraception 1995; 51:359–365.

17. Indian Council of Medical Research Task Force on IUD. Randomized trial with intrauterine devices levonorgestrel intrauterine device (LNG), CuT 380 Ag, CuT 220C and CuT 200B). A 36 month study. Contraception 1989; 39:37–52.
18. Scholten PC. The levonorgestrel IUD. Clinical performance and impact on menstruation. Dissertation, Utrecht, 1989.
19. Faundes A, Alvarez F, Diaz J. A Latin American experience with levonorgestrel IUD. Ann Med 1993; 25:149–153.
20. Sivin I, Stern J, Coutinho E, et al. Prolonged intrauterine contraception: a seven year randomized study of levonorgestrel 20 mcg/day (LNG 20) and copper Tcu 380Ag IUDs. Contraception 1991; 44:473–480.
21. Croxatto HB, Ortiz ME, Valdez E. IUD mechanisms of action. In: Bardin CW, Mishell DR Jr., eds. Proceedings from Fourth International Conference on IUDs. Newton, MA: Butterworth-Heinemann, 1994, 44–62.
22. Nilsson CG, Luukkainen T, Arko H. Endometrial morphology of women using a *d*-norgestrel-releasing intrauterine device. Fertil Steril 1978; 29:397–401.
23. Nilsson CG, Haukkamaa M, Vierola H, Luukkainen T. Tissue concentrations of levonorgestrel in women using a levonorgestrel-releasing IUD. Contraception 1982; 17:529–536.
24. Jänne O, Ylöstalo P. Endometrial estrogen and progestin receptors in women bearing a progesterone-releasing intrauterine device. Contraception 1980; 22:19–23.
25. Pekonen F, Nyman T, Lähteenmäki P, Haukkamaa M, Rutanen EM. Intrauterine progestin induces continuous insulin-like growth factor-binding protein-1 production in the human endometrium. J Clin Endocrinol Metabol 1992; 75:660–664.
26. WHO Task Force on Intrauterine Devices for Fertility Regulation. Microdose intrauterine levonorgestrel for contraception. Contraception 1987; 35:363–379.
27. Nilsson CG, Lähteenmäki P, Luukkainen T. Ovarian function in amenorrheic and menstruating users of a levonorgesrel-releasing intrauterine device. Fertil Steril 1984; 41:52–55.
28. Tang GWK, Lo SST. Levonorgestrel intrauterine device in the treatment of menorrhagia in Chinese women: efficacy versus acceptability. Contraception 1995; 55:231–235.
29. Thiery M, Van der Pas H, Delberge W, Van Kets H, Das Levonorgestrel-Intrauterinpessar. Geburtsh Frauenheilk 1989; 49:186–188.
30. Masters T, Miskry T, Lowe D, et al. Report of ectopic pregnancies associate with the levonorgesrel intrauterine system. Br J Fam Plann 1997; 23:25–26.
31. Luukkainen T, Nielsen N-C, Nygren K-G, Pyörälä T. Nulliparous women, IUD and pelvic infection. Ann Clin Res 1979; 11:121–124.
32. Andersson K, Odlind V, Rybo G. Levonorgestrel-releasing and copper-releasing (Nova T) IUDs during five years of use. A randomized comparative trial. Contraception 1994; 49:56–72.
33. Luukkainen T, Allonen H, Nielsen N-C, et al. Five year's experience of intrauterine contraception with Nova-T and Copper-T-200. Am J Obstet Gynecol 1983; 147:885–892.
34. Toivonen J, Luukkainen T, Allonen H. Protective effect of intrauterine release of levonorgestrel on pelvic infection: three year's comparative experience of

levonorgestrel- and copper-releasing intrauterine devices. Obstet Gynecol 1991; 77:261–264.
35. Luukkainen T, Allonen H, Haukkamaa M, et al. Five year's experience with levonorgestrel–releasing IUDs. Contraception 1986; 33:139–148.
36. Diaz J, Faundes A, Diaz M, Marchi N. Evaluation of the clinical performance of a levonorgestrel-releasing IUD, up to seven years of use, in Campinas, Brazil. Contraception 1993; 47:169–175.
37. Peterson HB, Xia Z, Hughes JM, Wilcox LS, Tylor LR, Trussell J. The risk of pregnancy after tubal sterilization: findings from the U.S. Collaborative Review of Sterilization. Am J Obstet Gynecol 1996; 174:1161–1170.
38. Peterson HB, Xia Z, Hughes JM, Wilcox LS, Tylor LR, Trussell J. The risk of ectopic pregnancy after tubal sterilization. N Engl J Med 1997; 336:762–767.
39. Hillis SD, Marchbanks PA, Tylor LR, Peterson HB. Tubal sterilization and long-term risk of hysterectomy: findings from the United States Collaborative Review of Sterilization. Obstet Gynecol 1997; 89:609–614.
40. Andersson K, Batar I, Rybo G. Return to fertility after removal of a levonorgestrel-releasing intrauterine device and Nova-T. Contraception 1992; 46:575–584.
41. Faundes A, Alvarez F, Brache V, Tejada AS. The role of levonorgestrel IUD in the prevention and treatment of iron deficiency anemia during fertility regulation. Int J Gynecol Obstet 1988; 26:429–433.
42. Andersson K, Rybo G. Levonorgestrel-releasing intrauterine device in the treatment of menorrhagia. Br J Obstet Gynaecol 1990; 97:690–694.
43. Milsom I, Andersson K, Andersch B, Rybo G. A comparison of flurbiprofen, tranexamic acid and levonorgestrel-releasing intrauterine contraceptive device in the treatment of idiopathic menorrhagia. Am J Obstet Gynecol 1991; 164:879–883.
44. Arnold LL, Ascher SM, Scruefer JJ, Simon JA. The nonsurgical diagnosis of adenomyosis. Obstet Gynecol 1995; 86:461–465.
45. Fedele L, Portuese A, Bianchi S, et al. Treatment of adenomyosis-associated menorrhagia with a levonorgestrel-releasing intrauterina device. Fertil Steril 1997; 68:426–429.
46. Puolakka J, Nilsson CG, Haukkamaa M, et al. Conservative treatment of excessive uterine bleeding and dysmenorrhea with levonorgestrel intrauterine system as an alternative to hysterectomy. Acta Obstet Gynaecol Scand Suppl 1996; 75:82.
47. Smith DC, Prentice R, Thompson DJ, Herrmann WL. Association of exogenous estrogen and endometrial carcinoma. N Engl J Med 1975; 293:1164–1167.
48. Robboy SJ, Bradley R. Changing trends and prognostic features in endometrial cancer associated with exogenous estrogen therapy. Obstet Gynecol 1979; 54:269–277.
49. Cushing KL, Weiss NS, Voigt LF, et al. Risk of endometrial cancer in relation to use of low-dose, unopposed estrogens. Obstet Gynecol 1998; 91:35–39.
50. Studd JWW, Thom MH, Paterson MEL, et al. The prevention and treatment of endometrial pathology in postmenopausal women receiving exogenous oestrogen. In: Pasetto N, Paoletti R, Ambrus JL, eds. The Menopause and Postmenopause. Lancaster, UK: MTP Press 1980, 127–139.

51. Hammarback S, Backstrom T, Holst J, et al. Cyclical mood changes as in the premenstrual syndrome during sequential estrogen–progestogen postmenopausal replacement therapy. Acta Obstet Gynecol Scand 1985; 64:393–397.
52. Andersson K, Mattsson L-O, Rybo G, Stadberg E. Intrauterine release of levonorgestrel—a new way of adding progestogen in hormone replacement therapy. Obstet Gynecol 1992; 79:963–967.
53. Raudaskoski TH, Lahti EI, Kauppila AJ, Apaja-Sarkkinen MA, Laatikainen TJ. Transdermal estrogen with a levonorgestrel-releasing intrauterine device for climacteric complaints: clinical and endometrial responses. Am J Obstet Gynecol 1995; 172:114–119.
54. Suhonen S, Holmström T, Lähteenmäki P. Three-year follow-up of the use of a levonorgestrel-releasing intrauterine system in hormone replacement therapy. Acta Obstet Gynecol Scand 1997; 76:145–150.
55. Sivin I, Stern J. Health during prolonged use of levonorgestrel 20 μg/d and the copper TCu 380 Ag intrauterine contraceptive devices: a multicenter study. Fertil Steril 1994; 61:70–77.
56. Suhonen S, Allonen H, Lähteenmäki P. Sustained-release estradiol implants and a levonorgestrel-releasing intrauterine device in hormone replacement therapy. Am J Obstet Gynecol 1995; 172:562–567.
57. Ferguson K, Hoegh C, Johnson S. Estrogen replacement therapy. A survey of women's knowledge and attitudes. Arch Intern Med 1989; 149:133–136.
58. Sivin I, Alvarez F, Diaz J, et al. Intrauterine contraception with copper and with levonorgstrel. A randomized study of the TCu 380 Ag and levonorgestrel 20 mcg/day devices. Contraception 1984; 30:443–456.
59. Pakarinen P, Luukkainen T, Elomaa K, et al. A 12-month comparative clinical investigation of a levonorgestrel-releasing intracervical device situated in the uterine cavity or cervical canal. Contraception 1996; 54:187–192.
60. Shain RN, Ratsula K, Toivonen J, et al. Acceptability of an experimental intracervical device: results of a study for selection bias. Contraception 1989; 39:363–379.

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Contraceptive Vaginal Rings

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I. INTRODUCTION

It has been known for decades that steroids, when placed into the vagina are rapidly absorbed through the vaginal epithelium and enter the circulation (1). In 1965, it was demonstrated that when steroids were placed within polysiloxane tubes or solid discs, they pass through the polysiloxane at a constant rate (2). Combining these principles led to the development of contraceptive vaginal rings (CVRs) containing various progestins, with and without estrogen, which are placed in flexible, doughnut-shaped polysiloxane rings (3). The steroids are released from the polysiloxane and are transported through the vaginal epithelium into the circulation.

The magnitude of release of steroids from a CVR is directly proportional to the area of its outer surface (2) and inversely proportional to the thickness of its outer wall. The duration of action of the CVR is determined by the amount of steroid contained within the reservoir of the device. Cylindrical CVRs are usually made with an outside diameter of 50–58 mm. The diameter of the polysiloxane cylinder varies between 5 and 10 mm. CVRs, which are slightly smaller than the diaphragm which ranges from 60–80 mm in diameter, may be placed anywhere within the vaginal cavity. Unlike the diaphragm, they do not need to cover the cervix to be effective. Constant low levels of steroids diffuse directly through the vaginal epithelium and circulate systematically, thereby, avoiding the first-pass effect through the liver that occurs after ingestion of steroids.

There are three major designs of CVRs: homogeneous, shell, and core (Fig. 1). In the homogeneous CVRs, the steroids are mixed throughout the

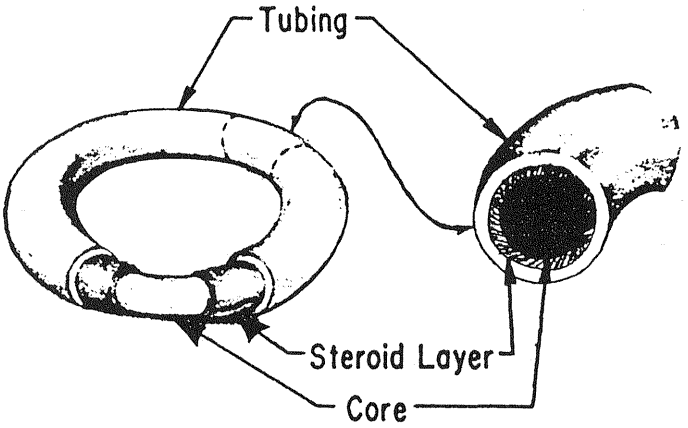

Figure 1 Schematic drawing of three-layer "shell" design of contraceptive vaginal ring. (From Ref. 12.)

polysiloxane in a homogeneous manner. The homogeneous design is no longer used because it is associated with an initial burst of steroid release, followed by a rapid decline as the outer portions of the ring are depleted.

In the shell design CVR, the steroid-containing portion is a thin coating that is located outside a nonmedicated central core and covered by a thin coating of polysiloxane The steroid in this design is contained in a narrow zone so that the difference between the initial diffusion rate and that seen after depletion of the steroid is small (4). In the core design, the drugs are placed in silicone rods which are placed in the core of a polysiloxane ring. The rate of release of the steroids is controlled by the distance the steroid has to diffuse through the ring to get to the surface. Contraceptive rings that are currently used in clinical trials are all of the core design. Both the shell and core design permit a fairly constant release of steroid over 3–6 months, and thus are now the most commonly used designs. There are two different types of CVR formulations. CVRs containing both an estrogen and progestin are similar to combination oral contraceptive pills (OCs), as they inhibit ovulation and administer active medication for 3 out of 4 weeks. Rather than taking a daily oral pill, however, combination CVRs are left in the vagina for 3 weeks and removed for 1 week to allow withdrawal bleeding. Progestin-only CVRs release a small dose of progestin without estrogen and are used either in an intermittent schedule, similar to combination CVRs, or are left continuously in the vagina for 3–6 months. Either type of CVR may be removed for up to 3 h for intercourse if so desired.

II. VAGINAL RING FORMULATIONS

A. Progestin-Only for Intermittent Use

1. *Medroxyprogesterone Acetate, Norethindrone, and Norgestrel*

The original CVR was a 65-mm CVR containing 100 mg of medroxyprogesterone acetate (MPA). The device was inserted on day 5 of the menstrual cycle and removed on day 26 to allow withdrawal bleeding. Ovulation was suppressed in all cycles, and breakthrough bleeding was minimal (3). Use of CVRs containing 100 and 200 mg of norethindrone (NET) were associated with a high incidence of bleeding and ovulation. CVRs with 50 and 100 mg of norgestrel (NG), also were associated with more breakthrough bleeding and lack of withdrawal bleeding compared with MPA-containing CVRs (5).

B. Progestin-Only for Continuous Use

1. *Levonorgestrel*

The CVRs that release levonorgestrel (LNG) were developed by the World Health Organization (WHO) and have undergone extensive clinical investigation. This type of CVR releases 20 μg of LNG daily resulting in a total release in 1 month (30 days) of 600 μg. This ring releases nearly constant amounts of LNG for 3 months and is worn continuously for this time period (6).

In a large multinational clinical study, conducted by WHO and involving more than 1000 subjects, after 1 year of continuous use of the LNG-releasing CVR the overall pregnancy rate was 4.5:100 women, with a method failure rate of 3.7:100 (7). The main reason for discontinuation was bleeding problems, resulting in a termination rate of 17.2:100 at 1 year (Table 1).

Unlike combination CVRs, this progestin-only CVR does not consistently suppress ovulation, as about one-third of treatment cycles are ovulatory. Similar to other widely used progestin-only methods, most progestin-only CVRs rely heavily on various other mechanisms, such as alterations in cervical mucus and the endometrium, for contraceptive action.

In other studies with this CVR, the 1-year continuation rates ranged between 50 and 75%, which are rates similar to those of OCs (7–8). In these studies, although the number of days of bleeding was increased compared with control cycles, the total blood loss was decreased. In one study, monthly menstrual blood loss decreased from 64.7 mL before insertion to 48.7 after 12 months of use, and hemoglobin levels increased after 6 and 12 months of use (8).

Table 1 Cumulative Discontinuation Rates of Levonorgestrel-Releasing CVRs at 1 Year

Reason for discontinuation	Number of events	Rate at 1 yr per 100 women
Pregnancy	32	4.5
Menstrual problems	138	17.2
Other medical reasons	109	14.0
Nonmedical reasons	77	10.8
Expulsion	57	7.1
Loss to follow-up	99	12.7

Source: Ref. 7.

2. Progesterone

Studies using a CVR releasing between 2.4 and 4.8 mg progesterone (P) per day reported unsuccessful use in normally cycling women owing to unacceptable bleeding patterns. However, use of a ring releasing 5–10 mg/day of P in lactating women appears to be an acceptable, effective method (9). In a comparison study between the P-releasing CVR, a copper T-200 IUD, and breastfeeding alone, no deleterious effects on lactation or infant growth were noted in any study group. The rings and IUDs were inserted on day 60 postpartum in women who were exclusively breastfeeding. The CVR was replaced every 3 months. There was one pregnancy in 739 women-months of CVR use in a subject who failed to reinsert the CVR after intercourse. In the untreated nursing women, there were 19 pregnancies in 677 women-months and no pregnancies in the IUD group (9).

3. Megestrol

As with most progestin-only CVRs, the Shanghai megestrol-releasing vaginal ring blocks ovulation in only a small percentage of cycles. The mechanism of action is primarily at the level of the cervical mucus and endometrium. The cervical mucus remains thick and impenetrable to sperm, and the blockage of estrogen receptor synthesis prevents endometrial growth. The pregnancy rate is reported to be 6.0:100 women-years (10).

C. Estrogen Plus Progestin Rings for Intermittent Use

Because higher levels of steroids are released daily, the bleeding patterns and pregnancy rates in these combination CVRs are superior to progestin-only CVRs.

1. Norethindrone Plus Estradiol

A CVR releasing 850 μg NET plus 200 μg estradiol (E_2) inhibited ovulation and was associated with a 10% (insignificant) decrease in low-density lipoprotein (LDL) cholesterol, a 6.9% (insignificant) decrease in high-density lipoprotein (HDL) cholesterol and a 5.4% decrease in total cholesterol (11). However, in one report, bleeding control was poor, as five of eight cycles (14% of treatment days) were associated with breakthrough bleeding (11).

2. Levonorgestrel Plus Estradiol

The Population Council developed a CVR releasing 280–300 μg LNG and 180 μg E_2 daily and studied it extensively in multicenter clinical trials. The ring is inserted and retained for 3 weeks and removed for 1 week to allow withdrawal bleeding. With use of this CVR, the serum levels of LNG range from 1–3 ng/mL and remain relatively constant during each treatment cycle. This level was sufficient to inhibit ovulation, as documented by consistently low endogenous progesterone levels (12). During use of this CVR, there were no significant changes in glucose tolerance and liver function, although a small decrease in levels of alkaline phosphatase was reported (13). Although E_2 levels fluctuated, levels remained high enough to support the endometrium, and low levels of breakthrough bleeding were reported during the treatment. The levels of LNG during use of this CVR were similar to those following ingestion of an OC containing 0.3 mg LNG and 0.3 mg ethinyl estradiol (EE_2) (14). Withdrawal bleeding after removal of the ring occurred within 1–5 days, with a mean of 2.6 days, and lasted from 3 to 7 days, with a mean of 4.5 days (12). Breakthrough bleeding or spotting with the CVR in place occurred in only 7% of cycles.

Two sizes of shell CVRs, 50 and 58 mm, containing LNG and E_2 were compared with an OC containing 150 μg LNG and 30 μg EE_2 in a large multicenter study in eight clinics involving 1103 ring users and 533 OC users (15). The net 1-year–pregnancy rates in the CVR users were 1.8:100 and 1.0:100 women for the two sizes, which was not significantly different from the pregnancy rate of 2.0 among the OC users (Table 2). The continuation rate at 1 year with both sizes of CVRs was 50%, significantly better than the 38% that occurred with the OC users. Terminations for the CVR were mainly for vaginal problems, whereas those for OCs were mainly for headache and nausea. In this study, only 13% of sexual partners complained about the ring, and only one-fourth of users removed the ring at any time for coitus, compared with pretreatment (16). Following treatment, compared with pretreatment, differences in body weight, hemoglobin levels, and blood pressure among both the CVR users and the OC users were small and insignificant.

Table 2 One-Year Termination Rates for Different Reasons per 100 Acceptors by Women Using Levonorgestrel–Estradiol and Women Ingesting an Oral Contraceptive Containing Levonorgestrel and Ethinyl Estradiol

	Net rates (CVR)			Gross rates (CVR)		
Rate	50 mm	58 mm	Nordette	50 mm	58 mm	Nordette
Pregnancy	1.8	1.0	2.0	2.4	1.4	3.3
Medical termination	23.5	22.5	18.7	29.2	27.0	24.7
Use-related termination	6.6	4.4	2.0	8.4	5.4	3.6*
Personal termination	8.8	9.7	11.2	12.2	13.7	18.5
Moving	2.0	2.1	2.0	2.8	3.2	3.2
Loss to follow-up (LFU)	8.5	9.9	25.9	9.5	11.3	31.0**
Continuation, LFU a termination	48.8	50.4	38.2	48.8	50.4	38.2**
Continuation, LFU not a termination	54.0	56.8	55.4	54.0	56.8	55.4
Type of termination	Events			Standard errors		
Pregnancy	9	5	10	0.8	0.7	1.1
Medical reasons	119	115	95	2.3	2.2	2.3
Use-related reasons	35	23	10	1.4	1.1	1.2
Personal reasons	45	47	55	1.8	1.9	2.4
Moving	10	10	10	0.9	1.0	1.1
Loss to follow-up	46	53	133	1.4	1.5	2.3
All	264	253	313	2.3	2.3	2.2
No. of women enrolled	547	556	553			
No. of women at risk, month 12	239	220	193			

*$p < 0.01$; **$p < 0.001$. Tietze Method, May 1980 cutoff.
Source: Ref. 16.

Table 3 Extent of Coronary Artery Atherosclerosis in Monkeys with TPC/HDL Cholesterol Ratio <15[a,b]

	Mean TPC/HDL cholesterol	Coronary artery intimal area
Males ($n = 14$)	11.0 = 1.5	0.532 = 0.114[c]
Control females ($n = 14$)	10.3 = 1.2	0.329 = 0.085[d]
OC ($n = 9$)	12.0 = 2.2	0.059 = 0.034
IVR ($n = 11$)	15.0 = 1.7	0.503 = 0.156[c]

[a]Groups included controlled males and females and animals ingesting an oral contraceptive (OC) and intravaginal ring (IVR) releasing levonorgestrel estradiol.
[b]Values are mean = standard error of the mean.
[c]Different from OC, $p < 0.03$.
[d]Different from OC, $p < 0.05$.
Source: Ref. 20.

Although the major advantage of this CVR is its contraceptive effectiveness and minimal side effects, the major disadvantage is its androgenic dominance owing to the use of a 19-nortestosterone derivative with an estrogen of low potency. Use of this ring was associated with a significant decrease in HDL cholesterol, 18–32%, and a small decrease in LDL cholesterol, 0–11%, resulting in large increases in the LDL/HDL ratio (16–19). In a study using this CVR in macaque monkeys, a significant increase in the amount of coronary artery atherosclerosis compared with controls was noted after 2 years of use (20) (Table 3). These findings motivated researchers to design CVRs that did not have the unacceptable androgenic effect

3. *Nestorone Plus Ethinyl Estradiol*

One of the new synthetic progestins with lowered androgenic activity is Nestorone previously termed ST-1435. Nestorone is inactive orally, but its use as a nonoral progestin is promising. When administered alone by subcutaneous implants, Nestorone has no adverse effect on lipoproteins and no androgenic or estrogenic side effects (21). A CVR releasing 100 μg of Nestorone and 30 μg/EE_2 daily was tested in two small studies. Use of the ring was associated with good bleeding control and minimal side effects (22,23). Serum P values remained low, indicating complete suppression of ovulation. Mean serum levels of Nestorone were 289 pmol/mL (100 pg/mL), which are higher than the minimum levels needed for suppression of ovulation (50 pg/mL). Overall serum HDL cholesterol and total cholesterol increased 40% with no change in LDL cholesterol. The conclusion of this study was that this ring was easy to use, well

accepted by the users, and could be further improved by decreasing the steroid dose.

4. 3-Keto-Desogestrel and EE_2

CVRs releasing 75, 100, and 150 μg of 3-keto-desogestrel (DSG) per day and 15 μg of EE_2 per day in a core of ethylene vinyl acetate, instead of polysiloxane, showed very promising results. Of the three doses of steroid in this ring, the combination of 150 μg 3-keto-DSG, and 15 μg EE_2 was associated with the best bleeding control and ovulation suppression. When used in 11 women for three cycles, this combination CVR inhibited ovulation in all cycles and was associated with good bleeding control (24). Similar to OCs with this combination of steroids, there was a significant increase (up to 93%) of sex hormone-binding globulin (SHBG) levels, indicating a low androgenic, high estrogenic effect of this CVR formulation.

Pharmacokinetic studies of these 3-keto-DSG–EE_2 CVRs demonstrated a relatively constant release rate of 3-keto-DSG (Fig. 2) for the 3 weeks the ring was in place (25). A CVR with a daily release rate between 100 and 150 μg/day of the progestin in combination with 15 μg/day EE_2 is a promising contraceptive option and clinical trials have taken place. Extended use of a CVR containing 150 μg EE/120 μg DSG for up to 84 days consistently suppressed ovarian activity (26).

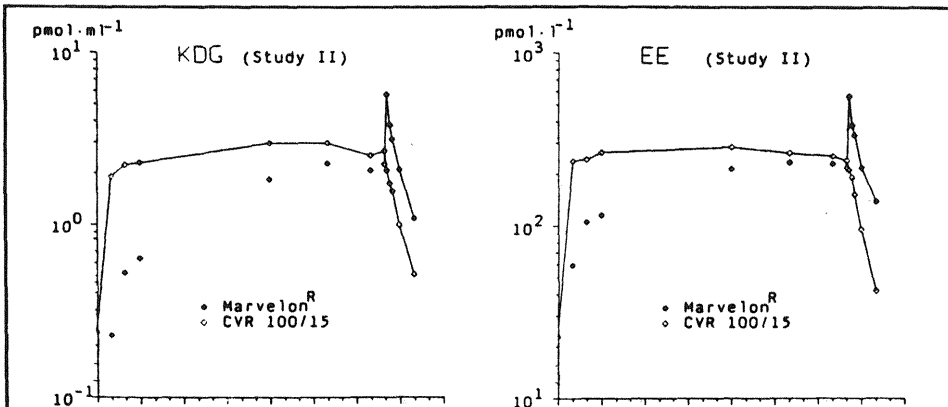

Figure 2 Mean KDG and EE serum levels for Marvelon and CVR 100/15. (From Ref. 27.)

5. *NET Acetate Plus EE_2*

CVRs releasing between 600 μg and 2 mg/day NET acetate (NETA) plus 20–65 μg/day EE_2 have also undergone clinical trials (27,28). The combination of release rates of NETA 650 μg/day plus 20–30 μg/day of EE_2 offers both excellent suppression of ovulation and good bleeding control. In a comparative trial, a core design CVR (releasing 10, 20, 30, or 65 μg EE_2 and 650 μg NETA) provided better control of uterine bleeding than did an OC containing 30 μg EE_2 and 1.5 mg NETA, and side effects were comparable (27). The 20 and 30 μg/day EE_2-releasing CVRs caused an increase in serum HDL cholesterol and triglycerides, with minor changes of angiotensinogen and SHBG. Once-weekly mean levels of circulating norethindrone and EE_2 levels are shown in Fig. 3. The

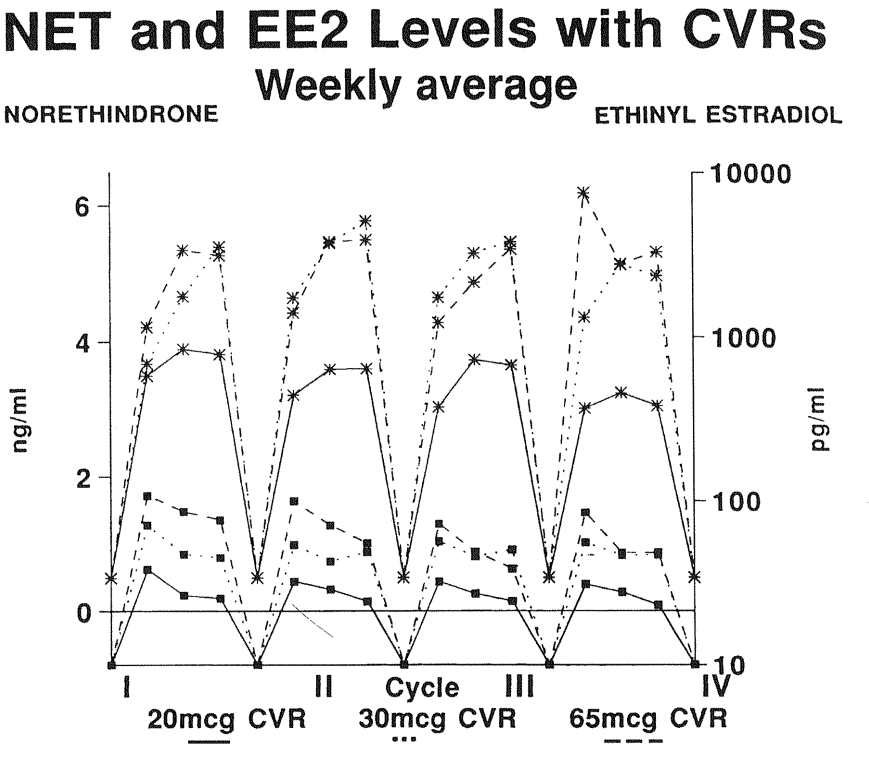

Figure 3 Mean weekly norethindrone (NET) and ethinyl estradiol (EE) levels during four cycles of CVR use. Line styles represent the release rates of CVRs with different amounts of ethinyl estradiol: ____ 20 μg, ... 30 μg; --- 65 μg EE. (*) represents weekly NE levels (■) represents weekly EE levels. (From Ref. 28.)

conclusion of this study was that this new CVR offers an excellent contraceptive alternative with the best performance provided by the 30 μg EE_2 dose (27).

III. ADVERSE EFFECTS

The major concerns associated with CVR use are related to its placement in the vagina.

A. Vaginal Infections

Because the CVR is a foreign device that remains inside the vagina for long time periods, the effect on the vaginal flora has been of concern. In many women, there is an increase in vaginal secretions with CVR use. In one large multicenter study, 23% of CVR users complained of vaginal discharge as compared with only 14.5% in OC users (16). The discharge is probably a result of mechanical effects of the ring on the vaginal epithelium (29), rather than a change in vaginal flora (30). Cultures from the posterior fornix showed no greater incidence of pathogens in CVR users than in OC users, and no significant changes in vaginal flora, including *Lactobacillus*, *Candida*, *Gardnerella vaginalis*, and *Neisseria gonorrhoeae*, and aerobic and anaerobic cultures (30). If a vaginal infection occurs with the core in place, it can be treated without removing the CVR.

B. Local Effects

Concern has also been raised about the effect of a vaginal device left in place for long time periods on cervical cytology. After 2 years of CVR use, however, no cervical changes were noted on colposcopic examination (31). One report of cervical erosion was noted in one study, but the lesion disappeared spontaneously when the CVR was removed.

C. Other Vaginal-Related Problems

Other use-related problems vary, but are usually low in frequency. Most women are comfortable leaving the CVR in place during coitus and complaints during intercourse are reported in only a small percentage of users (1–2%). About 0.5% of users discontinue use because of odor and from 1 to 23% for discomfort (31).

Spontaneous expulsion can occur on occasion. It is usually associated with laxity of the vaginal wall in multiparous patients and often occurs during squatting or defecation. The ring is usually replaced by the user after cleaning.

IV. ACCEPTABILITY

To evaluate the acceptability of the CVR in both rural small towns and urban areas, four clinics in Brazil and the Dominican Republic offered the CVR as a new method of birth control (32). The CVR was described as similar to the pill, but placed in the vagina for 3 weeks each month. Of the total birth control acceptors in the four clinics, 3, 8, 9, and 12.5% selected the CVR compared with 30.6% who preferred OCs. The anticipated use-related problems were most often mentioned by the women as reasons for not selecting the CVR. Following its use, however, the ''ease of use'' was mentioned in 55% of ring users as the ''most-liked'' characteristic (32). In the CVR users, no coital difficulties were experienced by 82% of sexual partners, discomfort was reported in 30%, and only three partners (2%) described coitus as feeling different. Seventy-seven percent never removed the CVR for coitus, and 10% removed it every time (30).

V. CONCLUSION

In conclusion, the CVR offers another route for administration of contraceptive steroids that is user controlled. Although the CVRs offer the same high contraceptive efficacy of OCs, the ease of use and the once-monthly insertion are advantages over the daily requirements of OCs. Another advantages over OCs are the attainment of constant steady-state blood levels of steroid, with less effect on hepatic globulins. Because of the similar mechanisms of action between CVRs and OCs, the noncontraceptive health benefits associated with OCs are also expected to be shared with CVR users. There are many different CVR formulations undergoing clinical trials that have excellent bleeding control and contraceptive efficacy. It is hoped that one of these formulations will soon be marketed so that women can have another contraceptive option.

REFERENCES

1. Macht DIJ. The absorption of drugs and poisons through the vagina. J Pharmacol Pathol 1918;10:509.
2. Dzuik PJ, Cook B. Passage of steroids through silicone rubber. Endocrinology 1966; 78:208.
3. Mishell DR Jr, Talas M, Parlow AF, et al. Contraception by means of a Silastic vaginal ring impregnated with medroxyprogesterone acetate. Am J Obstet Gynecol 1970; 107:100–107.
4. Jackanicz TM. Levonorgestrel and estradiol release from an improved contraceptive vaginal ring. Contraception 1981; 24:323.

5. Mishell DR Jr, Lumkin ME, Jackanicz T. Initial clinical studies of intravaginal rings containing norethindrone and Norgestrel. Contraception 1975; 12:253–260.
6. Landgren BM, Johannsson E, Masironi B, et al. Pharmacokinetic and pharmacodynamic investigations with vaginal devices releasing levonorgestrel at a constant, near zero order rate. Contraception 1982; 26:567.
7. Koetsawang S, Gao J, Krishna U, et al. Task Force on Long-Acting Systemic Agents for Fertility Regulation Special Programs of Research, Development and Research Training in Human Reproduction. Microdose intravaginal levonorgestrel contraception: a multicenter clinical trial. Contraception 1990; 41:105.
8. Ji G, Jong-zhu S, Gui-ying S, et al. Clinical investigation of low-dose levonorgestrel-releasing vaginal ring. Fertil Steril 1986; 46:626.
9. Diaz A, Jackanicz TM, Herraros C, et al. Fertility regulation in nursing women. VIII: progesterone-releasing vaginal ring. Contraception 1985; 32:603.
10. Ji-hua Z, Jun-min, Fu-qin S, et al. Clinical investigation of megestrol silicone vaginal ring. Shanghai Med J 1984; 7:156.
11. Ahren T, Victor A, Lithelik H, et al. Ovarian function, bleeding control and serum lipoproteins in women using contraceptive vaginal rings releasing five different progestins. Contraception 1983; 28:315.
12. Mishell DR Jr, Moore DF, Roy S, et al. Clinical performance of endocrine profiles with contraceptive vaginal rings containing a combination of estradiol and *d*-norgestrel. Am J Obstet Gynecol 1978; 130:55.
13. Ahren T, Victor A, Lithell A, et al. Comparison of the metabolic effects of two hormonal contraceptive methods: an oral formulation and a vaginal ring I. Carbohydrate metabolism and liver function. Contraception 1981; 24:415.
14. Brenner PF, Mishell DR Jr, Stanczyk FS, et al. Serum levels of *d*-norgestrel luteinizing hormone, follicle-stimulating hormone, estradiol and progesterone in women during and following ingestion of combination oral contraceptives containing *d*-norgestrel. Am J Obstet Gynecol 1979; 129:133.
15. Sivin I, Mishell DR Jr, Victor A, et al. A multicenter study of levonorgestrel–estradiol contraceptive vaginal rings I—use of effectiveness. An international comparative study. Contraception 1981; 4:342.
16. Sivin I, Mishell DR Jr, Victor A, et al. A multicenter study of levonorgestrel–estradiol releasing contraceptive vaginal rings II—subjective and objective measures of effects. An international comparative trial. Contraception 1981; 24:359.
17. Ahren T, Lithell H, Victor A, et al. Comparison of the metabolic effects of two hormonal contraceptive methods; an oral formulation and a vaginal ring II. Serum lipoproteins. Contraception 1981; 24:451.
18. Roy S, Krauss RM, Mishell DR Jr. The effect on lipids and lipoproteins of a contraceptive vaginal ring containing levonorgestrel and estradiol. Contraception 1981; 24:426.
19. Robertscn DN, Alvarez F, Sivin I, et al. Lipoprotein patterns in women in Santo Domingo using a levonorgestrel/estradiol contraceptive ring. Contraception 1981; 24:469.
20. Adams MR, Clarkson TB, Koritnik, DR, et al. Contraceptive steroids and coronary artery atherosclerosis in cynomolgus macaques. Fertil Steril 1987; 47:1010–1018.

21. Haukkamaa M, Laurikka-Routti M, Heikinheimo O, Moo-Young A., Contraception with subdermal implants releasing the progestin ST-1435: a dose-finding study. Contraception 1992; 45:49-55.
22. Alvarez-Sanchez F, Brache V, Jackanicz T, Faundes A. Evaluation of four different contraceptive vaginal rings: steroid serum levels, luteal activity, bleeding control and lipid profiles. Contraception 1992; 46:387–398.
23. Laurikka-Routti, M, Haukkamaa M, Heinkinheimo O. A contraceptive vaginal ring releasing ethinyl estradiol and the progestin ST-1435; bleeding control, serum steroid concentrations, serum lipids and serum chemistry. Contraception 1990; 42:111.
24. Apter D, Cacciatore B, Stenman U-H. Clinical performance and endocrine profiles of contraceptive vaginal rings releasing 3-keto-desogestrel and ethinyl estradiol. Contraception 1990; 42:285.
25. Olsson SE, Odlin V. Contraception with a vaginal ring releasing 3-keto-desogestrel and ethinyl estradiol. Contraception 1990; 42:563.
26. Davies G, Feng LX, Newton JR. Ovarian activity and bleeding patterns during extended continuous use of a combination contraceptive vaginal ring. Contraception. 1992; 46:269–278.
27. Timer CJ, Apter D, Voortman, G. Pharmaco-kinetics of 3-keto-desogestrel and ethinyl estradiol released from different types of contraceptive vaginal rings. Contraception 1990; 42:629.
28. Ballagh SA, Mishell DR, Lacarra M, Shoupe D, Jackanicz TM, Eggena P. A contraceptive vaginal ring releasing norethindrone acetate and ethinyl estradiol. Contraception 1994; 50:517.
29. Ballagh SA, Mishell DR, Jackanicz TM, Lacarra M, Eggena P. Dose-finding study of a contraceptive ring releasing norethindrone acetate/ethinyl estradiol. Contraception 1994; 50:535.
30. Schwan A, Ahren T, Victor A. Effects of contraceptive vaginal ring treatment upon vaginal bacteriology and cytology. Contraception 1983; 28:341.
31. Roy S, Wilkins J, Mishell DR Jr. The effect of a contraceptive vaginal ring and oral contraceptives on the vaginal flora. Contraception 1981; 24:481.
32. Roy S, Mishell DR Jr. Vaginal ring clinical studies update. In: Zatuchni, GL, Goldsmith A, Shelton JD, Sciarra JJ, eds. Long Acting Contraceptive Delivery Systems. Philadelphia: Harper & Row, 1984, pp 581.
33. Faundes A, Hardy E, Reyes Q, et al. Acceptability of the contraceptive vaginal ring by rural and urban populations in two Latin American countries. Contraception 1981; 24:393.

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Progestins for Emergency Contraception

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Emergency contraception is defined as any drug or device that can be used after intercourse to prevent pregnancy (1). Many methods of hormonal emergency contraception have been used since 1960s when it was first introduced (2). Initially, a 5-day regimen of high-dose ethinyl estradiol was used, with a high incidence of severe side effects (nausea and vomiting). In 1977, Yuzpe proposed the method that was named after him, employing a combination of 0.1 mg ethinyl estradiol and 1 mg of *dl*-norgestrel, given twice, 12 h apart, within 72 h after intercourse, allowing fewer side-effects than the estradiol alone method. This method has been widely used since, despite the absence of a dedicated preparation in most countries: the physicians usually prescribe off-label the amount of commercially available combined oral contraceptive (e.g., Ovlane or Stediril) 2 × 2 tablets 12 h apart. In some countries a dedicated product has been licensed for several years (PC4, containing 4 tablets of 0.5 mg ethynil estradiol plus 0.5 mg *dl*-norgestrel, or Tetragynon, containing 4 tablets of 0.5 mg ethynil estradiol plus 0.25 mg *l*-norgestrel).

Other hormonal contraceptives have been used or at least evaluated, such as danazol (3) or the antiprogesterone mifepristone (4,5). For the latter, encouraging results published initially have not been confirmed by larger studies, which nevertheless indicate that the efficacy of the product is equivalent to the Yuzpe method, but fewer less side-effects. However, the abortifacient nature of the molecule makes it unsuitable for large-scale use of emergency contraception, especially in countries where pregnancy termination is illegal, or subject to well-defined legal requirements.

The mode of action of emergency contraception is not fully understood, and most probably involves more than one mechanism (6), such as (a) inhibition or disruption of ovulation, which might be a common mechanism to all the agents tested so far, although its reality has not been unequivocally demonstrated with the regimens used in emergency contraception; this mechanism is probably the most important because the probability of fertilization is maximal during the days immediately preceding ovulation (7,8); (b) direct effect on fertilization; (c) modifications of luteal-phase progesterone secretion; (d) inhibition of implantation. Apart from mifepristone, none of the other agents (estrogens, progestins, danazol) are able to interrupt an early pregnancy once implantation has taken place, which clearly differentiates emergency contraception with these agents from early pregnancy termination.

Progestins, such as levonorgestrel (*l*-norgestrel), alone at high doses have been used for many years for emergency contraception (9–11), prescribed either off-label (intake of 25 Microval tablets, twice, 12 h apart), or as Postinor or as Norlevo, dedicated preparations containing 2 tablets of 0.75 mg levonorgestrel. However, only recently has the efficacy of levonorgestrel alone been demonstrated. Two studies sponsored by the World Health Organization (WHO) (12,13) have shown that levonorgestrel alone, given at the dose of 0.75 mg twice, 12 h apart, within 72 h following a supposedly fertilizing intercourse, is at least as efficient and better tolerated than the Yuzpe regimen. This could constitute the future reference method for hormonal emergency contraception (14). The main findings of these studies are reviewed here, as well as the available pharmacokinetic data, with the recommended levonorgestrel dosage for this indication.

Methodological aspects are of high importance for the efficacy assessment of emergency contraceptive methods: the proper efficacy evaluation of the Yuzpe method has been a matter of concern (15). Indeed, in most of the earlier publications, the efficacy was evaluated by the calculation of the failure rate (observed pregnancies divided by the number of patients treated), yielding efficacy rates as high as 95–99%. However, this is an inappropriate measure because in most publications, the timing of drug intake relative to the probable date of ovulation was not recorded or reported. This leads to an overestimation of the efficacy rate. (In large trials, it is assumed that many women have received the drug at a time when their risk of pregnancy was minimal). On the other hand, underestimation of the efficacy rate could have derived from inappropriate inclusion or monitoring of the patients; for example, if the patient had several acts of unprotected intercourse before treatment, or if she underwent other unprotected intercourses after it. Therefore, for accurate efficacy assessment (16) (a) women should have had only one act of unprotected intercourse since the last menstrual period; (b) this supposedly fertilizing intercourse should have taken place within 72 h before drug administration; and (c) subsequent to treatment, women should

abstain or use condoms until the results of therapy could be verified. In addition, accurate recording of intercourse timing, usual menstrual cycle length, and date of last menstrual period are mandatory to allow an estimation of the expected number of pregnancies using the Dixon's table (7). This table gives an estimation of the probability of being pregnant after a single act of intercourse relative to the date of ovulation. The probability of pregnancy can also be derived from the more recent work of Wilcox et al. (8), which confirms that the fertile days of the menstrual cycle are the 6 days immediately preceding ovulation, and that the likelihood of fertilization is almost nil from the days after ovulation onward.

Literature survey of trials using this method indicates that the efficacy rate of the Yuzpe method (defined as the number of observed pregnancies divided by the number of expected pregnancies derived from the Dixon's table) ranges from 55 to 94%. Pooling the largest studies give an average efficacy rate of 74%, which can be viewed as the reference efficacy rate for the Yuzpe method (17).

The two WHO studies reviewed here fulfill the methodological criteria indicated in the foregoing. In addition, as they include a control arm versus the reference Yuzpe method, they permit some comparison with the other published studies, thereby strengthening their conclusions.

The first study took place in the Family Planning Association of Hong Kong (12). In this open study, 880 women were randomly allocated to the levonorgestrel or to the Yuzpe groups (440 in each group). In both groups, the most common request for emergency contraception was breakage or slippage of condom (46 and 50% in the Yuzpe and levonorgestrel groups, respectively), and unprotected intercourse (46 and 40%, respectively). The second (international) study (13) is a double-blind, randomized comparison of Yuzpe and levonorgestrel for emergency contraception, and constitutes an extension of the Hong Kong study. It was performed in 1998 women over 21 centers in 14 countries. Eligible women were randomly assigned to Yuzpe method (4 tablets) or to levonorgestrel (0.75 mg twice) with a placebo. The first dose was taken in the prescribing center under supervision, whereas the second dose was taken at home, less than 24 h after the first intake.

For both studies, the primary outcome measure was unintended pregnancy, expressed as both crude and adjusted rates, as well as the estimated reduction in expected pregnancies. The probabilities of conception by cycle day were estimated from the works of Dixon (Hong Kong study (7) or of Wilcox et al. (8) (international study), taking into account clinical pregnancies only (the chemical pregnancies were excluded).

In the Hong Kong study, 15 pregnancies (3.5%) occurred in the Yuzpe group and 12 (2.9%) in the levonorgestrel group (no statistically significant difference). Of these, 6 in the Yuzpe group and 4 in the levonorgestrel occurred in women who had further acts of intercourse and, therefore, can been considered

as protocol violations. After excluding these patients, the failure rate was 2.7% (95% confidence interval [CI], 1.0–4.1%) in the Yuzpe group, and 2.4% (95% CI, 0.8–4.1%) in the levonorgestrel group. The difference was not statistically significant. Table 1 compares the number of expected pregnancies derived from Dixon's table and the number of actually observed pregnancies after treatment in each group for women with reliable menstrual data. As shown by the table, in both groups, the number of pregnancies observed after the treatment was significantly lower than expected from Dixon's table (7) ($p < 0.05$, chi-square test).

In the international study, levonorgestrel prevented 85% of expected pregnancies, a figure significantly higher than that after Yuzpe (57%). In addition, the efficacy of both methods was highest when they were used as early as possible after intercourse. However, at each time, levonorgestrel was more efficient than Yuzpe.

Table 2 indicates the efficacy rate for both groups in the international study, and the effect of time since unprotected coitus. The pregnancy rate was significantly higher—3.2% (95% CI 2.2–4.5) among women assigned the Yuzpe method—than in those assigned levonorgestrel alone—1.1% (0.6–2.0). Identical results were obtained after adjustment for center, cycle day on which intercourse took place, age, body-mass index, and reason for requesting emergency contraception. All pregnancies were intrauterine; five women continued their pregnancies with normal outcomes, the other opted to have induced abortion.

Table 2 also shows that the earlier emergency contraceptive is given, the greater its efficacy, in each group. However, at each time, levonorgestrel was associated with a significantly higher efficacy rate than Yuzpe. In this study, a further act of intercourse (with or without a barrier method) after treatment was associated in a decline in efficacy of both methods, which was more marked with the Yuzpe method. The difference between the two methods persisted in a subgroup of women who met all the criteria for correct use of the assigned treatment: the pregnancy rate was 1.9% (95% CI interval, 1.0–3.4) in the Yuzpe regimen and 0.9% (0.3–2.0) in the levonorgestrel regimen. Conversely, in patients without all criteria of correct use, the efficacy rate declined more in the Yuzpe than in the levonorgestrel group.

In both studies, the incidence of complaints (nausea and vomiting) was significantly lower after levonorgestrel alone than after the Yuzpe regimen (Table 3 summarizes results obtained in the international WHO study).

In both studies, the time of resumption of menses was similar for women in the two groups ($p = 0.67$). For instance, in both groups combined in the large international study, 13% of women had a delay of more than 7 days beyond the anticipated onset of next menses; 15% had a delay of 3–7 days; 57% had menses return within 3 days of the expected day; and 15% had an early onset. The mean duration of next menses was 4.7 (SD 1.4) days for both groups.

Table 1 Efficacy of Yuzpe and Levonorgestrel Methods

Day of cycle[a]	Probability of pregnancy	Yuzpe			Levonorgestrel		
		Number of women	Number of pregnancies		Number of of women	Number of pregnancies	
			Expected	Observed		Expected	Observed
<−8	0	30	0	0	32	0	0
−8	0.001	10	0	0	11	0	0
−7	0.007	8	0	0	10	0	0
−6	0.025	17	0	0	15	0	0
−5	0.055	18	1	1	18	1	1
−4	0.104	25	3	1	19	2	1
−3	0.146	26	4	1	22	3	0
−2	0.169	24	4	0	21	4	0
−1	0.173	22	4	1	24	4	4
0	0.141	21	3	0	20	3	1
1	0.091	21	2	0	12	1	0
2	0.049	16	1	2	25	1	0
3	0.019	26	0	1	13	0	0
4	0.005	12	0	0	12	0	0
5	0.001	9	0	0	20	0	0
>5	0	56	0	2	57	0	1
Total		341	22	9	331	20	8

[a]Days of cycle: 0 = day of ovulation.
Source: Ref. 12.

Table 2 Pregnancy Rate per Treatment Group and Time Since Unprotected Coitus

No. of pregnancies		Yuzpe	Levonorgestrel
All patients	Observed/expected	31/72	11/75
	% prevented (95% CI)[a]	57 (39–71)	85 (74–93)
≤ 24 h	% prevented	77	95
25–48 h	% prevented	36	85
49–72 h	% prevented	31	58

[a]CI = 95% confidence interval.
Source: Ref. 13.

Table 3 Side Effects Reported in Both Groups in the International WHO Study

	% with symptom (95% CI)		
Side-effect	Yuzpe ($n = 979$)	Levonorgestrel ($n = 977$)	p
Nausea	50.5 (47.3–53.6)	23.1 (20.5–25.9)	<0.01
Vomiting	18.8 (16.4–21.4)	5.6 (4.3–7.3)	<0.01
Dizziness	16.7 (14.4–19.1)	11.2 (9.3–13.3)	<0.01
Fatigue	28.5 (25.7–31.4)	16.9 (14.5–18.4)	<0.01
Headache	20.2 (17.8–22.9)	16.8 (14.5–19.3)	0.05
Breast tenderness	12.1 (10.1–14.3)	10.8 (8.8–12.9)	0.4
Low abdominal pain	20.9 (18.4–22.6)	17.6 (15.3–20.1)	0.07
All other adverse effects[a]	16.7 (14.4–19.1)	13.5 (11.4–15.8)	0.06

[a]Mostly diarrhea and some irregular bleeding or spotting.
Source: Ref. 13.

Pharmacokinetics data for levonorgestrel at the dose used for emergency contraception (0.750 mg orally) is limited (18). Levonorgestrel absorption is rapid, with an absorption half-life of less than 1 h, the mean time taken to achieve maximal serum concentrations being approximately 2 h. In blood, levonorgestrel binds to both albumin and sex hormone-binding globulin (SHBG). Sex steroids can modify SHBG concentration: after a single administration of 0.750 mg levonorgestrel, plasma SHBG is decreased by 25%. However, with the dosage regimen for emergency contraception, the relevance of such finding, if any, should be clinically insignificant.

Similarly, only few studies provide information on the pharmacological effects of levonorgestrel given at the dose of 0.750 mg. In a study by Shi Yong-en et al. (19), six healthy women were given levonorgestrel at the dose of 0.75 mg

orally for 7 days during the periovulatory phase. During the study, four of the six women ovulated. In the remaining two, estradiol levels increased, suggesting some degree of follicular activity, but this was not followed by an increase of progesterone secretion. Prolactin remained in the normal range throughout the study in all women. In a multicenter study (Sweden, India, China) sponsored by WHO (18), 72 volunteers received 0.75 mg levonorgestrel every 2 days for treatment periods of 4 days each. Each treatment period took place at different times during the menstrual cycle: follicular phase (days 2–8), early periovulatory phase (days 9–15), late periovulatory phase (days 11–19), or luteal phase (days 16–22). When levonorgestrel was given during the follicular phase, the mean cycle length was significantly prolonged during treatment, compared with pre- and posttreatment cycles. The prolongation of cycle length was due to an increase in the follicular phase, but was not accompanied by suppression of ovarian function. When levonorgestrel was given in the early periovulatory phase, overall no modifications of the mean cycle length was observed, despite some site-related differences (cycle shortening in Swedish and Indian women, cycle prolongation in Chinese women). In this group, 3 women showed follicular activity only, 7 exhibited follicular activity followed by insufficient luteal function, and 7 women ovulated normally.

Very similar findings were observed in the group given levonorgestrel at the late periovulatory phase: there was overall no change in cycle length. Seven women out of 18 in this group did ovulate during the treatment, 6 women had follicular activity followed by inadequate luteal function, and 5 exhibited follicular activity only. In the posttreatment group, 1 woman did show follicular activity only, 1 showed follicular activity, followed by insufficient luteal function, and 16 ovulated normally. When levonorgestrel was given during the luteal phase, no effect on cycle length or ovarian function was observed. Quantitative reading of endometrial samples in a subgroup of patients showed that in all but one patient (who showed a late proliferative endometrium), all pretreatment biopsies showed normal secretory endometrium. In all women given levonorgestrel during the follicular phase, biopsies (taken on cycle days 20–22) were proliferative during treatment, probably reflecting the prolongation of the follicular phase. In women given the treatment during early and late periovulatory phase, all biopsies were irregular secretory or proliferative, none was normal secretory. In women given levonorgestrel during the luteal phase, three biopsies were irregular secretory and three were normal secretory.

The effect of treatment on the number of glands, diameter of glands, and glandular epithelial height has also been quantitated. When given during the follicular phase, levonorgestrel significantly decreased the number of glands, suggesting a suppression of the proliferative activity at the endometrial level. Similarly, the glandular diameter was significantly smaller than in the control cycle in women given levonorgestrel during the follicular phase, or in the early

periovulatory phase. The treatment had no effect on the glandular epithelial height. Taken together, these results indicate that the effects of levonorgestrel on ovarian function depend on the date of administration during the cycle, the late follicular period being the most sensitive to the progestin treatment. This could explain the efficacy of this regimen as an emergency contraceptive agent, because this preovulatory period corresponds to the highest probability of fertilization in case of intercourse (7). The observed changes are compatible with some degree of ovarian functional suppression. At the endometrial level, administration of levonorgestrel during the follicular phase is associated with suppression of the proliferative activity. When given during the luteal phase, levonorgestrel does not induce any significant endometrial change. Finally, the posttreatment cycle appears unmodified by the treatment, whatever the date of administration.

In conclusion, available data confirm that the use of progestin alone, as 0.75 mg levonorgestrel twice, 12–24 h apart constitutes a mean of emergency contraception more efficient and significantly better tolerated than the reference Yuzpe method, when taken within 72 h after unprotected intercourse. The additional advantages of an estrogen-free emergency contraceptive are obvious and twofold. On one hand, the absence of estrogen explains why there are much fewer side effects with the levonorgestrel-alone product. On the other hand, contrarily to preparations containing estrogen, which theoretically should be contraindicated or used cautiously in women at risk of vascular diseases, no such limitation exists with levonorgestrel alone. Emergency contraception is an efficient and cost-effective method (20) to prevent unwanted pregnancies and the consequences of induced abortion. However, until now, the use of emergency contraceptive methods has been hampered by the lack of an available dedicated product and by the side effects related to the estrogen component of the Yuzpe method.

Levonorgestrel is not an abortifacient, and it cannot be misused to induce medical abortion. A recent study (21) has suggested that making emergency contraception easily available may reduce the number of unwanted pregnancies: the high efficacy and good tolerance of levonorgestrel, and the absence of contraindication, make it the ideal candidate for large-scale use as an emergency contraceptive agent.

REFERENCES

1. A Glasier. Adv Contracep 12:167–172, 1996.
2. M Fasoli, F Parazzini, G Cecchetti, C La Vecchia. Contraception 39:459–468, 1989.
3. G Zuliani, UF Colombo, R Molla. Eur J Obstetr Gynecol Reprod Biol 37:253–260, 1990.

4. A Glasier, KJ Thong, M Dewar, M Mackie, D Baird. New Engl J Med 327:1041–1044, 1992.
5. AMC Webb, J Russell, M Elstein. Br Med J 305:927–931, 1992.
6. A Glasier. N Engl J Med 337:1058–1079, 1997.
7. GW Dixon, JJ Schlesselman, WO Howard, RP Blye. JAMA 244:1336–1339, 1980.
8. AJ Wilcox, CR Weinberg, DD Baird. N Engl J Med 333:1517–1521, 1995.
9. G Seregély. Ther Hung 30:72–78, 1982.
10. WHO Task Force on Post-Ovulatory Methods for Fertility Regulation, Contraception 36:275–286, 1987.
11. C He, Y Shi, J Xu, PFA Van Look. Int J Gynecol Obstet 36:43–48, 1991.
12. PC Ho, MSW Kwan. Hum Reprod 8:389–392, 1993.
13. Task Force on Postovulatory Methods of Fertility Regulation, Lancet 352:428–438, 1998.
14. J Guillebaud. Lancet 352:416–417, 1998.
15. L Silvestre, Y Bouali, A Ulmann. Lancet 338:39–41, 1991.
16. J Trussell, C Ellertson, G Rodriguez. Obstetr Gynecol 88:150–154, 1996.
17. J Trussell, C Ellertson, F Stewart. Fam Plann Perspect 28:58–87, 1996.
18. BM Landgren, E Johannisson, AR Aedo, A Kumar, Y Shi. Contraception 39:275–289, 1989.
19. Y Shi, S Zheng, Y Zhu, C He, P Yu, K Fotherby. Contraception 37:359–369, 1988.
20. J Trussell, J Koenig, C Ellertson, F Stewart. Am J Publ Health 87:932–937, 1997.
21. A Glasier, D Baird. N Engl J Med 339:1–4, 1998.

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Progestins in Hormonal Replacement Therapy and Prevention of Endometrial Disease

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I. EPIDEMIOLOGY OF ENDOMETRIAL DISEASE AND PROGESTIN USE

Since the mid-1970s, several retrospective studies have indicated that unopposed estrogen replacement therapy is associated with an increased risk of endometrial cancer in postmenopausal women (1,2). Since then, a better understanding of the role of progesterone on the endometrial tissue led to the demonstration of the protective effect of progestins against endometrial hyperplasia and cancer (see also Chap. 15).

The major benefit of progestins administration in postmenopausal women is nowadays well documented and recognized. It is undisputed that addition of a progestational agent to estrogens would oppose the proliferative effect of these steroids on the endometrial tissue.

Long-term epidemiological studies have clearly demonstrated that progestins, when administered for more than 10 days per month of estrogen therapy, would prevent the excess risk of development of endometrial hyperplasia and of endometrial cancer associated with use of estrogen alone (3). In all studies, use of estrogen with a progestin was associated with a lower relative risk of endometrial cancer than use of estrogen alone, but the risk was not reduced below that of untreated women. Both studies that presented results according to the duration of progestin use each month found that the relative risk was lower

in women using progestin for 10 or more days per month than in women using progestins for fewer than 10 days per month (3) (See also Chap. 15).

II. STUDIES ON THE ROLE OF PROGESTINS IN PREVENTING ENDOMETRIAL HYPERPLASIA

From previous retrospective studies, it was clear that the duration of progestin addition had an important influence on the rate of endometrial hyperplasia. Although unopposed estrogens were able to induce hyperplasia in up to 30% of subjects, 7 days of progestins would still lead to 4% and 10 days to 2% of hyperplasia (4). More recent prospective studies have better defined the necessary dose and duration of the progestin course to oppose the proliferative effect of estrogens.

A. The PEPI Trial

The Postmenopausal Estrogen–Progestin Interventions (PEPI) Trial provided definite results (5). Among the 875 women enrolled in that trial, 596 (68%) had a uterus. These women were followed for up to 3 years and underwent annual endometrial biopsies following ultrasonographic assessment of the endometrium. The women were randomly assigned to receive placebo or one of four active treatments for 3 years. All active treatments included daily conjugated equine estrogens (CEE) at a dose of 0.625 mg/day. One group received unopposed CEE and three groups received a progestin, either medroxyprogesterone acetate (MPA), given at a dose of 2.5 mg daily on a continuous combined regimen, or on a cyclic regimen at a dose of 10 mg daily for 12 days per month. In one group the progestin was micronized progesterone administered at the dose of 200 mg daily for 12 days in a cyclic regimen.

The results of this 3-year prospective study were clear-cut. Of the women receiving unopposed estrogens, 62.2% developed some type of endometrial hyperplasia. Hyperplasia was found in the endometrium of less than 1% of all groups of women receiving combined therapy. In 34 of 36 women with hyperplasia, the lesions reverted to normal after progestin therapy.

B. Other Midterm Trials

Several other progestins have been tested in shorter studies with at least 12-months follow-up (Table 1). As many as 42% of women in the groups receiving unopposed estrogens could develop endometrial hyperplasia, if they received a high estrogen dose. In that case medroxyprogesterone acetate (MPA) at a dose of 10 mg daily for 12 days was unable to totally counteract the high-dose

Table 1 Incidence of Endometrial Hyperplasia According to Various HRT Regimens

1st Author (yr)	No. of women	Duration of treatment (mo)	Estrogens type and dose (mg/d)	Progestins type and dose (mg/d × No. days)	Hyperplasia %
Clisham (6) (1992)	26	24	TD E_2 (0.1)	MPA 10 × 13 d	4
	20	24	ED E_2 (0.1)	None	42
*Moyer (7) (1993)	126	60	E_2 perc 1.5	MP 200 × 14 d	0
	23	60	E_2 perc 3	300 × 10 d	0
Thomas (8) (1993)	150	12	E_2 V (2)	NOM Ac 5 × 12 d	0.75
Foidart (9) (1994)	418	6	TDE_2 (0.05)	MPA 2.5/5/10 cc	0
Sturdee (12) (1994)	124	24	E_2 or E_2V	NETA 1 × 10 d or NG 0.5 × 10 d	2.4
	289	24	CEE 0.625 or TDE_2 0.05	NG 0.15 × 12 d or NETA 1 × 12 d	2.8
Woodruff (10) (1994)	283	12	CEE 0.625	None	21.0
	277	12	CEE 0.625	MPA 5 × 14 d	1.1
	272	12	CEE 0.625	MPA 10 × 14 d	0
	279	12	CEE 0.625	MPA 2.5 cc	0.7
	274	12	CEE 0.625	MPA 5 cc	0
Pepi (5) (1996)	119	36	Placebo	Placebo	2.5
	119	36	CEE 0.625	Placebo	62.5
	118	36	CEE 0.625	MPA 10 × 12 d	5.1
	120	36	CEE 0.625	MPA 2.5 cc	0.8
	120	36	CEE 0.625	MP 200 × 12 d	5

*Retrospective.

E_2, estradiol; E_2V, estradiol valerate; TDE_2, transdermal estradiol; CEE, conjugated equine estrogens; E_2 perc, percutaneous estradiol; MP, micronized progesterone; MPA, medroxyprogesterone acetate; NETA, norethisterone acetate; NG, norgestrel; NOMAc, nomegestrol acetate; cc, continuous combined; d, days; mo, months.

estrogen effect, leading to 4% hyperplasia (6). The lower doses of estrogens were all well opposed by micronized progesterone 200 mg/day, MPA at 10 mg daily, nomegestrol acetate at 5 mg daily, trimegestone at 0.5 mg daily, and didrogesterone at 20 mg/day (7–11).

The results were less clear when fixed combinations of estrogens and progestins were used. Adding progestins, such as levonorgestrel or norethindrone (norethisterone) acetate at very low doses would decrease the rate of hyperplasia, but not below the 1% threshold value (12). Therefore, the dose and the duration of the added progestin are of importance to totally control endometrial abnormalities.

C. Bleeding Patterns and Prediction of Endometrial Hyperplasia

From previous retrospective trials it was believed that regular bleeding occurring after day 11 of the 12-day progestin course reflected a normal secretory pattern of the endometrial tissue (13). However, further prospective trials did not confirm these findings, and no correlation could be established between bleeding patterns and histological findings (12). Nevertheless, most studies with cyclic administration of progestins, as aforementioned, showed a high percentage of regular withdrawal bleeding in women with normal secretory endometrium. The patterns were less reliable when continuous combined regimens were used. Those hormone replacement therapy (HRT) schedules were developed to avoid the withdrawal bleeding for better convenience. According to the type of HRT used, the observed bleeding patterns were indeed unpredictable.

The arguments for cyclic regimens allowing a regular reassuring withdrawal bleeding were indeed challenged, as no direct correlation could be established between occurrence of normal tissue and a regular-bleeding pattern. The prevalence of unscheduled uterine bleeding in users of cyclic or continuous combined (cc) progestin treatment was assessed on a long-term follow-up by Ettinger et al. (14) in 590 postmenopausal women. These authors also assessed the gynecological resources required to care for these women.

In the first few years of HRT, whatever the progestin schedule, women had a 1:3 chance of having unexpected bleeding and about 1:6 chance of having an endometrial biopsy (EB) to determine endometrial histology. No statistical difference in incidence of either of these events was found between women using either cyclic progestin or continuous combined progestin administration. However, among those women who used HRT for longer than 2 years, those using a continuous combined regimen had a lower rate of unexpected bleeding and of EB than those using the cycling regimen. These findings confirm several other studies showing a decreased rate of breakthrough bleeding with passage of time in women using the continuous, combined regimen.

III. THE MINIMAL EFFECTIVE DOSE OF PROGESTINS VERSUS SATISFACTORY BLEEDING PATTERNS

Although observational studies indicate that sequential progestin treatment of 12 days or more per month would substantially reduce the excess risk of endometrial cancer, associated with estrogen alone, the long-term effects of combined HRT are still not known. Controversy still exists over whether the minimum dose of progestin that is able to transform the endometrial tissue into a secretory state would be sufficient to ensure long-term protection against endometrial cancer.

To avoid the metabolic drawbacks or some progestins with androgenic properties, the trend is to decrease the progestational component of HRT to the minimal effective dose. Also, owing to the controversy over progestin's role on breast tissue (see also Chaps. 3 and 18) the scientific community recognizes the endometrial protection as the only rationale for adding progestin to estrogen replacement therapy.

The present regulations and guidelines about the evaluation of the efficacy of a progestational molecule to protect the endometrium, require assessment of one 12-month dose-ranging pivotal trial, comparing the selected unopposed estrogen dose, and the same dose associated with several doses of the progestin (15) (Table 2). The primary efficacy analysis must show a clinically and statistically significant reduction in the 1-year incidence of endometrial hyperplasia, in at least one combination estrogen–progestin group, compared with the equivalent dose of unopposed estrogen group. From a clinical perspective, the cut-off point should be about 1% of endometrial hyperplasia in the combined group.

Although the lowest effective dose might produce an adequate endometrial response, data suggest that uterine bleeding is often unacceptably prolonged with low progestin doses (11,12). Even though the regulatory authorities in North America tend to favor the lowest dose of progestin to prevent hyperplasia (15), it might be preferable to select the dose that also produces a satisfactory bleeding pattern (11). This would be more relevant for long-term compliance of

Table 2 FDA HRT Working Group Recommendations for Endometrial Protection in HRT Trials

One 12-month placebo-controlled trial
• Includes unopposed estrogen (E)/group
• Two progestin (P) doses for each E dose
• Need to demonstrate the minimum effective dose of P for each proposed E dose
• Endometrial biopsy at base line and at end of study
• Independent, replicated, blinded assessment of biopsy slides

Source: Ref. 15.

postmenopausal women. Other sequential regimens have been proposed, and one of their goals was to induce a lower number of bleeding episodes per year. Thus, once-every-3–months administration of progestins has been studied as a ''long-cycle'' regimen (16). The Scandinavian long-cycle study group has published the results of their long-term follow-up, which revealed adverse endometrial effects leading to the premature conclusion of their study (16).

Also, the selection of progestin molecules that would be metabolically weak, with no negative effect on the lipids, carbohydrate metabolism, or the vessels, but still be endometrially active would be highly relevant (17). Preliminary data (see Chaps. 5–8) indicate that molecules devoid of androgenic effects and with a high progestational activity would answer the foregoing requirements (11,18).

IV. MONITORING HRT

It would be preferable to monitor patients under long-term HRT with less expensive and invasive screening methods than a biopsy. Langer et al. (19) compared transvaginal ultrasonography with endometrial biopsy for the detection of endometrial disease. The women included in the PEPI trial underwent both assessments yearly at 48-h intervals. With a threshold value of 5 mm for the endometrial thickness, transvaginal ultrasonography had a poor positive predictive value, but a high negative predictive value for detecting serious endometrial disease in asymptomatic women. With this value, biopsies would have been indicated after more than half the ultrasonography examinations, but less than 10% of them would have revealed serious abnormalities. Therefore, the authors concluded that ultrasonography would offer no economic advantage, for half the women would need a second procedure, such as an EB. Nevertheless, ultrasonography appeared more useful for detecting endometrial hyperplasia than a more serious abnormality.

In practice, women receiving cyclic progestin therapy would have less than 1% risk of developing hyperplasia, and more than 60% would have regular withdrawal bleeding. Those women electing to use the continuous combined regimen are at a high risk of unscheduled uterine bleeding during the first 2 years, but this rate declines with time.

The endometrial biopsy would appear more reliable than ultrasonography in asymptomatic women. However, the frequency of this monitoring is still subject to debate, and a yearly biopsy would detect the 1–2% abnormal findings expected in women using combined HRT. Therefore, the recommendation should be for a yearly biopsy in all long-term users of HRT.

Progestins with a high antiestrogenic potency would be preferable, because they would oppose the estrogenic proliferative effect, at much lower doses than progestins with less antiestrogenic activity. Table 3 indicates the effective dose

Table 3 Progestins Classification, and Recommended Doses for Endometrial Protection in Hormonal Replacement Therapy (when used with various estrogens, sequential administration of progestins 10–14 days per month, of estrogen therapy)

Progestin type	Dose/oral tablet (mg)	Recommended dose (mg) for endometrial protection (α)
1. PREGNANES		
1.1. PROGESTERONE (P)		
• Micronized P	100	200–300
• Vaginal progesterone (cream)	45 or 90	45
• retro-progesterone (didrogesterone)	5 or 10	20
1.2. 17 HYDROXY PROGESTERONES		
• Chlormadinone acetate	2, 5	10
• Medroxyprogesterone acetate	2.5, 5, 10 (seq) 2.5 cc with CEE	10 (seq) 2.5 (cc)
• Cyproterone acetate	1 (with E2V 2mg) 50	1
1.3. 19-NORPROGESTERONES (β)		
• Promegestone (R5020)	0.125, 0.250	0.25–0.5
• Demegestone	0.5	1
• Nomegestrol Acetate	5	5–10
• Trimegestone	0.25, 0.5*	0.5*
• Nestorone (CVR or TTS)	0.05, 0.075, 0.1*	0.05–0.1*
• Medrogestone	5	5–10
2. TESTOSTERONE DERIVATIVES		
2.1. ESTRANES		
• Norethisterone	0.35, 5	1
• Norethisterone acetate	5–10 (TTS 0.14 or 0.25)	1 (oral); 0.25 (TTS)
• Ethynodiol di-acetate	2	2–4
• Lynestrenol	0.5, 5	—
2.2. GONANES		
• L-Norgestrel	0.075	0.15–0.5
• Desogestrel	*	
• Norgestimate	*	
• Gestodene	0.025–0.05	0.05 with E_2 2mg
• Dienogest (Hybrid progestin)	2, 3, 4*	3 and 4*

α. Data derived from references 4, 5, 8, 10–13.
*not yet registered for HRT.
Doses tested with various estrogens CEE 0.625 mg; E2V 2mg; micronized E2 2mg.
β. This new class of progestogens is not yet available in all countries.
Abbreviations: CEE, conjugated estrogens; E2, estradiol; E2V, estradiol valerate; CVR, contraceptive vaginal ring; TTS, transdermal system; P, progesterone; cc, continuous combined; seq, sequential.

of the progestins presently available for clinical use according to the published literature. Several continuous therapies are also available, but not included in the table.

Also progestins administered transdermally are available in combination transdermal systems. Their effect on the endometrium at doses as low as 0.250 mg of norethindrone (norethisterone) acetate (or even less in another system) appears sufficient to balance the endometrial estrogen stimulation (20). However, given the levels of estradiol (E_2) reached in some women, it has been recommended to perform an endometrial biopsy once a year especially in those women with high E_2 values (20).

Other routes of administration of progestins for HRT have also been proposed such as medicated IUDs or vaginal rings or gels (see Chaps. 4, 11, 12).

V. CONCLUSION

Several factors have to be taken into consideration for selecting a progestational molecule for HRT. According to the estrogen dose selected, the type of progestin, its antiestrogenic potency, the dose, and the duration of its administration per month of estrogen exposure, should be considered. Also molecules with a better safety profile are to be preferred to enhance long-term compliance of postmenopausal women with HRT.

REFERENCES

1. Ziel JK, Finkle WD. Increased risk of endometrial carcinoma among users of conjugated oestrogens. N Engl J Med 1975; 293:1167–1170.
2. Smith DC, Prentice R, Thompson DJ, Herrmann WL. Association of exogenous oestrogen and endometrial carcinoma. N Engl J Med 1975; 293:1164–1167.
3. Voigt LF, Weiss NS, Chu J, Daling JR, McKnight B, Vanbelle G. Progestogen supplements of exogenous oestrogens and risk of endometrial cancer. Lancet 1991; 338:274–277.
4. Whitehead MI, Fraser D. Controversies concerning the safety of estrogen replacement therapy. Am J Obstet Gynecol 1987; 156:1313–1322.
5. The writing group for the PEPI Trial. Effects of hormone replacement therapy on endometrial histology in postmenopausal women. JAMA 1996; 275:370–375.
6. Clisham PR, Cedars MI, Greedale G, Fu YS, Gambone J, Judd HL. Long-term transdermal estradiol therapy: effects on endometrial histology and bleeding patterns. Obstet Gynecol 1992; 79:196–201.
7. Moyer DL, de Ligniéres B, Driguez P, Pez JP. Prevention of endometrial hyperplasia by progesterone during long-term estradiol replacement: influence of bleeding pattern and secretory changes. Fertil Steril 1993; 59:992–997.

8. Thomas JL, Bernard AM, Denis C. Postmenopausal hormone replacement therapy with estrogens and nomegestrol acetate. A multicentric study. 7th International Congress on the Menopause, Stockholm, Sweden, June 1993, abstr 372.
9. Foidart JM, Kivinen S, Dombrowicz N, Wacquez M, Wälstrom H, Ibarra-Palacios P, Sitruk-Ware R. Optimal dose of medroxyprogesterone acetate (MPA) given continuously with Estraderm TTS 50 to provide amenorrhoea and endometrial control. Int J Gynecol Obstet 1994; 46 (suppl. 1): abstr P016.6.
10. Woodruff JD, Pickar JH for the Menopause Study Group. Incidence of endometrial hyperplasia in postmenopausal women taking conjugated estrogens (Premarin) with medroxyprogesterone acetate or conjugated estrogens alone. Am J Obstet Gynecol 1994; 170:1213–1223.
11. Ross D, Godfree V, Cooper A, Pryse-Davies J, Whitehead MI. Endometrial effects of three doses of trimegestone, a new orally active progestogen, on the postmenopausal endometrium. Maturitas 1997; 28:83–88.
12. Sturdee DW, Barlow DH, Ulrich LG, Wells M, Gydesen H. Is the timing of withdrawal bleeding a guide to endometrial safety during sequential oestrogens/progestogen replacement therapy? Lancet 1994; 344:979–982.
13. Padwick ML, Pryse-Davies J, Whitehead MI. A simple method for determining the optimal dosage of progestin in postmenopausal women receiving oestrogens. N Engl J Med 1986; 315:930–934.
14. Ettinger B, De-Kun Li, Klein R. Unexpected vaginal bleeding and associated gynecologic care in postmenopausal women using hormone replacement therapy: comparison of cyclic versus continuous combined schedules. Fertil Steril 1998; 69:865–869.
15. FDA HRT Working Group Guidance for clinical evaluation of combination estrogen/progestin-containing drug products used for hormonal replacement therapy of post-menopausal women. Menopause 1995; 2:131–136.
16. Cerin A, Heldaas K, Moeller B, for the Scandinavian Long Cycle Study Group. Adverse endometrial effects of long cycle estrogen and progestogen replacement therapy. N Engl J Med, 1996; 334:668–669.
17. Andrews WC. FDA guidance for development of combination estrogen–progestin products [editorial]. Menopause 1995; 2:121–122.
18. Williams JK, Adams MR. Estrogens, progestins and coronary artery reactivity. Nat Med 1997; 3:273–274.
19. Langer RD, Pierce JJ, O'Hanlan KA, Johnson SR, Espeland MA, Trabal JF, Barnabei VM, Merino MJ, Scully RE, for the Post-menopausal Estrogen/Progestin Interventions Trial. Transvaginal ultrasonography compared with endometrial biopsy for the detection of endometrial disease. N Engl J Med 1997; 337:1792–1798.
20. Jasonni VM, Naldi S, Amadori A, Santini D, Caccarelli C, Possati G, D'Anna R, Leonardi J. Transdermal progestins in hormone replacement therapy. Ann NY Acad Sci 1997; 828:352–365.

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Progestins in Postmenopausal Women: Epidemiological Data on Relationships with Endometrial and Breast Cancer Risk

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I. INTRODUCTION

Epidemiological studies published in the mid-1970s showed that hormone replacement therapy (HRT) with estrogen alone increases the risk for endometrial cancer, and this has been confirmed in many subsequent studies (1,2). Clinical studies have shown that combined treatment with an estrogen and a progestin can prevent the development of endometrial hyperplasia (3,4), and it is now standard practice to give combined treatment to postmenopausal women who have a uterus, with the intention of reducing the risk for endometrial cancer. There are strong theoretical reasons for predicting that combined hormone replacement therapy will reduce or eliminate the increase in risk for endometrial cancer caused by treatment with estrogen alone, but randomized, controlled trials to test this have not been conducted, and current knowledge is based on a few relatively small epidemiological studies.

Hormone replacement therapy with estrogen alone increases the risk of diagnosis of breast cancer as well as endometrial cancer; the relative risk associated with estrogen replacement therapy is smaller for breast cancer than for endometrial cancer, but breast cancer is more common than endometrial cancer and survival is poorer. There is no reason to add progestins to hormone replacement therapy for the benefit of the breasts, and it has been suggested that progestins might cause a further increase in breast cancer risk (5). As for

endometrial cancer, current knowledge of the effects of progestins on breast cancer risk in postmenopausal women is based on relatively few data from epidemiological studies.

II. LIMITATIONS OF THE EPIDEMIOLOGICAL DATA

The data available are important but they do have limitations. The use of progestins as part of hormone replacement therapy has only recently become widespread, and in most of the existing epidemiological studies the majority of women have used estrogens alone. Furthermore, even among women who have used progestins, this use may have been of short duration, or the information available on patterns of use may be scanty. One particular problem with studies of hormone replacement therapy is that such use commonly begins at menopause, and there is usually a close association between duration of use and time since menopause. Failure to adjust properly for this association can produce serious underestimation of the risks associated with hormone use (6).

III. ENDOMETRIAL CANCER

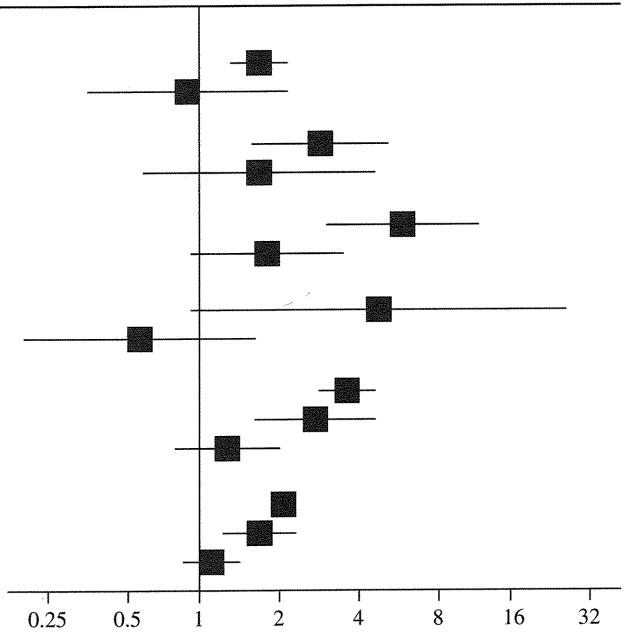
The effects of hormone replacement therapy with estrogen alone on the risk for endometrial cancer are now reasonably well defined. Risk increases with increasing duration of use, reaching a relative risk of about 10 after 10 or more years of use; the elevation in risk declines after cessation of use, but the risk is still significantly raised 5 or more years after last use (7).

There are fewer data in relation to combined hormone replacement therapy with an estrogen and a progestin. The results of six epidemiological studies are shown in Table 1 (8–13). For each study, the results plotted are the relative risks, compared with women not using any HRT, for women using estrogen alone, and for women using estrogen combined with a progestin. All six studies found a substantial increase in relative risk in association with use of estrogen alone, and this increase was statistically significant in all except the very small study of Jick (11). In all six studies, use of estrogen with a progestin was associated with a lower relative risk than use of estrogen alone, although in four out of the six studies (including the two largest studies) the relative risk was slightly, but not significantly higher than in untreated women. Both studies that presented results according to the duration of progestin use each month found that the relative risk was lower in women using progestins for 10 or more days per month than in women using progestins for fewer than 10 days per month.

The data are thus completely consistent in showing that addition of a progestin reduces the increase in risk of developing endometrial cancer that is

Table 1 Relative Risks of Endometrial Cancer, Compared with Never Users, in Relation to Hormone Replacement Therapy Comprising Estrogen alone or Estrogen with Progestin in Six Studies

Study and treatment[a]	Number of cases	Relative risk (95% confidence interval)
Persson et al. (8)[b]		
E	48	1.4 (1.1–1.9)
E + P	7	0.9 (0.4–2.0)
Brinton et al. (9)		
E	60	3.4 (1.8–6.3)
E + P	11	1.8 (0.6–4.9)
Jick et al. (10)[c]		
E	32	6.5 (3.1–13.3)
E + P	18	1.9 (0.9–3.8)
Jick 1993 (11)[d]		
E	4	5.1 (0.9–30.2)
E + P	4	0.6 (0.2–1.9)
Beresford et al. (12)[e]		
E	324	4.0 (3.1–5.1)
E + P <10 d	25	3.1 (1.7–5.7)
E + P 10–21 d	25	1.3 (0.8–2.2)
Pike et al. (13)[f]		
E	422	2.2 (1.9–2.5)
E + P <10 d	74	1.9 (1.3–2.7)
E + P ≥10 d	79	1.1 (0.8–1.4)



[a]E, estrogen alone; E + P, estrogen with progestin, including duration of progestin per month where available.
[b]Case–cohort analysis.
[c]Relative risks for use currently or within the year before diagnosis or pseudodiagnosis. Some overlap with study of Voigt et al. (14).
[d]Relative risks for current use.
[e]This study is an extension of that of Voigt et al. (14). The relative risks given here for progestin use exclude cases in the earlier paper.
[f]Relative risks are per 5 years of use. Reference group includes never users and users of other types of hormone replacement therapy.

Table 2 Relative Risk of Endometrial Cancer, Compared with Never Users, in Users of Estrogen Combined with Cyclic Progestin

	Duration of hormone therapy		
Progestin use	6–35 mo	36–59 mo	≥60 mo
Progestin < 10 days/mo	2.1 (0.9–4.7)	1.4 (0.3–5.4)	3.7 (1.7–8.2)
Progestin 10–21 days/mo	0.8 (0.4–1.8)	0.6 (0.2–1.6)	2.5 (1.1–5.5)

Source: Ref. 12.

due to estrogen replacement therapy. However, the most important question is whether adequate use of progestins can completely eliminate the increase in risk caused by estrogen, or can even reduce the risk below that of untreated women. The first four studies summarized in Fig. 1 are small and do not provide information on the number of days per month for which progestins were used. The recent studies of Beresford et al. and Pike et al. (12,13) are more informative and the results of these studies, therefore, are described in more detail.

The study of Beresford et al. (12) is a population-based study of 832 cases and 860 controls (the first part of this study was reported in an earlier paper; 14). As shown in Table 1, the relative risk in current users was substantially increased among women who used estrogen alone and in women who used progestins for fewer than 10 days per month, but only slightly and insignificantly increased among women who used progestins for 10–21 days per month. However, further analysis by duration of use of combined HRT showed that the relative risk was significantly increased (relative risk [RR] 2.5; 95% CI, 1.1–5.5) among women who had used combined treatment with a progestin for 10–21 days per month for 5 years or more, as well as in women with long-term use of combined treatment with a progestin for less than 10 days per month (Table 2).

Pike et al. (13) conducted a population-based study of 833 cases and 791 controls. As shown in Table 1, the relative risk was significantly increased in women who used estrogen alone (RR per 5 years = 2.2; 95% CI, 1.9–2.5) and in women who used combined treatment with a progestin for fewer than 10 days per month (RR per 5 years = 1.9; 95% CI, 1.3–2.7). However, women who used combined treatment with a progestin for 10 days or more per month had no significant increase in risk (RR per 5 years = 1.07; 95% CI, 0.8–1.4), nor did women who used combined replacement therapy with continuous use of a progestin (RR per 5 years 1.07; 95% CI, 0.8–1.4; not shown in Table 1). These results appear to differ from those of Beresford et al. (12), and more data are needed to clarify the effects of long-term use of combined treatment with

a progestin for 10 days or more per month. More data are also needed on the type and dose of progestin (15).

IV. BREAST CANCER

The epidemiology of breast cancer suggests that endogenous estrogens increase risk: risk is increased by early menarche, late menopause, and postmenopausal obesity, and recent prospective studies have shown that high serum concentrations of estradiol are associated with an increase in breast cancer risk in postmenopausal women (16). These observations suggest that postmenopausal hormone replacement therapy with estrogen alone may also increase breast cancer risk. Furthermore, whereas both progesterone and synthetic progestins stop the endometrial cell division that is stimulated by estrogen, there is no evidence that this occurs in the breast; therefore, there is no reason to predict that progestins might be protective for breast cancer. It is also possible that progestins might augment the mitotic effect of estrogens and, thereby, increase breast cancer risk (5,17).

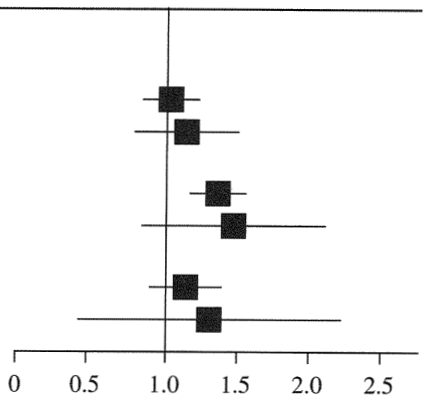
For many years, researchers have debated the interpretation of epidemiological studies of HRT and breast cancer risk; some studies did show an increase in risk with increasing duration of use, but others appeared to show no association. Much of this controversy was probably due to two features of these studies: (a) the effect of HRT on breast cancer risk is relatively small, much smaller than the effect of estrogen replacement therapy on the risk for endometrial cancer; therefore, it is difficult to detect; (b) there is strong confounding owing to menopause—women with early menopause are at reduced risk for breast cancer, but are also likely to begin HRT therapy at a young age and to continue treatment for a long time.

The report in 1997 from the Collaborative Group on Hormonal Factors in Breast Cancer (6) has overcome these problems and produced definite answers to some of the outstanding questions. This group conducted a collaborative reanalysis of data from 51 epidemiological studies of 52,705 women with breast cancer and 108,411 women without breast cancer. The large size of this data set made it possible to produce precise estimates of risks, and a particular feature of the main analyses was that women were only included if there was a reliable estimate of age at menopause, and all results were adjusted for the number of years since menopause.

The main findings of this analysis were as follows: (a) among current users of hormone replacement therapy or those who ceased use 1–4 years previously, the relative risk of having breast cancer diagnosed increased by 2.3%/year of use; (b) 5 or more years after cessation of use of hormone replacement therapy

Table 3 Relative Risk of Breast Cancer, Compared with Never Users, in Relation to Hormone Replacement Therapy Comprising Estrogen Alone or Estrogen with Progestin (or Progestin Alone), by Time Since Last Use and Duration of Use

Treatment	Number of cases	Relative risk (95% confidence interval)
Last use < 5 years before diagnosis		
Duration < 5 years		
Estrogen alone	498	0.99 (0.83–1.15)
Estrogen and progestin, or progestin alone	136	1.15 (0.78–1.52)
Duration ≥ 5 years		
Estrogen alone	558	1.34 (1.16 –1.52)
Estrogen and progestin, or progestin alone	58	1.53 (0.88–2.18)
Last use ≥ 5 years before diagnosis		
Estrogen alone	310	1.12 (0.90–1.34)
Estrogen and progestin, or progestin alone	21	1.30 (0.40–2.20)



Source: Ref. 6.

there was no significant excess of breast cancer diagnosis overall or in relation to duration of use; (c) cancers diagnosed in women who had ever used HRT tended to be less advanced clinically than those diagnosed in women who had never used HRT.

These findings were for all use of any type of HRT in the 51 studies; most of the data were from the United States and most of hormone replacement therapy was estrogen alone, predominantly conjugated equine estrogens. Information on the specific hormonal constituents of the therapy used was available for 22 of the 51 studies. This information was used to ascertain the preparation used most by each woman, and women were grouped according to whether they had used predominantly preparations containing estrogens alone, preparations containing both estrogen and progestin (or progestin alone), or preparations containing estrogen together with some other compound.

Table 3 shows the results for women known to have used predominantly estrogen alone and for women who had used predominantly estrogen and progestin (or progestin alone). For use of estrogen alone, the relative risk of breast cancer was significantly raised (1.34; 95% CI, 1.16–1.52) among women who had recently used HRT for at least 5 years, but there was no significant increase in risk in women who had recently used hormone replacement therapy for fewer than 5 years or in women who had used hormone replacement therapy 5 or more years previously (irrespective of the duration of use). The results for therapy including progestins are based on only 215 cases altogether. All three relative risks were above 1.0 and were higher than the corresponding relative risk in women who had used predominantly estrogen alone, but none of these results was statistically significant.

V. CONCLUSIONS

Addition of progestins to hormone replacement therapy reduces the risk for endometrial cancer relative to women receiving estrogen alone. The reduction is greater if the progestin is used for 10 or more days per month than if it is used for fewer than 10 days each month. More data are required to determine the optimum type, duration, and dose of progestin use per month, and to determine whether optimal use can completely eliminate the increase in risk.

There are few data for breast cancer and firm conclusions cannot be drawn except that progestins do not protect against breast cancer. Analyses of all HRT show that risk increases with duration of use while the therapy is being used, but then falls after cessation of use. In the data available, breast cancer risk is slightly higher among women receiving HRT containing a progestin than in women receiving estrogen alone. This difference is not statistically significant, but use of combined HRT is now very common, and more epidemiological data are urgently needed.

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REFERENCES

1. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Vol 21. Sex Hormones (II). Lyon: IARC, 1979.
2. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Suppl. 7. Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs Vols. 1–42. Lyon: IARC, 1987.
3. Paterson ME, Wade-Evans T, Sturdee DW, Thom MH, Studd JW. Endometrial disease after treatment with oestrogens and progestogens in the climacteric. Br Med J 1980; 22:822–824.
4. The Writing Group for the PEPI Trial. Effects of hormone replacement therapy on endometrial histology in postmenopausal women. JAMA 1996; 275:370–375.
5. Key TJA, Pike MC. The role of oestrogens and progestagens in the epidemiology and prevention of breast cancer. Eur J Cancer Clin Oncol 1988; 24:29–43.
6. Collaborative Group on Hormonal Factors in Breast Cancer (writing and analysis committee: Beral V, Reeves G, Bull D, Peto R, Key T, Doll R). Breast cancer and hormone replacement therapy: collaborative reanalysis of data from 51 epidemiological studies of 52,705 women with breast cancer and 108,411 women without breast cancer. Lancet 1997; 350:1047–1059.
7. Grady D, Gebretsadik T, Kerlikowske K, Ernster V, Petitti D. Hormone replacement therapy and endometrial cancer risk: a meta-analysis. Obstet Gynecol 1995; 85:304–313.
8. Persson I, Adami H-O, Bergkvist L, Lindgren A, Pettersson B, Hoover R, Schairer C. Risk of endometrial cancer after treatment with oestrogens alone or in conjunction with progestogens: results of a prospective study. Br Med J 1989; 298:147–151.
9. Brinton LA, Hoover RN, the Endometrial Cancer Collaborative Group. Estrogen replacement therapy and endometrial cancer risk: unresolved issues. Obstet Gynecol 1993; 81:265–271.
10. Jick SS, Walker AM, Jick H. Estrogens, progesterone, and endometrial cancer. Epidemiology 1993; 4:20–24.
11. Jick SS. Combined estrogen and progesterone use and endometrial cancer [letter]. Epidemiology 1993; 4:384.
12. Beresford SAA, Weiss NS, Voigt LF, McKnight B. Risk of endometrial cancer in relation to use of oestrogen combined with cyclic progestagen therapy in postmenopausal women. Lancet 1997; 349:458–461.
13. Pike MC, Peters RK, Cozen W, Probst-Hensch NM, Felix JC, Wan PC, Mack TM. Estrogen–progestin replacement therapy and endometrial cancer. J Natl Cancer Inst 1997; 89:1110–1116.

14. Voigt LF, Weiss NS, Chu J, Daling JR, McKnight B, van Belle G. Progestagen supplementation of exogenous oestrogens and risk of endometrial cancer. Lancet 1991; 338:274–277.
15. Grady D, Ernster VL. Hormone replacement therapy and endometrial cancer: are current regimens safe? J Natl Cancer Inst 1997; 89:1088–1089.
16. Thomas HV, Reeves GK, Key TJA. Endogenous estrogen and postmenopausal breast cancer: a quantitative review. Cancer Causes Control 1997; 8:922–928.
17. Pike MC, Spicer DV, Dahmoush L, Press MF. Estrogens, progestogens, normal breast cell proliferation, and breast cancer risk. Epidemiol Rev 1993; 15:17–35.

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Progestins in Hormonal Replacement Therapy and Cardiovascular Risk

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I. INTRODUCTION

The main benefit for progestin use in hormonal replacement therapy (HRT) is the protection of the endometrium by opposing the proliferative effect of estrogens. On the other hand, several risks are attributed to progestins as a class-effect, although the molecules used in HRT vary and do not induce the same side effects according to their different pharmacological properties. Among the alleged risks of progestins in HRT, cardiovascular risk has been one of the most debated issues. This chapter will address both risks and benefits of progestins in HRT and analyze the available data that led to opposing views on the risk of cardiovascular disease (CVD).

It is now well established that CVD represents the major cause of death in women, just as in men, but at a later age. In women the incidence of CVD increases after the menopause, and the risk of atherosclerosis increases by three- to fourfold after a natural menopause (1). Several epidemiological studies performed since the early 1980s indicate about a 50% reduction in cardiovascular morbidity and mortality in women using estrogens after the menopause (2–4). However, controversy is ongoing over a possible selection bias in the studies (i.e., healthier women receive estrogens for long-term use more often than women at risk) (3). This hypothesis has been reinforced by the recent results from the Heart and Estrogen/Progestin Replacement Study (HERS) research group, indicating that in postmenopausal women with established CHD, an HRT regimen with conjugated estrogens (CEE) and medroxyprogesterone acetate (MPA), does

not reduce mortality compared with untreated controls (5). These findings were disappointing, but not unexpected, in the light of previous animal experiments.

Indeed, a better understanding of the mechanisms underlying the protective effect of sex steroids was provided by animal studies using the monkey model (6), as well as studies of postmenopausal women, using surrogate markers of cardiovascular risk (7,8). There is now evidence that estrogens will improve the endothelial function and, hence, the vascular tone (8), as well as improve the lipid profile (9), the carbohydrate metabolism (10), and hemostatic parameters (11).

The controversy was again raised over the role of progestins, prescribed together with estrogens to protect the endometrium, and some of the most prescribed molecules were shown to partially oppose the beneficial cardiovascular effect of estrogens (12). Unfortunately, that concern was directed toward progestins as a class effect, although several categories of progestins are often prescribed, and striking differences exist according to the type of molecules that have been tested (13–15). Obviously, natural progesterone and some of its derivatives, such as the 19-norprogesterone molecules, do not exert any androgenic effects (13,15); hence, no negative effect on the lipid levels, whereas the 19-nortesterone derivatives and even some 17-hydroxyprogesterone molecules exert a partial androgenic effect (13,16), explaining some of the negative effects observed on cardiovascular risk factors or on surrogate markers of risk.

Given these class differences, it would appear inappropriate to claim that progestins in general compromise the cardioprotective effects of estrogens without specifying which of these progestins reverse the estrogenic effects and those that do not as indicated by the existing data.

II. EPIDEMIOLOGICAL DATA

A. Coronary Heart Disease

Barrett-Connor (4) reported the results of her metanalysis of 25 published studies on the relation between HRT and coronary heart disease (CHD). The relative risk (RR) for estrogen users was 0.7 (confidence interval [CI], 0.65–0.75) and in the studies that assessed the combined HRT, the RR was 0.66 (CI, 0.53–0.84). Among the studies included in the metanalysis, an epidemiological study analyzing the relative risk of myocardial infarction of estrogen–progestin users versus nonusers, indicated a ''protective'' effect for all therapies, as compared with no treatment, even in the group using levonorgestrel, the most androgenic of the progestins used so far in HRT (17).

Grodstein et al. (18) reported the relation between cardiovascular disease and HRT in 59,337 women followed for up to 16 years. Compared with the

Table 1 Key Ongoing Trials Focusing on Primary or Secondary Prevention of CHD and Including Progestin Treatment in Combined HRT

	Steroids studied	Population	Expected results
Primary prevention			
WHI (Women's Health Initiative)	CEE/MPA	164,000	2005
MRC–HRT study (Medical Research Council)	CEE/MPA	34,000	2006
Secondary Prevention			
HERS (Heart and Estrogen/Progestin Replacement Study)	CEE/MPA	2,763 with CHD	1998 (Ref. 25)
Coronary atherosclerosis			
WELL–HART (Women's E/P Lipid Lowering Hormone Atherosclerosis Regression Trial)	E_2/MPA	with CHD	2001?

risk of major coronary heart disease for women who did not use hormones, the relative risk was 0.6 (95% CI, 0.43–0.83) for women using estrogens alone and even lower at 0.39 (CI, 0.19–0.78) for those using combined hormones. The authors found no association between stroke and use of combined hormones. For this study, conducted in the United States, the progestin used was nearly always medroxyprogesterone acetate. Although this large observational study confirms the previous results of Falkeborn et al. (17) in Sweden and Psaty et al. (19) in the United States, the possibility of a selection bias is raised again. Women who stay on long-term HRT are usually healthier, on average, than those who do not, with lower blood pressure, lower weight, and they exercise more often. Grodstein et al. (18) have adjusted for these confounding factors as did the other researchers. However, only the long-term, randomized, controlled trials, will bring a definite conclusion (Table 1).

B. Primary and Secondary Prevention Trials

Several ongoing epidemiological trials focus on primary or secondary prevention of CHD (5,20). From all the large ongoing studies, HERS has published the first results (5). The other trials will be completed just after the turn of the century (see Table 1).

The Women's Health Initiative in the United States and the Medical Research Council—HRT study, in Europe are designed as long-term primary pre-

vention trials and would enroll postmenopausal women without coronary disease. The long-term follow-up under treatment or placebo will help answer the questions of the presumed protective effects of HRT in preventing the occurrence of CVD and CHD. In women using estrogen alone, observational studies indicated an 80% reduction of risk, also in secondary prevention, in women with documented coronary disease (4). In the latter women, it was not clear, however, whether estrogen plus progestin would improve survival or not until the results of HERS appeared.

Among the secondary prevention trials addressing that question, the Heart and Estrogen/Progestin Replacement Study (HERS) recently gave a negative answer (5). The study was designed to determine whether estrogen (conjugated equine estrogen; CEE) plus continuous progestogen (medroxyprogesterone acetate; MPA) is better than placebo in preventing recurrent events in women with documented coronary disease. In this randomized, double-blind, placebo-controlled trial, 2763 women have been followed up for 4.1 years on average. The main objective of the study was to determine whether HRT would reduce the morbidity and mortality of cardiovascular disease in this high-risk group, and the primary outcome was the occurrence of nonfatal myocardial infarction (MI) or CHD death.

After 4 years of follow-up the same number of events were recorded in both the active and the placebo groups, indicating that the combined HRT regimen selected did not reduce the overall rate of coronary events in postmenopausal women with previous CHD. Their relative hazard (RH) for a further event was 0.99 (95% CI, 0.8–1.2). Also, there was no significant difference between groups in any of the secondary outcomes, despite a net decrease in low-density lipoprotein (LDL) and an increase in high-density lipoprotein (HDL) cholesterol levels in the hormone group. A more detailed analysis is ongoing to explain why the therapy did not bring the expected protective effect of HRT. Nevertheless, the authors concluded that, although they do not recommend starting HRT for the secondary prevention of CHD, they would find it appropriate for women receiving it to continue, given the favorable pattern of CHD events after several years of treatment.

Angiographic trials are also ongoing comparing estrogens with and without progestins with placebo on the vessels of women with previously documented CHD. These studies will answer the question of whether HRT can slow or even reverse the accumulation of atherosclerotic plaque in the coronary arteries by providing direct evidence of the arteries' anatomical changes. It is obvious that different molecules may bring along different results; unfortunately, most of the large ongoing trials have selected the same HRT regimen for their study design, and we will not have an answer about a possible beneficial effect of other treatment regimens.

C. Stroke and Venous Thromboembolism

Hormone replacement therapy is not consistently associated with a reduced or increased risk of stroke, either of hemorrhagic or thromboembolic origin (4). In the large cohort studies, and in mainly the Nurses Health Study (18), no significant association has ever been found between stroke and hormones. In recent observational studies current users of HRT have been found to be at an increased risk of venous thromboembolism (22–25). Three papers published in *Lancet* on 12 October 1996, led to a published statement from the Committee on Safety of Medicines of the MCA in the United Kingdom (26). The articles express concern, because all studies showed an increased risk of deep venous thrombosis or pulmonary embolism in women currently taking HRT. The relative risks ranged from 2.1 to 3.5 according to the study (Table 2). That the risk appears to be concentrated in the first year would suggest that some women who are more sensitive, or with predisposing factors, would develop thrombotic events with any HRT and then stop therapy, whereas the other women who tolerate it better remain in the longer-term users group. The recent results of HERS indicate that combined treatment with CEE and MPA did increase the rate of thromboembolic events in women with previous CHD, as compared with placebo (5) (RH 2.89; 95% CI, 1.5–5.6). This double-blind radomized, placebo-controlled study confirmed the results of the aforementioned observational studies. The role of the progestin in this apparently negative effect has to be evaluated. Therefore, it is of the utmost importance to investigate the effects of the various sex steroids on the hemostatic parameters to select therapy with those agents devoid of unwanted effects.

Although the large prospective trials are the only way to answer definitely the role of HRT in CVD and CHD in postmenopausal women, several short-term

Table 2 Studies of Venous Thromboembolism with HRT

First Author (yr)	Study population	Relative risk (95% CI)
Daly (23) (1996)	VTE case–control	3.5 (1.8–7.0)
Jick (24) (1996)	VTE case–control	3.6 (1.6–7.8)
Grodstein (25) (1996)	PE cohort	2.1 (1.2–3.8)
Perez-Gutthan (22) (1997)	VTE case–control	Adjusted (odd ratios)
	Unopposed	1.9 (1.0–3.8)
	Opposed	2.2 (1.4–3.5)
	Current use	2.1 (1.4–3.2)

VTE, venous thromboembolic events; PE, pulmonary embolism; CI, confidence interval.

trials used surrogate markers of risk to compare the various molecules available for HRT. Those markers are well-known risk factors for CVD.

III. ROLE OF PROGESTINS ON THE RISK FACTORS FOR CARDIOVASCULAR DISEASE

Among the main cardiovascular risk factors recognized for both men and women, cigarette smoking, high cholesterol levels, hypertension, diabetes mellitus, and obesity may be preventable causes of coronary heart disease (28). The existence of atherosclerosis in the vascular tree definitely increases the risk of cardiovascular disease. In women, the estrogen deprivation following menopause may affect several of these risk factors, and it is now well accepted that estrogen replacement therapy (ERT) will improve cholesterol levels, diastolic blood pressure, insulin sensitivity, and some of the clotting factors (9–11,13). The beneficial effects of estrogens on the vasodilating endothelial factors, hence, on vasomotion (29), would definitely play a major role in the primary prevention of coronary heart disease in women.

A. Effects of the Progestins on Lipids and Lipoproteins

Most of the studies evaluating the effect of estrogens on the lipoproteins indicated a reduction of LDL-C levels and an increase of HDL-C levels by 10 to 15% (13) (see also Chap. 20). The addition of a progestin to ERT may affect the lipid formula, however, the effects differ according to the type of the progestogen. Progestogens with androgenic properties partially reverse the HDL-raising effect of estrogen (13,30), whereas natural progesterone and some 19-norprogesterone derivatives, such as nomegestrol acetate do not affect the HDL levels (13,31).

The results from various studies indicate that it is not the dose or route of administration that is the most important factor to consider for progestins' effect on HDL, but rather, the molecule from which they derive. Those progestogens derived from progesterone and devoid of androgenic properties, do not impede the beneficial estrogen effects. Therefore, as a pratical recommendation, one should conclude that, overall, the effects of most HRT regimens on lipids and lipoproteins would, on balance, seem to be beneficial (9), but the selection of the least androgenic progestins should be recommended for long-term therapy.

B. Effects of Progestins on Carbohydrate Metabolism

Glucose intolerance and hyperinsulinemia are well-known risk factors for cardiovascular disease. Postmenopausal women have an age-related deterioration of glucose metabolism (32) and have a reduced number of peripheral insulin re-

ceptors, compared with premenopausal women in the early follicular phase (33). Insulin is a potent stimulus to endothelial cell growth and also regulates LDL receptor activity (34,35). Therefore a reduction in fasting insulin levels may be important in controlling one of the mechanisms of CVD (see also Chap. 21).

In several studies using various progestins in combined HRT, variations in response to oral or IV glucose tolerance test are observed according to the androgenic or nonandrogenic properties of the progestin used, the latter being neutral toward carbohydrate metabolism (10,36–38).

C. Effects of Progestins on the Hemostatic Risk Factors

The suggested preventive effect of sex steroids on the development of atherosclerosis might be counteracted by their possible thrombogenic effect, indicated by recent studies among oral contraceptive users. Obviously, ethinyl estradiol contained in the contraceptive pill is no longer used in ERT, and the so-called third-generation progestins are not yet widely used for HRT.

In the large 3-year Progestin/Estrogen Postmenopausal Intervention Trial (PEPI trial) the combined regimen of equine estrogens plus MPA or plus progesterone lowered fibrinogen levels (13), one of the markers considered to be an independent risk factor for myocardial infarction and stroke (11). The plasma fibrinogen concentrations increase with age, especially during the menopausal transition. In a 2-year open, prospective study, 42 postmenopausal women received estradiol given transdermally (50 μg/day) and MPA (5 mg/day, 12 days every second month). The hemostatic risk factors were measured at baseline, at 3 months, and after 2 years of treatment, and compared with the results observed in an untreated control group of 18 postmenopausal women, as well as a reference group of 20 premenopausal women. Fibrinogen levels significantly decreased under HRT, whereas they slightly increased in the untreated women. Similarly, factor VII antigen and PAI-1 antigen decreased after 2 years of treatment, but slightly increased in the control group. There were no changes in antithrombin III (AT-III) or protein C values in any group. Therefore, a beneficial effect of the sex steroids used in the study was demonstrated on the hemostatic parameters involved as a defense system against thrombosis (11). Also with a progestin other than MPA, Basdevant et al. (38) showed no effect of nomegestrol acetate on plasminogen, fibrinogen, or proteins C and S. The only change observed was a significant increase in antithrombin III which indeed may not be considered as a negative effect.

D. Effects of Progestins on Blood Pressure

Menopause by itself has no influence on high blood pressure (BP) according to longitudinal studies (39). Whether normotensive or hypertensive women would

be at higher risk of increased BP under treatment has been questioned. Recently, Foidart et al. (40) have evaluated the effect of transdermal estradiol (50 μg/day) and sequential use of MPA (10 mg/day for 12 days/month) on the blood pressure of hypertensive women whose condition was therapeutically controlled. Only 1 patient out of 92 experienced an increase in diastolic blood pressure; no effect of treatment was detected on either the systolic or diastolic blood pressure of the remaining 91 patients. In another study, long-term treatment with a combined regimen of transdermal estradiol and medroxyprogesterone acetate in normotensive postmenopausal women was reported by Pang et al. (41). In this study, treatment was associated with a reduction in mean systolic and diastolic blood pressures. In neither study did combined HRT with MPA as the progestin component appear to affect blood pressure negatively.

IV. DIRECT EFFECTS OF PROGESTINS ON THE VESSELS

Much attention has been directed toward the effects of sex steroids on the vessels and, although estrogens exert beneficial effects on the vascular wall, it has been suggested that some progestins may reverse that benefit.

A. Atherosclerosis

The relevance of the lipid changes induced by sex steroids has been questioned, as the role of HDL changes in the alleged cardiovascular protective effects of estrogens accounts for 30%, to a maximum of 50%, of these effects (42). The results obtained by Adams et al. (14) in the female cynomolgous monkey denied a direct relation between lipid changes and atherosclerosis. These authors examined the effects of sex hormone replacement therapy on diet-induced coronary artery atherosclerosis, expressed as the plaque area developed in the vessels of female ovariectomized monkeys. The animals received either no therapy or estradiol, or combined estradiol and progesterone from subcutaneous implants. In parallel, the animals were fed with a highly atherogenic diet for 25 months. The plaque area was significantly decreased in both groups of animals that received steroids, although HDL and total cholesterol levels were not significantly different among groups. Their results suggest, first, that HRT has antiatherogenic effects independently of variation in the lipid formula, and second, that natural progesterone does not reverse the beneficial effect of estradiol. To date, these results have not been reproduced with other progestins (Table 3 and Fig. 1).

B. The Vessel Wall and Endothelial Factors

The most recent studies on the mechanisms by which estrogens may afford cardioprotective effects have examined their effect on endothelial function. The

Table 3 Coronary Artery Atherosclerosis (Plaque Area), Total Plasma Cholesterol (TC) and Plasma HDL Concentrations in Ovariectomized Monkeys With or Without Sex Steroid Treatment Given During 25 Months

Variable	No treatment $n = 17$	$E_2 + P$ $n = 20$	E_2 $n = 18$	p
Plaque area (mm^2)	0.204	0.105	0.109	$p = 0.002$
TC* (mg/dL)	463 ± 26	433 ± 30	448 ± 33	NS
HDL* (mg/dL)	34.9 ± 4.0	35.2 ± 3.3	35.9 ± 3.0	NS

*means ± SEM; NS, not significant; E_2, estradiol; p, progesterone (implants).
Source: Ref. 14.

presence of estrogen- and progesterone-binding sites in endothelial cells and the vessel wall has been documented in animal and human arteries (43). A direct role of estrogens on the endothelium has been suggested and may be related to binding of the steroids to their receptors.

The endothelium is actively involved in regulating vascular tone through the production of endothelial factors with vasodilating or vasoconstricting properties. Hayashi et al. (44) have found evidence of a greater production of nitric

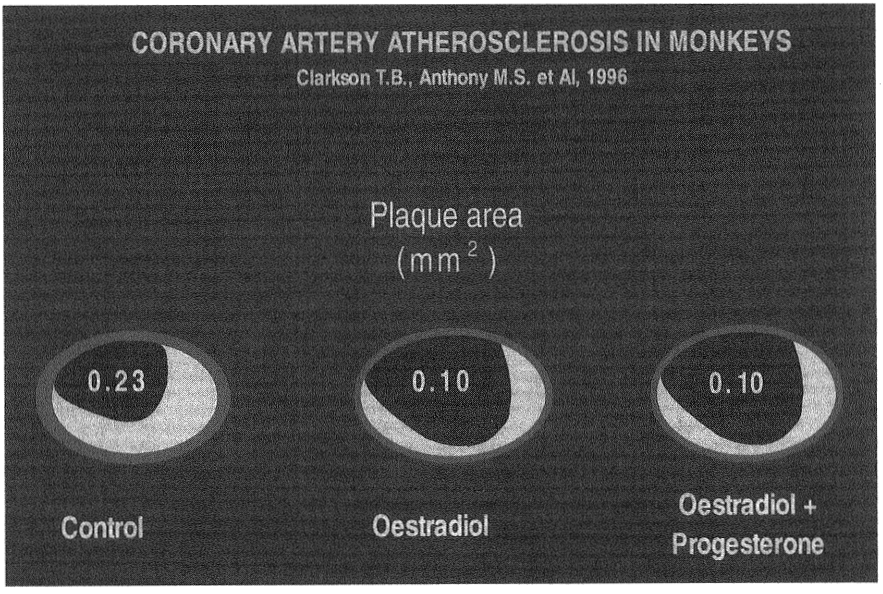

Figure 1 Effects of sex hormone replacement therapy on the coronary artery atherosclerosis (plaque area) in ovariectomized monkeys. (From Ref. 14.)

oxide in female than in male rabbit aorta, suggesting that estrogens might affect the release of this endothelial vasodilating factor. In postmenopausal women, the levels of vasodilatory factors, nitric oxide, and prostacyclin, as well as vasoconstrictive factors—endothelin-1 and thromboxane-A_2—vary under therapy. Ylikorkala et al. (45) have recently shown that especially women who smoke have high levels of endothelin-1. In these women, transdermal combined therapy, with estradiol and norethisterone acetate, was able to significantly decrease the levels of that potent vasoconstrictive agent.

C. Smooth Muscle Cells

Other recent studies performed in vitro, on vascular smooth muscle cells (VSCM) indicated that sex steroids, both E_2 and P, inhibit their proliferation (46). Progesterone at physiological levels inhibited DNA synthesis and proliferation in these cells in a dose-dependent manner (47).

D. Vasomotion

From the major studies performed in the primate model at the Winston-Salem, Bowman Gray School of Medicine, since the early 1990s, it is now well-known that sex hormones modulate the vasomotor response of the main arteries, particularly the coronary arteries.

In the cynomolgus monkey (6,18), 17β-estradiol modulated the responses of the coronary arteries of the animals to acetylcholine (ACh). Estrogen-deprived atherosclerotic monkeys were compared with animals receiving estrogen replacement therapy. The degree of coronary artery constriction following an infusion of acetylcholine was measured in both groups of animals. Paradoxical vasoconstriction occurred following ACh administration in the untreated animals, whereas estradiol therapy restored the normal endothelium-dependent vasodilation. The process occurred rapidly, vasomotion being restored to normal within 20 min of an intravenous injection of estrogens. Progesterone did not reverse the effects of estrogens. The authors concluded that estrogens preserved the normal endothelium-mediated dilation of coronary arteries, and natural progesterone did not reverse this potential cardioprotective mechanism.

Similar regulation of the vasomotion was found in women with coronary disease, those receiving ERT exhibiting a dose-dependent vasodilation in response to ACh, in contrast with the untreated women who exhibited a vasoconstriction (48). The changes observed in the vasomotion of the postmenopausal women appear to be as prompt as observed in monkeys (49). Collins et al. (50) showed that intracoronary estradiol decreased the ACh-induced vasoconstriction in nine postmenopausal women, but not in seven men of similar age.

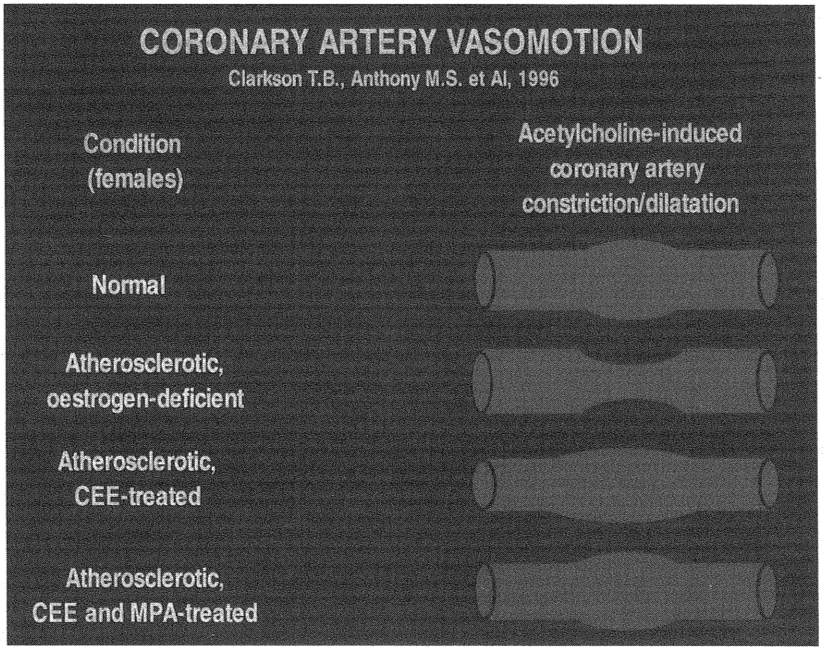

Figure 2 Coronary artery vasomotion in ovariectomized monkeys treated with sex steroids: CEE, Conjugated equine estrogens; MPA, medroxyprogesterone acetate. (From Ref. 6.)

It has been suggested that progestins would partially reverse the estrogenic effects based on the assumption that these molecules exert an antiestrogenic effect at several target levels. Sullivan et al. (12) studied the effects of conjugated equine estrogen, given alone during 21 days and with added progestin, MPA 10 mg for 10 days, on forearm vascular resistance in postmenopausal women. They found that resting vascular resistance and resistance after cold pressor stimulation rose significantly and at a higher level during combined treatment than after estrogen alone.

In the monkey model, as described earlier, the addition of cyclic or continuous MPA to estrogens inhibited ACh responses by 50% (51) (Fig. 2). Williams et al. (51) administered MPA to ovariectomized animals with diet-induced atherosclerosis. The progestin was given either continuously at a low dose, or at a cyclic, high-dose regimen. Both regimens were equivalent to the currently recommended regimens used in postmenopausal women. In both instances, MPA decreased the estrogen-induced dilator response to ACh. However, other progestins exert different effects on the vascular tone.

Miyagawa et al. (52) comparing the effects of progesterone and MPA on the same model, from the standpoint of coronary artery vasospasm, showed that progesterone plus estradiol protected, but MPA plus estradiol failed to protect, allowing vasospasm. Conversely, Williams et al. (53,54) indicated that a nonandrogenic progestin, nomegestrol acetate, does not diminish the beneficial effects of estrogen on the coronary dilator response in monkeys.

Although these studies differed in design and in duration of follow-up, it appears that not all progestins act similarly on vasomotion. Different HRT combinations may affect the vascular reactivity in different ways. From the data now available, nonandrogenic molecules would appear to be safer in that respect.

V. CONCLUSION

Although the benefits of progestins are undisputed as far as endometrial protection is concerned, the controversies related to the potential risks have to be reassessed according to the type of progestins considered. Progesterone itself and natural derivatives of the molecules do not display androgenic properties; hence, they behave differently on the vascular wall than androgenic progestins. Further epidemiological data are needed that consider separately the different types of steroid used in clinical practice, which vary from country to country.

REFERENCES

1. Wittemen J, Grobbee D, Kof F, Hofman A, Valkenburg H, Barret-Connor E. Increased risk of atherosclerosis in women after the menopause. Br Med J 1989; 298:642–644.
2. Grady D, Rubin SM, Petitti DB, Fox CS, Black D, Ettinger B, Ernster VL, Cummings SR. Hormone therapy to prevent disease and prolong life in postmenopausal women. Ann Intern Med 1992; 117:1016–1037.
3. Meade T, Berra A. Hormone replacement therapy and cardiovascular disease. Br Med Bull 1992; 48/2:276–308.
4. Barret Connor E. Hormone replacement therapy. Br Med J 1998; 317:457–461.
5. Hulley S, Grady D, Bush T, Furberg C, Herrington O, Rigges B, Vittinghoff E. Heart and Estrogen/Progestin Replacement Study (HERS) research group. Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. JAMA 1998; 280:605–613.
6. Clarkson TB, Anthony MS, Potvin Klein K. Hormone replacement therapy and coronary artery atherosclerosis: the monkey model. Br J Obstet Gynaecol 1996; 103(S13):53–58.
7. Sullivan JM. Hormone replacement therapy and cardiovascular disease: the human model. Br J Obstet Gynaecol 1996; 103(S13):59–67.

8. Holdright DR, Sullivan AK, Wright CA, Sparrow JL, Cunningham D, Fox KM. Acute effect of estrogen replacement therapy on treadmill performance in postmenopausal women with coronary artery disease. Eur Heart J 1995; 16:1566–1570.
9. Stevenson JC. Are changes in lipoproteins during HRT important? Br J Obstet Gynaecol 1996; 103(S13):39–44.
10. Godsland IF, Gangar K, Walton C, Cust MP, Whitehead MI, Wynn V, Stevenson JC. Insulin resistance, secretion, and elimination in postmenopausal women receiving oral or transdermal hormone replacement therapy. Metabolism 1993; 42:846–853.
11. Lindoff C, Peterson F, Lecander I, Martinsson G, Astedt B. Transdermal estrogen replacement therapy: beneficial effects on hemostatic risk factors for cardiovascular disease. Maturitas 1996; 24:43–50.
12. Sullivan JM, Shala BA, Miller LA, Lerner JL, McBrayer JD. Progestin enhances vasoconstrictor responses in postmenopausal women receiving estrogen replacement therapy. Menopause 1995; 2:193–199.
13. The writing group for the PEPI trial. Effects of estrogen or estrogen/progestin regimens on heart disease risk factors in postmenopausal women: the Postmenopausal Estrogen/Progestin Interventions (PEPI) Trial. JAMA 1995; 273:199–208.
14. Adams MR, Kaplan JR, Manuck SB, Koritnik DR, Parks JS, Wolfe MS, Clarkson TB. Inhibition of coronary artery atherosclerosis by 17-beta estradiol in ovariectomized monkeys. Lack of an effect of added progesterone. Arteriosclerosis 1990; 10:1051–1057.
15. Duc I, Botella J, Bonnet P, Fraboul F, Delansorne R, Paris J. Antiandrogenic properties of nomegestrol acetate. Arzneimittelforschung Drug Res. 1995; 45:70–74.
16. Bradley DD, Wingerd J, Petitti DB, Krauss RM, Ramcharan S. Serum high-density-lipoprotein cholesterol in women using oral contraceptives, estrogens and progestins. N Engl J Med 1978; 299:17–20.
17. Falkeborn M, Persson I, Adami HO, Bergstrom R, Eaker E, Lithell H, Mohsen R, Naessen T. The risk of acute myocardial infarction after oestrogen and oestrogen-progestogen replacement. Br J Obstet Gynaecol 1992; 99:821–828.
18. Grodstein F, Stampfer MJ, Manson JE, Colditz GA, Willett WC, Rosner B, Speizer FE, Hennekens CH. Postmenopausal estrogen and progestin use and the risk of cardiovascular disease. N Engl J Med 1996; 353:453–461.
19. Psaty BM, Heckbert SR, Atkins D, Lemaître R, Koepsell TD, Wahl PW, Siscovick DS, Wagner EH. The risk of myocardial infarction associated with the combined use of estrogens and progestins in postmenopausal women. Arch Intern Med 1994; 154:1333–1339.
20. Spencer CP, Cooper AJ, Stevenson JC. Clinical trials in progress with hormone replacement therapy. Exp Opin Invest Drugs 1996; 5:739–749.
21. The Women's Health Initiative Study Group. Design of the Women's Health Initiative Clinical Trial and Observational Study. Controlled Clin Trials 1998; 19:61–109.

22. Perez-Gutthann S, Garcia-Rodriguez LA, Castelsague J, Duque-Oliart A. A Population Based Case-Control Study. HRT and the risk of venous thromboembolic event. BMJ 1997; 314:796–800.
23. Daly E, Vessey MP, Hawkins MM, Carson JL, Gough P, Marsh S. Risk of venous thromboembolism in users of hormone replacement therapy. Lancet 1996; 348:977–980.
24. Jick H, Derby LE, Myers MW, Vasilakis C, Newton KM. Risk of hospital admission for idiopathic venous thromboembolism among users of postmenopausal estrogens. Lancet 1996; 348:981–983.
25. Grodstein F, Stampfer MJ, Goldhaber SZ, Manson JE, Colditz GA, Speizer FE, Willett WC, Hennekens CH. Prospective study of exogenous hormones and risk of pulmonary embolism in women. Lancet 1996; 348:983–987.
26. Committee on Safety of Medicines. Risk of venous thromboembolism with hormone replacement therapy. Curr Prob Pharmacovigilance 1996; 22:9–10.
27. Herrington DM, Potvin-Klein K. Estrogen replacement and prevention of coronary artery disease in postmenopausal women. Menopause Manage 1998; 7:8–19.
28. Rich-Edwards JW, Mason JE, Hennekens CH, Buring JE. The primary prevention of coronary heart disease in women. N Engl J Med 1995; 332:1758–1766.
29. Williams JK, Adams MR, Klopfenstein HS. Estrogen modulates responses of atherosclerotic coronary arteries. Circulation 1990; 81:1680–1687.
30. Crook D, Cust MP, Gangar KF, Worthington M, Hillard TC, Stevenson JC, Whitehead MI, Wynn V. Comparison of transdermal and oral estrogen–progestin replacement therapy: effects on serum lipids and lipoproteins. Am J Obstet Gynecol 1992; 166:950–955.
31. Conard J, Basdevant A, Thomas JL, Ochsenbein E, Denis C, Guyene TT, Degrelle H. Cardiovascular risk factors and combined estrogen–progestin replacement therapy: a placebo-controlled study with nomegestrol acetate and estradiol. Fertil Steril 1995; 64:957–962.
32. Jackson RA. Mechanisms of age-related glucose intolerance. Diabetes Care 1990; 13(S2):9–19.
33. De Pirro R, Fusco A, Bertoli A, Greco AV, Lauro R. Insulin receptors during the menstrual cycle in normal women. J Clin Endocrinol Metab 1978; 47:1387–1389.
34. Stout RW, Bierman EL, Ross R. Effect of insulin on the proliferation of cultured primate arterial smooth muscle cells. Circ Res 1975; 36:319–327.
35. Chait A, Bierman EL, Albers JJ. Low density lipoprotein receptor activity in cultured human skin fibroblasts. Mechanisms of insulin-induced stimulation. J Clin Invest 1979; 64:1309–1319.
36. De Cleyn K, Buytaert P, Coppens M. Carbohydrate metabolism during hormonal substitution therapy. Maturitas 1989; 11:235–242.
37. Dorangeon P, Thomas JL, Lumbroso M, Hazard MC, Dorangeon S. Effects of nomegestrol acetate on carbohydrate metabolism in premenopausal women. XIIIth World Congress of Gynecology and Obstetrics (FIGO). Singapour, 1991, abstr 0636.

38. Basdevant A, Pelissier C, Conard A, Degrelle H, Guyenne TT, Thomas JL. Effects of nomegestrol acetate (5 mg/d) on hormonal, metabolic and hemostatic parameters in premenopausal women. Contraception 1991; 44:599–605.
39. Casiglia E, d'Este D, Ginocchio G, Colangeli G, Onesto C, Tramontin P, Ambrosio GB, Pessina AC. Lack of influence of menopause on blood pressure and cardiovascular risk profile a 16-year longitudinal study concerning a cohort of 568 women. J Hypertens 1996; 14:729–736.
40. Foidart JM. The effects of estraderm TTS 50 + medroxyprogesterone acetate on blood pressure in hypertensive post-menopausal women. In: Samsioe G, ed. Cardiovascular Disease and HRT. New Perspectives. Carnforth, UK: Parthenon, 1991; pp 41–44.
41. Pang SC, Green Dale GA, Cedars MI, Cambone AC, Lozano K, Eggena P, Judd HL. Long term effects of transdermal estradiol with and without medroxyprogesterone acetate. Fertil Steril 1993; 59:76–82.
42. Bush TL, Barrett-Connor E, Cowan LD, Criqui MH, Wallace RB, Suchindran CM, Tyroler HA, Rifkind BM. Cardiovascular mortality and noncontraceptive use of estrogen in women: results from the Lipid Research Clinics Program follow-up study. Circulation 1987; 75:1102–1109.
43. Bergqvist A, Bergqvist D, Ferno M. Estrogen and receptors in vessels walls. Acta Obstet Gynecol Scand 1993; 72:10–16.
44. Hayashi T, Fukoto AM, Ignarro LA, Chaudhuri G. Basal release of nitric oxide from aortic rings is greater in female rabbits than in male rabbits, implications for atherosclerosis. Proc Natl Acad Sci 1992; 59:11259–11263.
45. Ylikorkala O, Cacciatore B, Paakkari I, Tikkanen MJ, Viinikka L, Toivonen J. The long-term effects of oral and transdermal postmenopausal hormone replacement therapy on nitric oxide, endothelin-1, prostacyclin, and thromboxane. Fertil Steril 1998; 69:883–888.
46. Morey AJ, Pedram A, Razandi M, Prins BA, Hu RM, Biesiada E, Levin ER. Estrogen and progesterone inhibit vascular smooth muscle proliferation. Endocrinology 1997; 138:3330–3339.
47. Lee WS, Harder JA, Yoshizumi M, Lee ME, Haber E. Progesterone inhibits arterial smooth muscle cell proliferation. Nature Med 1997; 3:1005–1008.
48. Herrington DM, Braden GA, Williams AK, Morgan TM. Endothelial-dependent coronary vasomotor responsiveness in postmenopausal women with and without estrogen replacement therapy. Am J Cardiol 1994; 73:951–952.
49. Rosano GMC, Sarrel PM, Poole-Wilson PA, Collins P. Beneficial effects of estrogen on exercise-induced myocardial ischaemia in women with coronary artery disease. Lancet 1993; 342:133–136.
50. Collins P, Rosano GMC, Sarrel PM, Ulrich L, Adamopoulos S, Beale CM, McNeil AG, Poole-Wilson PA. 17beta-Estradiol attenuates acetylcholine induced coronary arterial constriction in women but not men with coronary heart disease. Circulation 1995; 92:24–30.
51. Williams AK, Honore EK, Washburn SA, Clarkson TB. Effects of hormone replacement therapy on reactivity of atherosclerotic coronary arteries in cynomolgus monkeys. J Am Coll Cardiol 1994; 24:1757–1761.

52. Miyagawa K, Rösch J, Stanczyk F, Hermsmeyer K. Medroxyprogesterone acetate interferes with ovarian steroid protection against coronary vasospasm. Nature Med 1997; 3:324–327.
53. Williams JK, Adams MR. Estrogens, progestins and coronary artery reactivity. Nature Med 1997; 3:273–274.
54. Williams JK, Delansorne R, Paris J. Estrogens, progestins and coronary artery reactivity in atherosclerotic monkeys. J Steroid Biochem Mol Biol 1998; 65:219–224.

17

Control of Estrogen's Effects by Progestins in Breast Cancer

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I. INTRODUCTION

Recent statistical information indicates that in the United States 1:8 women will be at risk of developing breast cancer during her life; the values are 1:12 for countries of the European Community, and 1:80 for Japan. Two-thirds of breast cancers are manifested during the postmenopausal period. The great majority (approximately 95%) are initially hormone-dependent, for which estradiol plays a predominant role in their development and evolution (1–4). The remaining 5% of breast cancers, denoted BRCA-1 and BRCA-2, are considered hereditary; the gene BRCA-1 is localized on chromosome 17q21 (5,6) and gene BRCA-2 on chromosome 13q12-13 (7,8).

Three main enzymatic mechanisms are involved in the last steps of estradiol formation: the "sulfatase pathway," which transforms estrone sulfate into estrone; the "aromatase pathway," which converts androgens into estrogens; and the 17β-hydroxysteroid dehydrogenase (17β-HSD) mechanism, which transforms estrone into estradiol.

Figure 1 gives a schematic representation of the enzymatic process involved in the formation and transformation of estrogens in breast cancer.

There is a huge amount of information showing that breast cancer tissues contain the enzymes necessary for the formation of estradiol (E_2) from circulating precursors; these processes include sulfatases (9–15), aromatase (16–18), and 17β-HSD (19–22) activities. Breast cancer tissues also have the capacity to form estrogen sulfates from unconjugated estrogens. Estrogen sulfotransferase

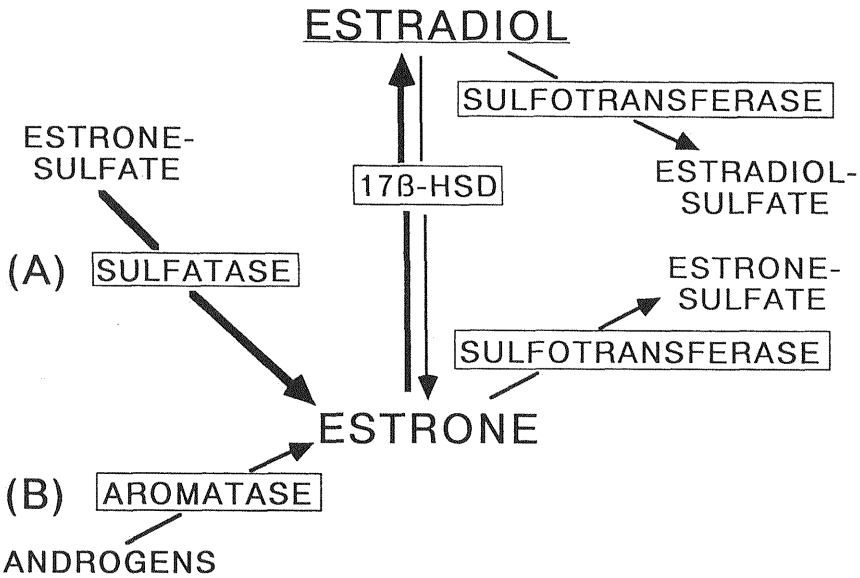

Figure 1 Enzymatic mechanisms involved in the formation and transformation of estrogens in human breast cancer. The sulfatase pathway (A) is quantitatively 10–500 times higher than that of the aromatase pathway (B): 17β-HSD; 17β-hydroxysteroid dehydrogenase.

activity was demonstrated in breast tumors (11,23), as well as in isolated human breast cancer cells (24).

After a period that can span several years, the breast tumor becomes hormone-independent by a very complex process, still under scrutiny. One explanation for the progression toward hormone-independence may be the presence of estrogen receptor (ER) mutants (25,26). As the cancer cell evolves, mutations, deletions, and truncations appear in the receptor gene (27–29), the ER becomes ''nonfunctional'' and, despite estrogen binding, the cancer cells do not respond to the hormone; this might explain why 35–40% of breast cancer patients who are ER-positive fail to respond to antiestrogen therapy (30,31).

II. ESTROGEN CONCENTRATIONS IN BREAST CANCER PATIENTS

Estrone sulfate (E_1S) is quantitatively the most important circulating estrogen in the menstrual cycle and in postmenopausal women (32,33). Because during the postmenopausal period, two-thirds of breast cancers are manifest, and in

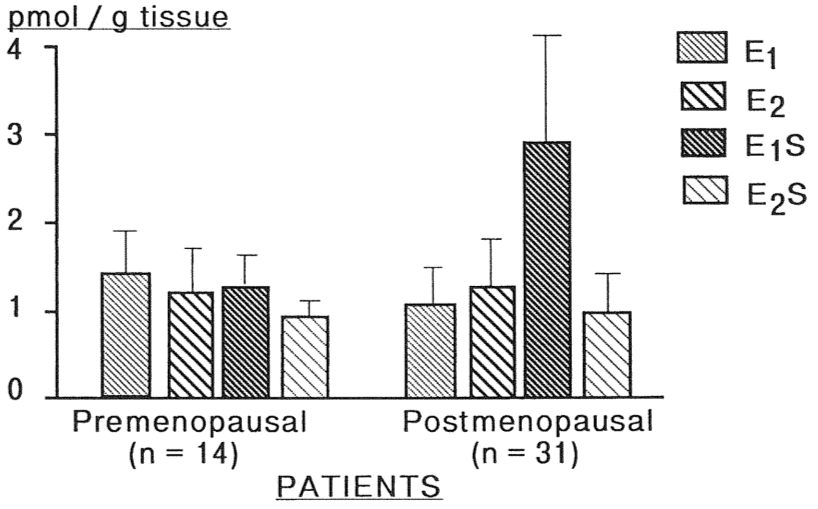

Figure 2 Mean concentration of estrone (E_1), estradiol (E_2), and their sulfates (E_1S, E_2S) in the tumoral tissue of pre- and postmenopausal patients with breast cancer (vertical bars = S.E.).

that period, as a consequence of the ovary being nonfunctional, the circulating estrogens are very low, it was interesting to evaluate the tissue levels of the various estrogens in postmenopausal patients with breast cancer.

Figure 2 gives the concentration values of E_1, E_2, and their sulfates (E_1S, E_2S) in the breast tissues of pre- and postmenopausal patients with breast cancer. The high estrogen concentrations in the tumor of postmenopausal patients are noteworthy, in particular that of E_1S.

Table 1 Ratio[a] Concentration Between the Tumor Tissue and Plasma of Estrone (E_1), Estradiol (E_2), and Their Sulfates (E_1S, E_2S) in Human Breast Cancer

Patients	E_1	E_2	E_1S	E_2S
Premenopausal	7	5	0.3	2
Postmenopausal	6	22	9.0	4

[a]The ratio corresponds to values obtained with the tissue concentration of each estrogen (pmol/g) divided by the plasma concentration of the respective estrogen (pmol/mL). The data represent the average values obtained from 10–15 patients.

Source: Ref. 34.

Table 1 gives the tumor/plasma concentration ratios of the various estrogens. As observed, the gradient of tumoral tissues to blood increases very significantly for E_2 and E_1S when pre- and postmenopausal patients are compared.

III. SULFATASE VERSUS AROMATASE ACTIVITIES IN BREAST CANCER

As the two main pathways of estrogen formation in the breast tumor are the transformation of androgens by aromatase and the conversion of estrone sulfate to estradiol by sulfatase, the quantitative evaluation of these activities in the breast tumor is the most interesting. Figure 3 shows that the tumoral sulfatase activity is many times higher than that of aromatase in both pre- and postmenopausal women (34). Figure 4 shows data on the activities of sulfatase (A) and aromatase (B) in individual patients. The values of both enzymes are significantly higher in postmenopausal than in premenopausal patients (34).

IV. EFFECT OF PROGESTINS ON THE PROLIFERATION OF HUMAN BREAST CELLS

A. In Normal Breast

Despite that development of human breast starts during fetal life, the breast is one of the few organs of the body that is not completely developed at birth. Its growth, with lobule formation, occurs during puberty, but the development and differentiation are completed only at the end of puberty and then regress in the postmenopausal period. During the menstrual cycle, the volume and morphology of the breast is a function of the fluctuations of gonadal steroids (mainly estrogens and progesterone). The maximal epithelial mitosis is found between 22 and 26 days of the cycle, which corresponds to the high serum levels of estradiol and progesterone (35). During pregnancy, it is suggested that the elevated values of circulating progesterone are responsible for the induction of the lobular–alveolar development, to prepare the breast for lactation (36).

A possible "direct effect" of progesterone was extensively explored in in vitro studies using organ culture, transplantation of normal human breast to nude mice, or primary cell culture. The data on the effect of progesterone on breast

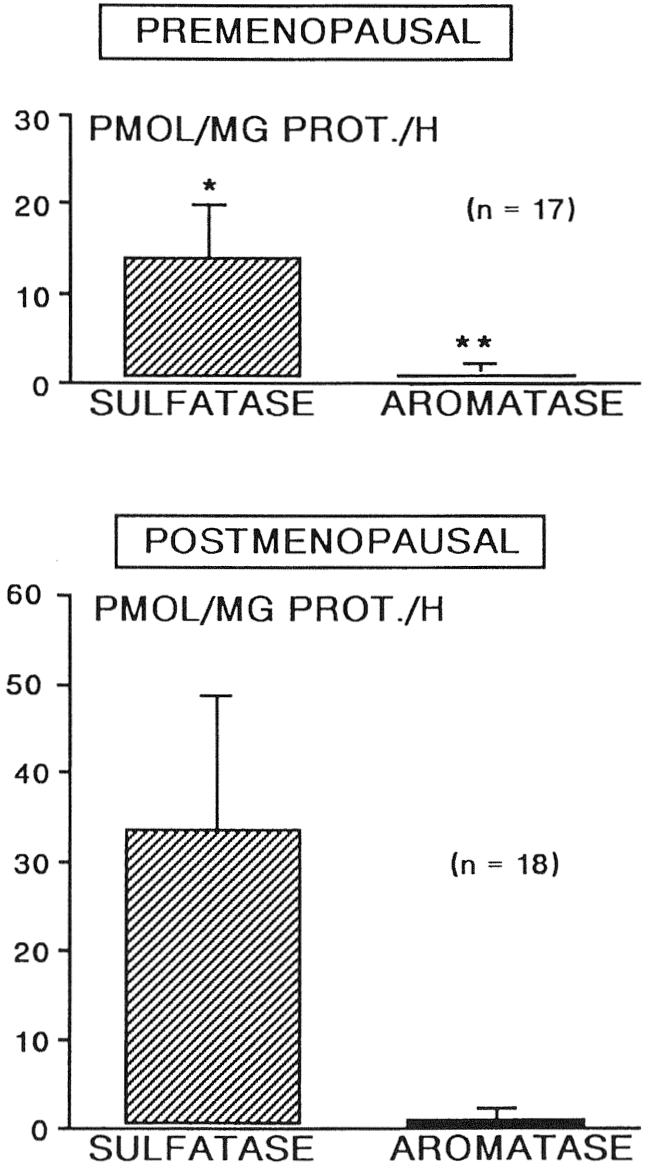

Figure 3 Estrone sulfatase and aromatase activities in breast cancer tumors of pre- and postmenopausal patients. Values are expressed as the mean ± SEM. *, $p \leq 0.001$ vs. E_1S-sulfatase in postmenopausal patients; **, $p \leq 0.001$ vs. aromatase in postmenopausal patients. (From Ref. 34.)

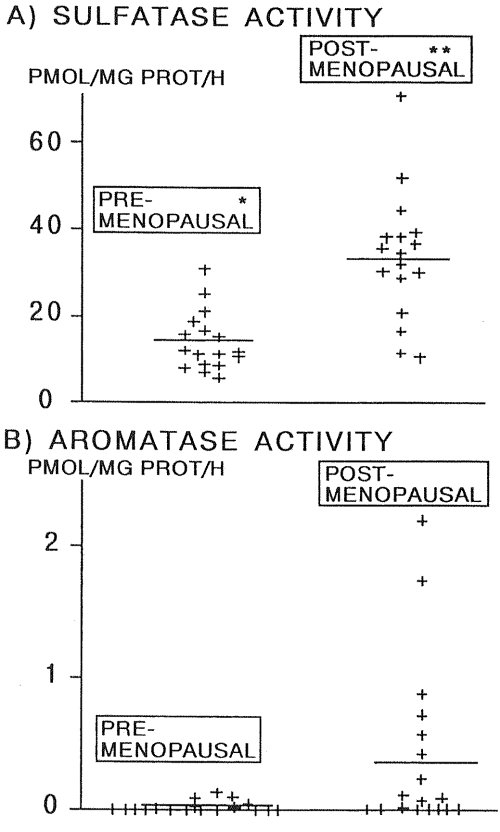

Figure 4 Individual determination of E_1S-sulfatase (A) and aromatase (B) in pre- and postmenopausal patients with breast cancer (horizontal line is mean value). (From Ref. 34.)

epithelial proliferation are contradictory. Progesterone increases DNA synthesis in normal breast epithelium in organ culture (37). However, other studies show that progesterone either decreases or has no effect on the proliferation of normal mammary epithelium explanted into nude mice (38,39). In normal epithelial human breast cells, the progestin, promegestone (R-5020) decreases cell proliferation (40).

Progestins can inhibit (40) or not (38,41) the stimulatory effect provoked by estradiol. The present information clearly demonstrates that, in normal breast, gonadal steroids can act directly in the epithelium, but a possible paracrine mechanism involving stromal factors is to be explored.

B. In Breast Cancer

The effects of progestins on cell proliferation in in vivo studies in patients with breast cancer are very limited. Most of the data were observed after a combined treatment of estrogens plus progestins. Interesting information was obtained after administration of progesterone alone in patients with breast cancer, in whom a decrease in growth was found in four of six tumors; however, in the other two there was stimulation of growth (42). The same authors reported that in a combined treatment of estradiol plus progesterone, growth increased in four of seven patients at low doses, but treatment with 10- to 100-fold higher concentrations of both hormones invariably led to a decrease in proliferation.

The most important information on the effect of progestins in breast cancer was explored with isolated models: cell lines, organ culture, or transplantation of breast cancer cells in nude mice. The data are contradictory, as it was reported that progestin can either inhibit (43–47), stimulate (48–52), or have no effect (53) on the proliferation of breast cancer cell lines.

The structure of the progestins is an important parameter because, in addition to their progestagenic activity, they can possess estrogenic, androgenic, and glucomineralocorticoid properties (54–56). 19-Norprogestins (such as those derived from testosterone: norethindrone, norgestrel, or norethynodrel) possess a weak estrogenic activity and can stimulate (at 10^{-6} M) the proliferation of ER^+, but not ER^- breast cancer cells (48). Interesting data were obtained with nomegestrol acetate (Lutenyl), a 19-norprogestin derivative. This compound does not possess estrogenic activity and is exclusively antiproliferative in MCF-7 and T-47D cells. It was postulated that the estrogenic activity is determined mainly by the 17α-hydroxyl group associated with estrogenic progestins, rather than by the absence of a methyl group at the C-19 position (57). Differences in the effects of progestins in cell proliferation could also be due to the experimental conditions (source of cell lines, media, sera, presence of phenol red, insulin, duration of treatment) as well as to dose concentration (58–63).

The effects of progestins alone or in combination with estradiol on the proliferation of tumor cell lines are markedly different. A series of data indicate that progestins can stimulate cell proliferation in an estrogen-free environment and induce inhibition of cell growth when cells are cultivated in an estrogenic environment (58,64). This observation could be explained by the well-known fact that estradiol is necessary to induce the progesterone receptor (PR) (65). However, Horwitz and Freidenberg (45) show that R-5020 can inhibit the growth of the ER^- T-47Dco antiestrogen-resistant cell line, in which the two PR isoforms are constitutively expressed.

The process by which progestins are involved in the regulation of cell growth includes steroid receptors (66,67), growth factors and their receptors,

oncogenes (68–71), cell cycle (72,73), and the effect on the enzymes implicated in the formation and transformation of estrogens (see Sec. V).

V. EFFECT OF PROGESTINS ON THE ENZYMES INVOLVED IN THE FORMATION AND TRANSFORMATION OF ESTROGENS IN NORMAL AND CANCEROUS BREAST CELLS

A. Effect of Progestins on Estrone Sulfatase

The estrone sulfatase activity in mammary tumors is significantly higher in postmenopausal than in cycling patients, and this activity is increased in the tumor tissue, compared with the surrounding area or in areas considered as "normal" (34,74). No correlation has been found between sulfatase activity levels and estrogen receptor status (75,76). However, in intact breast cancer cells in culture, a marked difference in sulfatase activity exists between hormone-dependent (MCF-7, T-47D) and hormone-independent (MDA-MB-231, MDA-MB-468) cells. The former possess high activity, whereas the latter have little effect on the hydrolysis of estrone sulfate (77,78); however, when these hormone-independent cells are homogenized, the sulfatase activity is restored (14,79). The data suggest that, in the mechanism involved in the hydrolysis of the sulfate, "stimulatory factors" necessary for the enzyme activity are present in the hormone-dependent cells, but they could be absent in the hormone-independent cells.

The effect of progestins on the sulfatase activity in breast cancer has, as yet, been explored in only in vitro studies. In breast tumors, progestins, such as demegestone or chlormadinone acetate, at 10^{-5} M, inhibit by 25–50% the sulfatase activity (80,81). In breast cancer cells (MCF-7, T-47D), promegestone (R-5020), nomegestrol acetate, medrogestone, norethisterone, as well as danazol, at a range of concentrations between 5×10^{-7} and 5×10^{-6} M decrease the sulfatase activity by 40–70% (82–87). Another compound, tibolone and its metabolites (Org 4094, Org 30126, and Org OM38) at low doses (5×10^{-8} M) are very potent antisulfatase agents, for they inhibit the activity of this enzyme by 80–90% in these cell lines (88). Table 2 gives a comparative study on the effects of the conversion of estrone sulfate to estradiol by various progestins in the MCF-7 and T-47D cells.

B. Effects of Progestins on 17β-Hydroxysteroid Dehydrogenase

17β-Hydroxysteroid dehydrogenase (17β-HSD) catalyzes bidirectional reactions (oxidative activity: $E_2 \rightarrow E_1$, and the reductive activity: $E_1 \rightarrow E_2$) and can be

Table 2 Effect of Various Progestins as well as Tibolone on the Conversion of Estrone Sulfate (E_1S) to Estradiol (E_2) by the MCF-7 and T-47D Human Breast Cancer Cells (% Inhibition)[a]

	MCF-7 cells			T-47D cells		
	(Progestin concentration)					
Progestins	0.5 μM (% inhibition)	50 μM	IC_{50}[b] (μM)	0.5 μM (% inhibition)	50 μM	IC_{50}[b] (μM)
Nomegestrol acetate	16	77	6.10	45	71	2.20
Medrogestone	37	84	2.50	45	88	0.35
Tibolone	93	98	0.02	93	98	0.04
Norethisterone	74	93	7.30	72	93	7.30

[a] E_1S (5×10^{-9} M) was incubated with or without the progestins or tibolone for 24 h. Data are the average of three duplicate experiments.
[b] IC_{50}, progestin concentration for 50% of inhibition.
Source: Refs. 82, 84, 88.

involved in both the inactivation or the synthesis of E_2, respectively. It is now known that the enzyme 17β-HSD consists of several isoenzyme forms (I–VII) that express different activities, substrate specificities, regulations, and localizations (91). This aspect is very complex because the orientation of the enzymatic activity (oxidative or reductive) is dependent on the nature and concentration of the cofactors [NAD(P) or NAD(P)H] produced by the cells (92,93).

In normal breast tissue, it was observed that the oxidative 17β-HSD activity ($E_2 \rightarrow E_1$) is the preferential direction, and that this activity is more intense during the secretory phase of the menstrual cycle (19). The data is confirmed using epithelial cells of a normal breast. In these cells, it was observed that the progestin, promegestone, can increase the 17β-HSD activity in the $E_2 \rightarrow E_1$ direction; this stimulatory effect of the progestin depends on preliminary sensitization by the estrogens (94).

Immunochemical studies indicate that the cytosolic type I 17β-HSD is essentially expressed in breast cancer tissues and in estrogen-sensitive breast cancer cell lines (MCF-7; T-47D; ZR-75-1; R-27). This isoform is specific to estrogens and possesses a preferential reductive activity (91,95). However, types II and IV, with oxidative preferential activity, have also been detected at low levels in several cell lines (T-47D; BT-20; MDA-MB-231; MDA-MB-436) (95). Studies on estrogen metabolism have demonstrated that the 17β-HSD reductive activity is very high in hormone-dependent breast cancer cells (MCF-7; T-47D), whereas in hormone-independent cells (MDA-MB-231; MDA-MB-468) the oxidative activity is preferential (22,96), suggesting that there is a change in 17β-HSD phenotype in neoplastic cells (15). Figure 5 schematizes the inter-

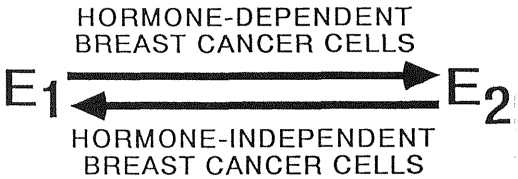

Figure 5 Interconversion of estrone (E_1) and estradiol (E_2) in human breast cancer cells.

conversion of estrone and estradiol in both dependent and independent human breast cancer cells.

A series of progestins have been tested in breast tumors or in breast cancer cells for their capacity to act on 17β-HSD to decrease the production of E_2. Breast tumors from postmenopausal patients receiving lynestrenol display higher oxidative 17β-HSD activity than tumors from untreated patients. The activity depends on the ER or PR status of the tumor (97).

Interesting results show that nomegestrol acetate, medrogestone, as well as tibolone significantly reduce the reductive 17β-HSD activity in hormone-dependent breast cancer cells (82,84,98). This effect is more intense with the PR-rich T-47D cells. Promegestone (R-5020) has no effect on this reductive activity but can increase the oxidative activity ($E_2 \rightarrow E_1$) of 17β-HSD in T-47D cells (22,96). The relative inhibitory effects of various progestins on the conversion of estrone to estradiol by the MCF-7 and T-47D breast cancer cells are shown in Table 3.

Other authors have obtained an increase of both the reductive and the oxidative 17β-HSD activities with progesterone, medroxyprogesterone acetate (MPA), levonorgestrel, and norethisterone in MCF-7 cells (99,100). MPA also stimulates these dual activities in ZR-75-1 cells, whereas Org 2058 increases the oxidative direction in T-47D cells (101,102).

C. Effect of Progestins on Sulfotransferase Activities

The formation of estrogen sulfates by the action of sulfotransferase is of particular importance because: (a) these conjugates can be utilized as reserve material for the biosynthesis of estradiol through the action of endogenous sulfates; (b) estrogen sulfates can be one of the ways to protect the hormone, as it is well documented that estrogen sulfates do not bind to the estrogen receptor and have no estrogenic activity; therefore, the increase of sulfotransferase activities can diminish the estrogenic stimulation.

In breast cancer cells, several isoforms are present; the estrogen sulfotransferase (EST) is specific to estrogens and acts at nanomolar concentrations. Hydroxysteroid sulfotransferase (HST) and phenol sulfotransferase (PST) can

Table 3 Effect of Various Progestins as well as Tibolone on the Conversion of Estrone (E_1) to Estradiol (E_2) by the MCF-7 and T-47D Human Breast Cancer Cells (in % Inhibition)[a]

	MCF-7 cells			T-47D cells		
	(Progestin concentration)					
Progestins	0.5 μM (% inhibition)	50 μM	IC_{50}[b] (μM)	0.5 μM (% inhibition)	50 μM	IC_{50}[b] (μM)
Nomegestrol acetate	5	46	202.7	35	85	1.1
Medrogestone	33	55	12.0	57	79	0.4
Tibolone	27	61	10.6	30	73	2.1

[a]E_1 (5×10^{-9} M) was incubated with or without the progestins or tibolone for 24 h. Data are the average of three duplicate experiments.
[b]IC_{50}, progestin concentration for 50% of inhibition.
Source: Refs. 82, 84, 98.

also transform estrogens, but at micromolar concentrations (103–106). In general, sulfotransferase activities are higher in hormone-dependent (MCF-7; T-47D; ZR-75-1) than in hormone-independent breast cancer cells, although in MDA-MB-468 cells, sulfotransferase activity is very elevated (24).

Little information is available on the regulation of EST expression in breast cancer. Recent data have shown that, in hormone-dependent breast cancer cells (MCF-7; T-47D), low concentrations (5×10^{-7} M) of promegestone (R-5020) can increase the enzyme activity, whereas higher concentrations (5×10^{-5} M) decrease this activity. This dual effect is correlated with the mRNA expression of EST that is modulated by promegestone in a similar manner (107). A stimulatory effect on sulfotransferase activity was also found with low, but not with high, concentrations of medrogestone in MCF-7 and T-47D cells (Fig. 6) (108).

D. Effects of Progestins on Aromatase

The aromatase cytochrome P-450 catalyzes aromatization of androgens to estrogens; biochemical and immunocytochemical studies have revealed the presence of this enzyme in the adipose stromal cells of breast cancer tissues. Although levels of aromatase activity are relatively low in breast, this local production of estrogens ''on site'' can contribute to the pathogenesis of estrogen-dependent breast cancers. Aromatase inhibition by antiaromatase (e.g., by aminoglutethimide, 4-OH-androstenedione, vorozole, letrozole, anastrozole [Arimidex]) is a well-defined second-line therapeutic treatment for breast cancer in postmenopausal women (109–112).

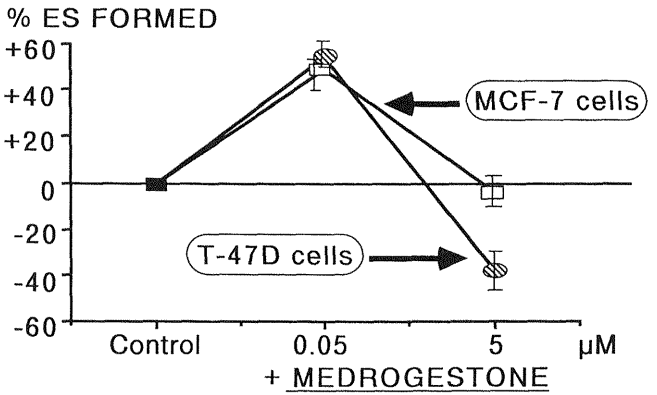

Figure 6 Dose–response effect of medrogestone (Prothil) on the conversion of estrone (E_1) to estrogen sulfates (ES) by the action of the sulfotransferase in human MCF-7 and T-47D breast cancer cells. (From Ref. 108.)

The role of progestins on aromatase activity in breast is not well understood. Perel et al. (18) observed that progestins can inhibit the aromatase activity in cultured breast carcinoma cells.

VI. CONCLUSIONS AND PERSPECTIVES

Recent findings in this laboratory and others demonstrate very clearly that human breast cancer tissue contains the enzymes necessary for the formation of E_2: sulfatase, aromatase, and 17β-HSD. Sulfotransferases, which transform estrogens into their sulfates, are also present in this tissue. Evaluation of various estrogens: E_1, E_2, and their sulfates, in cancer tissues (see Fig. 2) of postmenopausal patients shows that the concentrations of these estrogens are relatively high. This accumulation of estrogens in the carcinoma tissue at a period in which the ovary is nonfunctional is very intriguing, and complementary information is required for clarification and better understanding.

Comparative data show that, in breast cancer tissues, the sulfatase activity is 100–500 times higher than the aromatase, suggesting that the main pathway for the formation of E_2 in this tissue is through the hydrolysis of E_1S.

In another series of studies, it was well established that mRNA of sulfatase is present in different breast cancer cells and that the expression of this messenger is correlated with sulfatase activity. However, the capacity to hydrolyze E_1S in some cell lines does not reflect the levels of sulfatase activity; for instance, for the hormone-independent MDA-MB-231 cells the sulfatase activity is very low

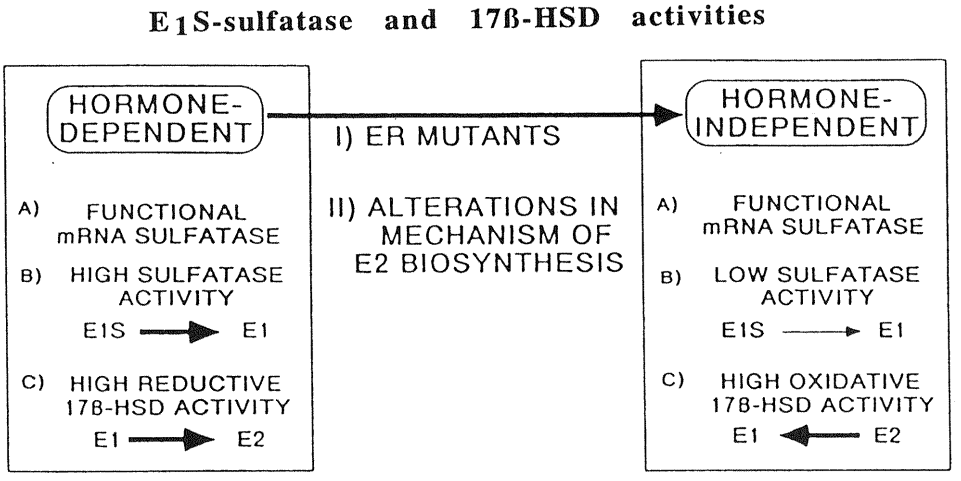

Figure 7 Hypothetical concept of the factors involved in the transformation of breast cancer from hormone-dependence to hormone-independence. The transformation of breast cancer cells from hormone-dependent to hormone-independent is a very complex mechanism for which only limited information is now available. One interesting discovery is the presence of ER mutants; the ER evolve to nonfunctional and the hormone becomes inoperative. Another attractive aspect concerns the enzymes involved in E_2 formation. The sulfatases are very active in the intact hormone-dependent (HD) cells but, despite the presence of the enzyme and its mRNA, the sulfatase activity in hormone-independent (HI) cells is very low, suggesting either the presence of activator factor(s) in HD or repressive factor(s) in HI. Similarly, in the interconversion of $E_1 \rightarrow E_2$, the factors for the formation of E_2 (the biological form) are very active in HD, whereas in HI the factor(s) act to the formation of the less active estrogen E_1. E_1S: estrone sulfate; E_1: estrone; E_2: estradiol; 17β-HSD: 17β-hydroxysteroid dehydrogenase.

in the intact cells, but becomes very active after homogenization of the cells. The data indicate that the presence of the enzyme and its mRNA are not sufficient, suggesting that other "factors" are involved in the mechanism for the hydrolysis of E_1S; these factors could be absent from hormone-independent cells.

Another example in the alteration of the enzyme activities implicated in the formation of E_2 as the breast cancer evolves from hormone-dependent to hormone-independent concerns 17β-HSD. It was observed that in the hormone-dependent cells, the activity of the 17β-HSD is formation of E_2, whereas in the hormone-independent cells, the tendency is to the oxidative form (formation of E_1). All this information, and the interesting finding of ER mutants, suggests that the evolution of cancer to hormone-independence is a very complex mechanism. A present hypothetical concept of this transformation is represented in Fig. 7.

In the exploration of sulfatase inhibitors using isolated breast cancer cells, interesting findings were obtained with the progestins; nomegestrol acetate, promegestone, tibolone, and medrogestone. These drugs can also block the conversion of E_1 to E_2 by inhibition of 17β-HSD activity.

More recently it was found that some progestins (promegestone, medrogestone) can stimulate sulfotransferase activity. This is an important point in the physiopathology of this disease because the estrogen sulfates are biologically inactive.

The utilization of various progestins in trials with breast cancer patients, showing an inhibitory effect on sulfatases and 17β-HSD, and a stimulatory effect on sulfotransferases, will provide a new possibility in the treatment of this disease.

REFERENCES

1. Segaloff A. Hormones and mammary carcinogenesis. In: McGuire WL, ed. Breast Cancer 2: Advances in Research and Treatment. New York: Plenum, 1978; 1–22.
2. Kirschner MA. The role of hormones in the development of human breast cancer. In: Breast Cancer 3: Advances in Research and Treatment, Current Topics. New York: Plenum, 1979; 199–226.
3. Osborne CK, Hobbs K, Clark GM. Effect of estrogens and antiestrogens on growth of human breast cancer cells in athymic nude mice. Cancer Res 1985; 45:584–590.
4. Henderson BE, Ross R, Bernstein L. Estrogens as a cause of human cancer: the Richard and Hinda Rosenthal Foundation Award Lecture. Cancer Res 1988; 48:246–253.
5. Hall JM, Lee MK, Newman B, Horrow JE, Anderson LA, Huey B, King MC. Linkage of early-onset familial breast cancer to chromosome 17q21. Science 1990; 250:1684–1689.
6. Cropp CS, Nevanlinna HA, Pyrhönen S, Stenman U-H, Salmikangas P, Albertsen H, White R, Callahan R. Evidence for involvement of BRCA1 in sporadic breast carcinomas. Cancer Res 1994; 54:2548–2551.
7. Wooster R, Neuhauson SL, Mangion J, et al. Localization of a breast cancer susceptibility gene, *BRCA2* to chromosome 13q12-13. Science 1994; 265:2088–2090.
8. Wooster R, Bignell G, Lancaster J, et al. Identification of the breast cancer susceptibility gene *BRCA2*. Nature 1995; 378:789–792.
9. Dao TL, Hayes C, Libby PR. Steroid sulfatase activities in human breast tumors. Proc Soc Exp Biol Med 1974; 146:381–384.
10. Vignon F, Terqui M, Westley B, Derocq D, Rochefort H. Effects of plasma estrogen sulfates in mammary cancer cells. Endocrinology 1980; 106:1079–1086.

11. Tseng L, Mazella J, Lee LY, Stone ML. Estrogen sulfatase and estrogen sulfotransferase in human primary mammary carcinoma. J Steroid Biochem 1983; 19:1413–1417.
12. Pasqualini JR, Gelly C, Lecerf F. Biological effects and morphological responses to estriol, estriol-3-sulfate, estriol-17-sulfate and tamoxifen in a tamoxifen-resistant cell line (R-27) derived from MCF-7 human breast cancer cells. Eur J Cancer Clin Oncol 1986; 22:1495–1501.
13. Pasqualini JR, Gelly C, Nguyen B-L, Vella C. Importance of estrogen sulfates in breast cancer. J Steroid Biochem 1989; 34:155–163.
14. Pasqualini JR, Schatz B, Varin C, Nguyen B-L. Recent data on estrogen sulfatases and sulfotransferases activities in human breast cancer. J Steroid Biochem Mol Biol 1992; 41:323–329.
15. Pasqualini JR, Chetrite G, Nguyen B-L, Maloche C, Delalonde L, Talbi M, Feinstein M-C, Blacker C, Botella J, Paris J. Estrone sulfate-sulfatase and 17β-hydroxysteroid dehydrogenase activities: a hypothesis for their role in the evolution of human breast cancer from hormone-dependence to hormone-independence. J Steroid Biochem Mol Biol 1995; 53:407–412.
16. Abul-Hajj YJ, Iverson R, Kiang DT. Aromatization of androgens by human breast cancer. Steroids 1978; 33:205–222.
17. Lipton A, Santner S, Santen RJ, Harvey HA, Feil PD, White-Hershey D, Bartholomew MJ, Antle CE. Aromatase activity in primary and metastatic human breast cancer. Cancer 1987; 59:779–782.
18. Perel E, Daniilescu D, Kharlip L, Blankenstein M, Killenger DW. Steroid modulation of aromatase activity in human cultured breast carcinoma cells. J Steroid Biochem 1988; 29:393–399.
19. Pollow K, Boquoi E, Baumann J, Schmidt-Gollwitzer M, Pollow B. Comparison of the in vitro conversion of estradiol-17β to estrone of normal and neoplastic human breast tissue. Mol Cell Endocrinol 1977; 6:333–348.
20. Abul-Hajj YJ. Relationship between estrogen receptors, 17β-hydroxysteroid dehydrogenase and estrogen content in human breast cancer. Steroids 1979; 34:217–225.
21. Vermeulen A, Deslypere JP, Paridaens R, Leclercq G, Roy F, Heuson JC. Aromatase, 17β-hydroxysteroid dehydrogenase and intratissular sex hormone concentrations in cancerous and normal glandular breast tissue in postmenopausal women. Eur J Cancer Clin Oncol 1986; 22:515–525.
22. Nguyen B-L, Chetrite G, Pasqualini JR. Transformation of estrone and estradiol in hormone-dependent and hormone-independent human breast cancer cells. Effects of the anti-estrogen ICI 164 384, danazol, and promegestone (R-5020). Breast Cancer Res Treat 1995; 34:139–146.
23. Raju U, Blaustein A, Levitz M. Conjugation of androgens and estrogens by human breast tumors in vitro. Steroids 1980; 685–695.
24. Pasqualini JR. Steroid sulphotransferase activity in human hormone-independent MDA-MB-468 mammary cancer cells. Eur J Cancer 1992; 28A:758–762.
25. Raam S, Robert N, Pappas CA, Tamura H. Defective estrogen receptors in human mammary cancers: their significance in defining hormone dependence. J Natl Cancer Inst 1988; 80:756–761.

26. Fuqua SAW, Fitzgerald SD, Chamness GC, Tandon AK, McDonnell DP, Nawaz Z, O'Malley BW, McGuire WL. Variant human breast tumor estrogen receptor with constitutive transcriptional activity. Cancer Res 1991; 51:105–109.
27. McGuire WL, Chamness GC, Fuqua SAW. Abnormal estrogen receptor in clinical breast cancer. J Steroid Biochem Mol Biol 1992; 43:243–247.
28. Castles CG, Fuqua SAW, Klotz DM, Hill SM. Expression of a constitutively active estrogen receptor variant in the estrogen receptor-negative BT-20 human breast cancer cell line. Cancer Res 1993; 53:5934–5939.
29. Murphy LC, Dotzlaw H, Leygue E, Douglas D, Coutts A, Watson PH. Minireview: estrogen receptor variants and mutations. J Steroid Biochem Mol Biol 1997; 62:363–372.
30. Edwards DP, Chamness GC, McGuire WL. Estrogen and progesterone receptors in breast cancer. Biochim Biophys Acta 1979; 560:457–486.
31. Litherland S, Jackson IM, Antiestrogens in the management of hormone-dependent cancer. Cancer Treat Rev 1988; 15:183–194.
32. Loriaux DL, Ruder HJ, Lipsett MB. The measurement of estrone sulfate in plasma. Steroids 1971; 18:463–472.
33. Roberts KD, Rochefort JG, Bleau G, Chapdelaine A. Plasma estrone sulfate levels in postmenopausal women. Steroids 1980; 35:179–187.
34. Pasqualini JR, Chetrite G, Blacker C, Feinstein M-C, Delalonde L, Talbi M, Maloche C. Concentrations of estrone, estradiol, and estrone sulfate and evaluation of sulfatase and aromatase activities in pre- and postmenopausal breast cancer patients. J Clin Endocrinol Metab 1996; 81:1460–1464.
35. Longacre TA, Bartow SA. A correlative morphologic study of human breast and endometrium in the menstrual cycle. Am J Surg Pathol 1986; 10:382–393.
36. Topper YJ, Freeman CS. Multiple hormone interactions in the developmental biology of the mammary gland. Physiol Rev 1980; 60:1049–1106.
37. van Bogaert LJ. Effect of hormone on human mammary duct in vivo. Horm Metab Res 1978; 10:337–340.
38. McManus MJ, Welsch CW. The effect of estrogen, progesterone, thyroxine, and human placental lactogen on DNA synthesis of human breast ductal epithelium maintained in athymic nude mice. Cancer 1984; 54:1920–1927.
39. Laidlaw IJ, Clarke RB, Howell A, Owen AWMC, Potten CS, Anderson E. The proliferation of normal human breast tissue implanted into athymic nude mice is stimulated by estrogen but not progesterone. Endocrinology 1995; 136:164–171.
40. Gompel A, Malet C, Spritzer P, Lalardrie J-P, Kuttenn F, Mauvais-Jarvis P. Progestin effect on cell proliferation and 17β-hydroxysteroid dehydrogenase activity in normal human breast cells in culture. J Clin Endocrinol Metab 1986; 63:1174–1180.
41. Longman SM, Buehring GC. Oral contraceptives and breast cancer. In vitro effect of contraceptive steroids on human mammary cell growth. Cancer 1987; 59:281–287.
42. Jones B, Russo J. Influence of steroid hormones on the growth fraction of human breast carcinomas. Am J Clin Pathol 1987; 88:132–138.
43. Botella J, Duranti E, Duc I, Cognet AM, Delansorne R, Paris J. Inhibition by nomegestrol acetate and other synthetic progestins on proliferation and proges-

terone receptor content of T-47D human breast cancer cells. J Steroid Biochem Mol Biol 1994; 50:41–47.
44. Vignon F, Bardon S, Chalbos D, Rochefort H. Antiestrogenic effect of R-5020, a synthetic progestin in human breast cancer cells in culture. J Clin Endocrinol Metab 1983; 56:1124–1130.
45. Horwitz KB, Freidenberg GR. Growth inhibition and increase of insulin receptors in antiestrogen-resistant T-47Dco human breast cancer cells by progestins: implication for endocrine therapies. Cancer Res 1985; 45:167–173.
46. Murphy LC, Dotzlaw H, Alkhalaf M, Coutts A, Millar T, Wong MSJ, Gong Y, Murphy LJ. Mechanisms of growth inhibition by antiestrogens and progestins in human breast and endometrial cancer cells. J Steroid Biochem Mol Biol 1992; 43:117–121.
47. Musgrove EA, Lee CS, Sutherland RL. Progestins both stimulate and inhibit breast cancer cell cycle progression while increasing expression of transforming growth factor alpha, epidermal growth factor receptor, c-*fos* and c-*myc* genes. Mol Cell Biol 1991; 11:5032–5043.
48. Jeng M-H, Parker CJ, Jordan VC. Estrogenic potential of progestins in oral contracteptives to stimulate human breast cancer cell proliferation. Cancer Res 1992; 52:6539–6546.
49. Kalkhoven E, Kwakkenbos-Isbrücker L, de Laat SW, van der Saag PT, van der Burg B. Synthetic progestins induce proliferation of breast tumor cell lines via the progesterone or estrogen receptor. Mol Cell Endocrinol 1994; 102:45–52.
50. Papa V, Reese CC, Brunetti A, Vigneri R, Siiteri PK, Goldfine ID. Progestins increase insulin receptor content and insulin stimulation of growth in human breast carcinoma cells. Cancer Res 1990; 50:7858–7862.
51. Hissom JR, Moore MR. Progestin effects on growth in the human breast cancer cell line T-47D—possible therapeutic implications. Biochem Biophys Res Commun 1987; 145:706–711.
52. Catherino WH, Jeng M-H, Jordan VC. Norgestrel and gestodene stimulate breast cancer cell growth through an estrogen receptor mediated mechanism. Br J Cancer 1993; 67:945–952.
53. Schatz RW, Soto AM, Sonnenschein C. Effects of interaction between estradiol-17β and progesterone on the proliferation of cloned breast tumor cells (MCF-7 and T-47D). J Cell Physiol 1985; 124:386–390.
54. Markiewicz L, Hochberg RB, Gurpide E. Intrinsic estrogenicity of some progestagenic drugs. J Steroid Biochem Mol Biol 1992; 41:53–58.
55. Markiewicz L, Gurpide E. Estrogenic and progestagenic activities coexisting in steroidal drugs: quantitative evaluation by in vitro bioassays with human cells. J Steroid Biochem Mol Biol 1994; 48:89–94.
56. Botella J, Porthe-Nibelle J, Paris J, Lahlou B. Interaction of new 19-norprogesterone derivatives with progestagen, mineralocorticoid and glucocorticoid cytosolic receptors. J Pharmacol 1986; 17:699–706.
57. Catherino WH, Jordan VC. Nomegestrol acetate, a clinically useful 19-norprogesterone derivative which lacks estrogenic activity. J Steroid Biochem Mol Biol 1995; 55:239–246.

58. van der Berg B, Kalkhoven E, Isbrücker L, de Laat SW. Effects of progestins on the proliferation of oestrogen-dependent human breast cancer cells under growth factor-defined conditions. J Steroid Biochem Mol Biol 1992; 42:457–469.
59. Kloosterboer HJ, Schoonen WGEJ, Deckers GH, Klijn JGM Effects of progestagens and Org OD14 in in vitro and in vivo tumor models. J Steroid Biochem Mol Biol 1994; 49:311–318.
60. Schoonen WGEJ, Joosten JWH, Kloosterboer HJ. Effects of two classes of progestagens, pregnane and 19-nortestosterone derivatives, on cell growth of human breast tumor cells. I. MCF-7 cell lines. J Steroid Biochem Mol Biol 1995; 55:423–437.
61. Schoonen WGEJ, Joosten JWH, Kloosterboer HJ. Effects of two classes of progestagens, pregnane and 19-nortestosterone derivatives, on cell growth of human breast tumor cells. II. T-47D cell lines. J Steroid Biochem Mol Biol 1995; 55:439–444.
62. Braunsberg H, Coldham NG, Leake RE, Cowan SK, Wong W. Actions of the progestagen on human breast cancer cells: mechanisms of growth stimulation and inhibition. Eur J Clin Oncol 1987; 23:563–571.
63. Poulin R, Baker D, Poirier D, Labrie F. Multiple actions of synthetic ''progestins'' on the growth of ZR 75-1 human breast cancer cells: an in vitro model for the simultaneous assay of androgen, progestin, estrogen, and glucocorticoid agonistic and antagonistic activities of steroids. Breast Cancer Res Treat 1990; 17:197–210.
64. Hissom JR, Bowden RT, Moore MR. Effects of progestins, estrogens and antihormones on growth and lactate dehydrogenase in the human breast cancer cell line. Endocrinology 1989; 125:418–423.
65. Nardulli AM, Greene GL, O'Malley BW, Katzenellenbogen BS. Regulation of progesterone receptor messenger ribonucleic acid and protein levels in MCF-7 cells by estradiol: analysis of estrogen's effect on progesterone receptor synthesis and degradation. Endocrinology 1988; 122:935–944.
66. Alexander IE, Shine J, Sutherland RL. Progestin regulation of estrogen receptor messenger RNA in human breast cancer cells. Mol Endocrinol 1990; 4:821–828.
67. Nardulli AM, Katzenellenbogen BS. Progesterone receptor regulation in T-47D human breast cancer cells: analysis by density labelling of progesterone receptor synthesis and degradation and their modulation by progestins. Endocrinology 1988; 122:1532–1540.
68. Goldfine ID, Papa V, Vignerie R, Siiteri PK, Rosenthal S. Progestin regulation of insulin and insulin-like growth factor-I (IGF-I) receptors in cultured human breast cancer cells. Breast Cancer Res Treat 1992; 22:69–79.
69. Murphy LC, Dotzlaw H. Endogenous growth factor expression in T-47D human breast cancer cells associated with reduced sensitivity to antiproliferative effects of progestins and antiestrogens. Cancer Res 1989; 49:599–604.
70. Alkhalaf M, Murphy LC. Regulation of c-*jun* and *jun*-B by progestins in T-47D human breast cancer cells. Mol Endocrinol 1992; 6:1625–1633.
71. Moore MR, Zhou J-L, Blankenship KA, Strobl JS, Edwards DP, Gentry RN. A sequence in the 5′ flanking region confers progestin responsiveness on the human c-*myc* gene. J Steroid Biochem Mol Biol 1997; 62:243–252.

72. Sutherland RL, Hall RE, Pang GYN, Musgrove EA, Clarke CL. Effect of medroxyprogesterone acetate on proliferation and cell cycle kinetics of human mammary carcinoma cells. Cancer Res 1988; 48:5084–5091.
73. Groshong SD, Owen GI, Grimison B, Schauer IE, Todd MC, Langan TA, Sclafani RA, Lange CA, Horwitz KB. Biphasic regulation of breast cancer cell growth by progesterone: role of the cyclin-dependent kinase inhibitors, p21 and $p27^{Kip}1$. Mol Endocrinol 1997; 11:1593–1607.
74. Chetrite G, Pasqualini JR. Unpublished data.
75. Wilking N, Carlström K, Gustafsson SA, Skoldefors H, Tollborn O. Oestrogen receptors and metabolism of oestrone sulfate in human mammary carcinoma. Eur J Cancer 1980; 16:1339–1344.
76. Evans TRJ, Rowlands MG, Law M, Coombes RC. Intratumoral oestrone sulphatase activity as a prognostic marker in human breast carcinoma. Br J Cancer 1994; 69:555–561.
77. Pasqualini JR, Chetrite G, Nguyen B-L, Blacker C, Feinstein M-C, Maloche C, Talbi M, Delalonde L. Control of estrone sulfatase activity and its expression in human breast cancer. In: Motta M, Serio M, eds. Sex Hormones and Antihormones in Endocrine-Dependent Pathology: Basic and Clinical Aspects. Excerpta Medica International Congress Series—ICS 1064. Amsterdam: Elsevier Science, 1994, pp 257–265.
78. Pasqualini JR, Chetrite G, Le Nestour E. Control and expression of oestrone sulphatase activities in human breast cancer. J Endocrinol 1996; 150:S99–S105.
79. Pasqualini JR, Nguyen B-L. Estrone sulfatase activity and effect of antiestrogens on transformation of estrone sulfate in hormone-dependent vs. -independent human breast cancer cell lines. Breast Cancer Res Treat 1991; 18:93–98.
80. Prost-Avallet O, Oursin J, Adessi GL. In vitro effect of synthetic progestogens on estrone sulfatase activity in human breast carcinoma. J Steroid Biochem Mol Biol 1991; 39:967–973.
81. Santner SJ, Santen RJ. Inhibition of estrone sulfatase and 17β-hydroxysteroid dehydrogenase by anti-estrogens. J Steroid Biochem Mol Biol 1993; 45:383–390.
82. Pasqualini JR, Ebert C, Chetrite G. Control of estradiol in human breast cancer. Effect of medrogestone on sulfatase, 17β-hydroxysteroid dehydrogenase and sulfotransferases activities in human breast cancer cells. Proceedings IV European Congress on Menopause. Paris: Editions ESKA, 1998; in press.
83. Pasqualini JR, Varin C, Nguyen B-L. Effect of the progestagen R-5020 (promegestone) and of progesterone on the uptake and on the transformation of estrone sulfate in the MCF-7 and T-47D human mammary cancer cells: correlation with progesterone receptor levels. Cancer Lett 1992; 66:55–60.
84. Chetrite G, Paris J, Botella J, Pasqualini JR. Effect of nomegestrol acetate on estrone-sulfatase and 17β-hydroxysteroid dehydrogenase activities in human breast cancer cells. J Steroid Biochem Mol Biol 1996; 58:525–531.
85. Nguyen B-L, Fermé I, Chetrite G, Pasqualini JR. Action of danazol on the conversion of estrone sulfate to estradiol and on the sulfatase activity in the MCF-7, T-47D and MDA-MB-231 human mammary cancer cells. J Steroid Biochem Mol Biol 1993; 46:17–23.

86. Carlström K, Doberl A, Pousette A, Rannevik G, Wilking N. Inhibition of steroid sulfatase activity by danazol. Acta Obstet Gynecol Scand Suppl 1984; 123:107–111.
87. Evans TRJ, Rowlands MG, Jarman M, Coombes RC. Inhibition of estrone sulfatase enzyme in human placenta and human breast carcinoma. J Steroid Biochem Mol Biol 1991; 39:493–499.
88. Chetrite G, Kloosterboer HJ, Pasqualini JR. Effect of tibolone (Org OD14) and its metabolites on estrone sulphatase activity in MCF-7 and T-47D mammary cancer cells. Anticancer Res 1997; 17:135–140.
89. Chetrite G, Varin C, Delalonde L, Pasqualini JR. Effect of promegestone, tamoxifen, 4-hydroxytamoxifen and ICI 164,384 on the oestrone sulphatase activity of human breast cancer cells. Anticancer Res 1993; 13:931–934.
90. Pasqualini JR, Maloche C, Maroni M, Chetrite G. Effect of the progestagen promegestone (R-5020) on mRNA of the oestrone sulphatase in the MCF-7 human mammary cancer cells. Anticancer Res 1994; 14:1589–1594.
91. Andersson S, Moghrabi N. Physiology and molecular genetics of 17β-hydroxysteroid dehydrogenases. Steroids 1997; 62:143–147.
92. Penning TM. Molecular endocrinology of hydroxysteroid dehydrogenases. Endocrinol Rev 1997; 18:281–305.
93. Tait GH, Newton CJ, Reed M.J, James VHT. Multiple forms of 17β-hydroxysteroid oxidoreductase in human breast tissue. J Mol Endocrinol 1989; 2:71–80.
94. Prudhomme JF, Malet C, Gompel A, Lalardrie JP, Ochoa C, Boue A, Mauvais-Jarvis P, Kuttenn F. 17β-Hydroxysteroid dehydrogenase activity in human breast epithelial cells and fibroblast cultures. Endocrinology 1984; 114:1483–1489.
95. Poutanen M, Moncharmont B, Vihko R. 17β-Hydroxysteroid dehydrogenase gene expression in human breast cancer cells: regulation of expression by a progestin. Cancer Res 1992; 52:290–294.
96. Malet C, Vacca A, Kuttenn F, Mauvais-Jarvis P. 17β-Hydroxysteroid dehydrogenase (E2DH) activity in T47D cells. J Steroid Biochem Mol Biol 1991; 39:769–775.
97. Fournier S, Brihmat F, Durand JC, Sterkers N, Martin PM, Kuttenn F, Mauvais-Jarvis P. Estradiol 17β-hydroxysteroid dehydrogenase, a marker of breast cancer hormone dependency. Cancer Res 1985; 45:2895–2899.
98. Chetrite G, Kloosterboer HJ, Pasqualini JR. Control by four Organon compounds: tibolone and its metabolites (Org-4094, Org-30126, Org-OM38) on the formation of estradiol in human breast cancer cells [abstr]. 79th Ann Mtg Endocr Soc 1997.
99. Adams EF, Coldham NG, James VHT. Steroidal regulation of oestradiol-17β-hydrogenase activity of the human breast cancer cell line MCF-7. J Endocrinol 1988; 118:149–154.
100. Coldham NG, James VHT. A possible mechanism for increased breast cell proliferation by progestins through increased reductive 17β-hydroxysteroid dehydrogenase activity. Int J Cancer 1990; 45:174–178.
101. Couture P, Thériault C, Simard J, Labrie F. Androgen receptor-mediated stimulation of 17β-hydroxysteroid dehydrogenase activity by dihydrotestosterone and

medroxyprogesterone acetate in ZR-75-1 human breast cancer cells. Endocrinology 1993; 132:179–185.
102. Poutanen M, Isomaa V, Kainulainen K, Vihko R. Progestin induction of 17β-hydroxysteroid dehydrogenase enzyme protein in the T-47D human breast cancer cell line. Intl J Cancer 1990; 46:897–901.
103. Anderson E, Howell A. Oestrogen sulphotransferases in malignant and normal human breast tissue. Endocr Relat Cancer 1995; 2:227–233.
104. Falany JL, Falany CN. Expression of cytosolic sulfotransferases in normal mammary epithelial cells and breast cancer cell lines. Cancer Res 1996; 56:1551–1555.
105. Falany JL, Lawing L, Falany CN. Identification and characterization of cytosolic sulfotransferase activities in MCF-7 human breast carcinoma cells. J Steroid Biochem Mol Biol 1993; 46:481–487.
106. Falany CN, Wheeler J, Oh TS, Falany JL. Steroid sulfation by expressed human cytosolic sulfotransferases. J Steroid Biochem Mol Biol 1994; 48:369–375.
107. Chetrite G, Le Nestour E, Pasqualini JR. Human estrogen sulfotransferase activities (hEST1) and its mRNA in various breast cancer cell lines. Effect of the progestin, promegestone (R-5020). J Steroid Biochem Molec Biol 1998; 66:295–302.
108. Pasqualini JR, Ebert C, Chetrite G. Control of estradiol by medrogestone (Prothil) in human breast cancer cells [abstr]. 80th Ann Mtg Endocr Soc 1998.
109. Harris AL, Mitchell MD, Smith IE, Powles TJ. Aminoglutethimide in the treatment of advanced postmenopausal breast cancer. Cancer Res Suppl 1982; 42: 3405–3408.
110. Coombes RC, Goss PE, Dowsett M, Hutchinson G, Cunningham D, Jarman M, Brodie AM. 4-Hydroxyandrostenedione treatment for postmenopausal patients with advanced breast cancer. Steroids 1987; 50:245–252.
111. de Jong PC, van de Ven J, Nortier HWR, Maitimu-Smeele I, Donker TH, Thijssen JHH, Slee PHTJ, Blankenstein RA. Inhibition of breast cancer tissue aromatase activity and estrogen concentrations by the third-generation aromatase inhibitor vorozole. Cancer Res 1997; 57:2109–2111.
112. Miller WR. Aromatase inhibitors. Endocr Relat Cancer 1996; 3:65–79.

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Epidemiology and Treatment of Benign Breast Disease: Effect of Progestins on Breast Disease

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I. INTRODUCTION

The label *benign breast disease* includes a large spectrum of physiopathological lesions in the epithelial, stromal, or other components (adipocyte, vascular) of the breast. The classification and analysis of benign breast disease are controversial owing to the lack of a clear-cut clinical and histopathological dividing line between the physiological and pathological changes in the breast. Much of the controversy concerning the relation between benign breast disease and the subsequent development of carcinoma may be related to this ill-defined terminology. However, a classification based on the work of Dupont and Page (1) was adopted at the 1985 consensus meeting of the College of American Pathologists. The concept of benign epithelial proliferative disease identifies the histological characteristics of benign breast disease that have a cancerous potential. Three groups of benign breast disease have been characterized; nonproliferative lesions, proliferative lesions without atypia, and atypical hyperplasia. However, most of the epidemiological studies have not used this new classification; consequently, two major conditions are often considered in the epidemiological literature: fibroadenoma and fibrocystic disease. It would also be valuable to include mastalgia as a first stage of benign breast disease, and as a potential link to subsequent breast cancer.

The etiology of benign breast disease appears to be multifactorial. Although the mechanisms of the breast pathological changes remain unclear, the role of hormonal factors appears essential. In the early 1980s, on the basis of both experimental animal studies and in vitro studies using human breast cells in culture, estrogen was commonly accepted as the major hormonal determinant of breast pathology. In contrast, progesterone appeared to oppose estrogen's effects on the breast epithelial cells both locally at the breast tissue level and centrally at the hypothalamopituitary level by its antigonadotropic effect (2,3). Whereas the antiestrogenic activity of progesterone has been well documented for the endometrium, only few data are available on the human mammary gland. Obviously, the difficulties in collecting normal breast tissue, unlike the endometrial tissue, have drastically limited the studies about the hormonal control of the normal human mammary gland. Therefore, several controversial articles appeared during the last decade about progesterone's action in the breast (4–6).

II. INCIDENCE OF BENIGN BREAST DISEASE

Benign breast disease (BBD) incidence is generally overestimated. Indeed, benign breast diseases are not always symptomatic, and most of the women do not come to medical attention. Women ending with a well-defined clinical or cytological diagnostic entity, represent a selected subgroup of all cases. However, the incidence of benign breast disease may be estimated by their prevalence rates in autopsy studies, as well as their cumulative incidence rates from cohort studies.

In a recent review of the postmortem studies, Goehring and Morabia (7) estimate that about one out of every two women may develop some degree of fibrocystic breast disease during her lifetime, and one out of every five women may develop fibroadenoma. In contrast, from cohort studies data, the cumulative incidence of biopsy-proved fibrocystic breast disease before the age of 65 years was 8.8%; the corresponding cumulative incidence of fibroadenoma was 2.2%. Thus, compared with autopsy studies only 10–20% of benign disease cases would be histologically diagnosed by biopsy. Therefore, interpreting epidemiological studies related to pathologically proved BBD is difficult as the cases included in the studies might be restricted to the most clinically advanced lesions.

III. HORMONE DEPENDENCE OF HUMAN BREAST: CONTROVERSIES

The human breast is a target organ for the main steroids secreted by the ovary: estradiol (E_2) and progesterone (P). The natural life cycle of the mammary

gland is incompletely understood. However, the menstrual cycles, pregnancy, breastfeeding, and menopause are periods of a woman's life-time when the breast tissue is exposed to variation in sex steroid secretion.

A. Experimental Data in Animals

Experimentally, there is indirect evidence to suggest synergy and antagonism between estradiol and progesterone on the breast tissue. Many investigators have shown that the mammary gland of different species responds differently to estrogens, according to its administration, either at physiological or supraphysiological doses, or in combination with progesterone (8–10).

High doses of estrogen, administered for a prolonged time to castrated female rats, induce proliferation and dilation of the lobules in the glandular tissue, with formation of cysts and overgrowth of the epithelium (9). Administration of estradiol to female rats results in a cascade of mammary changes (i.e., proliferation of tubular system secretion, dilation of ducts, formation of cysts, and fibrosis). These changes observed with supraphysiological doses of estrogen are comparable with human fibrocystic disease. In contrast, when estradiol is administered in combination with progesterone, complete and proper development of the mammary gland, with an extensive alveolar proliferation is observed, provided the ratio between estrogen and progesterone is adequate. Cowie et al. (8) found that a combination of estrogen and progesterone in castrated goats resulted in uniform development and secretion when the dose of estrogen remained low (0.25 mg/day). Therefore, maintenance of a balanced ovarian hormonal function would be important for a normal mammary development.

Which ovarian steroids stimulate normal human mammary epithelial cell proliferation is an important issue that can be addressed, using the athymic nude mice model. McManus and Welsch (11) and more recently Laidlaw et al. (12) have analyzed the effects of estradiol and progesterone on normal human breast tissue implanted into athymic nude mice. They showed that estradiol appears to be sufficient to stimulate human breast epithelial cell proliferation at physiologically relevant concentrations, and progesterone does not affect proliferation either alone or after estradiol priming. They also demonstrated a dose–response relation between proliferation and serum estradiol concentrations. Longman and Buehring (13) showed that progesterone or progestins, when added alone, did not stimulate cell growth in explants of normal mammary tissue. When added to ethinyl estradiol, progestins slow down cell growth (13).

Hormone-dependent mammary tumors can be induced in animal models by chemical carcinogens or radiation. In animal studies, induction of breast cancer by a chemical carcinogen is prevented when the mammary gland has been completely differentiated by a first full-term pregnancy before exposure to the

carcinogen (14). Experimentally, the dimethylbenzanthracene (DMBA)-induced tumor incidence was significantly decreased in animals previously treated with E_2, P, or E_2 + P, in that order of efficacy. Combined administration of E_2 + P induced tissue differentiation similar to that observed after a first full-term pregnancy (i.e., rapid and complete differentiation of all terminal end buds into alveolar buds, and these into lobules). The protective effect of progesterone against various carcinogens seems to be dependent on the time of administration relative to the carcinogen and is generally related to the stimulation by progesterone of terminal duct differentiation, thus protecting lobuloalveolar glands from carcinogens (14,15).

B. Studies in Primates

In in vivo studies performed in surgically postmenopausal female monkeys, it was suggested by Cline et al. (16) that medroxyprogesterone acetate (MPA), administered orally, at doses equivalent to those used in women for hormone replacement therapy (HRT), would increase the tissue proliferation. These authors indeed showed from morphometric studies that the mammary gland thickness was increased with therapy, and also the percentage of mammary gland occupied by glandular tissue was increased more markedly in the group of animals receiving HRT. This finding was interpreted as a marker of a mitogenic role of the progestin. However, there was no statistical difference in the proliferative index measured in animals receiving estrogens alone or in combination with MPA. In addition as E and P act synergistically to promote tissue growth and acini formation, the increase in glandular volume may also account for the findings of Cline et al. (16).

C. Biochemical Data

In experiments conducted on human breast adenoma cells, P regulated E_2 and P receptors in epithelial cells. Also, P stimulated the enzyme 17β-dehydrogenase activity, which converts E_2 into E_1, a less active metabolite (10,17). The hormone dependence of those adenomas was confirmed in tumors with a high epithelial content. Also, progestins that are 19-norpregnane derivatives decreased cell multiplication in normal human breast cells in culture. These findings, however, have been criticized as being obtained under experimental conditions.

In addition, another important effect of progestins is related to their ability to decrease the amount of estrone sulfate (E_1S) in breast tissue. Pasqualini et al. (18) have demonstrated that several progestins can block the conversion of E_1S to E_2 very significantly in hormone-dependent breast cells.

On the other hand, progestins exert different effects according to the steroid from which they are derived (e.g., pregnanes, estranes, or gonanes). Some of the

estrane derivatives are able to stimulate breast cell multiplication in vitro through an estrogen receptor (ER)-mediated pathway. From using human breast cancer cells lines in culture, Catherino et al. (19) described the estrogenic activity of some 19-nortestosterone derivatives, such as norgestrel and gestodene, which stimulate breast cancer cell growth through an estrogen receptor mechanism. Later, Catherino and Jordan (20) tested 19-norpregnane derivatives using the same model and showed that they inhibited cell growth and did not exhibit any estrogenic effect. Also, some of the pregnane derivatives stimulate the apoptotic process leading to cell death (21).

D. Role of Progesterone and Progestins on the Cell Cycle

Musgrove et al. (22) have performed elegant in vitro studies demonstrating that breast cells in the last phase of cell cycle activity, are initially driven to the S phase of DNA synthesis by progestins (see also Chap. 3). This effect is transient, and further application of progestins suppresses the cyclins, then halting the breast cell division in early G_1 phase. These experiments underline a dual effect of the progestins according to the duration of their application, and may reconcile both hypotheses for the role of progestins: stimulator or suppressor of breast cell mitoses (22,23).

E. Human Studies

In women, the effects of sex steroids on breast tissue are less well known than their effects on the endometrium, mainly because normal breast tissue is not easy to sample in humans. In vivo studies were designed to detect changes in breast structure and function during the menstrual cycle, and identify what might predispose it to malignant changes. The uptake of tritiated thymidine has been used for calculating a thymidine-labeling index as a measure of the proliferative activity of the normal breast tissue at different times of the human cycle in women undergoing breast surgery (23).

In some studies, the mitotic activity of breast epithelial cells was reported to be higher during the follicular than during the luteal phase of a normal cycle, whereas other authors have shown a peak of mitotic activity of epithelial cells and DNA synthesis in the late luteal phase (24). Because the midluteal phase of the cycle immediately follows the secretion peaks of both progesterone and estrogen, it was inferred that progesterone is involved in the promotion of breast epithelial cell mitoses, in contrast with the well-documented antiproliferative effect of progesterone on the endometrium. However, this hypothesis remains controversial, for the exact timing of the biopsy within the menstrual cycle was not always well defined. In addition, other authors argue that breast cells would react to the cumulative effect of estrogens and progesterone secreted over

several cycles, rather than to the change in hormonal secretion in the 24-h period preceding the biopsy (25,26).

The recent development of monoclonal antibodies against specific cell proliferation marker antigens has also made possible a measurement of proliferation in cytological breast samples. Recent studies have shown the feasibility of mammary fine-needle aspiration biopsy in healthy women (27,28). The study by Söderqvist et al. (27) analyzed proliferation of breast epithelial cells in healthy women during the menstrual cycle. Late luteal phase was associated with high-mitotic activity of epithelial cells, compared with early follicular phase. Furthermore, the putative specific role of progesterone, mainly based on a temporal relation, could not be separated from a possible cumulative effect of estrogen. Hence, the selection of the different times for mammary needle aspiration appears determinant to interpret data on breast epithelial cells in healthy women during the menstrual cycle.

Interesting results on the interaction and effects of estradiol and progesterone on breast cell multiplication have recently been obtained in vivo (29). Women undergoing surgery for benign breast disease were randomly treated with either estradiol, progesterone, or placebo, applied locally to the breast between day 11 and 13 of the follicular phase of the menstrual cycle, before surgery. The number of mitoses in the epithelial cells of the normal part of the breast was counted. After estradiol treatment, both estradiol concentrations in the breast and the number of mitoses were high. After progesterone administration, progesterone concentration in breast tissue was high, but the number of mitoses was low; in the placebo group, the number of mitoses was also low. The authors concluded that in vivo high intratissular concentrations of progesterone were able to decrease the mitotic activity of the normal lobular epithelial cells. Similar tests were conducted in postmenopausal women before breast surgery. Exposure to progesterone for 14 days reduced the estradiol-induced proliferation of normal breast epithelial cells in vivo (30).

IV. EPIDEMIOLOGICAL DATA

A. Oral Contraceptives and Risk of Benign Breast Disease

Previous epidemiological studies have suggested that oral contraceptives (OC) use is associated with a significant decrease in the fibroadenoma incidence. A significant duration effect has been observed. The large Royal College of General Practitioners Study, performed in the early 1970s, in 46,000 women, showed that for a fixed dose of ethinyl estradiol (EE) in the oral contraceptives, increasing doses of the same progestogen (a 19-nortestosterone derivative) led to a decreased risk of fibroadenomas in young women (31).

Only few studies have analyzed the effect of the new low-dose OC. In one study, the decreased risk observed with OC containing 50 μg of EE, was not found for pills with a lower content in EE (32). Controversial results have been observed for the relation between OC use and fibrocystic disease. A lower risk of fibrocystic disease has been observed with OC use (33). However, when pathological lesions associated with high cancerous potential are taken into account, OC use has no significant effect (33).

B. Hormonal Replacement Therapy and Benign Breast Disease

Although mastalgia is a relatively common side effect of postmenopausal estrogen therapy (ERT) sometimes related to overdosage, there are few data suggesting that postmenopausal women receiving estrogen replacement therapy may develop an increased incidence of benign breast disease.

A large metanalysis of epidemiological studies evaluating the relation between ERT and breast cancer found no association between ERT and breast cancer in women with a previous history of benign breast disease. The relative risk for users compared with nonusers was 1.16 (95% confidence interval [CI]; 0.89–1.5), which was not statistically significant (34).

Some discrepancies between the various epidemiological studies may be related to the fact that a previous history of benign breast disease is defined differently, according to the studies (34,35). Nevertheless, in women with a previous history of fibrocystic disease with atypia and a history of breast cancer in a first-degree relative (mother or sister), ERT should be prescribed only if the breast x-ray is normal, and monitoring should be made on a regular basis.

C. Postmenopausal Estrogen Therapy and Breast Cancer

The relation between long-term ERT and risk of breast cancer remains controversial. A large metanalysis has shown an increased risk (30%) in long-term users (5–10 years) (35). This result essentially concerns estrogen-only use. Few studies have focused on combined estroprogestin hormonal replacement therapy (HRT) and risk of breast cancer. This relates to the recent coprescription of progestins with estrogens for menopausal therapy, especially in the United States where most of the large epidemiological studies were conducted. Three most recent studies showed contradictory results. In the Nurses Health Study (36), concerning 70,000 women who were followed for up to 16 years, the relative risk (RR) of breast cancer was significantly increased among women who were current users of estrogens (RR, 1.32; CI, 1.14–1.54) or of combined estrogens and progestins (RR, 1.41; CI, 1.15–1.74). No statistically significant difference was observed between the two groups using either estrogens alone or combined

HRT. The main finding was of an earlier appearance of risk, after 5 years of use of HRT. In the two population-based control studies published shortly after the Nurses Health Study, no increase in risk of breast cancer, in any group of HRT long-term users was observed (37,38). A complete reanalysis of all these studies has been made by Valerie Beral et al. (39). The results have been published and are reported in Chapter 15.

These conflicting data have resulted in an active controversy over whether progestins should be added to estrogens in HRT and especially whether hysterectomized women would need progestins (6). Our experience has been to recommend combined therapy also in hysterectomized women.

D. Mastalgia and Risk of Breast Cancer

Cyclic pain (mastalgia or mastodynia) must be defined and differentiated from other thoracic or intercostal pain. Painful bilateral swelling of the breasts, beginning toward the end of the cycle and lasting at least 4 days and up to 3 weeks before menstruation, but is alleviated after menstruation, is most likely hormone-dependent (40). Sometimes, however, the pain and swelling can start 2 or more weeks before menstruation. The women are asked specifically about the characteristics and intensity of mastalgia to eliminate other causes of the painful symptoms.

A strong association between a previous history of cyclic mastalgia and increased breast cancer has been found in a case–control study (41). In that study, 210 premenopausal women, younger than 45 years old and with a recently diagnosed breast cancer, were matched with 210 controls (for age, age at first-full term pregnancy, and socioeconomic status) (40). The cyclic mastalgia was associated with a twofold increase of relative risk of breast cancer (2.1 [95% CI, 1.3–3.4], adjusted relative risk for a familial history of breast cancer, personal history of BBD, and age at menarche) with a significant symptom–duration effect. These results have been confirmed by Goodwin et al. (42), who showed by another methodological approach a significantly increased risk of breast cancer in women with severe mastalgia. Therefore, mastalgia appears to be a marker of breast cell susceptibility and should not be neglected, especially when women are incapacitated by severe pain.

V. TREATMENT OF BENIGN BREAST DISEASE

A. Rationale for Treatment of Benign Breast Disease

For many years, long-term prospective studies have indicated that women with a previous breast biopsy for benign breast disease (BBD) were at increased

risk of breast cancer. More recent epidemiological studies have indicated that the risk was higher in women who exhibited proliferative lesions associated with fibrocystic disease when atypia was identified, compared with women with nonproliferative disease.

Two controversial hypotheses prevailed during the last two decades, both related to the characteristics and the number of menstrual cycles before a first full-term pregnancy. On one hand, Korenman (4) suggested that anovulatory menstrual cycles, with an estrogen secretion unopposed by progesterone and with infertility, increased the woman's risk of breast cancer. His hypothesis was based on the role of estrogen–progesterone imbalance, leading to an "unopposed estrogen effect," favoring the development of breast disease. With this rationale, progestin treatment for various benign breast diseases and menstrual disorders has been proposed (3).

On the other hand, Key and Pike (5) proposed the "estrogen augmented by progestogen" hypothesis. Here, the cumulative number of ovulatory menstrual cycles may represent an important etiological factor and some epidemiological hormonal risk factors for breast cancer could be explained.

The treatment of benign breast disease should be considered only in terms of its ability to correct breast cell overproliferation. That only benign breast disease with proliferative changes increases the breast cancer risk argues for the necessity to correct these changes, to keep the breast cells in a low rate of proliferation.

B. Progesterone and Progestins for the Treatment of Benign Breast Disease

Several therapeutic options have been used in women with BBD (40,43). Among those are danazol, progestins, bromocriptine, GnRH analogues, antiestrogens, and primrose oil. For the purpose of this chapter, only the use of progestogens and their action on breast disease will be addressed.

The therapeutic approach is based on the fact that in vivo benign breast disease is characterized, at least at the beginning, by a hormonal milieu of inadequate luteal function. In addition, progesterone and progestogen administration in patients with fibroadenomas leads to an increase in 17β-hydroxysteroid dehydrogenase activity and to an increased retention of progesterone receptors in the nucleus of breast epithelial cells.

Estrane derivatives are particularly effective, acting both as antigonadotropic agents at the pituitary level and as antiestrogenic agents on the breast cell. Also, progesterone dissolved in a hydroalcoholic gel is absorbed through the skin and concentrated in the mammary tissue. Topical daily application of this gel throughout the cycle stimulates 17β-hydroxysteroid dehydrogenase activity in human breast fibroadenomas. More recently, in Europe, 19-norpregnane

derivatives, such as nomegestrol acetate or promegestone (R 5020), not only did not increase breast cell proliferation, but also acted to decrease it under experimental conditions. Those progestins that also act as antigonadotropic agents have been recommended for therapy. In our hands, the best treatment for benign breast disease consisted of 9 months of cyclic administration of a progestin with antigonadotropic effects (such as lynestrenol acetate at the dose of 10 mg/day), from day 10 to day 25 of the menstrual cycle. Normal cycles will return in most cases after therapy is withdrawn. With irregular cycles, there is a risk of relapse of mastalgia and, later, of breast nodularities, especially, if the hormonal imbalance persists for several months. A fresh course of progestin therapy is then advised, with careful follow-up of breast symptoms, lipid and high density lipoprotein (HDL) cholesterol levels in plasma, body weight, and blood pressure. To date, there have been no randomized, placebo-controlled trials to assess the efficacy of the different progestins in benign breast disease treatment. The long-term effects of such therapies has been evaluated in only one epidemiological study.

In that cohort study of 1150 premenopausal women with a diagnosis of benign breast disease, the use of 19-norsteroid derivatives was significantly associated with a lower risk of breast cancer, compared with untreated women

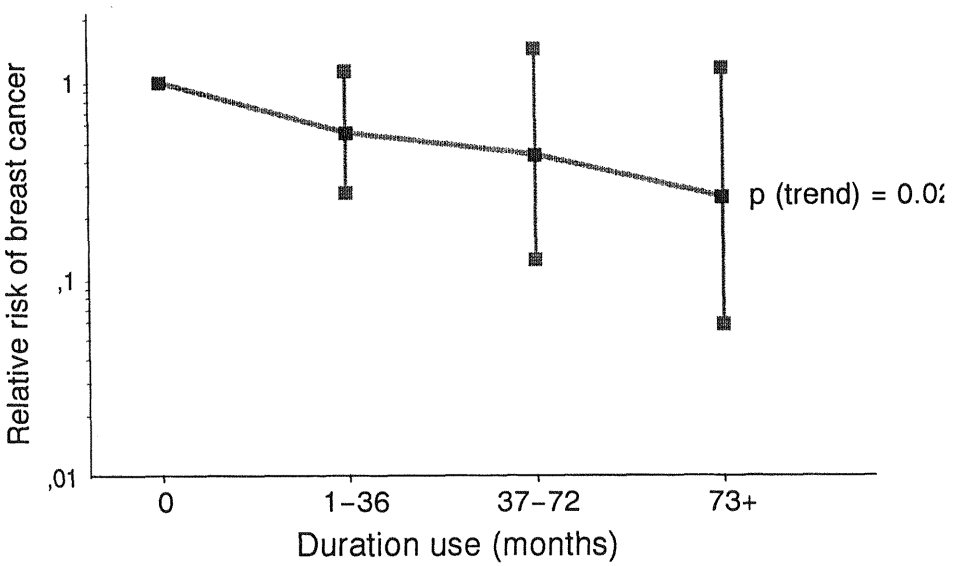

Figure 1 Risk of breast cancer according to 19-nortestosterone derivatives use. (From Ref. 44.)

(RR 0.48 [95% CI, 0.25–0.90]). In addition, there was a significant linear relation between the duration of use and the decrease in breast cancer risk (p for trend = 0.02) (Fig. 1) (44). These results should encourage further research in this direction.

As a summary, the role of progestins on the breast tissue has generated a great deal of controversies. The merit of the scientific debate has been to generate a large number of experimental and epidemiological trials. A better understanding of the dual role of progestins on the breast cells has recently emerged that may reconcile some apparently contradictory findings. Nevertheless, further long-term controlled studies should be conducted to determine the role of various progestational molecules on breast disease and breast cancer risk. The influence of these trials on women's health care in general would be enormous. The results of the Women's Health Initiative should bring some information about the role of MPA in postmenopausal women, but will not be available before the turn of the next century.

REFERENCES

1. Dupont WD, Page DL. Risk factors for breast cancer in women with proliferative breast disease. N Engl J Med 1985; 312:146–151.
2. Henderson BE, Ross R, Bernstein L. Oestrogens as a cause of human cancer. Cancer Res 1988; 48:246–253.
3. Mauvais-Jarvis P. Progesterone and progestins: a general overview. In: Bardin CW, Milgrom E, Mauvais-Jarvis P. eds. Progesterone and Progestins. New-York: Raven Press, 1983; 1–16.
4. Korenman SG. The endocrinology of breast cancer. Cancer 1980; 46:874–878.
5. Key TJA, Pike MC. The role of estrogens and progestogens in the epidemiology and prevention of breast cancer. Eur J Cancer Clin Oncol. 1988; 24:29–43.
6. Sitruk-Ware R. Estrogens, progestins and breast cancer risk in postmenopausal women; state of the ongoing controversy in 1992. Maturitas 1992; 15:129–139.
7. Goehring C, Morabia A. Epidemiology of benign breast disease, with special attention to histologic types. Epidemiol Rev 1997, 19:310–327.
8. Cowie AT, Folley ST, Malpress FH, Richardson KC. Studies on the hormonal induction of mammary growth and lactation in the goat. J Endocrinol 1952; 8:64–88.
9. Bassler R. The morphology of hormone induced structural changes in female breast. Curr Top Pathol 1970; 53:1–89.
10. Wang DY, Fentiman IS. Epidemiology and endocrinology of benign breast disease. Breast Cancer Res Treat 1985; 6:5–36.
11. McManus MJ, Welsch CW. The effects of estrogen, progesterone and human placental lactogen on DNA synthesis on human breast ductal epithelium contained in athymic mice. Cancer 1984; 54:1920–1927.

12. Laidlaw IJ, Clarke RB, Howell A, Owen AW, Potten CS, Anderson E. The proliferation of normal human breast tissue implanted into athymic nude mice is stimulated by estrogen, but not progesterone. Endocrinology 1995; 136:164–171.
13. Longman SM, Buehring GC. Oral contraceptives and breast cancer. In vitro effect of contraceptive steroids on human mammary cell growth. Cancer 1995; 59:281–287.
14. Russo J, Tay LK, Russo IH. Differentiation of the mammary gland and susceptibility to carcinogenesis. Breast Cancer Res Treat 1982; 2:5–73.
15. Labrie F, Li S, Bélanger A, Côté J, Mérand Y, Lepage M. Controlled release low dose medroxyprogesterone acetate (MPA) inhibits the development of mammary tumors induced by dimethyl-benz(*a*) anthracene in the rat. Breast Cancer Res Treat 1993; 26:253–265.
16. Cline JM, Soderqvist G, Von Shoultz B. Effects of hormone replacement therapy on the mammary gland of surgically postmenopausal cynomolgus macaques. Am J Obstet Gynecol 1996; 174:93–100.
17. Kuttenn F, Fournier S, Durand JC, Mauvais-Jarvis P. Estradiol and progesterone receptors in human breast fibroadenomas. J Clin Endocrinol Metab 1981; 52:1225-1229.
18. Pasqualini JR, Chetrite G, Nguyen BL, Maloche C, Delalonde L, Talbi M, Feinstein MC, Blacker C, Botella J, Paris J. Estrone sulfate-sulfatase and 17beta-hydroxysteroid dehydrogenase activities; a hypothesis for their role in the evolution of human breast cancer from hormone-dependence to hormone independence. J Steroid Biochem Mol Biol 1995; 53:407–412.
19. Catherino WH, Jeng MH, Jordan VC. Norgestrel and gestodene stimulate breast cancer cell growth through an oestrogen receptor mediated mechanism. Br J Cancer 1993; 67:945–952.
20. Catherino WH, Jordan VC. Nomegestrol acetate, a clinically useful 19-norprogesterone derivative which lacks estrogenic activity. J Steroid Biochem Mol Biol 1995; 55:239–246.
21. Desreux J, Noël A, Thomas JL, Bernard AM, Paris J, Delansorne R, Van Cauwenberge JR, Van Cauwenberge H, Francart D, Heinen V, Foidart JM. Progestin induced apoptosis of normal human breast cells: an in vivo and in vitro study. 8th International Congress on the Menopause, Sydney, Australia, November 1996, abstr F173, p 97.
22. Musgrove EA, Lee CS, Sutherland RL Progestins both stimulate and inhibit breast cancer cell progression while increasing expression of transforming growth factor receptor, c-*fos* and c-*myc* genes. Mol Cell Biol 1991; 11:5032–5043.
23. Ferguson DJP, Anderson TJ. Morphological evaluation of cell turnover in relation to the menstrual cycle in the resting human breast. Br J Cancer 1981; 44:177–181.
24. Plu-Bureau G, Touraine P, Mauvais-Jarvis P. Interactions between estradiol and progesterone in normal breast: implications for mammary carcinogenesis. In: Manni A, ed. Endocrinology of the Breast. Totowa, Humana Press 1999, pp 21–37.
25. Sitruk-Ware R, Plu-Bureau G. Progestogen use and breast cancer. In: BG Wren, ed. Progress in the Management of Menopause. Carnforth, UK: Parthenon, 1997, pp 203–210.

26. Wren BG. Hormonal replacement therapy and breast cancer. Eur Menop J 1995; 2:13–19.
27. Söderqvist G, Isaksson E, Von Schoulz B, et al. Proliferation of breast epithelial cells in healthy women during the menstrual cycle. Am J Obstet Gynecol 1997; 176:123–128.
28. Miller NA, Thomas M, Martin LJ, et al. Feasibility of obtaining breast epithelial cells from healthy women for studies of cellular proliferation. Breast Cancer Res Treat 1997; 43:201–210.
29. Chang KJ, Lee TTY, Linares-Cruz G, Fournier S, de Lignieres B. Influences of percutaneous administration of estradiol and progesterone on human breast epithelial cell cycle in vivo. Fertil Steril 1995; 63:785–791.
30. Foidart JM, Colin C, Denoo X, Desreux J, Béliard A, Fournier S, de Lignières B. Estradiol and progesterone regulate the proliferation of human breast epithelial cells. Fertil Steril 1998; 69:963–969.
31. Royal College of General Practitioners Oral Contraception Study. Effect on hypertension and benign breast disease of progestagen component in combined oral contraceptives. Lancet 1977; 1:624.
32. Sitruk-Ware R, Thalabard JC, Benotmane A, Mauvais-Jarvis P. Risk factors for breast fibroadenoma in young women. Contraception 1989; 40:251–268.
33. McGonigle KF, Huggins GR. Oral contraceptives and breast disease. Fertil Steril 1991; 56:799–819.
34. Dupont WD, Page DL. Menopausal estrogen replacement therapy and breast cancer. Arch Intern Med 1991; 151:67–72.
35. Steinberg KK, Thacker SB, Smith SJ, Stroup DF, Zack MM, Flanders D, Berkelman RL. A meta-analysis of oestrogen replacement therapy and the risk of breast cancer. JAMA 1991; 265:1985–1990.
36. Colditz GA, Hankinson SE, Hunter DJ, et al. The use of estrogens and progestins and the risk of breast cancer in postmenopausal women. N Engl J Med 1995; 332:1589–1593.
37. Newcomb PAA, Longnecker MP, Storer BE, et al. Long term hormone replacement therapy and risk of breast cancer in postmenopausal women. Am J Epidemiol 1995; 142:788–795.
38. Stanford JL, Weiss NS, Voigt LF, et al. Combined estrogen and progestin hormone replacement therapy in relation to risk of breast cancer in middle-aged women. JAMA 1995; 574:137–142.
39. Collaborative Group on Hormonal Factors in Breast Cancer (writing and analysis committee: Beral V, Reeves G, Bull D, Peto R, Key T, Doll R). Breast cancer and hormone replacement therapy: collaborative reanalysis of data from 51 epidemiological studies of 52,705 women with breast cancer and 108,411 women without breast cancer. Lancet 1997; 350:1047–1059.
40. Sitruk-Ware R. Benign breast disease. In: Bardin CW, ed. Current Therapy in Endocrinology and Metabolism. Philadelphia: BC Decker 1997; 396–400.
41. Plu-Bureau G, Thalabard JC, Sitruk-Ware R, Asselain B, Mauvais-Jarvis P. Cyclical mastalgia as a marker of breast cancer susceptibility: results of a case-control study among French women. Br J Cancer 1992; 65:945–949.

42. Goodwin PJ, Deboer G, Clark RM. Cyclical mastopathy and premenopausal breast cancer risk: results of a case-control study. Breast Cancer Res Treat 1994; 33:63–73.
43. Vorherr H, Fibrocystic breast disease: pathophysiology pathomorphology, clinical picture and management. Am J Obstet Gynecol 1986; 154:161–179.
44. Plu-Bureau G, Lê MG, Sitruk-Ware R, Thalabard JC, Mauvais-Jarvis P. Progestogen use and decreased risk of breast cancer in a cohort study of premenopausal women with benign breast disease. Br J Cancer 1994; 70:270–277.

19

Therapeutic Use of Progestins: Practical Recommendations

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I. INTRODUCTION

Different types of progestins have been synthesized, and they may be classified according to the parent compound from which they derive (see Chaps. 5–8). Except for the oral or vaginal preparations of progesterone, all the progestogens available to the prescriber differ from the natural hormone. However, they mimic some of its actions through the progesterone receptor (PR) and exert other effects according to their structure and binding capacities to other steroid receptors. Progesterone binds with other steroid receptors and not only exerts its main progestational activity, but also antiestrogenic, antiandrogenic, and antimineralocorticoid properties. Therefore, luteal deficiency often leads to symptoms related to the other missing actions of progesterone.

The potential therapeutic benefits of progestins are related not only to their progestational potency, but also to the other properties they may possess. Therefore, the clinical decision for prescribing one molecule or another would depend on the indication and the desired effect to be achieved. Owing to the binding of the progestins to the PR, most of them would exert the main action of progesterone. The correction of luteal deficiency, clinically expressed through secondary amenorrhea, or irregular cycles and abnormal uterine bleeding, could be ensured by any progestin. However, the molecules with a high, selective, progestational potency and no other parallel effects should preferably be used.

Should the therapeutic goal be to oppose the estrogenic effect on the target organs and to prevent endometrial hyperplasia, those progestins with a high antiestrogenic effect should be preferred. In clinical situations, for which ovulation suppression is required, progestins with high antigonadotropic potency should be selected. Usually, the molecules capable of suppressing the secretion of the gonadotropins also possess high antiestrogenic properties. Should the clinical symptoms include signs of hyperandrogenism, progestins with a potent antiandrogenic effect are recommended. Therefore, although any progestin would be able to correct luteal deficiency, the most appropriate molecule could be selected according to the required properties for relief of associated symptoms.

II. INDICATIONS FOR PROGESTINS

The progestational molecules available for clinical use have been registered for several indications. However, there are slight differences among the products, related to the specific properties beyond the main progestational action. The most logical use for the progestins remains the correction of luteal insufficiency that induces several clinical situations.

A. Luteal Insufficiency and Related Menstrual Disorders

Anovulatory cycles in the adolescent or during the perimenopausal years are most frequent. During these two extreme periods of the reproductive life the control of the gonadotropins and of the menstrual cycle may often be disturbed, leading to anovulatory or dysovulatory cycles. In those instances, clinical symptoms vary, from *polymenorrhea* to *oligomenorrhea*, with irregular menstrual cycles, of longer than 35–40 days. Less severe ovulatory dysfunction manifests itself at varying cycle lengths of 21–35 days and usually with dysmenorrhea and premenstrual sydrome.

Progesterone acts at several sites, under physiological conditions. It controls the pituitary storage of follicle-stimulating hormone (FSH), the FSH/LH ratio, and the selective increase in baseline FSH levels over those of luteining hormone (LH), by slowing the rate of pulsatility of gonadotropin-releasing hormone (GnRH). It thus indirectly induces the FSH-dependent recruitment of the follicles. At the endometrial level, it causes the well-known changes in the down-regulation of estrogen and progesterone receptors, leading to the secretory transformation of the estrogen-primed endometrium, thereby preventing hyperplasia.

During adolescence, a very small amount of progesterone produced by luteinized follicles is able to trigger the development of the cyclic function. A

failure in this process may lead to unruptured asymptomatic follicular cysts, excessive ovarian androgen production, and anovulation (1). The clinical symptoms include *irregular cycles with hypermenorrhea*, and often *acne and hypertrichosis*. Progesterone administration would be the most logical therapy to restore cyclicity, decrease endometrial thickness and, hence, decrease the amount of bleeding. It also can decrease the androgenic symptoms through the antiandrogenic properties of the native hormone; however, in these cases, should the hyperandrogenism be prominent, more potent antiandrogenic progestins would be appropriate.

Oral administration of micronized progesterone (200 mg daily for 12 days) or medroxyprogesterone acetate (MPA) at doses of 5–10 mg/day, or didrogesterone (20 mg daily) for 10–12 days per month (then from day 15 to 25 of the cycle) would restore regular withdrawal menses and decrease the amount of uterine bleeding. In addition, endometrial hyperplasia is prevented. In the anovulatory cycles, with high LH levels and enlarged ovaries, with clinical symptoms of hyperandrogenism, treatment able to inhibit the gonadotropin secretion would be more appropriate. This is also true in perimenopausal years in which anovulatory cycles, but with high FSH levels and high estradiol (E_2) levels lead to endometrial hyperplasia and hypermenorrhea.

Although combined estrogen plus progestogen preparations, such as combined oral contraceptives (OCs), have been recommended in those cases, progestin-only therapy, selecting molecules with high antigonadotropic potency, such as levonorgestrel, lynestrenol, or nomegestrol acetate among others, may be also appropriate.

Hypermenorrhea (or menorrhagia) can also be treated with local application of progestins. Lähteenmäkki et al. (2) have shown that the insertion of a levonorgestrel-releasing intrauterine device (LNG IUD), in patients with excessive uterine bleeding would decrease blood loss. In their randomized study comparing LNG IUD and medical treatment, 64.3% of the women in the IUD group cancelled their decision to undergo hysterectomy, against 14.3% in the control group. This method, would prevent surgery, although endometrial resection has also been well accepted by the women (3).

In women with *hyperandrogenism*, progestins with antiandrogenic properties are recommended. Cyproterone acetate (CPA) at doses of 50 mg/day for 20 days/cycle, and associated with percutaneous estradiol as an add-back therapy, have proved useful in hirsute patients (4). At much lower doses of 2 mg/day associated with 35 μg of ethinyl estradiol, the combination has a strong antiandrogenic action, increases sex hormone-binding globulin (SHBG), reduces LH and FSH secretion, and inhibits 5α-reductase activity. It has been recommended also in young adolescents with polycystic ovarian syndrome and hirsutism. This therapy should last for 12–18 months, with 6-month monitoring of ovarian volume and hypertrichosis.

In menstrual disorders of the *perimenopausal years*, the shut-off of the gonadotropins is recommended and progestins with potent antigonadotropic activity have been recommended and used successfully. In this period of life, nonandrogenic molecules are preferred to avoid unwanted lipid changes. The 19-norprogesterone molecules proved useful and devoid of metabolic side effects (5,6). Nomegestrol acetate at 5 mg/day or promegestone at 0.5 mg daily, have been used, administered for 3 weeks and 1 week off, to restore cyclic bleeding patterns. However, after several cycles, amenorrhea may occur, and an E_2 add-back therapy may be needed.

B. Premenstrual Syndrome and Dysmenorrhea

Although anovulatory cycles do not lead to premenstrual syndrome (PMS), or painful menses, dysovulatory cycles characterized by a short luteal phase lead to these clinical symptoms. *Primary dysmenorrhea* without organic pelvic disease is related to an increase production in prostaglandins, leading to painful cramping, sweating, nausea and vomiting, diarrhea, and dizziness. Abel and Baird (7), in previous experimental studies have indicated that an estrogen-primed endometrial tissue was unable to produce prostaglandins (PGs). However, low levels of progesterone transforming the tissue into a secretory phase triggered the estrogen-related PGs' production. Increasing the progestin levels reversed this effect, owing to the antiestrogenic action of progestogens. Therefore, it is assumed that progestins with high antiestrogenic properties may be useful in the treatment of dysmenorrhea.

An antiprogesterone molecule, such as mifepristone (RU 486), is able to induce increased sensitivity to prostaglandin in the myometrium (9). Conversely, progesterone would decrease this effect. Although nonsteroidal antiinflammatory drugs (NSAIDs) have been recommended as the treatment of primary dysmenorrhea, it appears more physiological to restore an adequate luteal phase.

Several progestins have been approved for the treatment of dysmenorrhea. In the adolescent, didrogesterone at doses of 20 mg/day from day 15 to 25 of the cycle has been recommended. Other nonandrogenic progestins, such as nomegestrol acetate or promegestone, have also been registered in a few countries for this indication. In patients with severe dysmenorrhea, it may be necessary to use antigonadotropic agents; progestins, such as norethisterone, lynestrenol, nomegestrol acetate, or promegestone, may be used for 15–20 days/cycle with success.

The use of progestins for the treatment of *premenstrual syndrome* has been very controversial. Although it has been considered ineffective by most authors (10), one double-blind, placebo-controlled trial indicated the efficacy of progesterone (11). Here again, contradictory results may have been related to different design, definition of symtoms, and selection of the progestin.

Several molecules have been registered for that indication in several European countries. It must be stressed however, that in severe PMS it may be necessary to suppress ovulation and estroprogestin combinations are more often prescribed. Antigonadotropic progestins such as norethisterone (NET), lynestrenol (LYN), nomegestrol acetate (NOMAc), or promegestone may be used for 15–20 days/cycle with success (12–14).

C. Progestins for Replacement Therapy

In several situations of *hormonal deprivation*, such as delayed puberty with primary amenorrhea, Turner's syndrome, or postmenopause, estrogen replacement therapy must be combined with progestins to avoid endometrial hyperplasia. Once the diagnosis of these hypoestrogenic states is made and estrogen substitutive therapy envisaged, progestogens would be selected for their antiestrogenic properties and the least metabolic effect. In those instances, progesterone itself in its micronized form (MP), didrogesterone (DDG), medroxyprogesterone acetate (MPA), and 19-norprogesterone, all have been used. The daily doses selected to oppose replacement doses of estrogen are of 10 mg of MPA sequentially (or 2.5–5 mg continuously), 200 mg of oral MP, 20 mg of DDG, 5 mg of NOMAc prescribed for 12–13 days each month of estrogen substitution. Also, norethisterone 1 mg has been recommended (see also Chap. 14).

D. Mastalgia and Benign Breast Disease

Mastalgia is one symptom of the premenstrual syndrome and has been considered the first step of further breast changes of benign nature (15). Although the role of progestins on human breast tissue has been highly controversial, some authors have published interesting results on the treatment of benign breast disease with progesterone and progestins (16) (see also Chap. 18).

E. Endometriosis

Progestins alone have been the most popular medical treatment of endometriosis for about 20 years (17). Other therapies, such as danazol and GnRH analogues, have shown good clinical response of the endometriosis lesions. However, these recent endocrine treatments are unable to achieve complete healing and disappearance of those lesions. No superiority of the more recent treatments over progestins has been found. Also, the progestational molecules, such as MPA at doses up to 100 mg/day, induce less harmful metabolic changes that danazol (17). Therefore, it may be more appropriate to prescribe progestins with potent antigonadotropic properties and nonandrogenic side effects in the treatment of

endometriosis. Several molecules meet these requirements, such as CPA, chlormadinone acetate (CLA), NOMAc, and more recently, dienogest.

The antiprogesterone molecule mifepristone also decreases endometriosis-related symptoms at doses of 50 mg daily (18). This effect may also be due to the partial agonistic action of the molecule. Katsuki et al. (19) have shown that mifepristone decreased the suppressive action of dienogest on endometrial cells, indicating the receptor-related mechanism of action of dienogest (see also Chap. 8). Therefore, progestins that are able to suppress the ovarian function, and act through progesterone receptors are potential agents for the treatment of endometriosis. Selection of molecules with less androgenic properties and, hence, less metabolic effect, would be appropriate for this indication.

F. Progestin Use in Cancer

Progestins have been used for many years as one element of the endocrine therapy of breast cancer. The main therapeutic strategy being the reduction of the estrogen amount available to the breast cells; the physiological antiestrogenic effect of progesterone and its derivatives has been proposed and widely used (20).

The two main progestins used in clinical practice, as treatment of advanced breast cancer, are medroxyprogesterone acetate (MPA) and megestrol acetate (MA). When used at high doses, these compounds exert an antigonadotropic effect that has been observed both in pre- and postmenopausal women. Serum levels of androgens are reduced to about 60% of basal levels and estradiol (E_2) and estrone (E_1) are also decreased by 30% during progestin treatment (21,22). A significant decrease in the sex hormone-binding globulin (SHBG) has been observed under progestins, likely related to the decrease in estrogen levels. Therefore, less E_2 is available for cellular uptake.

Another important effect of progestins is related to their ability to decrease the amount of estrone sulfate (E_1S) in breast tissue (23,24). Breast cancer cells transform E_1S into more active estrogens, such as E_1, then E_2. MPA and MA are able to significantly decrease E_1S in the circulation (21). More recently, Pasqualini et al. (24) have demonstrated that several progestins can block the conversion of E_1S to E_2 very significantly in hormone-dependent breast cells. Also, the ability of several progestins to stimulate the enzyme activity 17β-E_2 dehydrogenase, which converts E_2 into E_1, a less potent estrogen, has been described in human breast cells maintained in culture (25).

The other antiestrogenic effect of progestins is exerted through their ability to decrease the levels of progesterone receptors (PR), although nonreceptors pathways have been described (26). Therefore, potent progestational molecules may, in theory, be used in the endocrine treatment of breast cancer to decrease the amount of estrogens available to the cells. To date, only MPA and MA have

been used, and their effect on breast cancer response has been well documented (22). In the strategy of endocrine treatment, progestins are used only as second-line agents in advanced breast cancer.

That tamoxifen is used in the adjuvant setting of breast cancer is now well established. This antiestrogen is also used as first-line therapy in those patients with metastatic disease who have not received this agent as an adjuvant or have discontinued it for more than a year. Other patients receive either aromatase inhibitors or progestins. Those receiving aromatase inhibitors (AI) in first-line treatment would then be candidates for progestins in second-line therapy. A similar response rate has been observed when either MPA or MA is used. From an overall analysis of several clinical trials, performed in postmenopausal women, Lundgren (22) has published an overall response rate [complete response (i.e., complete tumor regression; CR) + partial response (i.e., partial tumor regression; PR)] of 26.7% with MPA used as second-line or later treatment, given orally at doses ranging from 600 to 1400 mg/day. The usual dose is 1000 mg/day. With MA given at doses of 160 mg/day and up to 1600 mg, similar responses were observed with 25.3% CR plus PR. Stabilization of disease (SD) is observed in 21.9% of patients with MPA and 34% with MA.

Megestrol acetate has a longer half-life than MPA, of 18 h, and can be used once daily. Because of the high dose of progestins needed to obtain a positive response in advanced breast cancer, several side effects were reported. The most frequent ones are weight gain and edema. Also Cushing-like symptoms are described with MPA. A partial glucocorticoid activity of MPA has been described (27) and may account for some of the reported unwanted side effects, when the steroid is used at high doses. Thromboembolic events have been reported in less than 1% of the cases with both progestins.

The results of progestin therapy used as first-line therapy is more encouraging, for 40.2% CR plus PR were reported with MPA and 29.8% with MA. However, the availability of several new aromatase inhibitors giving similar or better response rates with fewer side effects may lead to a decrease in the use of progestins, such as MPA and MA, in the management of breast cancer.

However, other potent progestins are now available and decrease the gonadotropin levels both in post- and premenopausal women and also decrease the production of E_2, E_1, and E_1S (24). Some derivatives of the 19-norprogesterone, which exhibit a potent antiestrogenic activity without androgenic or glucocorticoid effects, might be proposed in future trials. It must, however, be borne in mind, that second-line therapy is proposed in patients with advanced disease, whose prognosis is already compromised. The rate of response observed in those patients cannot be expected to be much higher than the 25% described in previous studies with MPA and MA. The only major advantage would be to use lower doses of those potent new molecules for better tolerability.

G. Progestins Used for Contraception

The properties of the progestins on the pituitary, the endometrium, and the cervical mucus have been used in combined oral contraceptives and also in progestins-only contraception. These aspects have been addressed in Chaps. 9 to 13. In addition, the progestin-only pills (POP) usually referred to as ''mini-pills'' exert a contraceptive effect through the peripheral actions of progestins. Under their action, cervical mucus shows a decrease in quantity and thickening, leading to decreased permeability in sperm penetration tests. It also transforms the endometrium and decreases tubal motility.

The POP available for contraception are usually estrane and gonane derivatives. Levonorgestrel (LNG) 30 μg daily, norethindrone (NET), 350 μg/day, and lynestrenol, 500 μg/day, have been available for this indication. The failure rate ranges between 1 and 3%, and irregular bleeding is the most common side effect (28). Long-acting systems for delivery of low doses of progestins have been developed to improve efficacy. They are described in Chaps. 10 to 12.

In addition, injectable contraceptives containing only a synthetic progestin have proved to be an effective, safe, and acceptable method suitable in national family planning programs. The two most widely used formulations are depot medroxyprogesterone acetate (DMPA) and norethisterone enanthate (NET-ET) with a use-effectiveness higher than that observed with oral contraceptives (29). More recently a new once-a-month injectable contraceptive, combining an estrogen, estradiol cypionate, and DMPA has been made available after a large development program conducted by the WHO.

H. Progestins Used in Male Contraception

Although male contraception is less used than female contraception, several methods have been used, including progestins (30). Molecules with antigonadotropic properties have been used to decrease FSH and inhibit spermatogenesis. The addition of testosterone is necessary to maintain secondary male sexual characteristics and libido.

I. Pregnancy Maintenance

Progesterone directly supports the pregnancy and women with luteal insufficiency may be infertile. Luteal phase support is necessary in patients with luteal phase defect or hypothalamic amenorrhea treated with GnRH. Progesterone is preferred to any other synthetic progestin as the most physiological therapy. Injectable progesterone (12.5 mg/day IM) has also been used and the treatment is recommended from the third postovulatory day to the tenth gestational week.

The success rate of this therapy is low with pregnancy rates of 10–50% per cycle (31).

The newly available vaginal delivery of progesterone appears to be the most practical route of delivery of high concentrations of progesterone directly to the uterus (see Chap. 4). Oral progesterone may also be selected (see Chap. 23).

III. SIDE EFFECTS OF PROGESTINS

Because of the different properties of progestational molecules, unwanted effects have been reported. The undesirable effects differ from one molecule to the other and it would be inappropriate to consider the side effects as a class effect of all the progestins. Also, according to the dose used for each category of progestins, some unwanted effects may appear only when high doses are used according to the indication.

A. Androgenic Side Effects

High doses of estrane or gonane molecules may induce acne and occasionally hypertrichosis. These effects are usually mild and occur only with the most androgenic derivatives. They are rarely reported with the pregnane or norpregnane derivatives.

B. Glucocorticoid Side Effects

Only a few progestins exhibit partial glucocorticoid activity (27). This secondary activity may induce water and salt retention, leading to bloating and weight gain.

C. Central Nervous System Side Effects

Progesterone is converted into 5α-reduced metabolites that are further transformed into the 3-OH derivatives (α and β), potent stimulators of the γ-aminobutyric acid type A ($GABA_A$) receptor complex. This may account for side effects, such as sleepiness, loss of vigilance, depressive mood, and other psychological symptoms often observed after oral administration of progesterone. These CNS effects induced by oral progesterone are reported in a low percentage of cases and may be related to high peak levels of plasma progesterone following oral intake. These side effects may also occur with molecules able to convert into these centrally active metabolites. A decreased libido has also been reported with antiandrogenic molecules, such as CPA.

D. Metabolic Side Effects

The unwanted metabolic effects are addressed in detail in other chapters of this book (see Chaps. 6, 9, 20, and 21). Undesirable changes in lipids with a decrease in HDL-2 fraction and an increase in LDL have been reported, mainly with the most androgenic molecules from the estrane and gonane groups (32). MPA, although belonging to the pregnane group, exhibits so-called synandrogenic activity at high doses. This may partially explain some of the negative effects observed on the lipid profile (33). Also insulin-resistance has been described with the estrane and gonane derivatives, the most androgenic molecules leading to the most significant decrease in insulin sensitivity.

E. Estrogenic Side Effects

Some estrane derivatives exert partial estrogenic activity that has been evidenced in breast cell cultures (34) (see also Chap. 5). Clinical symptoms such as mastalgia and bloating may be related to this estrogenic effect. However, it is not possible to determine any direct relation between these symptoms and progestins intake.

F. Risk–Benefit of the Progestins

Progesterone is an essential hormone in physiology. Therefore, luteal inadequacy leads to a variety of symptoms, the most severe of which are endometrial hyperplasia and cancer. It is essential to replace progesterone during luteal phase defects to prevent endometrial disease and also to correct the related symptoms of progesterone deficiency, as described in the foregoing, in the indications for progestin use. The only relevant side effects that may cause concern are the potential cardiovascular effects, on one hand, related to the unwanted metabolic changes of some progestins and, on the other hand, to the direct effect on the vessels of some androgenic progestins. The use of the lowest effective dose of the progestins and the selection of the nonandrogenic compounds should avoid the occurrence of metabolic and vascular undesirable effects. Therefore, whenever possible, preference should be given to the nonandrogenic molecules according to the expected effect in any given indication for progestins use.

REFERENCES

1. Flamigni C, Porcu E. Hypermenorrhea and anovulatory cycles in the adolescent. In: Bardin CW, ed. Current Therapy in Endocrinology and Metabolism. 6th ed. St. Louis, MO: Mosby-Year Book, 1997; pp 243–245.

2. Lahteenmäkki P, Haukkamaa M, Puolakka J, Riikonen U, Sainio S, Suvisaari J, Nilsson CG. Open randomised study of use of levonorgestrel releasing intrauterine system as alternative to hysterectomy. B Med J 1998; 316:1122–1126.
3. Crosignani PG, Vercellini P, Mosconi P, Oldani S, Cortesi I, DeGiorgi O. Levonorgestrel-releasing intrauterine device versus hysteroscopic endometrial resection in the treatment of dysfunctional uterine bleeding. Obstet Gynecol 1997; 90:257–263.
4. Kuttenn F, Rigaud C, Wright F, Mauvais-Jarvis P. Treatment of hirsutism by oral cyproterone acetate and percutaneous estradiol. J Clin Endocrinol Metab 1980; 51:1107-1111.
5. Dorangeon P, Thomas JL, Choisy H, Lumbroso M, Hazard MC. Effects of nomegestrol acetate on carbohydrate metabolism. Diabetes Metab 1993; 19:441–445.
6. Basdevant A, Pelissier C, Conard J, Degrelle H, Guyenne TT, Thomas JL. Effect of nomegestrol acetate (5 mg/d) on hormonal, metabolic and hemostatic parameters in premenopausal women. Contraception 1991; 44:559–560.
7. Osathanondh R. Dysmenorrhea. In: Bardin CW, ed. Current Therapy in Endocrinology and Metabolism. 6th ed. St. Louis, MO: Mosby-Year Book, 1997; pp 246–251.
8. Abel MH, Baird DT. The effect of 17β-estradiol and progesterone on prostaglandin production by human endometrium maintained in organ culture. Endocrinology 1980; 106:1599–1606.
9. Kelly RW, Smith SK. Progesterone and antiprogestins, a comparison of their effect on prostaglandin production by human secretory phase endometrium and decidua. Prostaglandins Leukot Med 1987; 29:181–186.
10. Mortola JF. Premenstrual syndrome. In: Bardin CW, ed. Current Therapy in Endocrinology and Metabolism. 6th ed. St. Louis, MO: Mosby-Year Book, 1997; pp 251–256.
11. Dennerstein L, Spencer-Gardner C, Gotts G, Brown JB, Smith MA, Burrows GD. Progesterone and the premenstrual syndrome: a double-blind cross-over trial. Br Med J 1985; 280:1617–1621.
12. Cameron IT, Fraser IS, Smith SK, eds. Clinical Disorders of the Endometrium and Menstrual Cycle. New York: Oxford University Press, 1998.
13. Labrie F, Ferland L, Lagace L. High inhibitory activity of R 5020, a pure progestin at the hypothalamoadenohypophyseal level on gonadotropin secretion. Fertil Steril 1977; 28:1104–1112.
14. Couzinet B, Young J, Brailly S, Chanson P, Thomas JL, Schaison G. The antigonadotropic activity of progestins (19-nortestosterone and 19-norprogesterone derivatives) is not mediated through the androgen receptor. J Clin Endocrinol Metab 1996; 81:4218–4223.
15. Plu-Bureau G, Thalabard JC, Sitruk-Ware R, Asselain B, Mauvais-Jarvis P. Cyclical mastalgia as a marker of breast cancer susceptibility: results of a case–control study among French women. Br J Cancer 1992; 65:945–949.
16. Mauvais-Jarvis P, Sitruk-Ware R, Kuttenn F. Benign breast disease. In Mac Guire W, ed. Breast Cancer. vol. 4. New York: Plenum Publishing, 1981; pp 51–94.

17. Kauppila A. Reappraisal of progestins in endometriosis therapy. Eur J Endocrinol 1998; 138:134–136.
18. Kettel LM, Murphy AA, Morales AJ, Ulmann A, Baulieu EE, Yen SS. Treatment of endometriosis with the antiprogesterone mifepristone (RU 486). Fertil Steril 1996; 65:23–28.
19. Katsuki Y, Takano Y, Futamura Y, Shibutani Y, Aoki D, Udagawa Y, Nozawa S. Effects of dienogest, a synthetic steroid, on experimental endometriosis in rats. Eur J Endocrinol 1998; 138:216–226.
20. Moghissi KS. Treatment of endometriosis with estrogen–progestin combination and progestin alone. Clin Obstet Gynecol 1988; 31:823–828.
21. Lundgren S, Lonning PE, Utaaker E, Aakvaag A, Kvinnsland S. Influence of progestins on serum hormone levels in post menopausal women with advanced breast cancer. I. General findings. J Steroid Biochem 1990; 36:99–104.
22. Lundgren S. Progestins in breast cancer treatment. A review. Acta Oncol 1992; 31:709–721.
23. Lundgren S, Lonning PE. Influence of progestins on serum hormone levels in post menopausal women with advanced breast cancer. II. A differential effect of megestrol acetate and medroxyprogesterone acetate on serum estrone sulfate and sex hormone binding globulin. J Steroid Biochem 1990; 36:105–109.
24. Pasqualini JR, Chetrite G, Nguyen BL, Maloche C, Delalonde L, Talbi M, Feinstein MC, Blacker C, Botella J, Paris J. Estrone sulfate-sulfatase and 17β-hydroxysteroid dehydrogenase activities: a hypothesis for their role in the evolution of human breast cancer from hormone-dependence to hormone-independence. J Steroid Biochem Mol Biol 1995; 53:407–412.
25. Fournier S, Kuttenn F, DeCicco N, Mauvais-Jarvis P. Estradiol 17β-hydroxysteroid dehydrogenase activity in human breast fibroadenomas. J Clin Endocrinol Metab 1982; 55:428–433.
26. Horwitz KB, Wei LL, Sedlacek SM, D'Arville CN. Progestin action and progesterone receptor structure in human breast cancer. A review. Recent Prog Horm Res 1985; 41:249–316.
27. Hellman I, Yoshida K, Zumoff B, Levin J, Kream J, Fukushima DK. The effect of medroxyprogesterone acetate on the pituitary–adrenal axis. J Clin Endocrinol Metab 1976; 42:912–917.
28. Fraser IS, Odlind V. Long-acting delivery systems. The answer for fertility control? In: Sitruk-Ware R, Bardin CW, eds. Contraception. Newer Pharmacological Agents, Devices, and Delivery Systems. New York: Marcel Dekker, 1992; pp. 1–39.
29. Fraser IS, Weisberg E. A comprehensive review of injectable contraception with special emphasis on depot-medroxyprogesterone acetate. Med J Aust 1981; 1(suppl):1–19.
30. Wu FCW, Aitken RJ. Suppression of sperm function by depot-medroxyprogesterone acetate and testosterone enanthate in steroid male contraception. Fertil Steril 1989; 51:691–697.
31. Ben-Ozer S, Morales AJ. Infertility in women. In: Bardin CW, ed. Current Therapy in Endocrinology and Metabolism. 6th ed. St. Louis, MO: Mosby-Year Book, 1997; pp 228–234.

32. Bradley DD, Wingerd J, Petitti DB, Krauss RM, Ramcharan S. Serum high-density-lipoprotein cholesterol in women using oral contraceptives, estrogens and progestins. N Engl J Med 1978; 299:17–20.
33. The writing group for the PEPI Trial. Effects of estrogen or estrogen/progestin regimens on heart disease risk factors in postmenopausal women: The Postmenopausal Estrogen/Progestin Interventions (PEPI) Trial. JAMA 1995; 273:199–208.
34. Catherino WH, Jordan C. Nomegestrol acetate, a clinically useful 19-norprogesterone derivative which lacks estrogenic activity. J Steroid Biochem Mol Biol 1995; 55:239–246.

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The Effect of Progestins on Plasma Lipids

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I. INTRODUCTION

The transport of water-insoluble lipids and cholesterol is made possible by their formation of macromolecular soluble complexes with proteins. This lipoprotein system takes care of the transport and distribution of triglycerides, free and esterified cholesterol, and phospholipids to various tissues. The major lipoprotein classes share a common structure. The surface layer consists of free cholesterol and phospholipids, as well as one or more protein components (apolipoproteins). The hydrophobic core region of lipoproteins consists of esterified cholesterol and triglycerides. Both dietary (exogenous) and endogenous lipids are transported by lipoproteins. Although endogenous lipid transport is effectively influenced by sex steroids, almost nothing is known about their possible effects on exogenous lipid transport.

Endogenous plasma lipid transport is coordinated by a complex interplay of several transport proteins and enzymes as well as lipoprotein receptors. Very low-density lipoproteins (VLDLs) are secreted by the liver and transport triglycerides, which are hydrolyzed in plasma by lipoprotein lipase (LPL), providing fatty acids to target organs, such as the adipose tissue. During LPL-mediated hydrolysis (lipolysis), the VLDL particle becomes smaller and enriched in cholesterol, first forming intermediate-density lipoprotein, and finally low-density lipoprotein (LDL). LDL transports cholesterol to all tissues by the LDL-receptor pathway, a large part of it returning to the liver for catabolism by

hepatic LDL-receptors. The cellular delivery of cholesterol occurs by internalization of LDL particles bound to LDL-receptors which recognize a receptor-binding sequence on apolipoprotein (apo) B-100, the apolipoprotein component of LDL.

High-density lipoproteins (HDL) take part in "reverse cholesterol transport," returning cholesterol from peripheral tissues to the liver. "Nascent" HDL particles are formed during LPL-mediated hydrolysis of VLDL when its cholesterol–phospholipid surface layer becomes partly detached from the lipoprotein particle, forming discoidal structures. These discoidal structures associated with lecithin-cholesteryl acyltransferase (LCAT) become acceptors of free cholesterol derived from peripheral cells. The LCAT reaction creates a gradient, which results in a constant flow of cholesterol esters formed on the surface of discoidal particles entering the hydrophobic core region. Following acquisition of apolipoprotein A-I these nascent particles become spherical and move into the HDL_3 density range. These particles are further matured by accumulation of cholesterol and other modifications into HDL_2. In a process mediated by cholesteryl ester transfer protein (CETP), esterified cholesterol may be transferred from HDL particles to VLDL and LDL, and returned to the liver through the LDL–receptor pathway.

Progestins and estrogens influence the plasma levels of the three major lipoprotein classes, VLDL, LDL, and HDL, by having effects on carrier proteins, regulatory enzymes, and receptors. The main actors in this interplay are the apoliproteins B-100 and A-I, as well as the LDL–receptor and hepatic lipase, all of which are sensitive to sex steroid effects. Because the plasma concentrations of LDL (cholesterol) and VLDL (triglyceride) are considered risk factors for coronary heart disease, and HDL concentration as a negative risk factor (protective factor), this field is of crucial importance when contraceptive or noncontraceptive hormone treatments are developed.

II. PROGESTINS USED IN CONTRACEPTION AND HORMONE REPLACEMENT THERAPY

Combined oral contraceptives (OCs) contain ethinyl estradiol (rarely, mestranol) plus one of a variety of progestin components (Table 1). Plasma lipid and lipoprotein effects are modified, depending on the estrogen–androgen balance of the formulation (i.e., the estrogen/progestin dose ratio), as well as the androgenic and antiestrogenic properties of the progestin component. Norethynodrel was contained in the first OC marketed in 1961, which is sometimes referred to as the "first-generation." OCs containing levonorgestrel and norethisterone are

Table 1 Progestins Approved for Hormonal Contraception or Replacement

Progestin	Androgenicity[a]
19-Nortestosterone-related progestins	
Gonanes	
Levonorgestrel	++
Desogestrel	(+)
Gestodene	(+)
Norgestimate	(+)
Estranes	
Norethisterone (Norethindrone)	+
Norethisterone (Norethindrone) acetate	+
Ethynodiol diacetate	+
Lynestrenol	+
Norethynodrel	+
17α-hydroxyprogesterone-related progestins	
Pregnanes	
Medroxyprogesterone acetate	(+)
Chlormadinone acetate	(+)
Megestrol acetate	(+)
Cyproterone acetate	(+)
Dydrogesterone	(+)

[a]Estimate based on currently used contraceptive or replacement dosages.

conventionally called "second-generation," and those containing desogestrel, gestodene, or norgestimate "third-generation" OCs (1). Third-generation OCs are all low-dose formulations containing 35 μg of ethinyl estradiol, or less. Their progestin components, although derived from levonorgestrel, an androgenic 19-nortestosterone-related gonane (see Table 1), exhibit very little androgenicity (2). Norethisterone and the other members of the estrane group of 19-nortestosterone-related steroids exhibit androgenic activity, although less than levonorgestrel (see Table 1).

In postmenopausal hormone replacement therapy (HRT), mainly two types of progestin are used. The 19-nortestosterone-derived progestins, such as levonorgetrel (D-norgestrel), norethisterone (norethindrone), and norethisterone acetate (norethindrone acetate) have significant androgenic properties (3,4). The C-21 progestins derived from 17α-hydroxyprogesterone, such as acetates of medroxyprogesterone, megestrol, and cyproterone, are less androgenic than the 19-nortestosterones (5,6).

III. EFFECTS OF PROGESTINS ON PLASMA LIPIDS AND LIPOPROTEINS

The issue of the effects of sex hormones on plasma lipids and lipoproteins was long confused by the difficulty of interpreting the results of a variety of early studies using combination OCs. Contradictory results for HDL cholesterol concentrations were a particular concern, as the plasma HDL concentration reemerged as an important negative risk factor for coronary heart disease in the 1970s (7). The conflicting results could later be explained when the individual effects of the estrogenic and progestinic components were investigated (8), and it became clear that "estrogen-dominant" and "progestin-dominant" contraceptive preparations had differing effects on plasma lipid and lipoprotein concentrations. Third-generation OCs, although considered to have beneficial lipid effects, may slightly, but significantly, increase venous thromboembolic disorders (9) (see also Chap. 9).

In HRT, the estrogen–progestin balance is influenced by the estrogen components (mainly conjugated equine estrogen [CEE] and estradiol) being weaker estrogens compared with ethinyl estradiol used in OCs. Accordingly, even progestins with little androgenicity may partly reverse estrogen effects in combined HRT regimens. Important information has been gained from the Postmenopausal Estrogen/Progestin Interventions (PEPI) Trial that compared the effects of unopposed estrogen (CEE), CEE plus cyclic medroxyprogesterone acetate (MPA), CEE plus consecutive MPA, CEE plus cyclic micronized progesterone, and placebo on coronary heart disease risk factors. A total of 875 healthy postmenopausal women, aged 45–64 years, participated in this 3-year randomized, double-blind, placebo-controlled trial (10). HDL cholesterol was among the main endpoints of PEPI, and plasma cholesterol, triglyceride, and LDL cholesterol were also monitored. These results will be referred to in the following sections discussing plasma lipid effects.

A. Very Low-Density Lipoprotein and Triglyceride

1. Underlying Mechanisms

The production of triglyceride-rich VLDL is enhanced by administration of contraceptive and noncontraceptive estrogens (11,12). Molecular mechanisms involve hepatocyte apoB mRNA stimulation by estrogens (13). Androgenic progestins have the opposite effects on VLDL production, and less androgenic progestins have less or no effect at all, and in combined estrogen–progestin preparations the "net effect" depends on the doses and properties of the steroids in question. Oxandrolone, an androgenic–anabolic steroid accelerates the removal of triglycerides from plasma in hypertriglyceridemic individuals (14,15), an ef-

fect also observed in some early studies with combination OCs (16,17). Studies employing norethisterone have indicated that both reduced splanchnic production and increased lipolytic clearance may be involved (18,19). Studies using a high dose (750 μg) of levonorgestrel and a low dose of estradiol (1 mg) in postmenopausal women indicated increased catabolism of VLDL (20).

2. *Progestin Effects on Plasma VLDL and Triglyceride Concentrations in Contraception and HRT*

In *contraception*, data available from studies comparing formulations containing androgenic second-generation progestins, with those containing nonandrogenic third-generation progestins indicate that estrogen-induced VLDL–triglyceride elevations are opposed more effectively by the former (e.g., levonorgestrel) than the latter (e.g., desogestrel) (21,22). A summary of the lipid effects of third-generation OCs has indicated that they exhibit an estrogen-dominant effect on triglyceride levels, with increases ranging from 15 to 38% (23).

In *HRT*, important information has been obtained in studies administering progestins alone to peri- and postmenopausal women (3). Farish and co-workers reported reductions in plasma triglyceride levels in perimenopausal women (24), whereas other reports suggested nonsignificant decreases with levonorgestrel and norethisterone acetate (25,26). Addition of the androgenic 19-nortestosterone-derivative levonorgestrel to estrogen regimens invariably caused statistically significant reductions in plasma VLDL and triglyceride levels (26–28). The triglyceride-lowering effect was also dependent on the initial triglyceride levels: norethisterone and norethisterone acetate did not reduce triglyceride levels significantly when combined with estradiol valerate (27,29), but did so when administered to women taking ethinyl estradiol, a synthetic estrogen with marked triglyceride-increasing properties (29,30). The CEE (0.625 mg/day) used in PEPI caused an elevation in triglyceride levels, which was not significantly attenuated by any of the progestin components employed: cyclic MPA 10 mg/day on 12 cycle days, continuous MPA 2.5 mg/day, or cyclic micronized progesterone 200 mg/day on 12 cycle days (10).

B. Low-Density Lipoprotein and Cholesterol

1. *Underlying Mechanisms*

Studies in experimental animals have demonstrated increased hepatic LDL–receptor mRNA transcription rates caused by estrogen administration (13). All types of estrogen produce significant reductions in plasma total and LDL cholesterol levels (31–33), and the magnitude of decrease is proportional to the baseline level (31). Very few studies have addressed the question of whether progestins counteract this effect. One theoretical possibility is that androgenic progestins,

by accelerating the lipolytic degradation of VLDL, could facilitate LDL formation, resulting in elevated plasma LDL levels (29,34).

C. Progestin Effects on Plasma LDL and Total Cholesterol Concentrations in Contraception and HRT

Studies employing administration of progestin alone in peri- and postmenopausal women suggested that levonorgestrel, desogestrel, and 17-hydroxyprogesterone derivatives did not significantly alter LDL cholesterol concentrations (3). However, longer-term administration of norethisterone to perimenopausal women resulted in a significant increase after 12 months of treatment (24). In *contraception*, the relatively small doses of progestins in currently used OCs had little effect on LDL cholesterol when administered alone (22) or in combination with ehinyl estradiol (21,22). Newer formulations containing desogestrel, gestodene, or norgestimate had, on the average, a slight LDL cholesterol-reducing effect (23). Although several studies have reported LDL cholesterol increases with second-generation OCs, and many studies with third-generation OCs have reported LDL cholesterol decreases (35); nonsignificant differences in LDL cholesterol levels between users of the newer low-dose OCs are the most common finding (21,22).

Addition of norethisterone to a preexisting estrogen regimen in *HRT* caused LDL cholesterol elevations in some studies, proposedly because of accelerated VLDL catabolism producing increased amounts of LDL particles (29,34). For some reason, studies employing levonorgestrel, an even more androgenic progestin, did not increase LDL cholesterol when added to an estrogen regimen (3). In the randomized PEPI trial, CEE combined with MPA or micronized progesterone was equally effective in LDL cholesterol lowering, as unopposed CEE (10). In summary, currently used doses of progestins do not appear to significantly blunt the estrogenic effect on LDL in HRT. The differences are small; therefore, it is probable that coronary risk is not altered much.

Lipoprotein(a) [Lp(a)] consists of one molecule of apolipoprotein(a) which is covalently bound to LDL–apolipoprotein B, thus forming an LDL-like particle. There are structural similarities between apoprotein(a) and plasminogen, suggesting that elevated Lp(a) levels could interfere with fibrinolysis, a possibility in line with its proposed role as an independent risk factor for coronary heart disease. Lp(a) levels tend to increase after normal or surgical menopause (36,37). Albers and co-workers were the first to report that a sex hormone, the anabolic–androgenic steroid stanozolol, reduced plasma Lp(a) levels (38). Significant reductions reportedly occurred with other steroids with androgenic and progestinic properties, including norethisterone (39), danazol (40), and tibolone (41). Also somewhat surprisingly, unopposed estrogen therapy reduces plasma Lp(a) levels (37), a finding that constitutes an exception to the general rule that

estrogens and progestins have opposite effects on lipoprotein fractions. According to many studies, combined HRT regimens also reduce plasma Lp(a) levels effectively (37), as would be expected. The underlying mechanisms have not been clarified (42).

D. High-Density Lipoprotein

1. *Underlying Mechanisms*

The role of HDL cholesterol as a negative risk factor for coronary heart disease has created interest in all pharmacological agents that influence its levels. In the 1970s, various OCs were reported to increase (43,44), decrease (45), or have no significant effect on HDL cholesterol (46). The apparently conflicting findings were later explained by differing contraceptive formulations: HDL cholesterol-lowering efficacy increased with increasing dose and potency of the progestin component (47), whereas the HDL cholesterol-elevating effect was increased with increasing estrogen dose. Depending on the dose, progestins with androgenic properties (second-generation 19-nortestosterones) tended to overwhelm the estrogen effect in OCs (3), whereas pills containing third-generation 19-nortestosterones, including desogestrel, gestodene, and norgestimate, exhibited estrogen dominance (23,48,49).

The mechanisms underlying the sex hormone-mediated regulation of plasma HDL cholesterol levels apparently involve both catabolism of the lipid components and production of apolipoprotein A-I. Hepatic lipase, a lipolytic enzyme located on the endothelial cell surfaces of liver sinusoids participates in the degradation of HDL particles (50). Hepatic lipase may be liberated into the circulation by an intravenous injection of heparin and the ''postheparin lipolytic activity'' in plasma serves as a measure of the activity of this enzyme. Hepatic lipase activity is down-regulated by exogenous (51) and endogenous estrogen (52), but androgens are powerful stimulators (53). Hepatic lipase can be up- and down-regulated in a consistent way by androgenic and estrogenic sex steroids, respectively (54–58). HDL_2 appears to be more sensitive to sex steroid effects than HDL_3. Thus, suppression of hepatic lipase by estrogen was, in some studies, associated with reduction in HDL_2 cholesterol, triglyceride, and phospholipid concentrations, whereas HDL_3 was not significantly influenced (55,59), Conversely, stimulation of hepatic lipase activity by levonorgestrel, administered alone (60) or in combination with estrogen (55,59), increased hepatic lipase activity, with concomitant reductions in HDL_2 cholesterol concentrations. Progestins with little or no androgenicity, such as medroxyprogesterone acetate and desogestrel, did not significantly influence hepatic lipase activity or HDL_2 levels (54,61). The findings indicating a consistent inverse relation between hepatic

lipase activity and HDL_2 levels during sex hormone administration (8) suggest an important regulatory role for hepatic lipase (62).

Early studies indicated that sex hormone effects on HDL apolipoprotein synthesis also participated in the regulation of plasma HDL levels. Androgenic steroids suppress HDL apolipoprotein synthesis in animals (63) and humans (58). On the other hand, ethinyl estradiol administration increased HDL apolipoprotein synthesis rate in fertile women (64), and both CEE and estradiol increased apolipoprotein A-I production in postmenopausal women (65).

2. *Progestin Effects on HDL Cholesterol Levels in Contraception and HRT*

Various OC preparations consistently tend to reduce plasma levels of HDL cholesterol in proportion to the dose and androgenicity of the progestin component (66). The effect of the progestin can be reduced by developing multiphasic preparations in which the progestin dose can be minimized. Third-generation OCs exhibit estrogenic effects because of the nonandrogenicity of their progestin components, showing greater HDL cholesterol elevations compared with second-generation preparations (67). As judged from the HDL cholesterol elevation, desogestrel-containing formulations are slightly more estrogen-dominant than those containing gestodene and norgestimate (23). The elevations in HDL cholesterol brought about by third-generation OCs ranged between 8 and 13% (23).

Marked decreases in HDL cholesterol concentrations were reported for HRT regimens containing androgenic progestins (3). In the PEPI trial, the HDL cholesterol elevation caused by unopposed CEE was partly reversed by MPA-containing regimens, but not by micronized progesterone (10). This is in line with other studies (3) suggesting that the effect of the relatively weak estrogens used in HRT can be counteracted not only by strongly androgenic, but also partially by the less androgenic, progestins.

E. Parenteral Administration of Progestins in HRT

The most common parenteral route of hormone administration is transdermal delivery, using sex steroid-containing patches or ointments that are applied over the skin. Transdermal estradiol is commonly used in many countries and can be combined with cyclic oral progestin, or cyclic progestin (e.g., norethisterone acetate) also administered transdermally. The route of administration may cause effects on plasma lipids that differ from those achieved using oral treatments. Oral administration results in supraphysiological hormone concentrations in the portal circulation and liver, which cause two phenomena: (a) extensive hepatic metabolism of the hormone (e.g., conversion of estradiol to estrone); and (b) pronounced effects on protein synthesis in the liver (e.g., synthesis of apolipopro-

teins B-100 and A-I). Thus, when oral and transdermal estradiol were administered giving comparable plasma levels and causing similar suppression of plasma follicle-stimulating hormone (FSH) levels, oral estradiol increased VLDL production and LDL catabolism, but transdermal estradiol did not (12,65). Further studies using endogenous labeling of apolipoprotein A-I with intravenous infusion of the nonradioactive amino acid tracer, trideuterated leucine, indicated that oral estradiol increased the production of apolipoprotein A-I mainly in HDL_2 and to a smaller degree in HDL_3, whereas transdermal estradiol had no effect (68). Accordingly, oral estrogen influences lipoprotein metabolism more than parenteral estrogen, although increasing doses of parenterally administered estrogen also alters plasma lipoprotein levels (65). Parenteral administration of progestins has been investigated relatively little. By analogy, the "first-pass" effect of oral administration probably results in more pronounced effects on lipids than parenteral administration of progestins. In two studies, transdermal cyclic administration of norethisterone acetate 250 μg/day combined with transdermal estradiol 50 μg/day decreased the levels of LDL and HDL cholesterol, as well as triglyceride levels in postmenopausal women (69,70).

IV. SUMMARY AND CONCLUSIONS

Estrogens and progestins influence the metabolism and plasma levels of all lipoprotein risk factors for coronary heart disease. Both contraceptive and noncontraceptive estrogens tend to increase the production of VLDL and its main protein component apolipoprotein B-100, as well as the production of HDL and its main protein component apolipoprotein A-I, while increasing the catabolic rate of LDL. Progestins tend to oppose these estrogenic effects, and the countereffect appears to be proportional to the degree of androgenicity of the progestin in question. However, for Lp(a), estrogens and progestins have qualitatively similar effects, both reducing Lp(a) levels. When conventional replacement doses are used, oral administration of female sex hormones results in alterations in plasma lipoproteins, whereas parenteral administration has a smaller or no significant effect, probably because of the higher intrahepatic hormone concentrations during oral treatment.

Hormonal preparations are continuously being developed to minimize adverse effects and to maximize beneficial effects on plasma lipoprotein risk factors. In this process, attention must also be focused on possible changes in nonlipid risk factors, such as those related to thromboembolic disorders. The first large randomized, double-blind cardiovascular prevention trial, the Heart and Estrogen/Progestin Replacement Study (HERS), did not show any reduction in cardiovascular events in postmenopausal women with coronary heart disease receiving CEE plus medroxyprogesterone acetate (71). Plasma LDL and

HDL cholesterol were influenced favorably, but there was an increased rate of thromboembolic events in HERS. Another great challenge is to define the roles of oral and transdermal delivery in HRT: What is lost and what is gained by using either one of the two alternative routes of administration?

REFERENCES

1. Robinson GE. Low-dose combined oral contraceptives. Br J Obstetr Gynaecol 1994; 101:1036–1041.
2. Rebar RW, Zeserson K. Characteristics of the new progestogens in combination oral contraceptives. Contraception 1991; 44:1–10.
3. Tikkanen MJ, Kuusi T, Nikkilä EA, Sipinen S. Postmenopausal hormone replacement therapy: effects of progestogens on serum lipids and lipoproteins. Maturitas 1986; 8:7–17.
4. Rijpkema AHM, van den Sanden AA, Ruijs AHC. Effects of postmenopausal oestrogen–progestogen replacement therapy on serum lipids and lipoproteins. Maturitas 1990; 12:259–285.
5. Neumann F. The physiological action of progesterone and the pharmacological effects of progestogens—a short review. Postgrad Med J 1978; 54(suppl 2):11–24.
6. Brenner PF. The pharmacology of progestogens. J Reprod Med 1982; 27:490–497.
7. Nikkilä EA. HDL in relation to the metabolism of triglyceride-rich lipoproteins. In: Miller NE, Miller GJ, eds. Clinical and Metabolic Aspects of High-Density Lipoproteins. Amsterdam: Elsevier Science, 1984; pp 217–236.
8. Tikkanen MJ, Nikkilä EA. Oral contraceptives and lipoprotein metabolism. J Reprod Med 1986; 31:898–905.
9. McPherson K. Third generation oral contraception and venous thromboembolism. Br Med J 1996; 312:68–69.
10. PEPI writing group. Effects of estrogen of estrogen/progestin regimens on heart disease risk factors in postmenopausal women. JAMA 1995; 3:199–208.
11. Tikkanen MJ. Role of plasma lipoprotein in the pathogenesis of atherosclerotic disease, with special reference to sex hormone effects. Am J Obstet Gynecol 1990; 163:296–304.
12. Sacks FM, Walsh BW. Sex hormones and lipoprotein metabolism. Curr Opin Lipidol 1994; 5:236–240.
13. Srivastava RAK, Baumann D, Schonfeld G. In vivo regulation of low-density lipoprotein receptors by estrogen differs at the post-transcriptional level in rat and mouse. Eur J Biochem 1993; 216:527–538.
14. Glueck CJ, Ford S, Steiner P, Fallat R. Triglyceride removal efficiency and lipoprotein lipases: effects of oxandrolone. Metabolism 1973; 22:807–814.
15. Olsson AG, Orö L, Rössner S. Effects of oxandrolone on plasma lipoproteins and the intravenous fat tolerance in man. Atherosclerosis 1974; 19:337–346.
16. Kekki M, Nikkilä EA. Plasma triglyceride turnover during use of oral contraceptives. Metabolism 1971; 20:878–889.

17. Kissebah AH, Harrigan P, Wynn V. Mechanism of hypertriglyceridemia associated with contraceptive steroids. Horm Metab Res 1974; 5:184–190.
18. Glueck CJ, Levy RI, Fredrickson DS. Norethindrone acetate, postheparin lipolytic activity, and plasma triglycerides in familial types I, II, IV and V hyperlipoproteinemia. Ann Intern Med 1971; 75:345–352.
19. Wolfe BM, Grace DM. Norethindrone acetate inhibition of splanchnic triglyceride secretion in conscious glucose-fed swine. J Lipid Res 1979; 20:175–182.
20. Wolfe BM, Huff MW. Effects of combined estrogen and progestin administration on plasma lipoprotein metabolism in postmenopausal women. J Clin Invest 1989; 83:40–45.
21. März W, Gross W, Gahn G, Romberg G, Taubert H-D, Kuhl H. A randomized crossover comparison of two low-dose contraceptives: effects on serum lipids and lipoproteins. Am J Obstet Gynecol 1985; 153:287–293.
22. Kauppinen-Mäkelin R, Kuusi T, Ylikorkala O, Tikkanen MJ. Contraceptives containing desogestrel or levonorgestrel have different effects on serum lipoproteins and post-heparin plasma lipase activities. Clin Endocrinol 1992; 36:203–209.
23. Speroff L, DeCherney A, the Advisory Board for the New Progestins. Evaluation of a new generation of oral contraceptives. Obstet Gynecol 1993; 81:1034–1047.
24. Farish E, Fletcher CD, Hart DM, Kitchener H, Sharpe GLM. A long-term study of the effects of norethisterone on lipoprotein metabolism in menopausal women. Clin Chim Acta 1983; 132:193–198.
25. Silfverstolpe G, Gustafson A, Samsioe G, Svanborg A. Lipid metabolic studies in oophorectomized women. Effects of three different progestogens. Acta Obstet Gynecol Scand Suppl 1979; 88:89–95.
26. Tikkanen MJ, Nikkilä EA, Kuusi T, Sipinen S. Effects of oestradiol and levonorgestrel on lipoprotein lipids and postheparin plasma lipase activities in normolipoproteinaemic women. Acta Endocrinol 1982; 99:630–635.
27. Hirvonen E, Mälkönen M, Manninen V. Effects of different progestogens on lipoproteins during postmenopausal replacement therapy. N Engl J Med 1981; 304:560–563.
28. Fåhraeus L, Larsson-Cohn U, Wallentin L. L-Norgestrel and progesterone have different influences on plasma lipoproteins. Eur J Clin Invest 1983; 13:447–453.
29. Silfverstolpe G, Gustafson A, Samsioe G, Svanborg A. Lipid and carbohydrate metabolism studies in oophorectomized women: effects produced by the addition of norethisterone acetate to two estrogen preparations. Arch Gynecol 1982; 231:279–287.
30. Gustafson A, Svanborg A. Gonadal steroid effects on plasma lipoproteins and individual phospholipids. J Clin Endocrinol Metab 1972; 35:203–207.
31. Tikkanen MJ, Nikkilä EA, Vartiainen E. Natural oestrogen as an effective treatment for type-II hyperlipoproteinaemia in post-menopausal women. Lancet 1978; 2:490–492.
32. Wallentin L, Larsson-Cohn U. Metabolic and hormonal effects of post-menopausal oestrogen replacement treatment, II. Plasma lipids. Acta Endocrinol 1977; 86:597–607.
33. Robinson RW, Lebeau RJ. Effect of conjugated equine estrogens on serum lipids and the clotting mechanism. J Atheroscler Res 1965; 5:120–124.

34. Silfverstolpe G, Gustafson A, Samsioe G, Svanborg A. Lipid metabolic studies in oophorectomized women: effects on serum lipids and lipoproteins of three synthetic progestogens. Maturitas 1982; 4:103–111.
35. Burkman RT. Lipid metabolism effects with desogestrel-containing oral contraceptives. Am J Obstet Gynecol 1993; 168:1033–1040.
36. Tikkanen MJ. The menopause and hormone replacement therapy: lipids, lipoproteins, coagulation and fibrinolytic factors. Maturitas 1996; 23:209–216.
37. Van der Mooren MJ, Mijatovic V, van Baal WM, Stehouwer CDA. Hormone replacement therapy in postmenopausal women with specific risk factors for coronary artery disease. Maturitas 1998; 30:27–36.
38. Albers JJ, Taggart HM, Applebaum-Bowden D, Haffner S, Chesnut CH, Hazzard WR. Reduction of lecithin-cholesterol acyltransferase, apolipoprotein D and the Lp(a) lipoprotein with the anabolic steroid stanozolol. Biochim Biophys Acta 1984; 795:293–296.
39. Farish E, Rolton HA, Barnes JF, Hart DM. Lipoprotein(a) concentrations in postmenopausal women taking norethisterone. Br Med J 1991; 303:694.
40. Crook D, Sidhu M, Seed M, Stevenson JC. Lipoprotein Lp(a) levels are reduced by danazol, an anabolic steroid. Atherosclerosis 1992; 92:41–47.
41. Rymer J, Crook D, Sidhu M, Chapman M, Stevenson JC. Effects of tibolone on serum concentrations of lipoprotein(a) in postmenopausal women. Acta Endocrinol (Copenh) 1993; 128:259–262.
42. Tuck CH, Holleran S, Berglund L. Hormonal regulation of lipoprotein(a) levels: effects of estrogen replacement therapy on lipoprotein(a) and acute phase reactants in postmenopausal women. Arterioscler Thromb Vasc Biol 1997; 17:1822–1829.
43. Rössner S, Larsson-Cohn U, Carlson LA, Boberg J. Effects of an oral contraceptive agent on plasma lipids, plasma lipoproteins, the intravenous fat tolerance and the postheparin lipoprotein lipase activity. Acta Med Scand 1971; 190:301–305.
44. Albers JJ, Wahl PW, Cabana WG, Hazzard WR, Hoover JJ. Quantitation of apolipoprotein A-I of human plasma high density lipoprotein. Metabolism 1976; 25:633–644.
45. Arntzenius AC, van Gent CM, van der Voort H, Stegerhoek CI, Styblo K. Reduced high-density lipoprotein in women aged 40–41 using oral contraceptives. Lancet 1978; 1:1221–1223.
46. Wallace RB, Hoover J, Barrett-Connor E, Rifkind BM, Hunninghake DB, Mackenthun A, Heiss G. Altered plasma lipid and lipoprotein levels associated with oral contraceptive and oestrogen use. Lancet 1979; 2:112–115.
47. Bradley DD, Wingerd J, Petitti DB, Krauss RM, Ramcharan S. Serum high-density lipoprotein cholesterol in women using oral contraceptives, estrogens and progestins. N Engl J Med 1978; 299:17–20.
48. Bergink EV, Borglin NE, Klottrup P, Liukko P. Effects of desogestrel and levonorgestrel in low-dose oestrogen oral contraceptives on serum lipoproteins. Contraception 1982; 25:477–485.
49. Samsioe G. Comparative effects of the oral contraceptive combinations 0.150 mg desogestrel + 0.030 mg ethinyl-estradiol and 0.150 mg levonorgestrel + 0.30 mg ethinyl-estradiol on lipid and lipoprotein metabolism in healthy female volunteers. Contraception 1982; 25:487–503.

50. Kuusi T, Saarinen P, Nikkilä EA. Evidence for the role of hepatic endothelial lipase in the metabolism of plasma high density lipoprotein$_2$ in man. Atherosclerosis 1980; 36:589–593.
51. Applebaum DM, Goldberg AP, Pykälistö OJ, Brunzell JD, Hazzard WR. Effect of estrogen on post-heparin lipolytic activity: selective decline in hepatic triglyceride lipase. J Clin Invest 1977; 50:601–608.
52. Tikkanen MJ, Kuusi T, Nikkilä EA, Stenman UH. Variation of postheparin plasma hepatic lipase by menstrual cycle. Metabolism 1986; 35:99–104.
53. Ehnholm C, Huttunen JK, Kinnunen PJ, Miettinen TA, Nikkilä EA. Effect of oxandrolone treatment on the activity of lipoprotein lipase, hepatic lipase, and phospholipase A_1 of human postheparin plasma. N Engl J Med 1975; 292:1314–1317.
54. Kuusi T, Nikkilä EA, Tikkanen MJ, Sipinen S. Effects of two progestins with different androgenic properties on hepatic endothelial lipase and high density lipoprotein$_2$. Atherosclerosis 1984; 54:251–262.
55. Tikkanen MJ, Nikkilä EA, Kuusi T, Sipinen SU. High density lipoprotein-2 and hepatic lipase: reciprocal changes produced by estrogen and norgestrel. J Clin Endocrinol Metab 1982; 54:1113–1117.
56. Tikkanen MJ, Kuusi T, Nikkilä EA, Sipinen S. Postheparin plasma hepatic lipase activity as predictor of HDL response to progestin therapy: studies with cyproterone acetate. Maturitas 1987; 9:81–86.
57. Tikkanen MJ, Nikkilä EA, Vartiainen E, Nikkilä EA. Treatment of postmenopausal hypercholesterolaemia with estradiol. Acta Obstet Gynecol Scand Suppl 1979; 88:83–88.
58. Haffner SM, Kushwaha RS, Foster DM, Applebaum-Bowden D, Hazzard WR. Studies of the metabolic mechanism of reduced high density lipoproteins during anabolic steroid therapy. Metabolism 1983; 32:413–420.
59. Tikkanen MJ, Nikkilä EA, Kuusi T, Sipinen S. Effects of oestradiol and levonorgestrel on lipoprotein lipids and postheparin plasma lipase activities in normolipoproteinaemic women. Acta Endocrinol 1982; 99:630–635.
60. Tikkanen MJ, Nikkilä EA, Kuusi T, Sipinen S. Reduction of plasma high-density lipoprotein$_2$ cholesterol and increase of postheparin plasma hepatic lipase activity during progestin treatment. Clin Chim Acta 1981; 115:63–71.
61. Tikkanen MJ, Nikkilä EA, Kuusi T, Sipinen S. Different effects of two progestins on plasma high density lipoprotein (HDL_2) and postheparin plasma hepatic lipase activity. Atherosclerosis 1981; 40:365–369.
62. Tikkanen MJ, Nikkilä EA. Regulation of hepatic lipase and serum lipoproteins by sex steroids. Am Heart J 1987; 113:562–567.
63. Solyom A. Effect of androgens on serum lipids and lipoproteins. Lipids 1972; 7:100–105.
64. Schaefer EJ, Foster DM, Zech LA, Lindgren FT, Brewer HB Jr, Levy RI. The effects of estrogen administration on plasma lipoprotein metabolism in premenopausal females. J Clin Endocrinol Metab 1983; 57:262–267.
65. Walsh BW, Schiff I, Rosner B, Greenberg L, Ravnikar V, Sacks FM. Effects of postmenopausal estrogen replacement on the concentrations and metabolism of plasma lipoproteins. N Engl J Med 1991; 325:1196–1204.

66. Wynn V, Niththyananthan R, Chem C. The effect of progestins in combined oral contraceptives on serum lipids with special reference to high-density lipoproteins. Am J Obstet Gynecol 1982; 142:766–771.
67. Godsland IF, Crook D, Simpson R, Proudler T, Felton C, Lees B, Anyaoku V, Devenport M, Wynn V. The effects of different formulations of oral contraceptive agents on lipid and carbohydrate metabolism. N Engl J Med 1990; 323:1375–1381.
68. Walsh BW, Li H, Sacks FM. Effects of postmenopausal hormone replacement with oral and transdermal estrogen on high density lipoprotein metabolism. J Lipid Res 1994; 35:2083–2093.
69. Lindgren R, Berg G, Hammar M, Larsson-Cohn U, Olsson AG. Plasma lipid and lipoprotein effects of transdermal administration of estradiol and estradiol/norethisterone acetate. Eur J Obstetr Gynecol Reprod Biol 1992; 47:213–221.
70. Crook D, Cust MP, Gangar KF, Worthington M, Hillard TC, Stenvenson JC, Whitehead MI, Wynn V. Comparison of transdermal and oral estrogen–progestin replacement therapy: effects on serum lipids and lipoproteins. Am J Obstet Gynecol 1992; 166:950–955.
71. Hulley S, Grady D, Bush T, Furberg C, Herrington D, Riggs B, Vittinghoff E for the Heart and Estrogen/Progestin Replacement Study (HERS) Research Group. JAMA 1998; 280:605–613.

21

Hormonal Replacement Therapy and Glucose Metabolism in Postmenopausal Women

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I. INTRODUCTION

The decrease in risk of having cardiovascular disease (CVD) observed in postmenopausal women receiving hormonal replacement therapy (HRT) varies between 50 and 70%, with the most pronounced effect in women predisposed to CVD (1–3). Well-defined risk factors for CVD are listed in Table 1. Among these are overt diabetes and even an abnormal glucose tolerance. Aging is associated with an increased incidence of hypertension, non–insulin-dependent diabetes (NIDDM), and CVD. Part of the reported preventive effect of HRT for the occurrence of CVD after the menopause, therefore, may be explained by an improvement of glucose tolerance associated with estrogen use. This chapter discuss the influence hyperinsulinemia has on the CVD process and the effect from sex steroids on the age-dependant decrease in glucose tolerance in normal women. Also, it addresses the glycometabolic control in women with non–insulin-dependent diabetes mellitus (NIDDM).

II. THE METABOLIC SYNDROME AND CVD

Epidemiological evidence for the relation between deterioration of glucose tolerance and CVD has been demonstrated in several investigations (4). Although

Table 1 Risk Factors for CVD

Dyslipedimia	Obesity
Hypertension	Smoking
Diabetes	Stress and personality

the epidemiological data cannot prove that insulin is atherogenic, the population studies imply insulin resistance and compensatory hyperinsulinemia as an independent risk factor for the occurrence of coronary heart disease (CHD). The combination predisposes to the development of a cluster of abnormalities, including some degree of glucose intolerance, an increase in plasma triglyceride (TG) and a decrease in high-density lipoprotein cholesterol (HDL-C) concentrations, high blood pressure, hyperuricemia, smaller denser low-density lipoprotein (LDL) particles, and higher circulating levels of plaminogen activator inhibitor 1 (PAI-1). The cluster of changes associated with insulin resistance has been defined as the *metabolic syndrome*, and all of the manifestations of the syndrome increase risk of CVD (5) (Table 2). Of particular interest is the effect of insulin on lipoprotein metabolism. Insulin stimulates the synthesis of very low-density lipoprotein (VLDL) in the liver, thus explaining why hypertriglyceridemia is associated with increased insulin resistance. Also restrained removal of TG-rich particles owing to decreased stimulatory action of insulin on lipoprotein lipase activity may contribute, and increased insulin resistance provides excess glucose and free fatty acids for the VLDL formation because of decreased glucose disposal and decreased suppression of lipolysis. The decreased HDL levels often observed concomitantly with increased VLDL levels during insulin resistance may be explained by increased hepatic lipase activity. Obesity and fat distribution are also associated with insulin resistance, the closest relation is with upper-body (android) obesity. Hyperinsulinemia may also directly affect the growth of vascular cells and promote formation of "fatty streaks." An established atherosclerotic plaque is characterized by excessive amounts of lipid and collagen, foam macrophages, and proliferated muscle cells. All of these constituents are affected by the plasma insulin concentration. Insulin and insulin-stimulated growth factors, such as insulin-like growth factor (IGF), facilitate proliferation of smooth muscle cells and migration of cells from media

Table 2 The Metabolic Syndrome

Resistance to insulin-mediated glucose uptake
Hypertension
Dyslipidemia
Decreased fibrinolysis

to intima. Moreover, hyperinsulinemia inhibits the reabsorption of the plaques once formed. Finally, atherosclerosis and thrombosis go hand in hand. Obliteration of the vessel lumina in an atherosclerotic artery is not established without activation of the hemostatic system. Carbohydrate disorders are accompanied by platelet hyperactivity, with an increase in von Willebrand factor; hypercoagulability, with increased fibrinogen and factor VII levels; and hypofibrinolysis, with increased plasma concentrations of plasminogen activator inhibitor (PAI-1). A particularly strong association exists between plasma insulin levels and PAI-1.

A reduction in the coronary vasodilatory reserve is common in patients with cholesterol narrowing of the epicardial coronary arteries. Whether hyperinsulinemia causes a reduction in nitric oxide (NO) synthesis, or a more generalized reduction in vascular reserve through the effect on the sympathetic nervous system, is not known. It is interesting, however, that insulin resistance often coexists with a condition referred to as the cardiological syndrome-X, consisting of microvascular angina in patients presenting with normal coronary arteriograms. The prevalence of this syndrome, with combined insulin resistance and endothelial dysfunction, is high in premenopausal women (6)

Several investigative techniques have been devised for in vivo quantitation of resistance to insulin-stimulated glucose uptake. All have limitations, and none is suitable for routine clinical use. The euglycemic clamp technique, which involves simultaneous infusions of insulin and glucose, has been used extensively and is considered the standard. The method is, however, complex, nonphysiological, and resource-demanding. A computer (minimal change) modeling of an intravenous glucose tolerance test (IVGTT) is easier to apply in larger series, although the differential equations necessitating simultaneous changing glucose and insulin concentrations are complex.

III. SEX STEROIDS AND CARBOHYDRATE METABOLISM

Several conditions suggest interrelation between insulin sensitivity and effects of sex steroids. Examples include the increased insulin resistance during the high estrogen and progesterone state of pregnancy, during the luteal phase of the menstrual cycle, and in women with high androgen production (polycystic ovarian syndrome, stromal hyperthecosis). In the context of sex steroids, during the fertile period, the insulin sensitivity to glucose is greater in women than in men. The overall association between estrogen levels expressed as sex hormone-binding globulin (SHBG) and glucose metabolism are as follows: SHBG is suggested to be a strong predictor of subsequent diabetes (NIDDM) in pre- and postmenopausal women, whereas low concentrations of SHBG are associated with insulin resistance and android obesity.

Postmenopausal women exhibit many features of the metabolic syndrome, with a rapid decline in insulin sensitivity following the menopause, which relates to menopausal, rather than chronological age (7). The effects of estrogens suggest that estrogen replacement therapy would have the potential to reverse the cluster of metabolic changes included in the metabolic syndrome and of importance for development of CVD. Thus, in the San Antonio Heart Study, SHBG was negatively correlated with fasting insulin and insulin and glucose responses to oral glucose (8). Some discrepancy, however, may exist between the physiological and pharmacological action of sex steroids. Sex steroids with estrogen and progestogen activity represent a wide spectrum of hormones with different molecular structure and, therefore, also differences in their pharmacokinetic properties and pharmacodynamic effects. The estrogens may be natural human or equine from genuine sources, or artificial, with or without steroid structure.

The progestogens represent an even wider selection of hormonal compounds, with progesterone-like activity, and still newer types will be introduced in the coming years. Moreover, patches, jells, implants, and vaginal pessaries, with no first-pass hepatic effect are available as exogenous sex steroids. One must remember that such differences in pharmacokinetic properties will also influence the metabolic effect of hormonal treatment.

In animal studies 17β-estradiol treatment enhances insulin secretion in islet cells, thereby augmenting the insulin response to glucose and, additionally, reducing insulin resistance. Also, natural progesterone may result in an increased insulin response to glucose, but probably because of decreased insulin sensitivity in target tissue, resulting in an increased beta cell responsiveness. Much of our knowledge about the effect of the exogenous sex steroids on glucose metabolism in women is derived from the oral contraceptive (OC) data. When administering OCs, a decrease in glucose tolerance is often observed, concomitant with a hyperinsulinemic response to the glucose load. The effect on glucose metabolism is clearly dose-dependent, and the newer types of combined OCs, therefore, have less influence on the glucose tolerance; most often there is no deterioration of the glucose curve, whereas hyperinsulinemia and decreased insulin sensitivity can still be observed (9). Following HRT administration of preparations with acetylated estrogens (i.e., ethinyl estradiol [EE] and mestranol [ME] combined with norethindrone [NET] or megestrol acetate [MGA]), there have been reports of abnormal glucose tolerance, similar to the effect of combined OCs. Luotola and co-workers have performed OGTT during sequential intake of estradiol 17β combined with NET. No influence was found on glucose or insulin values (10).

Barret-Connor and Laakso have published results from a comparative study on postchallenge glucose and insulin levels in women using no HRT, unopposed conjugated estrogen (CE), and CE plus medroxyprogesterone acetate (MPA). The glucose and insulin values were age and body mass index adjusted before comparison was made. Fasting glucose levels were significantly lower in the

estrogen-treated group compared with the untreated group. No difference was seen between the CE plus MPA group and the group with unopposed CE treatment, but fasting insulin values were lower in the estrogen-treated group than in the untreated group. This is interesting because fasting insulin is considered to be a fairly good marker for insulin sensitivity (11). In contrast Cagnacci et al. (12) found no significant effect on fasting insulin levels or beta cell sensitivity to oral glucose from oral intake of CE. This group compared oral intake of CE with transdermal administration of 17β-estradiol. Following the transdermal administration, fasting insulin levels decreased and beta cell response to glucose increased, indicating an increase in insulin sensitivity. The study is interesting because it evaluates the significance of the estrogen effect on the liver for glucose homeostasis, and in contrast to oral CE, the transdermal estrogen administration increased hepatic insulin clearance.

Godsland et al. (13) have compared insulin sensitivity (IVGTT, minimal model) during sequential intake of oral CE plus LNG and transdermal administration of estradiol 17β plus oral NET. Oral intake of CE plus LNG caused a decrease in glucose tolerance and an increased overall plasma insulin response. Insulin resistance was greater during the combined phase than during the estrogen-only phase. The transdermal regimen had relatively few effects on insulin metabolism, although the first-phase beta cell insulin secretion was enhanced. Both regimens increased hepatic insulin uptake. This study, therefore, indicates that the addition of a progestogen with high androgenicity, such as levonorgestrel, to estrogen therapy may markedly influence the effect on glucose metabolism, in parallel with the observations made during administration of OCs. Lindheim et al. demonstrated a bimodal effect of oral equine estrogens on insulin sensitivity with an improvement occurring with the lower dose of 0.625 mg, but with a deterioration with the dose of 1.25 mg. The authors suggest that this effect may be related to a first-pass hepatic–portal effect in that transdermal E_2 (0.1 mg), which may be equated more closely with the larger dose of oral estrogen (1.25 mg), improved insulin sensitivity. Progestin, however, appeared to attenuate the beneficial effects of transdermal estrogen and may alter the clearance of insulin (14–15).

We have examined the influence on glucose metabolism and insulin sensitivity from oral administration of 2 mg estradiol in combination with an intrauterine delivery system of levonorgestrel (LNS, Mirena) and compared the results with data obtained with a continuous, combined treatment with 2 mg estradiol plus 1 mg NETA (Kliogest). After 6 months, no changes were observed in the glucose tolerance tests nor in insulin sensitivity using the minimal model technique (Table 3) (14). Preliminary 12-month data, however, indicate a significant difference in estrogen and estrogen–progestin effect on insulin levels (data not shown).

Insulin resistance and its associated abnormalities are of utmost importance in the pathogenesis of NIDDM. However, estrogen replacement therapy is pre-

Table 3 HRT and Insulin Sensitivity (6 mo)

HRT	AUC glucose	AUC insulin	Insulin sensitivity
E_2 + LNS ($n = 20$) (local progestin)	↔	↔	↔
E_2 + NETA ($n = 21$) (cont. comb.)	↔	↔	↔

↔, no change.
Source: Ref. 14.

scribed only infrequently for patients with NIDDM. In a randomized controlled trial, Brussaard et al. (16) assessed the effect of oral 17β-estradiol during 6 weeks in 40 postmenopausal women with NIDDM. Glycated hemoglobin (HbA1c), insulin sensitivity, suppressibility of hepatic glucose production, lipoprotein profile, and parameters of fibrinolysis were determined. The estrogen-treated group demonstrated a significant improvement in the major glucometabolic variables and in hemostatic risk markers (16). Similarly, Andersson et al. (17) demonstrated that estrogen replacement therapy decreases hyperandrogenicity and improves glucose homeostasis and plasma lipids in postmenopausal women with NIDDM.

IV. CONCLUDING REMARKS

The association between deterioration of glucose metabolism and CVD has been observed in numerous studies. During more recent years, research has demonstrated that insulin resistance and hyperinsulinemia are common features in several conditions known to promote the atherosclerotic process (i.e., hypertension, android obesity, and dyslipidemia). In the vessel wall, insulin stimulates proliferation and migration of smooth muscle cells, binding of LDL to receptors in monocytes, and the activity of major enzymes in lipogenesis. Altogether, these changes result in formation of lipid-filled lesions preceding the atherosclerotic plaque. In any individual, insulin resistance is genetically determined and declines with age, but exogenous and endogenous sex steroids have a modulating effect. In postmenopausal women, exogenous estrogens decreases insulin resistance, although the effect may be blunted by increased synthesis of growth factors and decreased hepatic insulin clearance following the first-pass effect of orally administered hormones. Moreover, administration of progestogens, especially those with high androgenicity, together with estrogens may counteract the estrogen effects on insulin resistance. The estrogen modulation of the direct insulin action on the endothelial cell function also needs further elucidation. Specific studies on the effect of HRT on insulin resistance, therefore, are needed,

with proper adjustments for confounding factors, such as route of administration, configuration of the steroids, blood pressure, lipoprotein levels, exercise, and diet, before a proper conclusion can be made on the interrelation between HRT, insulin, and CHD.

REFERENCES

1. Welborn TA, Wearne K. Coronary heart disease incidence and cardiovascular mortality in Busselton with reference to glucose and insulin concentrations. Diabetes Care 1979; 2:154–160.
2. Pyorälâ K. Relationship of glucose tolerance and plasma insulin to the incidence of coronary heart disease: results from two population studies in Finland. Diabetes Care 1979; 2:131–141.
3. Fontbonne A, Charles MA, Thibault N, Richard JL, Claude JR, Warnet JM, Rosselin GE, Eschwege E. Hyperinsulinemia as a predictor of coronary heart disease mortality in a healthy population: the Paris Prospective Study, 15-year follow-up. Diabetologia 1991; 34:356–361.
4. Stampfer MJ, Colditz GA, Willett WC. Menopause and heart disease. A review. Ann NY Acad Sci 1990; 592:193–203.
5. Reaven GM. Pathophysiology of insulin resistance in human disease. Physiol Rev 1995; 75:473–486.
6. Dean JD, Jones CJH, Hutchinson J, Peters JR, Henderson AH. Hyperinsulinemia and microvasular angina. Lancet 1991; 337:456–457.
7. Walton C, Godsland I, Proudler A, Wynn W, Stevenson JC. The effects of the menopause on insulin sensitivity, secretion and elimination in nonobsese, healthy women. Eur J Clin Invest 1993; 23:466–473.
8. Goodman-Gruen D, Barrett-Connor E. Sex hormone-binding globulin and glucose tolerance in postmenopausal women. The Rancho Bernardo Study. Diabetes Care 1997; 20:645–649.
9. Skouby SO. Oral contraceptives: effect on glucose and lipid metabolism in previous gestational diabetic women and in insulin-dependent diabetic women—a clinical and biochemical assessment. Dan Med Bull 1988; 35:157–167.
10. Luotola H, Pyorälä T, Loikkannen M. Effects of natural oestrogen/progestogen substitution therapy on carbohydrate metabolism in postmenopausal women. Maturitas 1986; 8:245–253.
11. Barret-Connor E, Laakso M. Ischemic heart disease risk in postmenopausal women. Effects of estrogen use on glucose and insulin levels. Arteriosclerosis 1990; 10:531–534.
12. Cagnacci A, Soldani R, Carriero PL, Paoletti AA, Fioretti P, Melis GB. Effects of low doses of transdermal 17-beta estradiol on carbohydrate metabolism in postmenopausal women. J Clin Endocrinol Metab 1992; 74:1396–1400.
13. Godsland IF, Gangar K, Walton C, Cust MP, Whitehead MI, Wynn V, Stevenson JC. Insulin resistance, secretion, and elimination in postmenopausal women receiving oral or transdermal hormone replacement therapy. Metabolism 1993; 42:846–853.

14. Lindheim SR, Buchanan TA, Duffy DM, Vijod MA, Kojima T, Stanczyk FZ, Lobo RA. Comparison of estimates of insulin sensitivity in pre- and postmenopausal women using the insulin tolerance test and the frequently sampled intravenous glucose tolerance test. J Soc Gynecol Invest 1994; 1:150–154.
15. Lindheim SR, Duffy DM, Kojima T, Vijod MA, Stanczyk FZ, Lobo RA. The route of administration influences the effect of estrogen on insulin sensitivity in postmenopausal women. Fertil Steril 1994; 62:1176–1180.
16. Brussaard HE, Gevers Leuven JA, Frolich M, Kluft C, Krans HM. Short-term oestrogen replacement therapy improves insulin resistance, lipids and fibrinolysis in postmenopausal women with NIDDM. Diabetologia 1997; 407:843–849.
17. Andersson B, Mattsson LA, Hahn L, Marin P, Lapidus L, Holm G, Bengtsson BA, Bjorntorp P. Estrogen replacement therapy decreases hyperandrogenicity and improves glucose homeostasis and plasma lipids in postmenopausal women with noninsulin-dependent diabetes mellitus. J Clin Endocrinol Metab 1997; 82:638–643.

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Antiprogestins in Clinical Practice

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I. INTRODUCTION

Progesterone plays a critical role in mammalian reproduction. After the discovery of the progesterone receptor (PR) in 1970 (1), researchers realized that a progesterone receptor antagonist would have a major influence on female reproductive health. The search for such an antiprogestin extended over more than a decade. In 1981, Philibert et al. (2) reported on a newly synthesized glucocorticoid receptor antagonist, known as RU 38486 which also displayed marked antiprogestin activity. RU 38486 was subsequently abbreviated to RU 486 and is now currently known by the generic name mifepristone. Mifepristone has a relative binding affinity five times that of progesterone and three times that of dexamethasone at their respective receptors. It has very slight affinity for the androgen receptor, but does not bind to either estrogen or mineralocorticoid receptors (3).

Since the initial publication, over 400 additional antiprogestins have been synthesized, and several are undergoing preliminary clinical investigation. Full details on chemistry, pharmacology, and mechanism of action of antiprogestins have been reviewed elsewhere (4–9). This chapter highlights the clinical applications for treatment with antiprogestins. Because almost all studies in humans have used mifepristone, this will be the focus of this review.

II. CLINICAL APPLICATIONS

A. Pregnancy Termination

Progesterone is essential for the initiation and maintenance of pregnancy. It also promotes uterine quiescence by inhibiting myometrial contractions. Progesterone-induced relaxation of muscle cells is associated with hyperpolarization of the cell membrane, prevention of calcium ion influx, and suppression of cell coupling by gap junctions. When antiprogestins are administered, myometrial cell excitability increases, in association with establishment of gap junctions between cells and influx of calcium ions. This results in coordinated uterine contractions (10). Herrmann et al. (11) were the first to demonstrate that mifepristone had the ability to successfully terminate pregnancy in humans. Subsequent studies have shown that when 200–600 mg of mifepristone is administered to women with a gestational duration of 49 days or less, the rate for complete termination of pregnancy usually ranges from 64 to 85% (4,12–14). Clearly, this is inadequate for general clinical use.

The next major advance was made by Bygdeman and Swahn (15), who showed that the addition of a prostaglandin, administered 36–48 h after mifepristone significantly improves the success rate. Prostaglandins enhance uterine contractions and antiprogestins increase endometrial prostaglandin concentrations by inhibiting prostaglandin dehydrogenase, the progesterone-dependent enzyme that metabolizes the active prostaglandins, PGE_2 and $PGF_{2\alpha}$. As a consequence, there is accumulation of these active prostaglandins (16,17). Antiprogestins also increase the myometrial response to exogenous prostaglandins (15).

The first prostaglandin used in combination with mifepristone was the PGE_2 analogue sulprostone, which was administered by injection. However because of the occurrence of a death from myocardial infarction in a 38-year-old smoker and of cardiovascular side effects in other subjects, this agent was withdrawn (13). The prostaglandins currently used with mifepristone are the PGE_1 analogues, misoprostol (given by the oral or vaginal route) or the vaginal pessary, gemeprost. To date, no untoward cardiovascular events have been reported when these prostaglandins are used together with antiprogestins. The combination of either prostaglandin administered 36–48 h after mifepristone provides an effective method of medical termination of pregnancy (15,18–23). In women whose duration of gestation is less than 49 days, the success rate reaches 95% (18–23). With oral misoprostol, the rate of successful pregnancy termination decreases after 49 days (20,24). Vaginal misoprostol administration has been reported to give better results than the oral administration (23). Gemeprost is more potent than misoprostol (21), and if it is used, the window of application can be extended to a duration of gestation of up to 63 days, with excellent efficacy (21,22). The reasons for failure include incomplete abortion or ongoing preg-

nancy. Surgical intervention may also rarely be performed for excess or severe bleeding, other medical reasons, or because of a patient request.

With this method, expulsion usually occurs in 50–60% of women during the 4-h observation period following misoprostol, and the vast majority of the other women expel gestational tissue in the first 24 h after the prostaglandin is administered (18,19,24). All women who abort have vaginal bleeding. Other adverse events observed include abdominal pain, nausea, vomiting, and diarrhea. Most of these symptoms occur during the 4-h observation period after prostaglandin administration.

We have recently reported the results of the first large multicenter trial in the United States on the use of mifepristone and oral misoprostol to terminate pregnancy in women with a duration of gestation of up to 63 days (24). The success rates observed in this U.S. study ranged from 92% at 49 days to 77% at 63 days, somewhat lower than those previously described (18–23). This may be related to lack of experience with medical abortion in the United States, protocol design, and that oral misoprostol (rather than vaginal misoprostol or gemeprost) was used. The combination of mifepristone, 600 mg orally, and misoprostol, 400 μg orally, has been approved for use in pregnancy termination in France for up to 49 days gestation and in China for up to 63 days. In Sweden and the United Kingdom, mifepristone 600 mg orally, followed by gemeprost 1 mg per vaginum, is registered for up to 63 days of amenorrhea.

B. Cervical Softening

Antiprogestins have numerous other obstetrical and gynecological applications. In addition to their ability to enhance myometrial contractility, antiprogestins also dilate and soften the uterine cervix. The available data indicates that antiprogestins do not act on the cervix by stimulating endogenous prostaglandin production (25); rather, their action originates from inflammatory cells and chemotactic agents, such as cytokines (e.g., IL-8, IL-1β; 26). Indeed progesterone inhibits and mifepristone stimulates Interleukin-8 (IL-8) release in human choriodecidual cells in vitro (27). Because of its action on the uterine cervix, antiprogestins are useful in the preoperative preparation of women for first-trimester vacuum aspiration. Mifepristone is usually administered 48 h before surgical abortion, is as effective as prostaglandins, and has significantly fewer side effects (28). In second-trimester abortions, pretreatment with mifepristone reduced the interval between prostaglandin administration and expulsion. Furthermore, the dose of prostaglandin required was reduced, and the women experienced considerably less pain (29).

Mifepristone is very effective in inducing labor following intrauterine fetal death and it is used routinely in France for this purpose (31). It has also been

used to induce labor at the end of the third trimester (32). In this latter study, women at term received either 200 mg mifepristone or placebo daily for 2 days. In the mifepristone group, 50% of the women underwent spontaneous labor, compared with 25% in the placebo group. Although the mean time from onset of treatment and the start of labor was shorter in the mifepristone group, the number of women ultimately requiring cesarean section was similar in the two groups (32). Moreover, because this agent crosses the fetal placental barrier (33), further studies are required to ensure that no untoward effects are observed in the neonate.

C. Antiprogestins as Possible Contraceptives

In addition to its critical role in pregnancy, progesterone facilitates the luteinizing hormone (LH) surge, transforms the endometrium to a secretory state, and maintains endometrial integrity (7). Thus, an antiprogestin may have contraceptive potential by (a) inhibiting the LH surge and ovulation, (b) preventing secretory transformation of the endometrium and delaying the emergence of the implantation window, and (c) shedding of the endometrium resulting in menstrual bleeding and prevention of implantation.

Mid- or late follicular phase mifepristone administration blocks follicular development and delays the LH surge and ovulation by 12 days (7,34). To inhibit ovulation, mifepristone must be given either continuously or intermittently. Daily mifepristone doses of 2–10 mg for 1 month blocked ovulation, although this did not consistently occur with intermittent-dose schedules administered at weekly intervals (7,35–38). In all anovulatory cycles, estradiol levels were in the midfollicular phase range and associated with suppressed progesterone levels, raising the possibility of unopposed estrogen action on the endometrium. For this reason and because ovulation inhibition is not consistently observed, intermittent administration has been disbanded as a potential contraceptive method. Further studies with continuous low doses need to be conducted to determine if this could be developed as a potential contraceptive.

Because endometrial maturation and development is critically dependent on progesterone, it is possible that this may be altered by antiprogestins. In women, a single 200-mg dose of mifepristone on the second day after the LH surge resulted in retardation of endometrial development, but did not alter the length of the cycle or serum follicle-stimulating hormone (FSH) or progesterone levels (39). When the strategy was used as the only contraceptive method in 21 unprotected women for a total of 169 treatment cycles for up to 12 months, only one clinical pregnancy resulted (40). Similar results have been observed in monkeys (41,42).

These contraceptive effects are explained on the basis of a delay in endometrial maturation, with a shift of the implantation window (43). Very low

doses of mifepristone (0.5 mg daily or 2.5 mg once a week) can postpone endometrial maturation without altering bleeding patterns, ovulation, or hormonal profiles (7,44,45). These studies support the concept of endometrial contraception based on a delay in endometrial maturation, rendering it nonreceptive to implantation of a blastocyst. Further studies need be conducted to identify precise endometrial markers critical for development of the implantation window and to determine which are altered by antiprogestins.

With the fall in estradiol and progesterone at the end of the luteal phase, endometrial bleeding occurs. Indeed, one of the major effects of progesterone is to maintain endometrial integrity. Unlike the marked sensitivity of the endometrial morphology to antiprogestins, higher doses (50 mg or more) are required to produce endometrial shedding and bleeding (7,47), which occurs within 3 days of mifepristone administration.

When used occasionally after missed menses, mifepristone, together with a prostaglandin prevented pregnancy in women with menstrual delay of up to 11 days (48). Studies have also been conducted using mifepristone as a ''menses regulator'' (i.e., mifepristone was administered every month at the end of the cycle independently of whether or not the woman was pregnant). Unlike the efficacy of a single dose with menses delay, with repeated monthly use, the results are not so promising. In the largest studies yet published, the failure rate ranged from 17 to 19% (7,12,13). This is similar to the failure rate of mifepristone when used to terminate early pregnancy without a prostaglandin (11–14). This regimen is clearly not clinically acceptable, and further studies using other approaches need be undertaken to improve its efficacy.

D. Postcoital Administration

Mifepristone has also been used as an emergency contraceptive. Two randomized trials, involving a total of 597 women, compared 600 mg of mifepristone with the estrogen–progestin (Yuzpe) regimen (49,50). Mifepristone, given within 72 h of unprotected intercourse, was 100% effective in preventing pregnancy, whereas the estrogen–progestin regimen was associated with nine failures. Without treatment, it was predicted that 34 pregnancies would have occurred in the mifepristone group. Side effects, such as nausea, vomiting, headache, and breast tenderness, were less frequent among women given mifepristone; however, in one of the studies, 34% of women using mifepristone had a delay of more than 3 days in the onset of the next menstrual period (49). This occurred if mifepristone was given during the follicular phase of the cycle, which delays the LH surge and inhibits ovulation. This is clearly an obvious drawback of mifepristone, because the onset of menses reassures the woman who has used emergency contraception that she is not pregnant. Further studies are in progress to assess the response to lower doses of mifepristone.

E. Other Gynecological Indications

Under most circumstances, antiprogestins behave as classic progestin antagonists preventing the transformation of the endometrium to a secretory pattern. However, on occasion, antiprogestins inhibit endometrial proliferation and secretory activity (7,51). In view of these properties, antiprogestins have a role in the treatment of endometriosis, an estrogen-dependent condition (52). In clinical studies with daily mifepristone doses of 50 mg for 6 months, there was an improvement in pelvic pain and a decrease in the extent of disease as determined by laparoscopy (52). Antiprogestins have also clinical application in the treatment of uterine leiomyomas (52). Although this might also represent an antiproliferative effect of mifepristone, there is both clinical and in vitro evidence that progestins promote leiomyoma growth (53,54). Thus, under these conditions, mifepristone may be acting as a classic antiprogestin. After 3 months of daily treatment with mifepristone in doses of 25 and 50 mg, there were significant decreases in leiomyoma volume (52). Unlike treatment with gonadotropin-releasing hormone (GnRH) agonists, there was no decrease in bone mineral density in the treatment of patients with endometriosis and uterine fibroids (52).

F. Tumors Containing Progesterone Receptors

Antiprogestins have also been proposed in the treatment of tumors that contain steroid receptors. Many meningiomas contain progesterone receptors. Antiprogestins inhibit growth of meningioma cells in culture (55) and reduce the size of human meningioma implanted into nude mice (56). In one clinical trial, 200 mg was given daily for up to 62 months to a total of 28 patients with unresectable meningiomas. Eight subjects demonstrated objective responses, as shown by reduced tumor size on computed tomography (CT) or magnetic resonance imaging (MRI) scans and improvement in visual field examination (57). A randomized double-blind placebo-controlled phase 3 trial is currently underway to confirm the activity of mifepristone in unresectable meningioma (57). Three small clinical trials have been reported in advanced breast carcinoma. Two were conducted in patients who had failed other therapy (58,59). In the third trial, the subjects had received no previous medical therapy (60). The results in all three studies have been disappointing. Studies in animals have suggested that antiprogestins could be used in other tumors, including gliomas and ovarian, prostate, and endometrial cancer (13,61).

G. Antiglucocorticoid Activity

Mifepristone also binds to the glucocorticoid receptor and displays potent antiglucocorticoid properties. Higher doses of mifepristone are required to produce an

antiglucocorticoid effect than to produce an antiprogestin effect. This may be related to the high concentrations of cortisol in blood, to the fact that many glucocorticoid effects are permissive, or to feedback regulation of the hypothalamic-pituitary-adrenal axis (13). High-dose continuous mifepristone administration (5–22 mg/kg per day) has been used to treat Cushing's syndrome caused by ectopic corticotropin (adrenocorticotropic hormone, ACTH) secretion and adrenal carcinoma (62,63). Mifepristone normalized the cushingoid phenotype, ameliorated depression, decreased hypertension, eliminated abnormal carbohydrate metabolism, and corrected glucocorticoid-induced gonadal and thyroid hormone suppression (62,63). However, this drug cannot be used in Cushing's disease if the hypothalamic–pituitary–adrenal axis is intact, but is regulated at a higher setpoint. Under these circumstances the mifepristone-induced increase in ACTH and cortisol secretion may overcome the glucocorticoid receptor blockade (13). Mifepristone, however, could be used to prepare a patient for surgery. Moreover, it has few side effects, compared with those observed with other agents used to treat these patients (12,13).

There are other indications for these agents besides Cushing's syndrome (12,13,64). In general, the applications of mifepristone as an antiglucocorticoid may be thought of as local or systemic. Studies in rabbits provided a rationale for the local application of eye drops containing mifepristone to lower intraocular pressure in humans with glaucoma (65). Systemic administration of an antiglucocorticoid could be used for the antagonism of large doses of exogenous glucocorticoid, as well as for inhibition of the physiological effects of endogenous cortisol (13). For systemic administration to be effective, the antagonist would need to have highly selective actions on specific organs. For example, mifepristone blocked the loss of muscle and body weight induced by pharmacological doses of dexamethasone (66). In this instance, if mifepristone is to have a potential use in the treatment of steroid-induced myopathy, it must have a selective antagonistic effect on muscle, without attenuating the beneficial effects for which the glucocorticoid was administered in the first place. Little is yet known on whether mifepristone or any other antiglucocorticoid has selective antagonistic activity (13).

Systemic administration of mifepristone for neutralization of endogenous cortisol could be of benefit in a wide variety of disorders (64,67). For example, muscle atrophy associated with conditions as diverse as androgen withdrawal, denervation, and muscular dystrophy is associated with increased glucocorticoid receptors in muscle, thus suggesting that endogenous cortisol is a regulator of muscle mass. This postulate was confirmed with the demonstration that mifepristone could attenuate muscle loss following orchidectomy (68). Several studies showed that diseases caused by herpes and Maloney viruses in animals were prevented or inhibited by reducing glucocorticoid synthesis and that cortisol enhanced human immunodeficiency virus (HIV) and cytomegalovirus expres-

sion (64,67). These observations suggested that antagonism of the action of endogenous cortisol could prevent progression of several serious viral diseases possibly even against HIV (64,67). However, unless the specific action of cortisol that one wished to inhibit was sensitive to very low doses of mifepristone, the use of antiglucocorticoids to neutralize endogenous cortisol will be associated with a rise in ACTH and cortisol as the pituitary–adrenal axis responds to the functional decrease in cortisol as glucocorticoid receptors in these organs are blocked (13).

Other situations in which the antiglucocorticoid properties of this class of compound may prove useful are in the treatment of burns, glucocorticoid-dependent hypertension, depression, arthritis, and cataracts (7,13,64,67,69,70). It is thus evident that this new class of compounds have numerous proved and possible clinical applications.

III. UNTOWARD EFFECTS

The adverse events reported during single-dose mifepristone–misoprostol administration for pregnancy interruption are invariably due to the prostaglandin component of the regimen. They could also be related to the pregnancy and abortive process. Long-term administration of mifepristone in doses ranging from 100 to 200 mg/day is generally well tolerated (12,13). The most common side effect reported is fatigue, which develops in most of the subjects (12,13). Nausea, anorexia, and vomiting may also occur (12,13). That such symptoms improve with supportive therapy that includes glucocorticoids is not sufficient evidence to suggest glucocorticoid deficiency. Although hypoadrenalism, consequent to glucocorticoid antagonism with mifepristone, has been reported on occasion in long-term treatment with doses exceeding 200 mg/day, this has rarely been objectively proved and is an uncommon occurrence in humans with an intact pituitary–adrenal axis. Nevertheless, if the clinical picture does suggest hypoadrenalism, then glucocorticoid replacement is indicated.

Other side effects reported during long-term treatment include slight decrease in serum potassium, weight loss, skin rashes, cessation of menses in premenopausal women, intermittent hot flashes, transient thinning of the hair, biochemical hypothyroidism, development of Hashimoto's thyroiditis, and occasional decrease in libido and gynecomastia in males (12,13,71). The latter is presumably because mifepristone binds with low affinity to androgen receptors (3). When administered over a prolonged period to women in a dose of 50 mg daily or higher, there is progesterone suppression, and the endometrium is exposed to unopposed estrogen levels (7). The clinical consequences of this still remain to be fully evaluated. A single case of massive simple benign endometrial hyperplasia was reported in a young 19-year-old subject, with a variant

of Cushing's syndrome who was treated with mifepristone (400 mg) daily for intermittent intervals for a total of 1 year. This resolved completely on cessation of mifepristone treatment (72).

Because some women fail to abort and may continue with their pregnancy following mifepristone administration, it is important to determine if there are any teratogenic effects. There are isolated case reports of normal pregnancies and offspring when women have taken mifepristone alone or in combination with a prostaglandin, have not aborted, and have elected to continue their pregnancies (13,73). One woman's pregnancy was terminated at 18 weeks because ultrasonography revealed that the fetus had multiple severe congenital defects not thought to have been caused by mifepristone (74). In rabbits, skull deformities did occur and were attributed to mechanical effects secondary to uterine contractions because of the decrease in progesterone activity (75). However, the prostaglandins used in association with antiprogestins for pregnancy termination may be associated with teratogenic effects (76,77). Because of this, the manufacturer of mifepristone recommends that in the case of failure of the method, the pregnancy should be terminated by surgery.

IV. CONCLUSIONS

Antiprogestins are among the most controversial and yet the more interesting therapeutic compounds developed in the past 20 years. It is evident that antiprogestins have numerous proved and potential clinical applications. To consider antiprogestins exclusively as abortifacients is to grossly underestimate their usefulness. In addition to providing an effective and safe means of medical abortion, these agents may be used for other obstetric indications, in various gynecologic disorders, as postcoital contraceptives, as potential contraceptives, as well as in the treatment of patients with cancer, various forms of Cushing's syndrome, and in other miscellaneous disorders. It is of interest that few of these clinical applications were envisaged when antiprogestins were first described 15 years ago.

The initial clinical trial of mifepristone for medical termination of pregnancy was conducted in 1982 (11). Despite this, antiprogestins are available for pregnancy termination only in France, the United Kingdom, Sweden, and China. This is probably related to economic, and legal reasons, as well as opposition from antiabortion groups. It is expected that the U.S. Food and Drug Agency (FDA) will give approval for the use of mifepristone for medical termination of pregnancy in the United States during 1999. It is hoped that this will be an impetus for the introduction of mifepristone and other antiprogestins into other countries. Further studies can then be conducted to define the therapeutic values and benefits of these fascinating compounds.

REFERENCES

1. Sherman MR, Corvol PL, O'Malley BW. Progesterone-binding components of chick oviduct. 1. Preliminary characterization of cytoplasmic components. J Biol Chem 1970; 245:6085–6096.
2. Philibert D, Deraedt R, Teutsch G. RU 486: a potent antiglucocorticoid in vivo. VIII International Congress of Pharmacology. Tokyo, 1981. abstr 1463.
3. Philibert D. RU 38486: an original multifaceted antihormone in vivo. In: Agarwal MK, ed. Adrenal Steroid Antagonism. Berlin: Walter de Gruyter, 1984; pp 77–101.
4. Baulieu EE. Contragestion and other clinical applications of RU 486, an antiprogesterone at the receptor. Science 1989; 245:1351–1357.
5. Teutsch G, Philbert D. History and perspectives of antiprogestins from the chemist's point of view. In: Beier HM, Spitz IM, eds. Progesterone Antagonists in Reproductive Medicine and Oncology. vol 9 (suppl). Oxford: Oxford University Press, 1994; pp 12–31.
6. Robbins A, Spitz IM. Mifepristone: clinical pharmacology. Clin Obstet Gynecol 1996; 39:436–450.
7. Spitz IM, Croxatto HB, Robbins A. Antiprogestin: mechanism of action and contraceptive potential. Annu Rev Pharmacol Toxicol, 1997; 36:47–81.
8. Horwitz KB. The molecular biology of RU 486. Is there a role for antiprogestins in the treatment of breast cancer? Endocr Rev 1992; 13:146–163.
9. McDonnell DP. Unraveling the human progesterone receptor signal transduction pathways (insights into antiprogestin action). Trends Endocrinol Metab 1995; 6:133–138.
10. Garfield RE, Gasc JM, Baulieu EE. Effects of the antiprogesterone RU 486 on preterm birth in the rat. Am J Obstet Gynecol 1987; 157:1281–1285.
11. Herrmann W, Wyss R, Riondel A, Philibert D, Teutsch G, Sakiz E, Baulieu EE. Effet d'un steroide antiprogesterone chez la femme: interruption du cycle menstruel et de la grossesse au debut. CR Acad Sci Paris 1982; 294:933–938.
12. Spitz IM, Bardin CW. Mifepristone (RU 486)—a modulator of progestin and glucocorticoid action. N Engl J Med 1993; 329:404–412.
13. Spitz IM, Bardin CW. Clinical pharmacology of RU 486—an antiprogestin and antiglucocorticoid. Contraception, 1993; 48:403–444.
14. Couzinet B, Le Strat N, Ulmann A, Baulieu EE, Schaison G. Termination of early pregnancy by the progesterone antagonist, RU 486 (mifepristone). N Engl J Med 1986; 315:1565–1570.
15. Bygdeman M, Swahn ML. Progesterone receptor blockage. Effect on uterine contractility and early pregnancy. Contraception 1985; 32:45–51.
16. Cheng L, Kelly RW, Thong KJ, Hume R, Baird DT. The effects of mifepristone (RU486) on prostaglandin dehydrogenase in decidual and chorionic tissue in early pregnancy. Hum Reprod 1993; 8:705–709.
17. Cheng L, Kelly RW, Thong KJ, Hume R, Baird DT. The effect of mifepristone (RU 486) on the immunohistochemical distribution of prostaglandin E and its

metabolite in decidual and chorionic tissue in early pregnancy. J Clin Endocrinol Metab 1993; 77:873–877.

18. Silvestre L, Dubois C, Renault M, Rezvani Y, Baulieu EE, Ulmann A. Voluntary interruption of pregnancy with mifepristone (RU 486) and a prostaglandin analogue. N Engl J Med 1990; 322:645–648.
19. Peyron R, Aubeny E, Targosz V, Silvestre L, Renault M, Elkik F, Leclerc P, Ulmann A, Baulieu E-E. Early termination of pregnancy with mifepristone (RU 486) and the orally active prostaglandin misoprostol. N Engl J Med 1993; 328:1509–1513.
20. Aubeny E, Peyron R, Turpin CC, Renault M, Targosz V, Silvestre L, Ulmann A, Baulieu EE. Termination of early pregnancy (up to and after 43 days of amenorrhea) with mifepristone (RU 486) and increasing doses of misoprostol. Int J Fertil 1995; 40:85–91.
21. Baird DT, Sukcharoen N, Thong KJ. Randomized trial of misoprostol and Cervagem in combination with a reduced dose of mifepristone for induction of abortion. Hum Reprod 1995; 10:1521–1527.
22. Urquhart DR, Templeton AA, Shimewi F, et al. The efficacy and tolerance of mifepristone and prostaglandin in termination of pregnancy of less than 63 days gestation—U.K. multicenter study final results. Contraception 1997; 55:1–5.
23. El-Refaey H, Rajasekar D, Abdalla M, Calder L, Templeton A. Induction of abortion with mifepristone (RU 486) and oral or vaginal misoprostol. N Engl J Med 1995; 332:983–987.
24. Spitz IM, Bardin CW, Benton L, Robbins A. Early pregnancy termination with mifepristone and misoprostol in the United States. N Engl J Med 1998; 338:1241–1247.
25. Radestad A, Bygdeman M. Are prostaglandins mediators of mifepristone (RU 486)-induced cervical softening in early pregnancy? J Lipid Mediat 1993; 6:503–507.
26. Chwalisz K, Stockemann K, Fuhrmann U, Fritemeier KH, Einspanier A, Garfield RE. Mechanism of action of antiprogestins in the pregnant uterus. Ann NY Acad Sci 1995; 761:202–223.
27. Kelly RW, Leask R, Calder AA. Choriodecidual production of interleukin-8 and the mechanism of parturition. Lancet 1992; 339:776–777.
28. Henshaw RC, Templeton AA. Pre-operative cervical preparation before first trimester vacuum aspiration: a randomized controlled comparison between gemeprost and mifepristone (RU 486). Br J Obstet Gynaecol 1991; 98:1025–1030.
29. Rodger MW, Baird DT. Pretreatment with mifepristone (RU 486) reduces interval between prostaglandin administration and expulsion in second trimester abortion. Br J Obstet Gynaecol 1990; 97:41–45.
30. Gupta JK, Johnson N. Effect of mifepristone on dilatation of the pregnant and non-pregnant cervix. Lancet 1990; 335:1238–1240.
31. Cabrol D, Dubois C, Cronje H, Gonnet JM, Guillot M, Maria B, Moodley J, Oury JF, Thoulon JM, Treisser A, Ulmann D, Correl S, Ulmann A. Induction of labor with mifepristone (RU 486) in intrauterine fetal death. Am J Obstet Gynecol 1990; 163:540–542.

32. Frydman R, Lelaidier C, Baton-Saint-Mleux C, Fernandez H, Vial M, Bourget P. Labor induction in women at term with mifepristone (RU486): a double-blind, randomized, placebo-controlled study. Obstet Gynecol 1992; 80:972–975.
33. Hill NCW, Selinger M, Ferguson J, MacKenzie IZ. The placental transfer of mifepristone (RU 4860) during the second trimester and its influence upon maternal and fetal steroid concentrations. Br J Obstet Gynaecol 1990; 97:406–411.
34. Shoupe D, Mishell DR Jr, Page MA, Madkour H, Spitz IM, Lobo RA. Effects of the antiprogesterone RU 486 in normal women. II. Administration in the late follicular phase. Am J Obstet Gynecol 1987; 157:1421–1426.
35. Ledger WL, Sweeting VM, Hillier H, Baird DT. Inhibition of ovulation by low-dose mifepristone (RU 486). Hum Reprod 1992; 7:6–11.
36. Croxatto HB, Salvatierra AM, Croxatto HD, Fuentealba A. Effects of continuous treatment with low dose mifepristone throughout one menstrual cycle. Hum Reprod 1993; 8:201–207.
37. Batista MC, Cartledge TP, Zellmer AW, Merino MJ, Axiotis C, Loriaux DL, Nieman LK. Delayed endometrial maturation induced by daily administration of the antiprogestin RU 486: a potential new contraceptive strategy. Am J Obstet Gynecol 1992; 167:60–65.
38. Spitz IM, Croxatto HB, Salvatierra AM, Heikinheimo O. Response to intermittent RU 486 in normal women. Fertil Steril 1993; 59:971–975.
39. Swahn ML, Bygdeman M, Cekan S, Xing S, Masironi B, Johannisson E. The effect of RU 486 administered during the early luteal phase on bleeding pattern, hormonal parameters and endometrium. Hum Reprod 1990; 5:402–408.
40. Gemzell-Danielsson K, Swahn M-L, Svalander P, Bygdeman M. Early luteal phase treatment with mifepristone (RU 486) for fertility regulation. Hum Reprod 1993; 8:870–873.
41. Ghosh D, Sengupta J. Anti-nidatory effect of a single, early post-ovulatory administration of mifepristone (RU 486) in the rhesus monkey. Hum Reprod 1993; 8:552–558.
42. Katkam RR, Gopalkrishnan K, Chwalisz K, Schillinger E, Puri CP. Onapristone (ZK 98.299): A potential antiprogestin for endometrial contraception. Am J Obstet Gynecol 1995; 173:779–787.
43. Beier HM, Hegele-Hartung C, Mootz U, Beier-Hellwig K. Modification of the endometrial cell biology by progesterone antagonists to manipulate the implantation window. In: Beier HM, Spitz IM, eds. Progesterone Antagonists in Reproductive Medicine and Oncology. vol 9. Oxford: Oxford University Press, 1994; Suppl 1 pp 98–115.
44. Gemzell-Danielsson K, Westlund P, Johannisson E, Swahan M-L, Bygdeman M, Seppala M. Effect of low weekly doses of mifepristone on ovulation function and endometrial development. Hum Reprod 1996; 11:256–264.
45. Gemzell-Danielsson K, Swahan M-L, Westlund P, Johannisson E, Seppala M, Bygdeman M. Effect of low daily doses of mifepristone on ovarian function and endometrial development. Hum Reprod 1997; 12:124–131.
46. Shoupe D, Mishell DR Jr, Lahteenmaki P, Heikinheimo O, Birgerson L, Madkour H, Spitz IM. Effects of the antiprogesterone RU 486 in normal women. I. Single-

dose administration in the midluteal phase. Am J Obstet Gynecol 1987; 157:1415–1420.
47. Croxatto HB, Salvatierra AM, Romero C, Spitz IM. Late luteal phase administration of RU 486 for three successive cycles does not disrupt bleeding patterns of ovulation. J Clin Endocrinol Metab 1987; 65:1272–1277.
48. World Health Organization Task Force on Postovulatory Methods of Fertility Regulation. Menstrual regulation by mifepristone plus prostaglandin: results from a multicentre trial. Hum Reprod 1995; 10:308–314.
49. Glasier A, Thong KJ, Dewar M, Mackie M, Baird DT. Mifepristone (RU 486) compared with high-dose estrogen and progestogen for emergency postcoital contraception. N Engl J Med 1992; 327:1041–1044.
50. Webb AMC, Russell J, Elstein M. Comparison of Yuzpe regimen, danazol, and mifepristone (RU 486) in oral postcoital contraception. Br Med J 1992; 305:927–931.
51. Hodgen GD, van Uem JFHM, Chillik CF, Danforth DR, Wolf JP, Neulen J, Williams RF, Chwalisz K. Non-competitive antioestrogenic activity of progesterone antagonists in primate models. In: Beier HM, Spitz IM, eds. Progesterone Antagonists in Reproductive Medicine and Oncology. vol. 9. Oxford: Oxford University Press, 1994; Suppl 1 pp 77–81.
52. Yen SSC. Use of antiprogestins in the management of endometriosis and leiomyoma. In: Donaldson MS, Dorflinger L, Brown SS, Benet LZ, eds. Clinical Applications of Mifepristone (RU 496) and Other Antiprogestins. Washington, DC: National Academy Press, 1993; pp 189–209.
53. Friedman AJ, Barbieri RL, Doubliet PM, Fine C, Schiff I. A randomized double blind trial of a GnRH agonist with or without medroxyprogesterone in the treatment of leiomyomata uteri. Fertil Steril 1988; 49:404–409.
54. Matsuo H, Maruo T, Hamana S, Samoto T, Mochizuki M. Induction of the expression of Bc1-2 protein by progesterone in leiomyoma cells of the human uterus. J Clin Endocrinol Metab 1997; 82:293–299.
55. Olson JJ, Beck DW, Schlechte J, Loh PM. Hormonal manipulation of meningiomas in vitro. J Neurosurg 1986; 65:99–107.
56. Olson JJ, Beck DW, Schlechte JA, Loh PM. Effect of the antiprogesterone RU-38486 on meningioma implanted into nude mice. J Neurosurg 1987; 66:584–587.
57. Grunberg SM. Role of antiprogestational therapy for meningiomas. Hum Reprod 1994; 9(suppl 1):202–207.
58. Bakker GH, Setyono-Han B, Portengen H, de Jong FH, Foekens JA, Klijn JGM. Treatment of breast cancer with different antiprogestins: preclinical and clinical studies. J Steroid Biochem Mol Biol 1990; 37:789–794.
59. Romieu G, Maudelonde T, Ulmann A, Pujol H, Grenier J, Cavalie G, Khalaf S, Rochefort H. The antiprogestin RU 486 in advanced breast cancer: preliminary clinical trial. Bull Cancer 1987; 74:455–461.
60. Perrault D, Eisenhauer EA, Pritchard KI, Panasci L, Norris B, Vandenberg T, Fisher B. Phase II study of the progesterone antagonist mifepristone in patients

with untreated metastatic breast carcinoma: a National Cancer Institute of Canada Clinical Trials Group Study. J Clin Oncol 1996; 14:2709–2712.
61. Pinski J, Halmos G, Shirahige Y, Wittliff JL, Schally AV. Inhibition of growth of the human malignant glioma cell line (U87MG) by the steroid hormone antagonist RU486. J Clin Endocrinol Metab 1993; 77:1388–1392.
62. Nieman LK, Chrousos GP, Kellner C, Spitz IM, Nisula BC, Cutler GB, Merriam GR, Bardin CW, Loriaux DL. Successful treatment of Cushing's syndrome with the glucocorticoid antagonist RU 486. J Clin Endocrinol Metab 1985; 61:536–540.
63. Chrousos GP, Laue L, Nieman LK, Udelsman R, Kawai S, Loriaux DL. Clinical applications of RU 486, a prototype glucocorticoid and progestin antagonist. In: Mantero F, Takeda R, Scoggins BA, Biglieri EG, Funder JW, eds. The Adrenal and Hypertension: From Cloning to Clinic. vol. 57 of Serono Symposia. New York: Raven Press, 1989; pp 273–284.
64. Agarwal MK. The antiglucocorticoid action of mifepristone. Pharmacol Ther 1996; 70:183–213.
65. Phillips CI, Gore SM, Green K, Cullen PM, Campbell M. Eye drops of RU 486-6, a peripheral steroid blocker, lower intraocular pressure in rabbits. Lancet 1984; 1:767–768.
66. Konagaya M, Bernard PA, Max SR. Blockage of glucocorticoid receptor binding and inhibition of deamethsone-induced muscle atrophy in the rat by RU38486, a potent glucocorticoid antagonist. Endocrinology 1986; 119:375–380.
67. Regelson W, Loria R, Kalimi M. Beyond ''abortion'': RU-486 and the needs of the crisis constituency. JAMA 1990; 264:1026–1027.
68. Konagaya M, Max SR. A possible role for endogenous glucocorticoids in orchiectomy-induced atrophy of the rat levator ani muscle: studies with RU 38486, a potent and selective antiglucocorticoid. J Steroid Biochem 1986; 25:305–308.
69. Murphy BEP, Filipini D, Ghadirian AM. Possible use of glucocorticoid receptor antagonists in the treatment of major depression: preliminary results using RU 486. J Psychiatry Neurosci 1993; 18:209–213.
70. Fang C-H, James HJ, Ogle C, Fischer JE, Hasselgren P-O. Influence of burn injury on protein metabolism in different types of skeletal muscle and the role of glucocorticoids. J Am Coll Surg 1995; 180:33–42.
71. Heikinheimo O, Ranta S, Grunberg S, Lhteenmki P, Spitz IM. Alterations in the pituitary–thyroid and pituitary–adrenal axes—consequences of long-term mifepristone treatment. Metabolism 1997; 46:292–296.
72. Newfield RS, Spitz IM, Isaacson C, New MI. Mifepristone (RU486) and endometrial hyperplasia. Submitted for publication.
73. Lim BH, Lees DAR, Bjornsson S, Lunan CB, Cohn MR, Stewart P, Davey A. Normal development after exposure to mifepristone in early pregnancy. Lancet 1990; 336:257–258.
74. Pons J-C, Imbert M-C, Elefant E, Roux C, Herschkorn P, Papiernik E. Development after exposure to mifepristone in early pregnancy. Lancet 1991; 338:763.
75. Jost A. Animal reproduction. New data on the hormonal requirement of the pregnant rabbit: partial pregnancies and fetal anomalies resulting from treatment with

a hormonal antagonist, given at a sub-abortive dosage. CR Acad Sci Paris 1986; 303 (Ser 111):281–284.

76. Fonseca W, Alencar AJC, Mota FSB, Coelho HLL. Misoprostol and congenital malformations. Lancet 1991; 338:56.
77. Gonzalez CH, Vargas FR, Perez ABA, Kim CA, Brunoni D, MJ Marques-Dias, Leone CR, JC, Llerena JC Jr, Cabral de Almeida JC. Limb deficiency with or without Mbius sequence in seven Brazilian children associated with misoprostol use in the first trimester of pregnancy. Am J Med Genet 1993; 47:59–64.

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Pharmacokinetics of Progesterone Administered Orally and Parenterally

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I. INTRODUCTION

The search for an optional therapeutic agent for progesterone replacement or supplementation has led to the study of different progestogen formulations and routes of administration. In recent years, there has been considerable interest in the therapeutic use of the natural progestogen, progesterone. The objective of the present chapter is to discuss the methods used to measure progesterone levels and to summarize the existing data on the absorption, metabolism, and pharmacokinetic profiles of progesterone administered orally and parenterally.

II. ABSORPTION OF PROGESTERONE

The process of micronization has allowed the therapeutic use of progesterone by the oral route. When progesterone is administered orally in a crystalline form, it is poorly absorbed. However, when the particle size of crystalline progesterone is decreased by the process of micronization, oral absorption of progesterone is increased. Micronization gives rise to a greater surface area of the compound, allowing it to be dissolved in the aqueous medium of the intestine.

Increased surface area of a water-insoluble compound can also be achieved by dispersing the compound in water-soluble carriers such as sterols. Oral administration of a mixture of progesterone with cholesterol pivalate on a lactose carrier increases plasma progesterone levels, compared with administration of

progesterone without the sterol (1). A rat model has been used to provide evidence that progesterone mixed with a sterol is absorbed through the lymphatic system (1).

Although the processes just described allow progesterone to be readily absorbed, another obstacle that limits its bioavailability is the extensive intestinal and hepatic metabolism to which it is subjected. The progesterone molecule is particularly vulnerable to enzymatic reduction by reductases and dehydrogenases owing to its characteristic functional groups (i.e., ketone groups at C-3 and C-20, and a double bond between C-4–5). Reducing enzymes, as well as conjugating enzymes (sulfuryl and glucuronyl transferases), are present in both intestinal bacteria and the intestinal mucosa. These enzymes reduce a portion of ingested micronized progesterone to pregnanediones, pregnanolones, and pregnanediols, which are found as isomers with the hydrogen at C-5 and the hydroxyl groups at C-3 and C-20 either in the α- or β-orientation. In addition, the hydroxyl groups may be sulfated or glucuronidated. Subsequently, progesterone and its metabolites are absorbed through the intestinal mucosa and enter the portal vein blood, which perfuses the liver. The liver then transforms a substantial portion of the unmetabolized progesterone, as well as some of the progesterone metabolites, by its extensive and highly active reductases, dehydrogenases, hydroxylases, and conjugating enzymes. The extensive intestinal and hepatic metabolism of progesterone requires administration of large doses of this compound compared with other progestogens, when used therapeutically.

III. MEASUREMENT OF PROGESTERONE

Although progesterone is measured most commonly in serum or plasma, it can also be quantified in urine and saliva. Progesterone is measured by immunoassay methods that include radioimmunoassay, chemiluminescent immunoassay, fluoroimmunoassay, and enzyme immunoassay. In urine, progesterone is present in low concentrations and requires a purification step (e.g., organic solvent extraction) before quantification by an immunoassay (2). Instead, its main metabolite, pregnanediol glucuronide, is usually measured in urine. When serum levels of progesterone and urinary levels of pregnanediol glucuronide are measured daily throughout a woman's menstrual cycle, an excellent correlation exists between the two compounds (3). The concentrations of urinary pregnanediol glucuronide are about 1000 times higher than those of urinary progesterone during the follicular phase, and as much as 4000 times higher during the luteal phase. Although urinary pregnanediol glucuronide levels are considerably higher than urinary progesterone levels, pregnanediol glucuronide excretion is subject to large intrasubject and intersubject variability. The dramatic elevation and great variability

in urinary pregnanediol glucuronide levels is primarily attributable to the hepatic first-pass effect of progesterone.

Although "direct" immunoassays (i.e., without a purification step) are used most commonly for quantifying progesterone in serum or plasma, such assays should not be used when measuring progesterone in samples obtained following exogenous administration of progesterone. This is because high concentrations of both unconjugated and conjugated progesterone metabolites are found in serum following oral progesterone dosing, which results in overestimated progesterone values. To obtain absolute progesterone levels, it is essential to subject samples to purification (e.g., organic solvent extraction and chromatography) before quantification by immunoassay.

Serum progesterone levels also correlate highly with salivary progesterone levels (4). However, the latter levels are approximately 1/25–1/50 of circulating progesterone levels. Low progesterone levels in saliva can be difficult to measure by immunoassay if the assay lacks sensitivity or if there is insufficient volume of saliva for the assay.

When drawing a blood sample for measurement of progesterone, it is important to realize that endogenous steroid levels may be influenced by food intake. One study showed that endogenous progesterone levels were decreased by as much as 35% at 1 h after food intake (5).

Finally, sometimes, endogenous serum progesterone levels can be underestimated when direct immunoassays are used. This is most often due to lack of dissociation of progesterone from its specific plasma-binding protein, namely, corticosteroid-binding protein (CBG) in serum or plasma. Approximately 20% of circulating progesterone is bound with high-affinity to CBG (6). Most of the remaining portion is loosely bound to albumin; less than 5% is unbound.

IV. PHARMACOKINETICS OF ORAL PROGESTERONE

When a sufficient dose of micronized progesterone is administered to a woman, therapeutic serum levels of progesterone are attained. Dose proportionality of micronized progesterone has been evaluated in a three-way, crossover design study of 100-, 200-, and 300-mg doses in 15 healthy postmenopausal women (7). Each dose was ingested daily for 5 days, and blood samples were obtained at frequent intervals for 24 h after the last dose. The results showed that both the maximum concentration (C_{max}) and area under the curve from 0 to 24 h (AUC_{0-24h}) values for serum progesterone increased proportionately with increasing dose. Mean C_{max} values were 6.5, 13.8, and 32.2 ng/mL for the three different doses, respectively. However, the times to reach C_{max} (T_{max}) values were not very different for the three different groups; they ranged from 2.2 to

2.7 h. Large intersubject variability in serum progesterone levels was observed in all three groups.

Serum progesterone levels in the foregoing dose–response study were in the range observed during the luteal phase of a spontaneous menstrual cycle. These findings indicate that 100-, 200-, and 300-mg doses of micronized progesterone can be used clinically for supplemental and therapeutic needs.

Presently, the 200-mg dose of micronized progesterone is commonly recommended for preventing endometrial hyperplasia in postmenopausal women receiving estrogen replacement therapy. This dose was evaluated in a group of nine healthy postmenopausal women following its administration, and frequent blood samples were taken during a 24-h period (8). The mean C_{max} for serum progesterone was 17.0 ± 4.9 ng/mL at a mean T_{max} of 2.8 ± 0.35 h. In the same study, the C_{max} values were not significantly different from midluteal serum progesterone levels measured in control cycles of a group of premenopausal women; their levels averaged 14.1 ± 2.7 ng/mL. These observations indicate that a single oral 200-mg dose of micronized progesterone provides circulating progesterone levels similar to those normally found during the luteal phase.

The influence of food ingestion on the absorption of oral micronized progesterone has also been studied (7). Following a daily 200-mg dose of oral micronized progesterone, either before or immediately after a standardized meal, food intake increased C_{max} by about fivefold and AUC_{0-24h} about twofold. T_{max} also increased, but only to a minor extent.

V. PHARMACOKINETICS OF PROGESTERONE ADMINISTERED PARENTERALLY

Progesterone can be administered parenterally by a variety of different routes; these include the following: intramuscular, vaginal, percutaneous, intranasal, sublingual, and rectal. Although little is known about the pharmacokinetics of progesterone administered parenterally, some data are available pertaining to circulating progesterone levels by these routes. A major observation in these studies is the large intersubject variability in circulating progesterone levels. Some of these studies will now be summarized.

A. Intramuscular Route

Dose proportionality of progesterone has been evaluated in a study of six premenopausal women who received intramuscular administration of 10, 25, 50, and 100 mg of progesterone in oil (9). Peak concentrations of plasma progesterone for the different doses increased proportionally with increasing dose and were attained within 8 h of dosing; the peak levels were 7, 28, 50, and 68 ng/mL

for each of the doses, respectively. Elevated progesterone levels persisted for 24–48 h, which is consistent with a depot effect of progesterone after intramuscular administration. The data indicate that a single intramuscular injection of 25 mg of progesterone in oil can give rise to circulating progesterone levels that are comparable with those found during the luteal phase of a spontaneously occurring menstrual cycle.

In another study, a comparison was made between intramuscular and oral administration of progesterone (2). Three premenopausal women received a single oral 200-mg dose of micronized progesterone during days 2–5 of the menstrual cycle. Two days later, the same women received an intramuscular injection of 25 mg of progesterone in sesame oil. Blood and urine were collected at baseline and at 1, 4, 8, 12, and 24 h posttreatment. Progesterone was measured in both serum and urine, and pregnanediol glucuronide was measured in urine. Following the oral dose, serum progesterone levels rose rapidly, and peak levels ranging from 8.6 to 11.7 ng/mL were attained at 3–4 h after dosing. By 24 h, the peak progesterone concentrations fell to approximately 10% of the peak values. In contrast, urinary progesterone and pregnanediol glucuronide levels peaked later (i.e., about 4–8 h after dosing), and at 24 h the levels of both compounds decreased to 5–8% of the peak values. In comparison with the oral dosing, the results of the intramuscular administration showed that serum progesterone levels rose rapidly and were elevated at a level of about 10 mg/mL after 12 h in all three subjects. Urinary progesterone and pregnanediol glucuronide levels peaked later, and were still elevated, even 24 h after dosing. Both the oral and intramuscular routes of administration showed that urinary pregnanediol glucuronide concentrations were much higher and much more variable than those of urinary progesterone. The clinical significance of this study is that luteal phase serum progesterone levels can be achieved by both routes of administration; however, the intramuscular route provides a prolonged effect on these levels.

B. Intravaginal Route

Vaginal administration of micronized progesterone has been an effective, acceptable, and convenient alternative to intramuscular injections (10). In one study, 200 mg of micronized progesterone was administered intravaginally every 6 h to one group of 15 women and 50 mg of progesterone in oil was injected intramuscularly twice into a second group of women during a 24-h study period. After intramuscular administration there was a rapid rise in serum progesterone levels, with a plateau in levels of about 16 ng/mL after 4 h of treatment. In contrast, after vaginal administration of progesterone there was a slow rise in serum progesterone levels that reached a plateau after 4 h. In the same study, endometrial concentrations of progesterone were also measured after intravaginal

and intramuscular progesterone treatments. The endometrial progesterone concentrations following intravaginal treatment were considerably higher than after intramuscular treatment, even though serum progesterone levels were higher after intramuscular injection. The high endrometrial progesterone concentrations obtained by the intravaginal route indicate the potential importance of this route, since the endometriam is the most important site of progesterone action.

C. Intranasal Route

A relatively small number of studies have been performed with progesterone administered intranasally. In one study, serum progesterone levels were measured in ten postmenopausal women following administration of progesterone by nasal spray, using two spray doses per nostril for a total dose of 11 mg of progesterone (11). The progesterone levels rose rapidly, reaching peak levels of about 3.75 ng/mL 1 h following treatment, and then fell dramatically up to about 3 h after treatment. Thereafter, a secondary peak, which had a maximum concentration of about 28 ng/mL, was observed at about 4 h after treatment. The significance of the secondary peak is not known. These data suggest that this route of administration may require mutliple dosing each day for therapeutic effectiveness.

D. Rectal Route

Progesterone is sometimes administered rectally, and therapeutic circulating progesterone levels are achieved. In one study, plasma progesterone levels were measured in two groups of women in the follicular phase of the cycle, following rectal administration of progesterone in a suppository form. One group received a 25-mg dose, and the other group received a 100-mg dose (9). With both progesterone doses, peak plasma progesterone levels were attained within the first 8 h of dosing and varied widely. Peak values ranged from about 1.5 to 14 ng/mL with the 25-mg dose and 15 to 50 ng/mL with the 100-mg dose. The levels then fell dramatically, and after 24 h, ranged from 0.5 to 1.5 ng/mL with the 25-mg dose, and 0.5 to 2.5 ng/mL with the 100-mg dose.

E. Sublingual Route

Serum progesterone levels that are similar to those found in the luteal phase can be achieved following a single dose of progesterone sublingually. In one study, mean peak serum progesterone levels of 10.5 ± 1.8 and 17.6 ± 3.8 ng/mL were obtained at 1 h and 1–2 h following sublingual administration of 50-mg and 100-mg doses of micronized progesterone, respectively (12). Corresponding levels 8 h after dosing were 1.22 ± 0.18 and 2.34 ± 0.22 ng/mL, respectively.

These data indicate that the rapid absorption and disappearance of sublingual progesterone may require more than once-a-day use for it to be therapeutically effective.

F. Percutaneous Route

Limited data are available on circulating levels of progesterone administered percutaneously. Preliminary data were obtained in one study in which one of two different doses (30 or 100 mg) of progesterone gel was applied on the inner portion of the arm of postmenopausal women daily for 1 month (13). Blood samples were obtained at frequent intervals on the first day of treatment. Following administration of the 30-mg dose of progesterone gel, serum progesterone levels increased by 50–100%, but remained in the follicular phase range (<0.5 ng/mL). With the 100 mg progesterone dose, peak progesterone levels of 5.8–8.0 ng/mL were attained at 2–3 h after gel application. Thereafter, relatively uniform progesterone levels were achieved at 1, 2, and 4 weeks of treatment.

Progesterone can also be administered percutaneously in the form of a cream. Of the several different products that are commercially available as progesterone creams, some do not contain progesterone. Instead they contain wild yam extract that contains diosgenin and can be converted to progesterone by chemical degradation. However, this conversion does not occur in the body.

Percutaneous absorption of progesterone has been studied in postmenopausal women receiving transdermal estrogen replacement during a 5-week cycle, following administration of progesterone cream (14). The cream was applied during the first 2 weeks of the cycle, using 30 mg of progesterone in 1 gram of cream. The dosage was doubled during the last 2 weeks of the cycle. Blood samples were obtained at frequent intervals on days 1, 8, 15, 22, and 29 of the study. Mean serum progesterone concentrations ranged from 1.6 to 3.3 ng/mL. Progesterone concentrations were sustained for at least 8 h. Although there was an increase in progesterone levels after 4 weeks of treatment, compared with 2 weeks of application, no dose–response was observed.

VI. CONCLUSIONS

There are limited data on the pharmacokinetics of progesterone administered orally and parenterally. This is due to lack of studies and deficiencies in existing studies. Major deficiencies include use of unreliable progesterone assays, insufficient number of study subjects, and infrequent blood sampling. In addition, from the clinical perspective, data are lacking on the optimum dose of progesterone required for endometrial protection from the proliferative effects of estrogen in postmenopausal women receiving long-term hormone replacement therapy.

REFERENCES

1. Kincl FA, Ciaccio LA, Benagiano G. Increasing oral bioavailability of progesterone by formulation. J Steroid Biochem 1985; 44:622–626.
2. Stanczyk FZ, Gentzschein E, Ary BA, et al. Urinary progesterone and pregnanediol. Use for monitoring progesterone treatment. J Reprod Med 1997; 42:216–222.
3. Stanczyk FZ, Miyakawa I, Goebelsmann U. Direct radioimmunoassay of urinary estrogen and pregnanediol glucuronides during the menstrual cycle. Am J Obstet Gynecol 1980; I37:443–450.
4. Bolaji II, Tallon DF, O'Dwyer E, et al. Assessment of bioavailability of oral micronized progesterone using a salivary progesterone enzyme immunoassay. Gynecol Endocrinol 1993; 7:101–110.
5. Nakajima ST, Gibson M. The effect of a meal on circulating steady-state progesterone levels. J Clin Endocrinol Metab 1989; 69:917–919.
6. Westphal U. Steroid–Protein Interactions. Berlin: Springer-Verlag, 1986.
7. Simon JA, Robinson DE, Andrews MC, et al. The absorption of oral micronized progesterone: the effect of food, dose proportionality, and comparison with intramuscular progesterone. Fertil Steril 1993; 60:26–33.
8. Maxson WS, Hargrove JT. Bioavailability of oral micronized progesterone. Fertil Steril 1985; 44:622–626.
9. Nillius SJ, Johansson EDB. Plasma levels of progesterone after vaginal, rectal, or intramuscular administration of progesterone. Am J Obstet Gynecol 1971; 110: 470–477.
10. Miles RA, Paulson RJ, Lobo RA, et al. Pharmacokinetics and endometrial tissue levels of progesterone after administration by intramuscular and vaginal routes: a comparative study. Fertil Steril 1994; 62:485–490.
11. Cicinelli E, Ragno G, Cagnazzo I, et al. Progesterone administration by nasal spray. Fertil Steril 1991; 56:139–141.
12. Chakmakjian ZH, Zachariach NY. Bioavailability of progesterone with different modes of administration. J Reprod Med 1987; 32:443–448.
13. Bello SM, Mezrow G, Shoupe D, et al. Administration of progesterone by use of a percutaneous gel in postmenopausal women. Annual Meeting of the Pacific Coast Fertility Society 1997; Abstr. 0-10.
14. Hermsmyer K, Patton P, Toomey C, et al. Blood levels of progesterone achieved with a cream in postmenopausal women [abstr]. 3rd International Symposium, Women's Health and Menopause, 1998.

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