PROGRESS IN MEDICINAL CHEMISTRY

Volume 2

G. P. Ellis &
G. B. West

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Edited by

G. P. ELLIS, B.Sc., Ph.D., F.R.I.C.

Research Department, Benger Laboratories Limited
Holmes Chapel, Cheshire

and

G. B. WEST, B.Pharm., D.Sc., Ph.D.

School of Pharmacy, University of London

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PREFACE

In presenting the second volume of this series, we have continued the efforts made in Volume 1 to provide a collection of reviews on topics of importance to scientists interested in one or more of the many disciplines connected with drugs—from their discovery to their use in medicine.

The first chapter is devoted to the patenting of chemicals as drugs—a subject of some importance to chemists engaged in the synthesis of potential new drugs but one which is often too little understood. The review on the mechanisms of neuromuscular blockade, although necessarily written with a biological stress, is of value to the chemist in that it illustrates the complexity of the reactions involving voluntary movement. The testing and development of analgesic drugs represents an important section of medicinal chemistry and one on which much time and money has been spent in man's efforts to secure agents to relieve pain. The reviews on neuromuscular blockade and analgesics are to be followed in a future volume by others complementary to them, and these will include the chemical aspects.

The chapter on anaphylaxis contains the latest information in this field, written, it is hoped, in a way in which a non-biologist can form some idea of the complex biological basis of this phenomenon; so many violent and even fatal anaphylactic reactions in patients are recorded each year that it is essential for the chemist to understand some of the processes involved as he is often called upon to prepare antagonists to these. One of the mechanisms involving adrenergic blockade is dealt with in the review of halogenoalkylamines, but it is important to remember that newer compounds have now been shown to prevent the release of the neurohormones and may therefore be of even more value in hypertension than the halogenoalkylamines. All of the reviews are written by specialists and each reflects the author's chief interest.

We are grateful to reviewers of the first volume for the warm reception given to it and to the staff of Butterworths for their encouragement and help in many directions. We also wish to thank the authors, societies and publishers for permission to use illustrations and tables which have appeared in previous publications.

G. P. Ellis
G. B. West

November 1961

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G. E. DAVIES, B.Sc., *Imperial Chemical Industries Limited (Pharmaceuticals Division) Alderley Park, Macclesfield, Cheshire*

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THE PATENTING OF DRUGS

F. MURPHY

INTRODUCTION

THE word 'patent' is the short form of 'Letters Patent', a term derived from the Latin *literae patentes* meaning 'open letters'. The letters patent are so called because these documents are not sealed up but are exposed to view, with the Great Seal attached to the document.

Letters patent are used to make the grant of dignities, territorial titles, appointments to certain Offices of State, and privileges of various kinds including monopoly rights in inventions. Originally, letters patent for inventions carried the Great Seal of the United Kingdom, but since 1883 the seal of the Patent Office has been substituted for the Great Seal.

The grant of monopolies by the Crown extends far back in history, and the United Kingdom was one of the first countries to grant monopolies or patents in respect of inventions. The first Act which provided specifically for the grant of patents for inventions was the Statute of Monopolies passed in 1623 during the reign of King James I. In effect this Statute terminated the numerous monopolies previously granted in respect of normal articles of commerce, but provided that monopolies could still be granted for inventions. However, little development took place during the next 200 years, and the main growth of the patent monopoly system followed the Industrial Revolution and the accompanying development in science and technology. Thus, the Patent Law Amendment Act of 1852 provided for the filing of a provisional specification which could be followed by a complete specification, and the requirement of a description of his invention from the applicant. Before this, a patent was obtained on the title of the invention, such as: 'Certain improvements in machinery for spinning cotton and like fibrous substances'.

The next stage came with the Patents Act of 1883, in accordance with which the fee payable on the filing of an application was reduced to £1, and the fee on the filing of a complete specification, to £3. Furthermore, this Act provided for the establishment of the Patent Office in its present form.

Another important development was the passing of the Patents and Designs Act 1907, which introduced for the first time the examination of patent applications for novelty, this examination being restricted to British patents published in the preceding fifty years before the date of the application. The Act also prohibited the claiming of chemical compounds *per se* and included legislation for the grant of compulsory licences in respect of patents concerned with food or medicine.

The Patents and Designs Act was later amended by various Acts, for example those of 1919, 1928, 1932, 1938 and 1942, but remained in force until it was repealed and replaced by the Patents Act 1949. One of the most important changes introduced by the 1949 Act was that chemical compounds

per se may be claimed, thus reversing the situation which had existed since the 1907 Act.

A result of the long history of the patent laws in the United Kingdom is that since the major part of the inventions made during the nineteenth century were concerned with the mechanical and engineering fields, the provisions of the law have been designed more with reference to inventions of this character than to those in the chemical field. An inevitable corollary is that inventions of a chemical nature (including pharmaceutical inventions) bear various disadvantages not shared by those in the mechanical field.

Legislation covering the protection of patents is part of that concerned with the whole field of 'industrial property', a term used to cover patent, design, trade mark and copyright matters. An essential difference between the forms of protection obtainable in these spheres derives from the fact that under the Copyright Act protection exists automatically as soon as the matter in question has been published, whereas under the Patents Act, the Registered Designs Act and the Trade Marks Act it is necessary to make formal application, accompanied by payment of the appropriate fees, to secure the desired protection. The Patents Act which forms the subject of this paper is discussed at some length.

The Registered Designs Act which lies closest to the Patents Act provides for the protection of designs. The term 'design' here means the features of shape, configuration, pattern or ornament applied to any article by any industrial process or means, where these features in the finished article appeal to, and are judged solely by, the eye[1]. It is not possible to protect by registered design any method or principle of construction, or any feature of shape or configuration, which is dictated solely by the function which the article is to perform. The type of designs which may be protected under the Registered Designs Act are statues, plaques, wallpaper designs, and so on.

Trade marks, used by traders and manufacturers to identify the products sold by them, may be registered under the Trade Marks Act, and such registration gives clear and definite privileges. For example, the use by any other person of the registered mark or one closely similar to it, in respect of the goods in question, comprises an infringement which can be summarily stopped.

The Copyright Act provides for the protection of any work of art, and covers any original literary, dramatic, musical or artistic work. For example, the Act covers articles written for the scientific press and in respect of such articles the copryight is infringed if the whole or a substantial part thereof is copied or reproduced substantially in any way. As opposed to a work of fiction, there can be no copyright in the substance of a scientific article, as it must comprise or relate to scientific fact. There can, therefore, be no infringement of copyright if the substance of any article is abstracted or employed as the basis of any further publication. Trade literature, handbooks, advertisements and the like are protected by copyright.

The essential purpose of the patent monopoly system is to encourage the development of science and technology and to assist in the dissemination of new inventions. This is achieved because the grant of the patent involves the publication of the invention, the essential contract between the patentee and the Government being that on the one hand the inventor discloses his invention and on the other hand the Government grants to him a monopoly for a

limited number of years. During this monopoly period, although the invention has been published, the right to use the invention is reserved to the patentee. The patent monopoly system may be regarded by some as providing a source of revenue to the Government, but in fact this is not the case, and in the United Kingdom the level of fees is designed so that the Patent Office should merely pay its way. In the last few years, the Patent Office has been operating at a loss and some fees were increased in 1961 and there will be further increases in the future.

The importance attributed to the patent monopoly system in achieving the development of science and technology is illustrated by the fact that in the United Kingdom a patent may be granted where the patentee has 'obtained' the invention overseas. These provisions have a long history, as is illustrated by the following statements in the Report of the Clothworkers of Ipswich Case[2], decided in 1615.

> But if a man hath brought in a new invention and a new trade within the kingdom in peril of his life and consumption of his estate or stock, *etc.*, or if a man hath made a new discovery of anything, in such cases the King of his grace and favour in recompense of his costs and travail may grant by charter unto him that he shall only use such a trade or trafique for a certain time, because at first people of the kingdom are ignorant, and have not the knowledge and skill to use it. But when the patent is expired the King cannot make a new grant thereof.

The patent monopoly system, by encouraging manufacturers and others to publish their inventions rather than to maintain developments secret, serves a useful function in increasing the general stock of scientific knowledge. In many new fields of technology the major part of the relevant publications —in some cases the only publications—comprise patent specifications.

The grant of a patent is restricted to the territorial limits of the Government giving the grant; thus a United Kingdom patent gives a monopoly only within the territory of the United Kingdom, a French patent only within the territory of France, and so on. In consequence it is necessary to file patent applications for all those territories where it is desired to obtain patent protection. There is at the present time no such thing as a universal patent, and it appears unlikely that one will be achieved within the foreseeable future*. However, various international arrangements, such as the International Convention for the Protection of Industrial Property, simplify the task of obtaining patents in other territories. The terms of the International Convention provide *inter alia* that, where a patent application is filed in any one of the territories subscribing to the convention, an application filed within twelve months from that date, providing it is the first application in the convention country, may claim priority from that application date.

PATENTABLE INVENTIONS

What comprises a patentable invention varies from country to country. In the United Kingdom inventions which are patentable are defined[3] in the following way: ' "Invention" means any manner of new manufacture, the

* Since this paper was written considerable advances have been made towards establishment of a combined patent system for the Common Market countries. Although not yet in sight such a patent may well be achieved in the next five years, and would possibly extend to countries not now within the Common Market (e.g. United Kingdom). Once there is a Common Market patent, a universal patent will be within the bounds of probability.

subject of letters patent and grant of privilege within Section 6 of the Statute of Monopolies, and any new method or process of testing applicable to the improvement or control of manufacture, and includes an alleged invention.' The latter half of this definition referring to any new method or process of testing is reasonably clear, but the main part of the definition may only be understood by reference to the archaic Statute of Monopolies. Section 6 of this Statute, which as noted was passed in 1623, reads as follows:

> Provided also (and be it declared and enacted) that any declaration before mentioned shall not extend to any letters patent and grants of privilege for the term of fourteen years or under, hereafter to be made, of the sole working or making of any manner of new manufactures within this realm, to the true and first inventor and inventors of such manufactures which others at the time of making such letters patent and grants shall not use, so as also they be not contrary to the law or mischievous to the State, by raising prices of commodities at home, or hurt of trade, or generally inconvenient.

By this it is meant that an invention to be patentable must comprise a manner of new manufacture; in other words, the invention must relate to a manufacture and it must be new.

Both of these conditions present complications in interpretation, the more so as the limitations are of an arbitrary character. In essence the word 'new' implies that the invention was not published or generally known at the date of application; this, however, is a simplification of the exact position (see p. 6). To determine what comprises manufacture, it is necessary to take into account the precedents handed down by the courts. In the most general pronouncement in this connection Mr. Justice Morton (as he then was) set out the criteria to be adopted in the following words[4]: 'In my view a method or process is a manner of manufacture if it: (*a*) results in the production of some vendible products, or (*b*) improves or restores to its former condition a vendible product, or (*c*) has the effect of preserving from deterioration some vendible product to which it is applied.'

In this decision Mr. Justice Morton also made the following comment: 'There are many ingenious methods or processes which can be stated in the form of a claim but which result in no vendible product and are accordingly incapable of protection by a patent. I might instance methods of diagnosing or treating diseases, methods of chemical or physical testing, methods of repelling hostile aircraft, and so forth.'

It will be noted that, in accordance with the definition of invention in the Patents Act, methods of testing are now patentable, if these are applicable to the improvement or control of manufacture.

Essentially all processes and products of an industrial character are patentable. Thus any new chemical process is patentable, and so is any new chemical compound. Furthermore, any new composition, be it a pharmaceutical composition or a soldering flux, is also patentable. However, a new chemical compound which is found in nature is not patentable[5], and a pharmaceutical or food composition is not entitled to protection if its properties are merely the aggregate of the known properties of the components[6].

By tradition, in the United Kingdom inventions concerned in the medical and agricultural fields are not patentable. In other words, such processes are

not considered to comprise a manner of manufacture. As there is no precise definition laid down in the Act, the rather arbitrary limits which have been adopted, and which follow the various precedents, present a complex and anomalous picture. Thus, for example, fermentation processes are held to comprise a manner of manufacture and to be patentable[7], although such processes necessarily involve the growth of a living organism. Further, while a method for the treatment of soil to prevent subterranean fires[8] and a method for the treatment of soil with soil-conditioning agents[9] have been found to be patentable, a method for the treatment of the soil with insecticides to obtain insect-free soil[10] has been held to be unpatentable.

Inventions which have always been held to be unpatentable for the reason that they do not comprise a manner of manufacture include all methods concerned with the treatment or control of most living organisms, including human beings, animals and plants. Thus all the following have been held to be unpatentable: process for extracting lead from the human body[11]; process for the production of lupin seeds of high oil content by selective cultivation[12]; the defoliation of cotton before harvesting[13]; the treatment of sheep with thyroxine compounds to increase wool yield[14]; the artificial induction of hereditable variations in the properties of micro-organisms in general by electric shock treatment[15] (although the application of this technique to a particular organism—lactic streptococcus—is patentable); the stimulation of the growth of plants in greenhouses by means of carbon dioxide[16].

An interesting anomaly is that a method for producing a permanent wave in hair on the human head[17] has been considered to be patentable on the ground that 'a woman's hair is a vendible article which is not only capable of being sold on the head but has been so sold frequently abroad and less frequently in this country'. It thus appears that the use of an external remedy to produce curly hair would be patentable, but the use of an internal remedy for this purpose would be unpatentable.

Apart from this question of the patentability of inventions in the fields of agriculture and medicine, for an invention to be patentable it must be something more than a scheme or plan, however ingenious, even though this is put forward in a concrete shape[18]. For example, methods of marking buoys as an aid to navigation[19], systems of musical notation[20] and architects' plans[21] are not patentable. Sets of cards or other combinations of parts to form a game[22] are patentable, while mere printed sheets and the like are not, unless the object of the particular arrangement of the words on the sheet is to serve a mechanical purpose[23].

In connection with any inventions in the pharmaceutical field, it is possible to protect a medicament itself where this comprises either a new chemical compound or a new composition, and also to protect methods for the production of the pharmaceutical compound where such methods are novel. Protection on these lines provides sufficient protection in those cases where the invention is concerned with a new compound; however, if the discovery is that a known chemical compound possesses valuable therapeutic properties, unless that compound requires to be formulated in a special new composition, no effective protection can be obtained for this invention. This limitation in the protection which may be obtained for a pharmaceutical

invention may have a serious effect on research since commercial concerns prefer to direct their research to new chemical compounds where full protection may be obtainable, than to known chemical compounds where the protection which they may obtain will either be limited or non-existent.

Overseas it is generally true that an invention to be patentable must relate to a manner of new manufacture; however, the definition followed in each territory varies rather widely both as to what comprises a manner of manufacture and as to what is new. Thus in some countries it is possible to protect chemical products *per se*, pharmaceutical products and medical processes; whereas in other countries it is impossible to protect inventions of any of these types. However, it is possible to protect chemical processes in all countries.

The U.S.A. has the broadest definition as to what comprises an invention, and the definition reads as follows[24]: 'Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter or any new and useful improvement thereof, may obtain a patent therefor.' This definition covers almost all inventions—including medical and pharmaceutical processes. At the other extreme, in most European countries it is only possible to protect an industrial process or product.

On the issue of novelty all countries require the invention to be new, but exactly what is meant by this varies considerably. In some territories publication in any country of the world is effective to destroy novelty; in other countries publication only in the territory in question may be relevant to destroy novelty. Yet another issue relevant to the question of novelty is whether or not, in order to deprive an invention of novelty, it is permissible to combine a number of publications together to form what is known as a 'mosaic' of documents, within which a disclosure may be found, and also in what way and to what extent obviousness may be relied upon. The term 'obviousness' is self-explanatory and covers the circumstances where an invention is not disclosed in the literature but is obvious from what is known or published. In some countries, *e.g.* U.S.A., Netherlands, Germany, the two issues are considered together; in others such as the United Kingdom they are dealt with separately.

In order to give details of what may be patentable in some leading countries, *Table 1.1* has been prepared indicating whether or not it is possible to claim chemical products *per se* (column 2), pharmaceutical formulations *per se* (column 3) or medical processes (column 4), and on the issue of 'novelty' whether the requirement of novelty is satisfied merely by absence of publication in the territory in question or anywhere in the world (column 5). It will be seen from *Table 1.1* that in Italy, for example, it is not possible to obtain any protection for pharmaceutical compounds; this applies not only to the compounds *per se*, but also to methods for their production. Italian manufacturers may therefore manufacture drugs patented in other countries. A somewhat similar situation exists in Canada where it is only possible to obtain manufacturing process claims in respect of chemicals useful as food or medicine; additionally in any such case it is possible to obtain a compulsory licence.

In all countries it is possible to secure claims to processes for the production of chemical compounds. In most countries where claims to chemical products

are not allowable, the process claim gives effective cover for the product of the process.

Table 1.1. The scope of patent protection in various countries

Country	*Claims to chemicals allowable*	*Claims to pharmaceutical compositions allowable*	*Claims to medical processes allowable*	*Novel if not published in:*
Argentina	Yes	No	No	World
Australia	Yes	Yes	No	Australia
Austria	No	No	No	World
Belgium	Yes	Yes	Yes	World
Brazil	No (a)	No (a)	Yes	World (e)
Canada	Yes (b)	No (c)	No	World (f)
Ceylon	Yes	Yes	Yes	Ceylon
Chile	No	No	No	World
Denmark	No	No	No	World
Finland	No	No	No	Finland
France	Yes	Yes	No	World
Germany	No	No	No	World
Greece	Yes	No	No	Greece
India	No	Yes	No	India
Italy	Yes (b)	No	No	World
Japan	No	No	No	Japan
Netherlands	No	No	No	World
New Zealand	Yes	Yes	No	New Zealand
Norway	No	No	No	World
Pakistan	Yes	Yes	No	Pakistan
Portugal	Yes	No	No	World
Rhodesia and Nyasaland, Federation of	Yes	Yes	No	World
South Africa, Republic of	Yes	Yes	Yes	World
Spain	Yes	No	No	World
Sweden	No	No	No	World
Switzerland	No	No	No	World
United Kingdom	Yes	Yes	No	United Kingdom
U.S.A.	Yes	Yes	Yes	World
U.S.S.R.	No	No (d)	No (d)	World

Notes: (a) Providing that intrinsic properties, analysis or other examination reveals the method of manufacture. (b) Chemicals used for food or medicine and processes for their manufacture are not patentable. (c) Unless made by a non-chemical process. (d) But can be covered in author's certificate. (e) One year before application date. (f) Two years before application date.

PROCEDURE FOR SECURING A UNITED KINGDOM PATENT

General

In the United Kingdom a patent application is made by the filing of an application form (Patents Form No. 1) accompanied by a specification, either provisional or complete. The application form sets out the name, address and nationality of the applicant, the title of the invention, the name, address and nationality of the inventor, and the address for service in the United Kingdom, and it is signed by the applicant. Where the inventor is not the applicant or a co-applicant, it is also necessary to obtain the inventor's signature to a declaration of assent, which may be included on the application form or filed separately. The signature of the inventor does not need to be obtained on filing and may be presented within three months of the date of filing[25]. At the option of the applicant, the application may be accompanied

on filing either by the complete specification or the provisional specification. Where the application is accompanied by a provisional specification, the complete specification has to be filed within twelve months (or fifteen months on payment of additional fees) of the application date[26].

This procedure for filing a provisional specification followed by a complete specification is to be found only in the United Kingdom, in South Africa and some Commonwealth countries including India, Pakistan, Australia and New Zealand, and dates from the Act of 1852. The formal requirements are not the same for the two specifications, and this procedure is of substantial advantage to inventors in that the year between the date of the provisional and complete specifications may be used for the development and modification of the invention, and all such additional information may be included in the complete specification.

In the United Kingdom one of the most important features of a patent is its date, and this is determined exclusively by the date of filing of the provisional or the complete specification. It is impossible to antedate an application by reason of circumstances which take place before the actual filing of an application, except where a date is claimed from an earlier application, as for example with a divisional application. This is in contrast with practice in the U.S.A. where it is possible to claim the date of making the invention, established from the date of conception and reduction to practice of the invention (see p. 16).

The provisional specification has merely to describe the invention; the requirements for the complete specification are set out in the following terms[27]:

> Every complete specification (*a*) shall particularly describe the invention and the method by which it is to be performed; (*b*) shall disclose the best method of performing the invention which is known to the applicant and for which he is entitled to claim protection; and (*c*) shall end with a claim or claims defining the scope of the invention claimed.

Therefore, as soon as an invention is made, an application accompanied by a provisional specification should be filed, and further work should be completed within the following year to provide the information required for the filing of the complete specification.

The relationship of the complete specification to the provisional specification has been radically changed by the 1949 Patents Act. Before this Act, the complete specification was required to follow closely the subject of the provisional specification, and the only additional matter which might be included in the complete specification was governed by the doctrine of 'legitimate development'. In other words, the inventions described in both specifications had to be substantially the same, and the additional matter was essentially restricted to mechanical or chemical equivalents or modifications of a minor character.

This was changed by the 1949 Act, and under present practice the complete specification may be different from the provisional specification, and perhaps relate to a different invention. The examiner is not required to compare the two specifications, except for the purpose of establishing the effective date of a claim, but no doubt objections would be raised if there were no relationship

between the provisional and complete specifications. The claims contained in a complete specification have the date of this specification unless: (*a*) any claims are fairly based on the matter disclosed in a provisional specification, when the claims have the date of the provisional specification; (*b*) any claims are fairly based on the matter disclosed in an earlier application for protection in another country covered by the convention, when the claims are entitled to the date of that original application[28].

It is possible for a complete specification to be based on more than one provisional specification or on more than one convention application, and the joining of such applications together is described by the term 'cognation'. Thus, when following an invention an application accompanied by a provisional specification is filed, a further development made before the date of completion may be covered in a second application and provisional specification, provided that within twelve months from the date of the first application a complete specification is filed, based on matter contained in the two provisional specifications. In such a case the claims on the complete specification based on the matter disclosed in the first application would be entitled to the first date, and the claims in the complete specification based on the second application would be entitled to the second date. However, a claim may only have one priority date so that any claim which is drafted so as to relate to matter disclosed in the first application, as well as that appearing for the first time in the second application, will only have the date of the second application, or perhaps only the date of the complete specification, depending on the form of the specifications and the claims.

The priority date of the claims of the complete specification is only brought into consideration when there is a conflict concerned with an 'intervening' publication between the date of the relevant provisional specification and the complete specification, or an 'intervening' claim having an effective date between these dates. In such a case the claims are compared with the relevant provisional specification to determine if these claims are entitled to the earlier date, thus antedating the claims to a date earlier than that of the intervening publication or claim.

Examination

When the complete specification has been filed, the application is examined in the Patent Office. The examination is in respect of its formal character and includes a review of the specification from an editorial point of view and a novelty examination among the patents published during the previous fifty years. In the novelty search, the examiner is not required to consult the relevant technical literature, but he may do so at his discretion. Although the Patent Office Library is one of the leading technical libraries in the country, the Patent Office examining staff make little use of the facilities available, and in most cases the novelty search is restricted to United Kingdom patent specifications. Any objections arising from this examination for novelty concerning the formal aspects of the specification are reported to the applicant, who must amend the specification so as to meet these objections, or persuade the examiner that the objections are without substance. The powers of the examining staff of the Patent Office are limited, and in the

important novelty examination, the Office may only refuse an application where there is a clear and exact prior publication of the invention. The Office is not entitled to consider whether or not the invention is obvious, nor are they entitled to consider the issue of utility. Because of these limitations in the power of the Patent Office with regard to examination the Office is forced to accept applications in all cases where there is no exact prior publication, regardless of the level of invention.

When the examination has been successfully concluded, the application is accepted and up to this time the contents of the specification are known only to the Patent Office. The application must be placed in order within three years from the date of the complete specification, plus an extra three months on the payment of special fees, or it is declared void[29].

For a period of three months after the date of its acceptance, the application is open to opposition, and if none is filed, letters patent are sealed thereon. If an opposition is filed, the sealing of the patent is delayed until the opposition proceedings are overcome.

Opposition

Opposition proceedings are heard at the Patent Office before the Comptroller, and provide a simple and cheap method by which persons interested may contest the grant of the patent. The patent application may be opposed on any one of eight grounds[30]:

(*a*) The patent applicant obtained the invention from the opponent.

(*b*) The invention was published before the priority date of the relevant claims.

(*c*) The invention is claimed in another patent having an earlier priority date.

(*d*) The invention was used in the United Kingdom before the priority date of the claims.

(*e*) The invention is obvious in the light of publications available before the priority date.

(*f*) The invention is not a manner of new manufacture.

(*g*) The complete specification does not sufficiently and fairly describe the invention or the method by which it is to be performed.

(*h*) In the case of a convention application, the application was not filed within twelve months from the first application in a convention country. The words 'priority date' are used to indicate effective date.

In the first case it is not a ground for opposition that the applicant went abroad, obtained the invention from another there, returned, and filed the application in the United Kingdom. To be relevant for opposition purposes, the obtaining may have taken place either in the United Kingdom or overseas, but where overseas, this must have been based upon information derived in the first place from the United Kingdom.

In the case of prior publication, publications which are relevant include patent specifications published in the fifty years before the date of the complete specification as well as any other document published in the United Kingdom before the relevant priority date. An effective publication for opposition purposes must comprise a 'published document'; the meaning of

'published' is not capable of simple definition, but any document available in a public library is 'published'[31]. This does include a thesis deposited in a university library, and which is available for reading by students and others.

In connection with prior use in the United Kingdom, to be relied upon, this prior use must not be a secret one. But it does not follow that the use must be a public use; it is necessary only to show that it was not a secret one. Thus, while experiments carried out in a research laboratory would be regarded as a secret use, a process operated in a factory where no special secrecy precautions were observed, and where visitors were commonly shown round, would amount to use which is not secret. Between these two examples it is not possible to draw a clear dividing line between what does and what does not amount to secret use. In an early case[32] the objection was that the applicant, Dollond, was not the inventor of the claimed method of making new object glasses, but that a Dr. Hall had made the same discovery before him. However, it was held that in as much as 'Dr. Hall had confined it to his closet', and the public were not acquainted with it, Dollond was to be considered the inventor. It follows, therefore, that for such a prior use to be relevant the public must have had some opportunity of acquainting themselves with the discovery, although it is not relevant whether any member of the public has, in fact, done so.

In connection with the ground of obviousness, a patent application may only be refused where the invention is 'obvious and clearly does not involve any inventive step'[33]. In other words, the ground of opposition is not that the invention is obvious, but that the invention is clearly obvious. This ground for opposition was new in the 1949 Act, and enabled lack of subject matter to be relied upon in opposition proceedings for the first time. It was suggested to the Swan Committee, which between 1944 and 1947 considered modifications of the then existing Patents Act, that the Patent Office examining staff should consider both novelty and subject matter in their examination of patents. This is so in many patent offices overseas, and as a result the patents ultimately granted are more likely to be valid than is at present the case in the United Kingdom. It was decided, however, that the powers of the Patent Office in this respect were not to be extended, and this amendment was the only concession made in this connection. In order to succeed on this ground, it is necessary to show that the invention is clearly obvious, and this takes much of the sting out of this ground. Nevertheless, it is still possible to succeed on this ground where the invention is merely a simple variant of what was previously known.

It is also possible to file opposition proceedings before the Comptroller after the patent has been granted and these are commonly termed belated opposition[34]. The grounds which may be relied upon are the same as those for normal opposition proceedings. However, if there is any action for infringement or proceeding for revocation in any court, such belated opposition proceedings may only take place with the leave of the court.

Fees

The Government fees involved in the filing of an application are comparatively small. The cost of an application accompanied by a provisional

specification is £1; the cost of filing the complete specification is £10, and that of sealing the letters patent is £3, so that the total fees to grant amount to £14. After grant, renewal fees fall due on an annual basis, and increase with the life of the patent. The life of the patent is counted from the date of the complete specification, and no renewal fees fall due in respect of the first four years. The first fee is in respect of the fifth year and amounts to £5, and thereafter increases as follows:

Sixth year	£6
Seventh year	£8
Eighth year	£10
Ninth year	£12
Tenth year	£14
Eleventh year	£16
Twelfth year	£17
Thirteenth year	£18
Fourteenth year	£19
Fifteenth year	£20
Sixteenth year	£20

These fees[35] which are payable up to 1962 may be increased in the future and the Board of Trade have the power to increase them up to the statutory maximum. Even so, the fees involved are not very substantial.

PROCEDURE FOR SECURING AN OVERSEAS PATENT

General

A United Kingdom patent extends only to this country and to the territories comprising Basutoland, Bechuanaland and Swaziland. If it is desired to secure patent protection in any territory overseas, it is therefore necessary to file an application for protection in that territory.

No person resident in the United Kingdom may file a patent application overseas unless either an application for patent of the same invention has been made in the United Kingdom at least six weeks before the application overseas and no directions as to secrecy have been given, or written permission has been obtained from the Comptroller of the Patent Office[36]. These regulations are designed to prevent the publication of inventions which are considered relevant for defence purposes. Providing these requirements are fulfilled, applications may be filed in any overseas territory.

The majority of overseas territories subscribe to the International Convention which provides that where an application is filed in any one of the convention territories, an application filed in any other of the convention territories within one year from the first date of application is entitled to the date of that first application. This means that where an application accompanied by a provisional specification or a complete specification (if this is filed in the first place without a provisional specification) is filed in the United Kingdom, the applicant has one year in which to decide whether he wishes to file applications overseas, and if applications are filed in any convention country within twelve months these applications are entitled to the date of the basic British application.

In some countries which subscribe to the convention, only single priority dates are allowed, but in the majority of convention countries it is possible to claim both multiple and partial priorities. *Multiple priority* means that a single application may claim dates from more than one application filed in convention countries, although such applications must be filed within the twelve-month period. *Partial priority* implies that claims may be based both on matter disclosed in the convention application and matter described for the first time in the application in the country in question. India does not subscribe to the International Convention, but there is a reciprocal arrangement between India and the United Kingdom which fulfils substantially the same purpose.

The provisions of the law in countries overseas vary considerably from country to country in the form of the specification, the nature of the claims, whether or not the specification is examined for novelty, whether or not the application may be opposed after acceptance, the term or life of the patent, and whether or not renewal fees have to be paid in order to maintain the patent in force. *Table 1.2* sets out in simple form the practice followed in some of these respects in some of the more important countries.

The procedure adopted in overseas territories varies so widely that it is impossible to describe the procedure adopted in each country within any reasonable scope. Brief notes are therefore given on the procedure in three countries, namely the U.S.A., France and Germany, which differ from each other in many important respects.

United States of America

In the U.S.A. the formal requirements for filing a patent application have to be strictly adhered to or the filing date may be refused; where, for example, the papers are not properly ribboned, the filing date may be allowed but fresh papers are required. The specification and the claims must be in a special form. These must be accompanied by an oath, petition, and Power of Attorney duly executed before a Notary Public or United States Consul, and all the documents ribboned together and the ribbons taken under the seal of the witnessing officer. Where the application is signed outside the U.S.A., the signature of the Notary Public must also be legalized by a United States Consul. Additionally the application in the U.S.A. requires to be filed in the name of the inventor or inventors, who must execute the application documents. If an assignment is filed before grant of the patent, the patent is granted to the assignees.

After the filing, the application is subjected to examination by the Patent Office. In making his examination, the United States examiner is entitled to consider the allowability of the application in all respects, including not only novelty but the more difficult grounds of obviousness and utility[24,37]. Furthermore, it is accepted practice in the U.S.A. that a number of prior documents may be read in conjunction with one another in order to determine whether or not a claim is novel. However, such references should relate to the same art[38]. This reading together of documents is usually referred to as a 'mosaic of documents' and it is not permissible in the United Kingdom. The examination before the United States Patent Office is severe, not so much

Table 1.2. Summary of characteristics of United Kingdom and overseas patents

Country	*Convention*	*Novelty examination*	*Opposition proceedings*	*Printing*‡	*The 'date' of the patent is date of:*	*Term* years	*Renewal fees payable*
Argentina	No	Yes	No	No	Grant	5, 10 or 15	Annually
Australia	Yes	Yes	Yes	Yes	Application or priority	16	Annually after 4th year
Austria	Yes	Yes	Yes	Yes	Publication	18	Annually
Belgium	Yes	No	No	Yes	Filing	20	Annually
Brazil	Yes	Yes	Yes	No	Grant	15	Annually
Canada	Yes	Yes	No	Yes	Grant	17	None
Ceylon	Yes	Yes	Yes	No	Application or priority	14	Annually after 4th year
Chile	No	Yes	Yes	No	Grant	5, 10 or 15	None
Denmark	Yes	Yes	Yes	Yes	Filing	17	Annually
Finland	Yes	Yes	Yes	Yes	Filing	17	Annually
France	Yes	No	No	Yes	Filing	20	Annually
Germany	Yes	Yes	Yes	Yes	Filing	18	Annually
Greece	Yes	No	No	No	Application	15	Annually
India	No*	Yes	Yes	Yes	Application or priority	16	Annually after 4th year
Italy	Yes	No	No	Yes	Filing	15	Annually
Japan	Yes	Yes	Yes	Yes	Publication	15	Annually
Netherlands	Yes	Yes†	Yes	Yes	Grant	18	Annually
New Zealand	Yes	Yes	Yes	No	Application or priority	16	At 4th, 7th, 10th, and 13th years
Norway	Yes	Yes	Yes	Yes	Filing	17	Annually
Pakistan	No*	Yes	Yes	No	Application or priority	16	Annually after 4th year
Portugal	Yes	No	Yes	No	Grant	15	Annually
Rhodesia and Nyasaland, Federation of	Yes	No	Yes	No	Complete specification	16	Annually
South Africa, Republic of	Yes	No	Yes	No	Complete	16	Annually after 3rd year
Spain	Yes	No	No	No	Grant	20	Annually
Sweden	Yes	Yes	Yes	Yes	Filing	17	Annually after 4th year
Switzerland	Yes	Yes	Yes	Yes	Filing	18	Annually
United Kingdom	Yes	Yes	Yes	Yes	Complete specification	16	Annually after 4th year
U.S.A.	Yes	Yes	No	Yes	Grant	17	None
U.S.S.R.	No	Yes	No	Yes	Application	15	Annually

* Reciprocal arrangement with United Kingdom.
† Under new law only when demanded.
‡ Some countries do not make available *printed* copies of the specifications, but photocopies of the original specification are usually available.

because of the scope or efficiency of the search, but on account of the wide powers of the examiner. The examiner may search all available literature including not only United States but also foreign patent specifications and the appropriate literature, and with regard to patent specifications he is not limited to the fifty-year period as is the case in the United Kingdom.

The United States examiner frequently takes a strong stand on the issue of obviousness which may be difficult to overcome, particularly where a mosaic of references is relied upon. In many such cases it is necessary, in order to persuade the examiner of the patentability of the claims, to file the results of experiments comparing the invention with the prior art so as to show that there is novel and unexpected effect.

Another difficult and serious objection which may be raised by the United States examiner is that on the issue of utility, which is particularly relevant to chemical cases. This issue is met in many countries, and the essential principle behind the objection is that anyone may draw out new chemical formulae on paper, but that an invention has only been made when a use has been found for these chemicals, thus providing a reason why they should be made. This is true even in the United Kingdom, although here the requirements on the issue of utility are mild compared with those in the U.S.A.

It is current practice in the U.S.A. to require, in the case of any new chemical compound, a clear statement of utility comprising an identification of a definite use for the compounds claimed. It is not sufficient to state that the chemicals find application as intermediates, but it is necessary to identify a definite use. If the new compound finds utility as a chemical intermediate, it is necessary to identify a specific process employing that intermediate and leading to a compound of clear and definite utility. At one stage some examiners in the United States Patent Office required that the intermediates *per se* should have utility; however, this practice has now been overruled[39].

In the case of new chemical compounds where the utility is pharmaceutical, the requirements of United States practice are now particularly stringent. Until comparatively recently it was sufficient to indicate that such compounds possessed therapeutically useful properties, coupled with an indication of the nature of the use to which the compound might be applied. This is now no longer sufficient and in any case where utility in the pharmaceutical field is claimed for a new chemical compound it is necessary to provide evidence showing the value of that compound. It is doubtful whether results of *in vitro* trials are now sufficient, and it is desirable that the results of *in vivo* trials should also be included in the specification. In the case of pharmaceuticals, in general, the Patent Office requires clinical results[40]; these data, however, need not have been in the specification as filed. It is not possible to provide an indication as to what may be required by the United States Patent Office as the examiner has arbitrary powers and one examiner may view the matter rather differently from another. However, if the examiner for any reason doubts the usefulness of the new compound, he will require substantial proof in order to be satisfied on this issue (see Addendum, p. 42).

A further issue is to what extent evidence of clinical trials requires to be included in the United States patent specification irrespective of the objections

which may be raised by the examiner. The danger here is that if there is an insufficient disclosure in the specification of the use of the compound and if the examiner is not satisfied on the issue of utility he may reject the application and refuse to accept new evidence on this question. However, if the specification contains a statement of utility and a record of some trials, even if these should only be *in vitro*, where this is appropriate, there is no doubt that the examiner should ultimately accept the application on being satisfied with the presentation of further experimental evidence, which may include the results of clinical trials.

The statutory requirements for application in the U.S.A. are that: 'The specification shall contain a written description of the invention and of the manner and process of making and using it in such full, clear, concise and exact terms as to enable any person skilled in the art to which it appertains or with which it is most nearly connected to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention.' These requirements[41] have to be coupled with the definition of 'invention' which is 'any new and useful process, machine, manufacture or composition of matter'.

Practice on utility in the U.S.A. stems from the inclusion of the word 'useful' in the statutory definition of invention. Additionally the Patent Office have defended their attitude on the basis that they stand in the public interest and should not grant patents, particularly in the pharmaceutical field, where there is any doubt of their utility. Nevertheless, providing the specification as filed contains a statement of the utility coupled with some evidence, and is otherwise sufficient, the examiner should accept the evidence filed subsequently in support of the utility of the application.

One aspect of United States practice which is rather difficult to appreciate is the citation of co-pending applications, either alone or as part of a mosaic of documents, to deprive an application of novelty. Such co-pending applications may be by the same applicant where the actual inventors are different. This is because the statutory requirement is that an invention at the effective date of the application must not be known or used by others in the U.S.A. Consequently the determination of novelty extends beyond what is published and covers what is known in the U.S.A.

Another unique feature of United States practice is that within certain limits the first inventor is the person who first made the invention in the U.S.A. rather than the person who first filed an application for a patent to cover that invention. This follows the practice that the effective date of a United States application may be antedated to the actual date of making the invention; according to circumstance this may be the date of 'conception' or of 'reduction to practice' of the invention within the U.S.A. Reduction to practice means the actual carrying out of the invention. Therefore, so far as any United States applicant is concerned, he does not necessarily require to file his application as soon as he has made the invention but may continue development work until such time as he feels in a position to do so, in the knowledge that in the U.S.A. his application may be antedated in this way. This is not, of course, the position overseas and any corresponding foreign application, if for example filed under the International Convention, would only be entitled to the date of filing of the application in the U.S.A. For this

reason, therefore, the United States applicant, although he is in a preferential position in the U.S.A., may find himself at a disadvantage overseas.

In the U.S.A., when the examination has been successfully concluded, the application is accepted, and after payment of the final fee the patent is granted and published. The actual dates of grant and publication are the same. The United States patent remains in force for seventeen years from the date of grant, without payment of any renewal fees. There are no provisions in the U.S.A. for opposition proceedings, and the only analogous proceedings are the 'interference' proceedings. These concern the determination of relative priority between two or more applications in the name of different parties covering the same invention.

An interference may be declared either before or after grant of the patent, and where an interference is instituted before grant the examiner may ask for the inclusion of a claim identical with a claim in the conflicting patent or application, for interference purposes. The interference proceedings are designed to determine which inventor first made the invention and first reduced it to practice. In the case of inventions made in the U.S.A., the effective date of the application may be antedated by establishing the date on which the invention was made and reduced to practice. Where the invention is made abroad the application may only be antedated, in the case of a convention application, to the date of the application for protection in the convention country, or in certain cases to the date of the introduction of the invention into the U.S.A. The interference proceedings thus amount to a determination of effective priority date between conflicting applications.

In the U.S.A., on account of the wide definition of invention, it is possible to claim not only chemical products *per se*, but also pharmaceutical products and (following recent practice) medical processes, *i.e.* processes for the treatment of human beings with drugs. The U.S.A. is one of the few countries where medical processes are patentable.

Unlike many other countries, the patentee is not required to operate the invention in the U.S.A.[42], and there are no provisions for the grant of compulsory licences.

The cost of filing a patent application in the U.S.A. is $30; the grant fee is $30. As there are no renewal fees, the total official cost is only $60; however, prosecution costs are generally rather heavy in view of the difficulties met in the examination of the application.

France

The formalities attendant upon the filing of an application in France are the minimum; any person may file the application and it is not necessary to name the inventor. As in other countries, the specification must describe the invention, but it does not contain any claims, and concludes with what is termed the 'résumé'. The résumé does not define or limit the scope of the monopoly which the patentee may secure, but is a general indication of the scope of the patent.

In France the application is not subjected to any novelty examination, and after filing it is not possible to make any amendment to the specification. Providing the formal requirements are fulfilled, the application passes

automatically to grant and there are no provisions for opposition proceedings. After grant, if the patent is involved in litigation, the court will consider the state of the prior art and determine the exact contribution made by the invention, and in accordance with this it will decide the scope of the protection to which the patentees are entitled. This is essentially different from practice, for example, in the United Kingdom and the U.S.A., where the scope of the monopoly claim is to be found in the words employed in those claims.

It is now possible to claim chemical products *per se* and also pharmaceutical products, but medical processes are not patentable. Compulsory licences may be granted in France in the case of abuse of monopoly (*e.g.* non-use).

Germany

The formal requirements for an application in Germany are slightly more than those in France, but far simpler than those in the U.S.A. Any person may apply for a patent in Germany, but if the inventor is not the applicant, he must be named, and a statement included in the application that the invention has been assigned by the inventor to the applicant. The specification must describe the nature of the invention and conclude with a statement of claim. After filing, the application is subject to an examination both as to novelty and inventive merit. The German examiner may rely upon patent specifications or printed literature published anywhere in the world not more than 100 years previously.

In Germany it is not possible to claim chemical compounds *per se* but only to claim a process for making a chemical compound; however, mixtures of chemical compounds are patentable, providing these are not pharmaceuticals. For instance, alloys are patentable, but pharmaceutical products, foods and medical processes are not.

The utility of new chemical compounds is also important in Germany, although for not quite the same reason as in the U.S.A. In Germany it is not possible to claim a process for the production of a chemical compound, which is analogous to known methods for the production of similar compounds (*i.e.* where the method may be regarded as obvious) unless the product possesses unexpected properties. In order to support an analogy process claim, it is necessary to provide an identification of the utility of the product. A corollary to this is that, once a compound is known, it is only possible to protect processes for its manufacture which are not analogous to known methods. In consequence it is not possible to protect a process for making an intermediate, unless the method is not analogous to known methods or the intermediate *per se* possesses unexpected properties.

On completion of the examination the application is accepted and published, and opposition may be lodged against the application within three months from the date of publication. The opposition proceedings are somewhat similar to those in the United Kingdom, and on their conclusion the patent is granted. Since the life of the patent is eighteen years from the date of filing, a patent which has a lengthy examination and is then opposed may not have many years of its life left. However, patent protection commences with the date of publication, and the applicant can sue infringers after this

date. Where such an infringement action is commenced, the hearing may be suspended until the grant of the patent.

The claims of a German patent are interpreted in the light of the disclosure and having regard to the inventive merit of the invention. Where the invention represents a large advance in the field, particularly where the advance is such that the patent may be regarded as a 'pioneer patent', the claims are interpreted broadly to the benefit of the patentee. Correspondingly the claims to an invention of small inventive merit are interpreted narrowly.

Compulsory licences may be granted in Germany where there is an abuse of monopoly (*e.g.* non-use) or in the case of a dependent patent, where the proprietor of the main patent refuses to grant a licence to the dependent patentee, thus preventing the working of the latter's invention.

THE SPECIFICATION

The patent monopoly system is based on contract between the Government and an inventor, on the one hand the inventor disclosing his invention and on the other hand, the Government granting to the inventor a monopoly for a limited period. In consequence, the legislation provides that the inventor must give a clear and precise disclosure of his invention in such a way and in such terms as to enable any person, skilled in the art, on reading the specification to put the invention into practice. There are a number of other requirements which must be met, but this is the basic one to be fulfilled by patent specifications in most countries.

In the United Kingdom there are two types of specification, provisional and complete, and as these differ both in form and purpose they are considered separately.

The Provisional Specification

The provisional specification is not examined and serves merely as a document of record for establishing the priority date of the relevant claims. The only statutory requirement[43] is that it 'shall describe the invention and shall begin with a title indicating the subject to which the invention relates'. Apart from this, its function is to establish a priority date for the corresponding complete specification and for any corresponding applications which are filed overseas under the International Convention. Therefore, from a practical point of view, the form of the specification should fulfil not only the requirements of United Kingdom practice, but also those in respect of practice overseas.

To ensure that the claims in the complete specification ultimately filed will be entitled to priority from the provisional specification, the latter should be drafted in as broad terms as are consonant with the invention which has been made. For example, if the discovery is that in a certain reaction sodium iodide acts as a catalyst greatly increasing the yield of the desired product, then it is desirable that the provisional specification should cover the use not only of sodium iodide, but also of related compounds which it is reasonable to expect will also be useful for this purpose. Thus, in the absence of any facts indicating the contrary, the scope of the provisional specification should

cover the use at least of alkali metal halides, and perhaps even halide salts in general. The specification must also describe specifically the use of sodium iodide and contain an indication that this is a special feature of the invention. On the other hand, if the provisional specification refers to sodium iodide, and it is subsequently discovered that other alkali metal iodides behave similarly, and this is referred to for the first time in the complete specification, any claims covering the use of alkali metal iodides other than sodium iodide will be entitled to protection only from the date of the complete specification.

It is also desirable for the provisional specification to contain at least an example of one embodiment of the invention. This is not required for United Kingdom practice, but in the case of a corresponding application filed subsequently in the U.S.A., that Patent Office may refuse to sustain the claim to priority where the provisional specification from which the date is claimed does not contain a specific example. Further, with an invention relating to an apparatus or other device which is capable of illustration, it is desirable that the provisional specification should contain a drawing of that apparatus or device, together with related description.

There is a classic case[44] where a British applicant filed an application in the U.S.A. claiming priority from a British provisional specification. The invention concerned a television receiver, and the provisional specification did not contain a drawing of the television receiver layout, although the United States application did so. An interference was declared with another application filed by a United States applicant and having a date between that of filing of the British provisional specification and of the corresponding application in the U.S.A. In this action the United States Patent Office refused to accord to the British applicant the priority date from the provisional specification and in consequence his application was refused. The ground for this decision was that the disclosure in the British provisional specification was considered too meagre and incomplete to justify the grant of a patent thereon, and in consequence was insufficient to establish the entitlement to the earlier priority date. In this case the British applicant provided evidence to the effect that a fourteen-year-old boy was able without difficulty to make a television receiver on the basis of the information in the provisional specification, but the United States Patent Office refused to accept this evidence. The moral, therefore, is that provisional specifications should always contain as full and as detailed a description as possible, together with as many examples of various embodiments in the invention as are available, and, in the case of an invention capable of illustration, drawings illustrating the invention.

With inventions concerned with new compounds, particularly where these may be prepared by 'known' methods (and this is the case with most new compounds), it is necessary to disclose a use for that compound. This information must be disclosed in the complete specification; it is desirable for the information to be present in the provisional specification as well, so as to ensure that priority can be claimed from the provisional specification, not only for the British complete, but also for any corresponding applications filed overseas under the International Convention.

In respect of inventions in the pharmaceutical field, it is most desirable that the provisional specification should contain more than a mere identifica-

tion of the potential use of the compound or composition in question, and if possible should contain a record of trials illustrating the pharmacological advantages obtained. Once again this information is not required for British practice, although it may prove of importance if there should be a co-pending application of similar date and the resolution of priority date becomes a significant issue, but this information is essential if priority is going to be claimed from this application for others overseas, particularly in the U.S.A. and Germany.

In the case of a new compound, the provisional specification should disclose at least one method for the preparation of this compound together with a clear identification of the pharmacological purpose for which the compound may be used, and a record of some experimental trials illustrating the properties of the compound. Where appropriate, *in vitro* trials are sufficient for the purposes of the provisional specification and may suffice also for the purposes of claiming priority overseas. If details of *in vivo* or even clinical trials are available, these should be included. However, the likelihood of clinical results being available at the time of filing the provisional specification is somewhat remote; indeed, if clinical results are available, this can only mean that the filing of the application has been delayed to a hazardous extent.

As already indicated, the most important feature of a patent application is its date, so that where a chemical compound is found to possess pharmacological properties the provisional specification should be filed as soon as possible thereafter. If this is delayed pending the completion of a series of trials, the inventor may find that others working along the same lines have filed an application covering the use of such compounds in advance of him, and thus deprived him of the rights which he should have obtained in respect of his invention.

In a case where the invention is in respect of a known chemical compound which has been found to possess new and unexpected pharmacological properties, the requirements for the provisional specification are details of the pharmacological properties supported by such record of trials as are available, and also an identification of the types of composition or formulation in which the compound may be used. In the United Kingdom, as in most other countries, it is not possible to claim the *use* of a pharmaceutical, and protection may only be obtained for new compositions of matter (*i.e.* formulations) containing the known chemical compound.

The Complete Specification

The requirements for the complete specification are laid down in the Patents Act in the following terms[27,45]. Every complete specification shall: (*a*) particularly describe the invention and the method by which it is to be performed; (*b*) disclose the best method of performing the invention which is known to the applicant and for which he is entitled to claim protection; and (*c*) end with a claim or claims defining the scope of the invention claimed.

The claim or claims of a complete specification must relate to a single invention, must be clear and succinct, and must be fairly based on the matter disclosed in the specification. The same requirements apply to the specifications for applications in most territories overseas. The only difference

between a British complete specification and a specification for a patent application in any other country lies in the form and nature of the statement of claim at the end of the specification. To a considerable extent the form of the complete specification follows on from that of the provisional specification. Although there is no statutory requirement for this, the specification when prepared professionally tends to follow a standard form.

It is usual for the specification to commence with a short statement identifying the general field with which the invention is concerned. This is followed, where appropriate, with a reference to the state of the art, and then by an identification of the discovery which has been made. This passage is usually preceded by the words: 'It has now been found . . .'. This passage completes the preamble, and then follows what is known as the statement of invention, which commences with the words: 'Accordingly the present invention is for . . .'. The Patent Office requires that there be a statement of invention in this form, where the statement of claim includes an omnibus claim (see p. 24). After the statement of invention follows a description of aspects of the invention in general and specific terms; for example, process conditions, nature of reactants, properties of the products, and so on. The body of the specification usually concludes with the examples which illustrate the invention. At the end of the specification is the statement of claim.

A frequent criticism of patent specifications as technical literature is that these do not usually contain an adequate acknowledgment of the prior art. The reason for this is that a full statement of the prior art in the specification may be of substantial assistance to those wishing to attack the validity of the patent. In some cases it is necessary for validity to have an adequate statement of the prior art and a clear differentiation of the invention from this, but in general this is only required in the case of selection inventions (see p. 29).

As indicated above, the description in the specification must be such that any person, skilled in the art, on reading the specification will be able without difficulty to put the invention into practice. Over and above this requirement, the applicant must disclose the best method of performing the invention known to him at the date of the complete specification. If the best method is not disclosed, the resulting patent is incurably invalid.

On this question the following comment[46] of the Court of Appeal is particularly apt:

> But it is settled law that a Patentee must not throw upon the public the burden of experimenting in order to ascertain how the invention is to be carried out. The Plaintiffs are here in a dilemma from which there is no escape. The Patentee is bound to act towards the public *uberrima fide*, and to tell them all that he knows which is requisite to enable them to carry out the invention to the best effect. Now in this case the Patentee had either made the dyes with the naphthols or he had not. If he had, he must have known that the temperatures necessary to success were vastly higher than those he had given in the case of the phenols, and the fact that he has not given that knowledge to the public must invalidate his Patent. If, on the other hand, he had never made the dyes from the naphthols, he could not, in the then state of knowledge, know that they could be so produced.

As already noted, inventions concerned with new chemical compounds, where these are made by methods known *per se*, must show that the new

compounds possess a use. In the case of pharmaceutical inventions concerned with a chemical compound (whether new or old) possessing pharmacological properties, it is necessary for the specification to contain a clear identification of the advantageous properties, desirably supported by evidence. In the United Kingdom the level of evidence which is required is much lower than, for example, in the U.S.A., but in any important case a corresponding application in the U.S.A. must also be contemplated; therefore, it is an advantage that where additional evidence is thus provided for the corresponding United States case then the same evidence be included in the corresponding complete specification. Here again, so far as the United Kingdom is concerned, the results of *in vitro* experiments will be sufficient, where these are appropriate. However, in the U.S.A. the results of *in vivo* tests generally will be required, and probably also clinical trials. Whether clinical results must be available at the time of filing in the U.S.A. is a moot point, but if they are not available at this time, it should not be considered as a deterrent to filing in the U.S.A., and the application should be filed with the support of what evidence is available. If and when further evidence such as the results of clinical trials become available, this can either be presented to the Patent Office in the form of affidavits in support of the application, or in some circumstances it may be more appropriate to file a further application embodying the substance of the first application in the U.S.A. and amplifying it with the clinical results. Such a further application would be filed as a 'continuation in part' application, and would be entitled to the date of the prior application in the U.S.A. (and the priority date thereof, if any) for matter appearing in that specification.

The Claims

The statement of claim which follows the body of the specification is perhaps the most important part. In present-day practice in the United Kingdom, the scope of the monopoly granted to the patentee is to be found in the wording of the claims. This has not always been the case, and at the turn of the century specifications were only accompanied by rudimentary claims, and the specification as a whole was considered by the court. The following judicial observations illustrate present practice in this respect.

> It is not sufficient for the inventor to find his gold mine; he must also peg out his claim. Outside the pegs the gold, if it be there, is free to all[47].
>
> The function of the claims is to define clearly and with precision the monopoly claimed, so that others may know the exact boundaries of the area within which they will be trespassers. Their primary object is to limit and not to extend the monopoly. What is not claimed is disclaimed. The claims must undoubtedly be read as part of the entire document, and not as a separate document; but the forbidden field must be found in the language of the claims and not elsewhere[48].

It is essential for the claims to be drafted in broad terms to cover all aspects of the invention and so make it impossible for others to take advantage of the nature of the invention and yet operate outside the scope of the claims. If the claims are too broad, however, they run the serious risk of invalidity, and it has been said on at least one occasion that the drafter of claims must steer between the Scylla of too limited scope and the Charybdis of excessive

breadth. The drawbacks of insufficiently broad claims are obvious; the danger of broad claims may be illustrated by reference to the sulphathiazole case[49]. The complete specification described and claimed the manufacture of *p*-aminobenzenesulphonamido-thiazoles from large classes of chemical substances by any known process, and the claims, as construed by the court, covered many millions of products. The specification contained a statement that the 'new para-amino-benzene-sulphonamido-thiazoles find application in therapeutics. They have chemo-therapeutic activity in streptococci infections and similar illnesses.' However, only two compounds were exemplified, namely sulphathiazole and sulphamethylthiazole, together with evidence of their pharmacological activity and low toxicity. It was established in the action that apart from the two mentioned compounds none of the compounds possess any therapeutic value. The patent was therefore found invalid on the grounds of inutility, false suggestion, and that the invention was not a manner of new manufacture. The complete specification contained no specific claims to the two compounds of established value, and the patentees sought to amend their specification to introduce claims to these two compounds named in the specification. This application for amendment was refused on the grounds that the specification in its amended form would claim an invention substantially different from that claimed in its original form. The probability is that had the patent specification in question also contained specific claims to sulphathiazole and sulphamethylthiazole, these claims would have been found valid and the patentees could have amended their specification to delete the other broad claims. As it was, the patent was revoked.

It is desirable, therefore, that the statement of claim should contain both broad claims and narrow claims, so as to attempt to meet the pitfalls referred to above. This is the case despite the alterations to the provisions regarding amendments made by the 1949 Patents Act.

In addition to claims which themselves define the monopoly claimed, it is possible to have claims which define the invention by reference back to the specification. Such claims are termed 'omnibus' claims and these may either be drafted as broad claims referring to the whole of the specification (*e.g.* 'an oxidation process substantially as hereinbefore described') or as narrow claims limited to specific examples or as illustrations (*e.g.* 'an oxidation process substantially as hereinbefore described and illustrated in any of the preceding Examples 1–10'). Omnibus claims are not usually highly regarded, but were justified in a famous case[50] where all the other claims were found invalid, but the omnibus claim was found to be both valid and infringed.

THE INVENTOR

It is a truism to say that in respect of every invention there must be an inventor; nevertheless the importance of the inventor as an individual is substantially greater in some countries than in others.

In the United Kingdom up to 1949, the inventor had to be joined at least as a co-applicant in all applications, and it was not possible for an assignee to file the application in his own name, except in the case of convention

applications or communications from abroad. However, since the 1949 Act, an assignee may file a patent application directly in his own name, but it is still necessary for the inventor to be named on the application form, and he must also give his consent to the filing of the application. In any case where the inventor is not named as a co-applicant, his name is printed at the head of the specification, so publicizing that he is the inventor.

These new provisions reflect conditions as they exist today, and it has to be admitted that the day of the private inventor is nearly over. The majority of inventions are now made in the research and development departments of industrial concerns where inventors are employed in research or allied work, and where the rights in the inventions which are made belong in equity to the employers. However, it is important that the actual inventors should be named, since, in the United Kingdom, if the inventor is not named correctly the patent may be invalid. Although in one United States case[51] the court decided that with inventions made by company employees it does not matter if the wrong employee is nominated as inventor, it is not safe to rely upon this, even for the U.S.A.

The principles to be adopted in determining who is the inventor in respect of an invention are similar in most countries, although, as in all other aspects of patent law, there are differences in detail.

In the United Kingdom, the inventor is the person who is responsible for devising the invention in the sense of having been responsible for the solution to the problem which comprises the invention. It is not necessary for the inventor himself to have been involved in any practical work providing he has given sufficiently precise instructions to those who carried out the appropriate practical work. However, it does not necessarily follow that the person who lays down a general scheme for research is an inventor or joint inventor in respect of inventions made during that research. It is difficult to lay down a general test for inventorship, but in effect the inventor must be the person or persons responsible for the technical advance forming the subject of the invention. For example, where the research planner suggests that an alkali should be used in a certain reaction, and the practical worker finds that whereas sodium and potassium hydroxide are unsuitable the alkali metal carbonates are suitable, in respect of the invention comprising the use of alkali metal carbonates, the practical worker is the inventor. On the other hand, where the research planner proposes that a reaction should be carried out trying specifically sodium hydroxide, potassium hydroxide, sodium carbonate and potassium carbonate, he is the inventor of the invention relating to the use of the alkali metal carbonates. In many cases it is difficult to apportion the contribution made to an invention by various co-workers, and where there is any doubt it is reasonable that those concerned should be named as co-inventors.

A difficult situation arises where a research worker is engaged on or has found a solution to a problem, and one or more colleagues make suggestions for meeting the problem or improving the solution. Where such suggestions are of a casual nature or represent an encyclopaedic listing of alternatives, and the original research worker selects his research route essentially independently, then he is the sole inventor, even though the invention may embody one or more such suggestions. On the other hand, where a suggestion

is put forward with a reasoned exposition for its trial, and this is adopted, then the person making the suggestion is at least a co-inventor. In one United States case[52] it was noted that an inventor may receive any number of suggestions, but he is to be regarded as the sole inventor if he is the one who decides whether to adopt or reject a suggestion.

Another difficult situation arises when a chemist makes a new chemical compound to which he cannot ascribe a utility and a second worker by screening trials determines a utility for that compound. According to the precise circumstances the screener may or may not be a co-inventor. Thus where the chemist asks the screener to test the activity of the compound as a bactericide, and the screener finds the compound possesses such properties, then the screener cannot be regarded as a co-inventor. On the other hand, where the screener is not given any precise instructions, and he selects a number of tests to be applied, and by this means finds that the compound possesses analgesic properties, then he is a co-inventor. It is difficult to visualize cirumstances where the screener would be the sole inventor, to the exclusion of the chemist who synthesized the new chemical compound, unless the invention for which protection is sought is directed exclusively to the use of the compound. Ultimately, invention lies in the assignment of a useful property to a particular compound; where the synthesizer sends the chemical to the screener under a code number and receives the screening results also related to the code number, the synthesizer will be the sole inventor, since the screener is never in a position to assign the useful properties to a particular compound.

In the U.S.A., strict rules apply in the determination of inventorship, and for co-inventors to be properly named, they should have co-operated actively in the making of the invention. Thus, according to United States practice, where inventor A discovers fact 1 and inventor B independently discovers fact 2, if these workers do not co-operate it is not correct to treat them as joint inventors of the invention including facts 1 and 2. In the U.S.A. a patent application must be filed by the inventor; where an assignment is filed before grant the patent is granted to the assignee. In most other countries applications can be filed in the name of the assignee without joining the inventor as a co-applicant, although in a number of countries where this is done it is also necessary to file an assignment from the inventor to the assignee.

At the present time the majority of inventions are made by employees who are employed for this specific purpose. In other words, the employees are employed to carry out research or to design engineering equipment, and so on. Where there is a specific agreement between the employee and an employer regarding inventions, this controls the situation between them and the division and allocation of rights[53]. However, it is usual for this to be more or less in line with the position in equity. It is well established that where an invention is made by an employee in the course of his employment, and where there is no agreement between the employee and his employer, all rights in the invention belong to the employer[54]. In such a case the employee has been held to stand in the position of a trustee holding the rights in the invention to the benefit of his employer[55]. In the absence of an agreement to the contrary, it follows that where an employee makes an invention outside

the normal course of his employment, the rights in such an invention belong to him and not to the employer.

Where an employee makes an invention in the course of his employment, his duties to his employer in this connection do not end on the termination of employment, and at any time he must execute documents in relation to such an invention as the employer requires[56].

In order to simplify and cheapen litigation in the case of disputes regarding inventions between employees and employers, application may now be made to the Comptroller for his decision[57].

SCOPE OF THE MONOPOLY

In the United Kingdom the scope of the monopoly given by any patent is to be found in the wording of the claims, and it follows that what is not claimed is disclaimed. This factor is important both from the point of view of the patentee as well as from the point of view of a competitor who is seeking to avoid infringement of a patent.

In general there are two types of claims which are to be found in patent specifications, namely (*a*) product claims, and (*b*) process claims. In a sense product claims, if these are relevant, are the more important, since these will probably cover what is ultimately sold to the public and thus make it simpler for the patentee to decide whether or not there is an infringement.

Each of these main classes of claims may be readily subdivided, and in the case of product claims, these may either comprise independent product claims, for example claims directed to an article, an apparatus, a new compound or a new composition, or may comprise a process-dependent claim, and be in the form: 'the product when prepared by the process . . .'. The process-dependent product claims are of lesser value than the independent product claims, but in many cases they may be the only form of product claims which are obtainable. The scope of a process-dependent product claim is limited to the product of that process; in other words, such a claim is not infringed when the product is prepared by any other process. These circumstances may make it difficult for a patentee to determine whether or not there has been infringement, unless the nature of the process employed is evident by examination of the product (by reason, for example, of the presence of impurities).

A process claim covers the procedural step or steps which achieve a desired object and may thus be for a process for the manufacture of an article or a machine, or for a process for using an article or machine, providing in the latter case that the process is a manner of manufacture. For example, where the invention is for a new chemical compound which has been found suitable for use as a blowing agent for thermoplastic materials it will be possible to have examples of all these claims, as follows:

Independent product claims

1. Claims to the new chemical compound
2. Claims to a composition comprising a thermoplastic material and the new chemical compound

Process-dependent product claims

3. The new chemical compound when prepared by a definite process
4. An expanded thermoplastic material when prepared using the new compound as blowing agent

Process claims

5. A definite process for the manufacture of the new compound
6. A process for the expansion of thermoplastics using the new compound as blowing agent

By way of contrast, where a new compound is found to be of value as a pharmaceutical compound, it is not possible to include those claims relating to use, since, as already indicated, pharmaceutical uses are not patentable. In this case permission claims would be as follows:

Independent product claims

1. Claims to the new compound
2. Claims to a pharmaceutical composition containing the new compound

Process-dependent product claims

3. The new compound when prepared by a definite process

Process claims

4. Claims to a definite process for producing the new compound

Where these are obtainable, independent product claims are the most valuable, since these give effective cover for the product under all conditions of use and manufacture. In those cases where product claims are not obtainable, for example, where invention is concerned with a new use for a known compound, then the other types of claims have to be relied upon. In such a case, however, if the compound requires to be used in a special formulation, and providing that this formulation is new, then it is possible to claim the compound in that formulation as a new composition of matter.

It is important that the specification should contain all the types of claims which are possible, otherwise that part of the invention which is not so claimed may not be protected by the patent. Thus, where the invention is concerned with a process for the manufacture of a known chemical, as for example aniline, if the statement of claim only includes claims directed to the process itself, without any claims to the product when prepared by the process, then very likely it would be held that the scope of the monopoly did not extend to the product of the process as opposed to the process itself. Thus, if the product were manufactured overseas by the process, it could probably be imported into this country without infringing such a patent.

A similar situation has arisen recently in South Africa[58] concerning an application for amendment of a specification in which the statement of claim contained claims to a process for producing xylocaine and related compounds. The applicants sought to amend the specification to limit the claims to the preparation of xylocaine, excluding the other compounds, adding an explanation that the word halogen extended only to chlorine, bromine and iodine with the exclusion of fluorine, and to add a claim to the product made in accordance with the process. The Supreme Court of South Africa held that the amendment to limit claims to the preparation of xylocaine and to

explain the term halogen were allowable, but that the amendment to add a claim to the product was not allowable. No decision was given on the question as to whether a process claim covered the product, but the court observed that: 'there is considerable force in the contention that under our Patents Act a process claim does not in itself protect the process product'.

Today, it is uncommon to find a British patent specification which does not contain claims to the product of the processes claimed, where this is relevant; where such claims are missing the scope of the monopoly is defective.

The limitation of the scope of the monopoly in accordance with the wording of the claims is unique to the United Kingdom and certain Commonwealth countries, including the Federation of Rhodesia and Nyasaland. In a number of countries, the scope of the monopoly is determined to a greater or lesser extent by the form of the claims, but interpreting the claims within the doctrine of equivalence. For instance, this is the case in U.S.A., Republic of South Africa, India, Finland, Switzerland and Sweden. In some other countries, for example the Netherlands and Denmark, the specification is also taken into account; as noted earlier, in Germany the nature of the invention is also relevant.

The application of the doctrine of equivalence means that where the claim refers to, for example, a certain chemical, the scope of the monopoly will also be held to extend to other chemicals which are the known chemical equivalent thereof. For example, in the U.S.A. where a process claim cites chlorine, it is likely that the claim would be held to be infringed by the same process using bromine.

In some other countries, including Belgium, Brazil, France and Italy, the claims are not important and the court takes upon itself the determination of what invention has been made, and in the light of the prior art and other circumstances what protection should be accorded to the patentee.

It follows, therefore, that the patentee receives the most favoured treatment in the last-named countries, and that in the United Kingdom, U.S.A., Republic of South Africa, India, Finland, and so on, the public, as opposed to the patentee, is assisted as it may more readily determine what comprises an infringement and what processes or products it may employ without infringement. In those countries, such as Germany, where only process claims of chemical inventions are permissible, the protection obtainable from the process claims extends also to the products of the claimed process.

SELECTION INVENTIONS

A special class of inventions, which is frequently met in the chemical field, although not exclusively so, is that of 'selection inventions'. Here the invention is based on a selection either of compounds or of conditions from what is already known in the art. A reaction is often described and perhaps patented in general terms, and subsequent workers may find that when using some specific starting material or reaction conditions, a marked advantage or a special result may be obtained. In a case where a different result is obtained, the invention may in fact be a normal invention and not a selection invention. Thus, where the reaction of A and B together to form C is already described in the literature, and it is subsequently found that a desirable product D is

also produced, it is permissible to claim the process for the protection of D comprising the reaction of A and B together coupled with the step of isolating the compound D from the reaction product, as a normal invention and not as a selection invention. By way of contrast, a selection invention is involved where it is found that the reaction of A and B together results in the production of C, but that if the reaction is carried out at 80°C a much higher yield is obtained than when operating at any other temperature. The fact that inventions could lie in the selection of special conditions or reactants has been appreciated for a great number of years[59], but the most important case regarding selection was decided in 1930 when the criteria which a selection invention must fulfil to be patentable were set out[60]. The action was heard before Mr. Justice Maugham (as he then was) and his judgment is one of the most famous in the whole history of patent actions. The action involved three patents relating to the manufacture of azodyestuffs by the coupling of amides of 2,3-oxynaphthoic acid (3-hydroxy-2-naphthoic acid) with diazo compounds. The first patent claimed the use of the *o*-toluidides of 2,3-oxynaphthoic acid, although the use of the *p*-toluidide had been published as an example of the class described as arylamides in general. The second patent claimed the use of *o*-alkoxyanilides, although the use of the *p*-anisidide had already been published. The third patent claimed the use of *p*-toluidides or *p*-alkoxyanilides which are halogen substituted, although the use of the *p*-toluidide, *p*-anisidide, and chloro-substituted anilides had been described.

The observations of Mr. Justice Maugham have been so frequently quoted and misquoted that these are reproduced at some length. Dealing first with the question of selection patents, Mr. Justice Maugham said: 'This case seems to be the first which has arisen in these courts in which the question of the validity of a chemical selection patent has been directly considered. It may be observed that chemical patents in recent years have consisted of two sharply divided classes. The first class is that of patents based on what may be described as an originating invention, that is, the discovery of a new reaction of a new compound. Such patents may be called for brevity "originating patents". The second class comprises patents (the so-called selection patents) based on a selection of related compounds such as the homologues and substitution derivatives of the original compounds which presumably have been described in general terms and claimed in the originating patent.'

Having referred to the nature of the prior publications, Mr. Justice Maugham continued: 'It must be remembered, of course, that the selected compounds have not been made before, or the patent would fail for want of novelty. If the selected compounds, being novel, possess a special property of an unexpected character, for example if a mono azo dye were to be made by selecting components not hitherto employed which resulted for the first time in a green dye, I cannot see that the inventive step essentially differs from the step involved in producing a new result by a new combination of well-known parts or indeed from using the common and well-known factors (cranks, rods, toothed wheels and so forth) employed in mechanics in the construction of a new machine.

'In a sense it is still true to say that there is no prevision in chemistry. Any one of the millions of dyestuffs in question might be found to possess some unexpected and distinctive properties, either of colour or fastness, or to have

some other incidental advantage. There is no short cut to knowledge of this kind. A laborious and systematic investigation of a long series of combinations becomes necessary; and it is the fact that of recent years certain industrial organisations with enormous financial resources have established laboratories where numbers of chemists of high scientific attainments devote their lives to a systematic examination on scientific principles of a vast number of chemical substances.'

Mr. Justice Maugham's remark 'there is no prevision in chemistry' has become almost a battle cry in the struggle to obtain patents in the chemical field, not only in the United Kingdom, but also overseas.

Having considered the situation, Mr. Justice Maugham then set out the criteria which must be fulfilled for selection patents to be valid: 'Three general propositions may, however, I think, be asserted as true: First, a selection patent to be valid must be based on some substantial advantage to be secured by the use of the selected members. (The phrase will be understood to include the case of a substantial disadvantage to be thereby avoided.) Secondly, the whole of the selected members must possess the advantage in question. Thirdly, the selection must be in respect of a quality of a special character, which can fairly be said to be peculiar to the selected group.'

While the first condition is self-explanatory, the second criterion may be more difficult to appreciate. It means that where a selection is made in respect of a group of starting materials or a range of conditions, the advantage must be secured when using all of those materials or conditions. For instance, where the prior publication covers metal salts and a selection is made in respect of alkali metal salts, it is necessary for validity that the advantage is found with all alkali metal salts, and not merely some of them. This, of course, may be tempered with some reason, and where, for example, the selection consists of a group of a hundred compounds (chosen out of a much larger field) the selection will not be invalidated if perhaps one or two of the selected members did not provide the claimed advantage.

The third part of the test is also important, and means that where a selection is made of a part of a known field, the same advantage must not be attainable in a substantial number of other parts of that field. Thus, where it is known that a class of compounds bearing an alkyl substituent of 1 to 20 carbon atoms possesses useful therapeutic properties, and it is found that compounds of this type where the alkyl substituent is of 6 to 8 carbon atoms (these compounds being new, although members of a homologous series) exhibit greatly reduced toxicity, this requirement is not fulfilled if the homologues wherein the alkyl substituent is of 10 to 20 carbon atoms exhibit the same advantages. However, if the compounds with alkyl substituents of 6 to 8 carbon atoms possessed a much lower toxicity than any of the homologues, or alternatively that this lower toxicity was only matched by homologues with alkyl substituents of 14 to 16 carbon atoms, then this requirement is fulfilled, and a selection patent could be obtained.

Since selection inventions have to fulfil such rigid requirements, the position is usually that the patentee seeks to show that the invention is not a selection invention, and therefore that the tests need not be applied, whereas those who attack the patent attempt to establish that it is for a selection invention. In order that an invention should be treated as a selection

invention and thus be required to fulfil the prescribed conditions, the prior disclosures establishing the position must themselves also fulfil certain conditions[61]. Firstly, the publication of the field from which the selection is made must be contained clearly and unequivocally in a single document. It is not permissible to find the broad field from which the selection is made in a number of documents. However, this requirement is satisfied if it can be shown that the whole of the field from which the selection is made lies in the common general knowledge. Secondly, the disclosure of the general field must not be untrue to any substantial extent. Finally, in the case of an invention comprising a specific substitution product, if the basic disclosure is of substituted compounds in general (for example, 'substituted hydrocarbons') there is selection only when the prior disclosure gives examples of compounds containing substituents which are representative of the class of substituents present in the substituted compounds forming the selected invention. Thus, when a general disclosure is of substituted pyridines, illustrated only by methylpyridine and chloropyridine as useful for some purpose, this will be regarded as extending to alkyl and halogen substituted pyridines, and a discovery that nitropyridine is also useful for that purpose will be treated as a normal invention and not a selection invention. On the other hand, the discovery that bromopyridine and isobutylpyridine (if they are new) are useful would form the basis of a selection patent.

Where all the conditions for a selection invention are present, there is a final requirement that the selection must not be in respect of a substantial portion of the general field. For example, the selection of the use of one member out of a disclosure of two members, or the selection of two members out of a disclosure of three members, is not sufficient to support a patent[62].

This issue of selection inventions is, as already indicated, of special relevance in chemical cases. An example which is frequently met is where a class of chemical compounds is known, and may be represented by a general formula, and where one or perhaps a special group of compounds falling within the general formula are found to have special properties. If these were specifically mentioned in the prior publication, they cannot be protected as new compounds, as they are not novel; but the novel use of such compounds may be patentable.

Where the selected compounds are new and possess therapeutic properties, for example for the treatment of dermatitis, if it were already known that the general class of compounds could be used for the treatment of certain dermatological conditions, and if the new compounds did not differ in their effectiveness or utility, then there is no possibility of obtaining protection for the new compounds or compositions containing them. On the other hand, if the new compounds exhibited markedly higher activity than the known compounds of the same group, or alternatively if of similar activity have some other advantageous and unexpected property such as greatly reduced toxicity, then the requirements for a selection invention will be fulfilled, and it will be possible to claim new compounds *per se* as well as to protect pharmaceutical compositions containing them.

On the other hand, if the general class of compounds were not known to possess therapeutic properties, the discovery that the new compounds had such properties will support a claim to the compounds *per se*. It is immaterial

whether or not other compounds in the general class possess the same property, providing this were not known[63]. In addition to protecting the new compounds *per se*, it would also be possible to protect new compounds, *e.g.* dermatological creams, containing any of the general class of compounds.

A selection invention may also be made protecting a particular use of known chemical compounds. Thus, where it is known that a reaction may be carried out in the presence of a heavy metal halide, *e.g.* halide of iron, nickel or titanium, the discovery that greatly improved results may be obtained by the use of chromium chloride is a patentable invention which comprises a selection. On the other hand, if the discovery is that the process may be carried out using an alkali metal halide, the invention is patentable as an ordinary invention and the conditions for selection inventions do not have to be fulfilled.

In the case of a selection invention, it is essential that the advantage obtained by the selection, or the disadvantage avoided, is set out in the specification. If this is not done, then the patent will be invalid on the ground of insufficiency[64]. A selection patent is one of the few cases where a clear acknowledgment of the prior art is desirable.

THE PATENT AFTER GRANT

Infringement

The wording of the patent grant in the United Kingdom is in the following form:

> Elizabeth the Second by the Grace of God in the United Kingdom of Great Britain and Northern Ireland and of Her other Realms and Territories, Queen, Head of the Commonwealth, Defender of the Faith: To all to whom these presents shall come greeting:
>
> Know ye, therefore, that We, of our especial grace, . . . give and grant unto the said patentee our especial licence, full power, sole privilege, and authority, that the said patentee by himself, his agents, or licensees, and no others, may . . . . . make, use, exercise and vend the said invention within our United Kingdom of Great Britain and Northern Ireland, and the Isle of Man, and that the said patentee shall have and enjoy the whole profit and advantage from time to time accruing by reason of the said invention, during the term of sixteen years from the date hereunder written of these presents: AND to the end that the said patentee may have and enjoy the sole use and exercise and the full benefit of the said invention, We do by these presents for Us, our heirs and successors, strictly command all our subjects whatsoever within our United Kingdom of Great Britain and Northern Ireland, and the Isle of Man, that they do not at any time during the continuance of the said term either directly or indirectly make use of or put in practice the said invention, nor in anywise imitate the same, without the consent, licence or agreement of the said patentee in writing under his hand and seal, on pain of incurring such penalties as may be justly inflicted on such offenders for their contempt of this our Royal command, and of being answerable to the patentee according to law for his damages thereby occasioned.

Although the patent is granted for the sole privilege to use the invention coupled with a command to subjects of the United Kingdom not to directly or indirectly make use of the invention, the patent monopoly right is not

effective automatically, and if the patentee is to maintain his exclusive position, he must take action against any person infringing his rights. Moreover, although the patent grant states 'directly or indirectly make use of or put in practice the said invention', in fact infringement only occurs with direct use. The doctrine of contributory infringement is not followed in the United Kingdom[65], and before a person is sued for infringement he must himself have committed an act falling within the scope of the claims of the patent. In other words, where the claims of the patent are for a particular use of a compound, any person selling the compound with an indication that it may be used in that way is not an infringer, and no action may be taken against him by the patentee.

Although this position may appear somewhat daunting to the patentee, in practice most individuals and organizations respect the patents of others which they consider to be valid. Under modern conditions the operation of any industrial process, and particularly any chemical process, involves a substantial investment in plant, and probably also in development and marketing. Consequently the risks involved in the infringement of a patent may well be substantial.

An infringement which would be evident from the product which is sold is less likely to occur than if the patent covers a process where it is impossible to ascertain from the final product whether the patented process has been employed. However, if the patentee is able to make out a prima facie case of infringement, he will then be able to obtain discovery[66] (this may include not only disclosure of documents but also inspection of the relevant factory) which will bring to light the actual processes employed by the alleged infringer.

Licences

The patentee may use the invention exclusively or, if desired, he may license others in its use. Whichever course of action is followed, the patentee must take positive steps to secure the maximum return.

One requirement that patentees in most countries should fulfil is that referred to as 'working'. As previously noted, one of the functions of the patent monopoly system is to encourage the development of science and technology, and, in addition to securing this by publication of the invention, it is also desired for new inventions to be put into practice at the earliest possible date. In order to achieve this, most countries provide some form of penalty for the patentee if the invention is not put into operation in that country within a certain period from the date of grant. In the countries belonging to the International Convention this period is three years from the date of grant, and provision is made for the grant of compulsory licences where there is an abuse of monopoly; for example, by reason of failure to use the invention. The patent may only be revoked where the 'grant of compulsory licences shall not suffice to prevent these abuses'. If the patented invention is not operated in the country in question, the requirement for working may be met by an offer of the patent for licence irrespective of whether such offers are taken up. However, if the patentee is able to operate the invention either himself or by licensing others in the country in

question then this requirement is fulfilled. Since a further object of such provisions is the promotion of domestic industries, the requirement for working cannot usually be met by importation.

Where the patentee decides to grant a licence under his patent, this may comprise either an exclusive licence or a non-exclusive licence. In the case of the former, a special situation is created between the patentee and the licensee, and, in the absence of any arrangement to the contrary, the patentee surrenders his right to use the invention, and the licence granted is exclusive of all persons, including himself[31]. Such a licensee secures various rights, including that he may institute proceedings for infringement and apply for an extension of term of the patent.

A patentee who grants an exclusive licence usually receives payment of a minimum royalty, so as to secure that the licensee will use his best efforts to secure the exploitation of the invention. A provision of this character is usually the main difference between agreements for exclusive and non-exclusive licences. In general, all such agreements provide for the payment of a royalty, which may be calculated in a number of ways. It may be on a percentage basis of either cost or sale price of the product, or may be calculated as a fixed sum per unit manufactured, or may even comprise an annual fixed sum. Licences are not usually granted for a single lump sum, although it is of course possible to do this. A common provision in licence agreements is that the licensee will not contest the validity of the patents which are licensed. Further provisions which may be important relate to improvements, made either by the patentee or the licensee, and whether or not these are to be included in the licence.

Restrictive Conditions

In the United Kingdom a licence agreement under a patent should not contain certain clauses[67] which are termed 'restrictive clauses', unless the licensee is either offered reasonable terms without such clauses or is entitled to relieve himself of such liability on three months' notice. In any other case, these clauses are void in law and in the event of an infringement action it is a complete defence for the infringer to show that at the time of infringement there was a contract in force containing such a clause. A void restrictive clause: (*a*) requires the licensee to obtain 'non-patented articles' from the licensor or his nominee, or prohibits him from obtaining 'non-patented articles' from any specified person or from anyone except the licensor or his nominee; (*b*) prohibits the licensee from using either any article (patented or not) not supplied by the licensor, or any patented process which does not belong to the licensor, or (*c*) restricts the right of the licensee to use such articles or processes. However, a clause is not restrictive and void if it merely prohibits the sale of goods other than those of a specified person (for example, the setting up of an exclusive selling agency) or reserves the right to the licensor to supply new parts to keep the patented article in repair. These provisions apply equally to assignment or other dealings under the patent, but apply only to patent agreements; no breach is involved in the case of other agreements, for example 'know-how' agreements.

By way of contrast, in the U.S.A. it is possible to include in a patent licence

agreement clauses which are not permissible in other agreements by reason of the Anti-Trust Laws. Monopoly situations may only be created in the case of patented inventions; however, such restrictive clauses have to be related strictly to the patent grant.

Limited Licences

The patentee may grant a licence under his patent in any form; thus, it may be a full licence to 'make, use, exercise and vend' the invention, or it may be a licence limited by the imposition of conditions. For example, such a licence may provide for a territorial limitation (*i.e.* the licensee may only make or sell in certain parts of the country), or may be a limitation on the parts of the invention which are licensed, or may be a limitation on the price or condition in which the patented product is sold. The most usual condition of this sort limits the price of sale, stating, for example, that the patented product must be sold at a price not less than a certain figure, or must only be sold at a certain price. Resale price maintenance in respect of patented articles is completely in order.

Where a patented product is purchased from a patentee or his licensee, this normally gives an implied licence to use or sell that product in any way. However, where the sale of the patented product is accompanied by conditions, then the patent is infringed if those conditions are not observed. If the patentee has applied conditions to the sale of the patented product and someone purchases the patented article in good faith without being aware of these conditions, he may use the patented article in any way without infringement[68]. But if he was aware of the conditions, then any use or sale in contravention of those conditions would comprise infringement.

Label Licences

In the case of a known chemical compound which is subject to a patent covering its use, for example as a blowing agent, it is not unusual for the patentee to let it be known that anyone purchasing the compound from him will have an implied licence to operate within the patent. This is what is known as a 'label licence', but care has to be taken to ensure that this implied licence does not give rise to a restrictive condition. If the patent in question is generally available for licence, then it is clear that this 'label licence' procedure will not give rise to a restrictive condition. On the other hand, if the patent is not available for licence, then a restrictive situation may exist, since, in fact, the patentee is requiring the licensee to purchase a non-patented article from him in order to secure the required licence to operate the patented process.

Compulsory Licences

There are provisions in the United Kingdom Patents Act for the grant of compulsory licences in two types of circumstance. A compulsory licence may be granted where there has been an 'abuse of monopoly'[69], and, on the other hand, a compulsory licence may be obtained in the case of patents relating to food or medicine[70].

In the case of an 'abuse of monopoly' a compulsory licence may only be sought three years after grant and in general, the provisions ensure that a compulsory licence may be obtained in any case where the patented invention is not being operated or made in the United Kingdom. By way of contrast, in the case of inventions relating to food or medicine, a compulsory licence may be granted immediately after grant[71], and the Act instructs the Comptroller to grant such a licence in the following terms: 'The Comptroller shall, on application made to him by any person interested, order the grant to the applicant of a licence under the patent on such terms as he thinks fit, unless it appears to him that there are good reasons for refusing the application.' There have been only three such cases but these confirm the view that anyone proposing to manufacture in the United Kingdom will probably be able to obtain a compulsory licence, providing it appears that he is capable of operating the patent in process or making a patented product. One of the reported cases concerned a food[72], where the application for a licence was refused, since the applicant for the licence did not intend to manufacture in the United Kingdom but only to import from the Netherlands. The Comptroller said that had the applicant intended to manufacture in the United Kingdom he would have been granted a licence. The other two cases[71,73] concerned pharmaceuticals, and in each case the application for a compulsory licence was successful. The existence of these conditions has simplified the securing of a licence in respect of patents concerned with food or medicine, and the explanation for the small number of contested cases may well be that patentees in respect of such inventions are normally prepared to grant licences since if they refuse the prospective licensee can inevitably obtain a compulsory licence.

General

It is also possible for a patentee to have his patent endorsed 'licence of right'[74], by which is meant that any person may apply for and secure a licence; if the terms of such a licence cannot be agreed between the parties, the matter is settled by the Comptroller. One advantage of endorsing a patent 'licence of right' is that only half the renewal fees thereafter fall due.

In the United Kingdom the patentee is not required to mark his product 'patented'; however, if he does not so mark his products, and give the patent number, an infringer may claim as a defence that he was unaware of the patent and had no reasonable grounds for suspecting its existence[75]. This defence may also be relied upon in any case where the infringer can show that he had no reasonable ground for supposing the patent existed. The infringer in such cases is usually termed an 'innocent infringer', and no damages will be awarded against him in respect of his infringement. However, an injunction may be awarded against him, effectively preventing him from continuing to infringe. In the United Kingdom infringement only occurs by the actual operation, possession or sale of something falling within the claims. Where the claims cover a use of a particular compound, there is no infringement involved in the sale of that compound with instructions to use it in an infringing way[76]. In other words, there is no contributory infringement in the United Kingdom. There is one decided case[77] where a contributory infringement was

held to be an infringement, but the authority of this case is doubtful. By way of contrast, the doctrine of contributory infringement is followed in a number of overseas territories including the U.S.A., Denmark, the Netherlands, Switzerland and Sweden.

Where the patentee commences an infringement action, and is successful, he may secure (at his option) either damages or an account of profits[78] (*i.e.* the improper profits secured by the infringer), delivery up of infringing articles, and the award of an injunction against the offender requiring him to refrain from such infringement. If the infringer continues in the face of an injunction, he is guilty of contempt of court and is liable to imprisonment. Where the patentee believes that others are operating his invention, he must be careful not to threaten to take action or institute proceedings, or he may find himself sued for threats[79]. The only action which a patentee may take which does not amount to a threat is to give a mere notification of the existence of the patent to the alleged infringer. Where the patentee threatens the alleged infringer with an action for infringement, the latter may himself sue the patentee for 'groundless threats', and the patentee, if he is to defend this action, must establish that the threats were justifiable, and that the person threatened is in fact infringing his patent.

The drafting of specifications and claims is a difficult task, and it not infrequently happens that the specification which has been accepted and published is deficient or inaccurate in some respect or another. Before the acceptance of the application, rather wide limits of amendment are granted at the discretion of the Patent Office. After acceptance any amendments to the specification must conform with the requirements of the Act[69,80] which are that: 'No amendment (of the complete specification) shall be effected except by way of disclaimer, correction or explanation, and no amendment thereof shall be allowed, except for the purpose of correcting an obvious mistake, the effect of which would be that the specification as amended would claim or describe matter not in substance disclosed in the specification before the amendment, or that any claim of the specification as amended would not fall wholly within the scope of a claim of the specification before the amendment.'

These provisions are strictly applied, and when making any amendment it is necessary to indicate whether the amendment is by way of disclaimer, correction, or explanation[81]. The most usual form of amendment is by way of disclaimer which means that, in fact, the scope of the claims is narrowed. For example, if the claim covers the use of halogens, an amendment to restrict the claim to the use of chlorine could be regarded as a disclaimer and would be allowable, providing there had been a clear disclosure in the specification of the use of chlorine. The terms correction and explanation are self-explanatory, but the nature of permissible amendments is rather limited. It is thus permissible to remove any inconsistencies from the specification and claims which clearly are not in conformity with the general disclosure (providing this does not enlarge the scope of the monopoly[82]) or to remove any ambiguity in the specification[83]. However, where the draftsman makes errors of judgment and the document represents the intention of the draftsman at the time of drafting, the amendment of such errors cannot be regarded as correction[84].

The normal term of a patent is sixteen years from the date of filing of the specification[85]. However, it is possible for this term to be extended for two different reasons. Extension may be accorded when the patentee has been inadequately remunerated by the patent[86], and in considering any such application the court has regard to the nature and merits of the invention in relation to the public, the profits made by the patentee and all the circumstances of the case. Only comparatively few patents have been extended on this ground. The extension may be for up to five years, or in exceptional cases ten years. To secure an extension on this ground it is necessary to show that the invention is of importance and that the failure to achieve adequate remuneration is not due to any fault on the part of the patentee. Where an invention is of exceptional inventive ingenuity, is of exceptional benefit to the public, and is inherently of a character such that exploitation will be slow, causing inadequate remuneration, then an extension of up to ten years may be granted[87]. Extension of the patent may also be obtained on the ground of war loss[88]. This is more or less self-explanatory, and all that the patentee requires to show is that as a result of war conditions he suffered loss or damage, including loss of opportunity of dealing in or developing the invention. The merit of the invention is of no relevance in this connection, and applications of this type are dealt with more or less as a matter of course.

Revocation

It is possible to apply for the revocation of a patent on any one of a number of grounds[89], and in the case of an infringement action, the defendant may counterclaim invalidity upon any of the grounds upon which it may be revoked. The grounds for revocation are similar to the grounds upon which a patent may be opposed, but are far wider in their scope and effect.

A patent may be revoked on the following grounds:

(*a*) The existence of a prior claim
(*b*) The applicant was not entitled to apply for a patent
(*c*) The claims do not relate to a patentable invention
(*d*) The invention is not a manner of new manufacture
(*e*) The invention is obvious
(*f*) The invention is not useful
(*g*) The complete specification does not sufficiently describe the invention or the method by which it is to be performed
(*h*) The specification is ambiguous or contains false suggestions
(*i*) The invention is contrary to the law
(*j*) The existence of a prior secret user

The main extensions beyond the scope of the grounds of opposition are on the issues of obviousness, utility and sufficiency. It is a ground for revocation that the invention is obvious and does not involve any inventive step having regard to what was known or used before the priority date of the claim in the United Kingdom. The issue of obviousness is one of the most difficult to assess as this is essentially a subjective rather than an objective issue. There are no hard and fast rules which must be followed, but in general, where the prior art suggests without actually disclosing that a certain process may yield

a useful result, then it is obvious to carry out such a process and show that this useful result is obtained.

The second ground which is particularly difficult is that of utility. It has to be remembered that there are two separate issues referred to by this term. One concerns the usefulness of the invention, and in general according to British practice the level required for usefulness is low. The other, and usual, meaning is that the promise of the specification is not fulfilled. In other words, if the specification suggests that a certain result is obtained by the operation of the invention, the patent is invalid for want of utility if that result is not secured[90]. This is one reason for not including unnecessary promises when drafting specifications.

Finally, on the issue of insufficiency, the specification requires both to describe the invention, and also to disclose the best method of performing the invention known to the applicant for which he was entitled to claim protection.

The multitude of attacks which may be made on a patent may seem rather intimidating, and, in fact, of the patents which have been the subject of litigation, a majority have been found to be invalid. Nevertheless a significant number of patents have been found valid and infringed.

The situation as regards the revocation of patents in most overseas countries is similar to that in the United Kingdom. Obviously those patents granted in territories of strict examination, such as the U.S.A., the Netherlands and Germany, have a greater likelihood of validity than patents granted in countries without examination.

As previously noted, patent monopoly systems exist in most countries; there are, however, at least two exceptions, namely the Sudan and China, where there is no patent system. Anyone who wishes to may therefore operate any patented process in the Sudan or in China.

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ADDENDUM

In the U.S.A. the position with regard to utility of new chemical compounds which are claimed to possess pharmaceutical activity has been considerably ameliorated by two recent decisions of the Court of Customs and Patent Appeals (Bergel and Stock In *re* **130** *USPQ* 205; Krimmel In *re* **130** *USPQ* 215) and it follows from these decisions that animal results only are sufficient to establish utility for these compounds. In the Bergel and Stock case the court held that the rejection on the basis of lack of utility since the experimental results only showed 'the continued disease-free existence of rodents' was fallacious, and further that the success of the animal trials 'is a plain indication of utility'. In the Krimmel case the court made the following trenchant comment: 'We hold as we do because it is our firm conviction that one who has taught the public that a compound exhibits some desirable pharmaceutical property in a standard experimental animal has made a significant and useful contribution to the art even though it may eventually appear that the compound is without value in the treatment of humans.'

2

THE TESTING AND DEVELOPMENT OF ANALGESIC DRUGS

A. H. Beckett and A. F. Casy

INTRODUCTION

Opium preparations have been used for the relief of pain for more than a century. Alleviation of pain and induction of rest may be achieved, in most cases, by doses which produce little or no stupefaction. Morphine, the chief active ingredient of such preparations, is a powerful sedative and its analgesic action is rapid in onset, reliable and long-lasting. It is readily available and relatively inexpensive. Its use in therapy, however, is restricted by its side-effects, some of which are of a highly undesirable nature. With daily administration, tolerance to the analgesic actions of the narcotic usually develops within a few weeks and the dose needs to be gradually increased to produce the required effect. Tolerance is closely associated with addiction, the most serious drawback to the use of morphine. Addiction involves both physical and psychical dependence on the drug, and this has become an international social problem, necessitating rigid control of the use and distribution of narcotics. In the U.S.A. where the problem is particularly serious, the number of addicts[1] in 1960, for example, was about 45,000.

Respiratory depression is another undesirable effect of morphine. In man, respiration is depressed by doses which are below the narcotic threshold and this depressant effect is the prime cause of death with higher doses. Morphine therapy must therefore be used with particular care in obstetrics where foetal respiration may be affected and in respiratory ailments such as bronchial asthma.

Morphine has a retarding action upon the digestive system and small doses produce constipation. Its effect on the urinary bladder gives rise to urinary urgency but urination is made more difficult by increased tone of the vesical sphincter. Urine retention is observed even with therapeutic doses. Morphine has a direct stimulating action on the emetic trigger zone of the medulla, giving rise to nausea and vomiting. Other excitatory effects encountered are tremors and, more rarely, delirium. Carbohydrate metabolism may be deranged, resulting in hyperglycaemia and reducing substances in the urine, but such effects are rarely seen with therapeutic doses in man.

These side-effects of morphine have made the development of an analgesic lacking such properties a highly desirable objective. Many synthetic analgesics have been discovered, some of which are highly potent, but in most cases their use is attended by side-effects which are similar to those of morphine itself. Indeed, Schaumann[2] considers that the pharmacological effects of narcotic analgesics such as respiratory depression, liability to addiction and the actions on intestinal tone and motility are inseparable from their

analgesic action, both qualitatively and quantitatively. However, this view has not daunted workers in the field and evidence of at least quantitative separation of effects has recently been obtained. Further, nalorphine, an analgesic as active as morphine in man, has been shown to be relatively non-addicting. Many synthetic compounds may now be used to advantage in place of morphine, and the increasing scale upon which these are applied in therapy is reflected by world production figures published by the United Nations Organization[3]. Although figures for the production of morphine have almost doubled over the last decade (the present annual figure being over 100 tons) the bulk is converted into codeine and the licit use of morphine itself is falling (6 tons in 1948, 4·2 tons in 1959). In contrast, the annual production of pethidine, the most widely-used synthetic analgesic, increased from 5·5 tons in 1948 to 13 tons in 1955 and has since remained steady at this figure.

For the purposes of this review, analgesics are defined as central nervous depressants that alleviate or abolish pain without, at the same time, inducing loss of consciousness. They act at specific sites in the central nervous system and may thereby be differentiated from other central nervous depressants (*e.g.* barbiturates and anaesthetics) that act more generally.

An analgesic is capable of relieving moderate to severe pain, *e.g.* traumatic pain, chronic pain of cancer, obstetrical pain and post-operative pain. Drugs which are effective only against mild pain (*e.g.* headache and toothache) are not classified as analgesics in this review. The term 'antalgics' has been applied to drugs of this second type (*e.g.* aspirin and phenacetin) and their analgesic action is probably secondary to their anti-inflammatory effect. Some compounds that have been produced as analgesics and subsequently proved to be effective only against mild pain are considered in this review but, in general, only substances that have analgesic potencies equal to, or greater than, that of pethidine in animal tests, are discussed.

The following are included in this chapter: (*a*) a description of the tests used to measure analgesic activity in animals and in man, together with an account of the assessment of addictive liability, and (*b*) a description of the various classes of analgesics that have been developed, with particular emphasis upon more recent developments. Discussion of structure–action relationships, mechanisms of action, metabolism and analgesic antagonists, is deferred to a future volume of this series.

TESTS FOR ANALGESIC ACTIVITY

Pain is a human subjective experience, the perception of which may only be ascertained by direct interview of the individual concerned. In man, painful sensations may arise either from internal stimuli such as disease processes and injuries, or from external stimuli such as heat, pressure and electric shock. It is impossible to determine whether external stimuli produce sensations in animals which are similar to those experienced by man, but they result in typical changes in behaviour by which the animal attempts to escape from the noxious stimulus. Whatever their significance in relation to human pain, these responses are used for the pharmacological evaluation of potential analgesics. Results in animals usually give a fair guide to the

potency of the drug in man if analgesic activities equal to, or greater than, that of pethidine are involved. Marked discrepancies sometimes occur, a good example being nalorphine which is inactive in some animals as an analgesic but is as active as morphine in man. Methods have been devised for measuring analgesia in man by means of experimentally-induced pain but the ultimate criterion of usefulness of a new drug is necessarily the result of its clinical trial.

The literature on testing of analgesic drugs is extensive and several reviews are available[4–6]. Two procedures are generally followed, one based upon a graded response and one upon a quantal response. In both procedures groups of animals are given graded doses of the drug under test, and after a

Table 2.1. Comparison of analgesic and mydriatic activity in mice[7,8]

Drug	ED_{50} (mg/kg) *Analgesia (hot plate method)*	*Mydriasis*
Phenadoxone	2·45	3·08
Levorphanol	3·00	2·94
Methadone	5·18	4·75
Morphine	11·8	13·1
Pethidine	28·0	21·5
Haloperidol	0·84	Inactive

suitable time interval the response of each animal to a stimulus is determined. Graded responses are measured in terms of pain threshold or reaction time and a dose–response curve plotted. The dose required to produce a given increase in pain threshold is determined and compared with the dose of a reference drug required to produce a similar increase. In contrast, the quantal procedure involves qualitative and not quantitative data, and is quicker to perform. Animals in each dose group are divided into two divisions, those that respond to a given stimulus and those that fail to respond. The percentage of each group that has attained analgesia, as shown by failure to react to the stimulus, is determined. A dose–response curve is constructed and the dose of analgesic producing analgesia in 50 per cent of the animals is found (ED_{50} value). Relative analgesic potencies may then be obtained by comparison with the corresponding value of a reference drug. The determination of the quantal response is simple and clear cut requiring only a single observation instead of the stepwise determination of a pain threshold. A suitable intensity of stimulus and an optimum time interval between treatment and observation of response (usually 20 minutes to 1 hour) are essential for a satisfactory quantal procedure. In both techniques, the results may be analysed statistically.

With some compounds, particularly those of intermediate or weak potency compared with morphine, ED_{50} values may represent their depressant effects on the central nervous system and not their analgesic actions. Analgesia may be characterized with greater confidence if the action of the compound is reduced or abolished by an analgesic antagonist such as nalorphine. Further, Janssen and Jageneau[7] have shown that in many morphine-like analgesics there is a correlation between analgesic activity and mydriasis in mice (see *Table 2.1*). Other drugs which depress the central nervous system, such as tranquillizers, do not show this correlation.

Animal Tests

Radiant heat methods

D'Amour and Smith[9] modified the original method of Hardy, Wolff and Goodell[10] by using a 6 to 8 W lamp with reflector focused on the tip of a rat's tail placed in a grooved board some 6 inches below the heat source. The apparatus in this test includes a voltage regulator, rheostat and stopwatch operated by the same switch which makes and breaks the current. The operator places the rat's tail in the groove, switches on the light and waits for the response—a sudden, typical twitch of the tail when the animal feels the pain. A heat intensity producing a reaction in about 5 seconds is the most convenient. Pain thresholds may be measured in terms of the reaction time or the minimal heat intensity required for a response. Christensen and Tye[11] have described a quantal procedure based on this method.

In the method of Ercoli and Lewis[12], a shaved area of a rat's back is exposed to a constant heat stimulus. The animal is protected by a plastic shield in which a circular hole is cut and arranged in line with a shutter and heat source. Analgesia is determined by measuring the time between exposure to stimulus and response of the animal. The response is characterized by twitching of the skin, retraction of the body and attempts at escape from the stimulus area.

Pressure methods

The method of Bianchi and Franceschini[13] is claimed to be superior to radiant heat methods as the response is a reflex mechanism based on centres higher than those involved in the tail-flick or skin-twitch reactions. An artery clip with its arms enclosed in thin rubber tubing is applied for 30 seconds to the root of the tail of a mouse, which makes continuous attempts to remove the noxious stimulus by biting the clip. The mouse is then injected with the drug and after 30 minutes the clip is again applied for 30 seconds to determine if analgesia has been produced. The percentage of mice which are insensitive to the pressure of the clip is an estimate of analgesia.

In a pressure method developed by Green, Young and Godfrey[14], the tip of a rat's tail is subjected to pressure which may be increased at will. A syringe is mounted vertically with the head of the plunger just above the tail. The pressure on the tail is then steadily increased until the rat responds, first by struggling and then by squeaking. A manometer is included in the assembly and the pressure shown when the rat responds is taken as a measure of pain threshold. Threshold values are higher with older animals but individual variation is small when the age group is narrow. This procedure may be used for a graded response in which activity is assessed in terms of increased pain threshold pressures, or for a quantal response in which analgesia is indicated when the threshold is raised to at least twice the control value. The analgesic activity of methadone relative to morphine has been shown to be similar when tested by pressure and by radiant heat methods.

The hot plate method

The technique devised by Woolfe and Macdonald[15] requires a zinc plate maintained at constant temperatures from 55°C, on top of which stands a

wide glass cylinder. A mouse is dropped inside the cylinder and on to the plate, and its reaction time is noted. The first signs of discomfort are shown by the mouse sitting up on its hind legs and licking or blowing its front paws. Soon the back paws are unable to bear the pain and the mouse either kicks its hind legs and dances about or attempts to jump out of the restraining cylinder. Hind limb movement is generally used as the end-point. Plate temperatures of 55 to 70°C are used, with 5° increments.

In Eddy and Leimbach's modification[16], the hot plate is the top surface of a copper bath containing a mixture of equal parts of ethyl formate and acetone, which when boiled under reflux maintains a plate temperature of 55 to 55·5°C. This method of heating is said to produce more consistent reaction times. For example, the average reaction time for 2,000 mice was 9·51 seconds with a standard error of 1·02 seconds. Reaction time is determined at least twice before and at intervals up to 60 minutes after drug administration, and then half-hourly until it returns to its initial value. The estimate of analgesic effect is the difference between the average reaction time for the first hour after injection and that during the pre-injection period. If this difference exceeds twice the standard error of the control value, it is considered to be significant.

Janssen and Jageneau[17] have also made extensive use of the hot plate test with both mice and rats. In their tests, reaction time is the time interval between the moment the animal reaches the hot plate and when it either licks its feet or jumps out of the cylinder. The average reaction time for a group of 10,000 mice in their experiments was 4·96 seconds, a value significantly lower than that reported by Eddy and Leimbach. This difference may be due to the different end-point criteria. The hot plate method may also be adapted to a quantal response, the animals showing analgesia when they fail to respond within a given time, for instance, 15 seconds.

Electric shock methods

Methods of electro-dental stimulation in guinea-pigs have recently been discussed[5]. Holes are drilled in the lateral upper side of one upper incisor about 0·5 mm below the gingival margin and to a depth of 0·1 mm. The animals are attached to horizontal boards and electrodes are inserted in the tooth and in the mouth. After 10 to 15 minutes, the animals calm down and then the voltage of the stimulus necessary to produce a rapid upward thrust of the head is determined. In other methods using mice, electric shocks are given to the tail and the end-point is a squeak[18,19].

Considerable difficulties have been encountered in determining the analgesic action of drugs such as salicylic acid derivatives and antipyretics. Many compounds of this type fail to show any activity in animal tests, and Harris and Blockus[20], using tooth pulp stimulation in man, were unable to distinguish aspirin from a placebo. However, under suitable experimental conditions, dose–response data for many of these weak analgesics have been obtained using hot plate, tail pinch and writhing tests. The latter test, devised by Siegmund, Cadmus and Lu[21], depends upon the antagonism of a syndrome induced in mice by intraperitoneal injection of a phenylquinone (*e.g.* 2-phenyl-1,4-benzoquinone) in aqueous alcohol. The syndrome, characterized by intermittent contractions of the abdomen, turning of the

trunk and extension of the hind legs, begins 3 to 10 minutes after injection and persists for more than 1 hour. Only those mice that exhibit the syndrome repeatedly within 10 minutes after a control injection are used for the test. Graded doses of the analgesic drug are administered to the animals which are then observed for 5 minute-periods every 15 minutes. At the end of a given time, the number of animals which fail to show the writhing response at each dose level are counted, a dose–response curve is drawn and the ED_{50} of the drug is estimated.

In many conditions in which weak analgesic drugs are effective, *e.g.* arthritis pain in rheumatic fever, pain is due in large part to inflammation and the apparent analgesic effect may be secondary to the diminution of inflammation brought about by these agents. Randall and Selitto[22] found that, while pain thresholds are little affected by mild analgesics, consistently raised values are obtained when measurements are made on inflamed tissues. With a typically anti-inflammatory substance such as phenylbutazone, the pain threshold of the inflamed tissue is raised while that of the control tissue is unchanged. By contrast, centrally-acting analgesics such as alphaprodine increase the pain thresholds of *both* tissues.

Tests in Man

Tests for analgesia in man involve either experimentally-induced pain or natural (pathological) pain. The common procedure for the study of experimentally-induced superficial pain is the Hardy–Wolff–Goodell radiant heat technique[10]. Light from a 1,000 W bulb of variable intensity is focused through a fixed aperture on to a blackened spot upon the forehead of the subject for exactly 3 seconds. If no pain is experienced, the procedure is repeated every 30 to 60 seconds with increasing heat intensity until pain is felt. The heat intensity at this point is measured by a radiometer and is considered to be the minimum stimulus for pain. This value represents a measure of the pain threshold and its elevation after drug administration gives an assessment of analgesic potency. Although the method in animals gives consistent results, those obtained by different workers in man are often inconsistent. Kuhn and Bromiley[23], for example, found the pain threshold range in control subjects to be much wider than that reported by the original workers and the effective dose of morphine was much higher.

The methods using electro-dental stimulation and contraction of muscle deprived of its blood-supply (ischaemic muscle) have been applied to the study of deep pain. The latter method is claimed to be useful for assessing analgesic dose levels prior to clinical trials[24,25]. In other procedures[26,27], a sphygmomanometer cuff is applied to the upper arm and inflated rapidly to 220 to 230 mm mercury to prevent the circulation to the arm. Hand and forearm muscles are exercised by rhythmically compressing a rubber bulb. After 30 to 45 such compressions, an aching pain usually develops in the flexor muscles in the forearm and sometimes also in the hand. The degree of pain depends on the number of contractions and, after training, subjects distinguish grades of pain expressed in units of one (slight) to ten (intolerable). For analgesic testing, muscles are exercised to a degree that gives pain of value 4 to 5 units. The analgesic is administered and pain re assessed after

a similar amount of exercise. Alternatively, the number of contractions producing a given degree of pain before administration is compared with that after administration.

The cases in which visceral pain thresholds have been studied relate to patients who have had the gall bladder removed and a T-tube left in the common bile duct for drainage[28]. It is possible to distend the bile ducts by the introduction of water via the T-tube, and pain thresholds may then be measured in terms of the hydrostatic pressure necessary to produce pain before and after drug administration.

The value of the pain threshold as a reliable measure of analgesic potency has been doubted by several workers. For example, Andrews[29] found that doses of morphine which effectively relieved pain in post-addicts only raised the threshold of the same individuals by 15 per cent, while lowering it in others. Beecher[4] considers that the appraisal of analgesic power of a drug should be based on its ability to relieve natural pain since the pain experience in man consists not only of the perception of painful stimuli but also of its psychic modification, *i.e.* patients may still have their pain but the anxiety and panic associated with it are suppressed.

Clinical trials employing pathological pain have been adapted, especially in the U.S.A., to give quantitative data. Keats and co-workers[30,31], for example, compared the analgesic potency of various drugs with that of morphine sulphate in patients with post-operative pain. The unknown drug at various dose levels and morphine sulphate at 10 mg/70 kg body-weight were alternately given by subcutaneous injection to individual patients during the first 30 post-operative hours. The patients were interviewed before and after each medication to evaluate the degree of pain relief at 45 and 90 minutes after administration of each drug. A dose was considered analgesic when 'most of the pain' was relieved at both interviews. Only the first two pairs of doses were used for evaluation in each patient since pain after the fourth dose was usually relieved more easily. Some of the data for phenadoxone hydrochloride (Heptalgin) are recorded in *Table 2.2*, which shows that this compound is about 5 times less active than morphine in this

Table 2.2. Comparison of analgesic activity of phenadoxone and morphine in man[30]

No. of Patients	*Morphine*				*Phenadoxone*				*Difference in relief* %
	Dose mg/70 kg	*No. of doses*	*Effective doses*	*Relief* %	*Dose* mg/70 kg	*No. of doses*	*Effective doses*	*Relief* %	
42	10	66	55	83·3	6–10	66	36	54·5	−28·8
22	10	33	25	75·8	30	33	21	63·6	−12·2
40	10	60	43	71·7	50	60	43	71·7	0
12	10	19	16	84·2	70	19	17	89·5	+5·3

test. When testing metopon, Keats and Beecher[30] showed that screening may be simplified and a dose–effect curve obtained with only two dose levels, provided a placebo (*e.g.* saline) be used in the tests.

Lee[32] compared the minimal effective clinical analgesic dose in cancer patients with chronic pain with that effective in surgical patients with acute pain. He found the average dose of morphine for chronic pain (13·1 mg) was higher than that for acute pain (9·6 mg). Other workers have also noted the variation in dose levels necessary to give analgesia in various forms of pain.

Recently, Houde, Wallenstein and Rogers[33] have described a procedure for the clinical evaluation of analgesics in cancer patients in which measurements are assessed by the patients' estimates of pain intensity before and at hourly intervals after administration of a drug. These data allow comparison of drugs in terms of peak activity and total effects.

The supplementation of clinical basal anaesthesia with analgesics, a technique that has become increasingly common over the past few years, was adapted by Chang, Safar and Lasagna[34] to evaluate analgesic potency. Pethidine or anileridine was used to supplement nitrous oxide analgesia during a standardized operation (dilation of the cervix and uterine curettage). Pethidine and anileridine were each used in 15 patients in a double-blind experiment. After pre-operative medication (0·5 mg atropine) either 30 mg anileridine or 50 mg pethidine were given intravenously for induction. The patient was then anaesthetized by breathing oxygen followed by an oxygen–nitrous oxide mixture and, after 10 minutes, the operation was started. Additional intravenous injections of each drug were administered as often as needed to maintain the required degree of anaesthesia, the principal criteria for injection being movement and irregular breathing. Comparison of the amounts of the two drugs needed showed that anileridine was approximately twice as potent as pethidine, a result in agreement with that derived from post-operative pain studies[35].

Tests for Addictive Liability

Until recently, the only way to assess addictive tendencies of drugs was from evidence of their clinical use. Under these circumstances, dangerous addictive properties may come to light only after some considerable time. Over the past 25 years, however, the National Institute of Mental Health Addiction Research Centre at Lexington, Kentucky, U.S.A., has been developing methods by which addictive liabilities of new drugs may be determined in a relatively short time[36,37]. Observations have been made on addicts or post-addicts and, although the validity of conclusions drawn from results obtained from such populations has been questioned, predictions made from this work have been confirmed in clinical experience. More rapid methods of screening have recently been developed in animals by Deneau, McCarthy and Seevers[38] using monkeys as the test subjects, and in man by Eddy, Lee and Harris[39] using nalorphine.

With the availability of these techniques it may now be necessary to insist that the addictive liabilities of all new analgesic drugs be determined before they are considered for use in general practice.

Tests in man

Addicts are stabilized by the regular administration of morphine at a daily dosage of 200 to 300 mg. When the drug is abruptly withdrawn from such individuals, they exhibit signs of physical dependence (abstinence phenomena) the intensity of which may be evaluated by a point-scoring system. Arbitrary values are assigned to the various symptoms such as mydriasis, tremor, restlessness, and fever, and the sum of these values gives a semi-quantitative estimate of the intensity of the abstinence syndrome. At about

the 30th hour of withdrawal, the morphine abstinence syndrome reaches its peak and a dose of the new compound is then given at a predetermined level. The suppression, if any, of the abstinence syndrome is compared in degree and duration with that obtained using a 30-mg dose of morphine. If suppression is achieved, attempts are made to substitute the new drug for morphine using a dose and interval of administration which prevents the abstinence phenomena. After about a week of stabilization on the new drug, the latter is usually abruptly withdrawn and the intensity of the ensuing abstinence syndrome compared with that after withdrawal of morphine. The successful substitution of the new drug in an addict stabilized on morphine defines the compound as one of addiction. Relative physical dependence properties may also be determined by comparing doses equivalent to a given amount of morphine (*e.g.* 50 mg) for maintenance of addiction. Thus the physical dependence of heroin is greater, and anileridine less, than that of morphine, equivalent doses being 18, 143 and 50 mg respectively. Comparative figures for the speed of development and intensity of physical dependence may also be derived from such experiments. The methadone abstinence syndrome, for example, develops more slowly and has a less intense maximum than that of morphine although methadone has the greater physical dependence capacity (12 mg≡50 mg morphine).

A more definite comparison of the ability of a drug to substitute for morphine in maintaining the physical dependence produced by chronic administration of morphine is obtained by 24-hour substitution of an agent for the morphine upon which the addict has been stabilized. Observations for symptoms of abstinence are made hourly from the 14th to the 24th hour after the last dose of morphine. As there is little interruption of the physical dependence state, 24-hour substitutions in the same individual may be repeated at weekly intervals to permit cross-over observations.

Another test applied at Lexington is that of direct addiction. Post-addicts, free of drugs for some months, are treated at regular intervals with a new drug, the dosage of which is increased as rapidly as possible. After about 30 days the drug is withdrawn and the rate of development and intensity of abstinence phenomena observed.

The abstinence syndrome which results following abrupt drug withdrawal from addicts may take several days to reach its peak, depending on the drug concerned. With control patients requiring analgesic therapy, such a delay in assessing severity of physical dependence may not be accepted due to recurrence of pain. However, abstinence phenomena may be precipitated by an analgesic antagonist such as nalorphine, and its use enables the utilization of short withdrawal periods. In the 'allyl test' procedure[39], nalorphine is given periodically during analgesic administration for chronic pain, followed by assessment of the intensity of physical dependence. The analgesic drugs are administered on a double blind basis to cancer patients with chronic pain in a dose and at time intervals sufficient to control pain. However, before starting the coded medication, each patient is screened by an initial allyl test to determine any physical dependence already present. Subsequently, nalorphine is given at 2-week intervals and an evaluation made of the physical dependence signs that precipitate. As a check, a placebo is administered on alternate weeks. Physical dependence is detectable

by the allyl test in 2 to 4 weeks with morphine and in about 4 weeks with oxymorphone or anileridine. The results obtained by this procedure may be used as a measure of the rate of development of physical dependence.

Animal tests

The development of tolerance in rats and mice to the analgesic effect of new drugs is usually detected by the routine tests applied to assess analgesic effectiveness, *e.g.* by an increase in the ED_{50} value in the same group of individuals following continued drug administration. Since tolerance and addiction liability are closely linked, a new drug may be qualitatively identified as addictive at an early stage.

Rhesus monkeys are used as test subjects for physical dependence assessments[38] and procedures similar to those employed in man are used.

In dogs, the relative addictive liabilities of dextromoramide and morphine have been determined by comparing the degrees of agitation produced by the abrupt withdrawal of the drug after a period of habituation[40]. Degrees of agitation are assessed from recordings of motor activity.

In the mouse, a prominent action of morphine-like analgesics is the characteristic S-shaped erection of the tail (Straub tail effect). Shemano and Wendel[41] determined the minimal dose of analgesic drugs to produce this effect and then calculated the Straub Index, *i.e.* a ratio of toxicity value (LD_{50}) to the dose giving the Straub effect (ED_{50}). The values compared well with the addictive liabilities of the compounds, *e.g.* heroin has an index of 100, morphine 29, pethidine 5 and codeine 2. The Straub Index may therefore serve as a guide to the relative addictive liabilities of new analgesics.

CLASSES OF ANALGESIC DRUGS

Morphine Derivatives

Gulland and Robinson's formula for morphine[42], advanced in 1923, has been confirmed by syntheses[43,44] and the relative stereochemistry of morphine followed from X-ray investigations[45,46]. The knowledge of morphine structure has been completed by the establishment of the absolute configuration of (—)-morphine as shown in (*I*)[47,48]. The work of Gates and Tschudi[43] made synthetic routes available to (+)-morphine but this was found to be almost inactive as an analgesic in mice[49] (see Reynolds and Randall[50] for a recent review of the pharmacology of natural (—)-morphine and its derivatives).

Numerous modifications of the morphine molecule have been made and new derivatives continue to be reported (see (*I*)–(*XVI*) for structures and *Table 2.3* for the analgesic activities in mice and man). Structure–action relationships in this field have been reviewed[59,60], while Eddy, Halbach and Braenden[61] have compiled data based on clinical experience with morphine derivatives and other synthetic analgesics. Etherification or esterification of the phenolic hydroxyl group decreases activity although some ethers, *e.g.* codeine (*XIII*; R=Me) and ethylmorphine (Dionine, *XIII*; R=Et) are widely used for the relief of mild to moderate pain and as antitussive agents. The benzyl ether (Peronine, *XIII*; R=CH_2Ph) is intermediate between

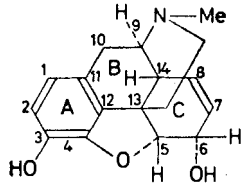
codeine and morphine in its pharmacological properties. A more recently prepared ether, pholcodine (*XIII*; R=β-4-morpholinoethyl) is valuable as an antitussive agent and is superior to codeine as a central sedative agent. Acetylation of the 3- and 6-hydroxyl groups gives diacetylmorphine (diamorphine, heroin, *II*) which although more potent than morphine is shorter acting, more toxic and has a higher addictive liability[61,62]. Addiction to it develops rapidly and it is the drug of choice of many addicts. The World Health Organization has therefore recommended that it be no longer used in therapy and the considerable fall in diamorphine production over the last few years (839 kg in 1954, 79 kg in 1959)[3] reflects world response.

Table 2.3. Relative analgesic activity of morphine derivatives in mice[36, 51, 52] and man[30, 32, 53–58]

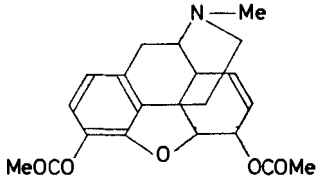
Compound	*Activity in mice* (morphine=1)	*Activity in man* (morphine=1)
Morphine (*I*)	1·0	1·0
Diacetylmorphine (*II*)	2·3	2–3·3
Dihydromorphine (*III*)	1·2	—
Hydromorphone (*IV*)	7·0	2–5
Oxymorphone (*V*)	0·17	10·0
Desomorphine (*VI*)	12·0	5–10
Metopon (*VII*)	4·2	2·8
Δ⁶-Deoxymorphines		
(*VIII*; R=H)	8·4	—
(*VIII*; R=Me)	19·4	—
(*VIII*; R=Et)	4·1	—
(*VIII*; R=Bu)	4·9	—
(*VIII*; R=Ph)	1·8	—
IX	82·0	—
X	16·0	—
XI	66·0	—
Codeine (*XIII*; R=Me)	0·15	0·08–0·16
Dihydrocodeine (*XIV*)	0·17	—
Hydrocodone (*XV*)	0·7	0·7
Oxycodone (*XVI*)	3·5	—

Many modifications of the morphine molecule involve changes in ring C since this is highly amenable to chemical transformations, and several useful drugs as potent as, or more potent than, morphine and with reduced side-effects have been prepared. World production figures[3] indicate the continued demand for some of these such as oxycodone (Eucodal, *XVI*), hydromorphone (Dilaudid, *IV*) and dihydromorphine (*III*). Metopon (*VII*, the structure is that given by Stork[63]) is considered to be one of the best morphine-type drugs developed; it is more potent than morphine on oral and subcutaneous administration, produces fewer side-effects such as nausea and vomiting, and makes the patient less drowsy; physical dependence develops less rapidly and is less severe than with morphine. Its use, however, is restricted by its difficult and expensive synthesis.

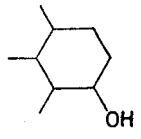
7,8-Dihydro-14-hydroxymorphinone (*oxymorphone, Numorphan, V*)—This compound, first prepared by Weiss[64], is reported[65] to be 12 to 15 times more active than morphine in animal tests, while Eddy and Lee[53] found a similar potency ratio in man with slightly fewer side-effects. A 14-hydroxy group also results in enhanced potency in the codeinone series, as for example compounds *XV* and *XVI* (*Table 2.3*). In a double blind cross-over study,



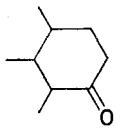
(I) (-)-Morphine



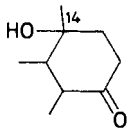
(II) Diacetylmorphine



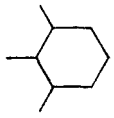
(III) Dihydromorphine



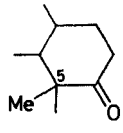
(IV) Hydromorphone



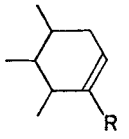
(V) Oxymorphone



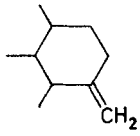
(VI) Desomorphine



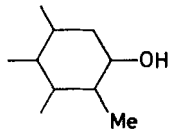
(VII) Metopon



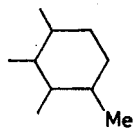
(VIII) 6-Substituted Δ^6-deoxymorphine



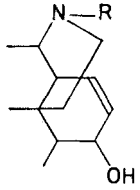
(IX)



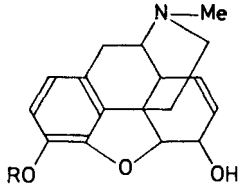
(X)



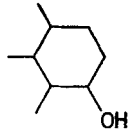
(XI)



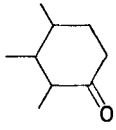
(XII) Substituted Normorphines



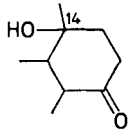
(XIII) Codeine (R = Me)



(XIV) Dihydrocodeine



(XV) Hydrocodone



(XVI) Oxycodone

Structure of morphine and its derivatives
(partial formulae depicting ring C of morphine or codeine)

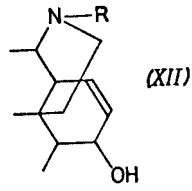
oxymorphone produced at least as much respiratory depression as morphine when given in comparable analgesic doses[66], and its addictive liability is high[67] (5 mg≡50 mg morphine for maintenance of addiction) but physical dependence detected by the allyl test did not develop any more rapidly[39].

6-Substituted Δ^6-deoxymorphines[51,68]—The most active member of this series, the 6-methyl compound (*VIII*; R=Me) has a somewhat briefer duration of action and is devoid of the emetic effect of morphine in mice.

6-Methylated dihydrodeoxymorphines[52,69]—The 6-methylene (*IX*) and 6-methyl (*XI*) compounds are highly active in mice and show promise as short-acting analgesics, with minimal side-effects.

It is significant that one of the more active derivatives of morphine is desomorphine (*VI*) in which ring C is unsubstituted and saturated. Its short duration of action, about half that of morphine, may be of value in obstetrics[70].

Table 2.4. Relative analgesic and antimorphine activities of *N*-substituted normorphine derivatives in rats[79]



(*XII*)

Derivative number	R	*Analgesic activity* (morphine=1)	*Antimorphine activity* (nalorphine=1)
1	Me	1·0	—
2	Et	<0·1	0
3	$CH_2{\cdot}CH{:}CH_2$	<0·1	1·0
4	Pr	0	1·0
5	Pr^i	<0·1	0
6	Bu	<0·1	<0·1
7	Bu^i	0	<0·1
8	n-C_5H_{11}	0·7	0
9	n-C_6H_{13}	0·7	—
10	$(CH_2)_2Ph$	6·1	—
11	$CH_2{\cdot}COPh$	<0·1	0

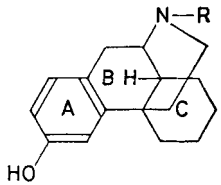
Normorphine (*XII*; *R=H*) *and N-substituted normorphines* (*Table 2.4*)—Interest in normorphine has been stimulated by the hypothesis that it may act as an intermediate in the mediation of analgesia. The analgesic effectiveness of normorphine relative to that of morphine varies considerably according to species and route of administration. Thus, it has been reported as one-eighth as active as morphine subcutaneously[71], one-seventh intraperitoneally[71], and of equal potency intracisternally in mice[19]; one-tenth intraperitoneally in rats[72]; of equal potency intravenously in dogs[72], and one-quarter as active subcutaneously in man[73]. It maintains addiction in morphine addicts, and its withdrawal after chronic administration results in abstinence phenomena that are milder in degree than those with morphine. In addicts, single doses of normorphine cause less sedation, depression of temperature, respiratory depression and pupillary constriction than do equivalent doses of morphine[74].

The replacement of the *N*-methyl group of morphine by *N*-allyl produces nalorphine (*XII*–3) a morphine antagonist. While nalorphine lacks analgesic properties in animals, it is an effective analgesic in man, being comparable in potency with morphine[75,76]. Attempts to produce addiction to nalorphine in former addicts have been unsuccessful, no symptoms of abstinence being shown after its withdrawal[77]. However, Schrappe[78] noted that chronic administration of large doses of nalorphine to patients led in some cases to the development of physical dependence, recognized by typical withdrawal phenomena of mild degree and short duration. The respiratory depressant properties of nalorphine and its disturbing psychic effects preclude its clinical use as an analgesic[61].

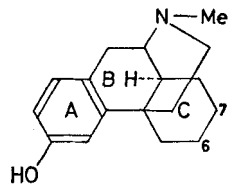
The antagonist properties of nalorphine have prompted the synthesis and examination of other variants of *N*-substitution in morphine and its related compounds. For example, Winter, Orahovats and Lehman[79] tested an extensive series[80] and found the length of the *N*-alkyl chain to be critical for exhibiting analgesic or anti morphine properties, a three-carbon chain being optimal for morphine antagonism. Lengthening the chains to *N*-pentyl and *N*-hexyl restored analgesic properties while chain branching gave inactive compounds in both respects[81] (*Table 2.4*). The *N*-phenethyl group gave a compound of particularly enhanced analgesic activity and this result foreshadowed the extensive work that has since been done on *N*-phenalkyl synthetic analgesics (see later). Recently Fry and May[82] reported the Mannich base derivative (*XII*; R = $(CH_2)_2$·CO Ph) to be several times less potent than the parent compound, whereas in the pethidine series the reverse is true.

Morphinans and Isomorphinans

Earlier work on morphinan derivatives culminated in the clinically valuable analgesic, racemorphan (*XVII*; R = Me) which has twice the activity of morphine, greater duration of effect and less frequent or severe side-reactions[50,61]. The activity of racemorphan is mainly in the (—)-isomer (levorphanol, Dromoran) and the clinical use of this isomer, which is highly effective by oral



(XVII)



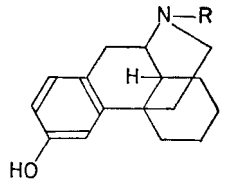
(XVIII)

administration, is preferred to that of the racemate (2 to 3 mg of levorphanol are equivalent to 10 mg morphine in man)[83,84]. The addiction liability of levorphanol, however, is as great as or greater than that of morphine[85]. As with morphine, methylation of the phenolic hydroxyl group of racemorphan results in a large decrease in activity. The (+)-isomer (dextromethorphan) is devoid of both analgesic activity and addictive liability[85] and is used as an antitussive agent.

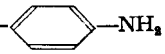
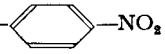
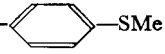
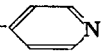
Compounds of considerably enhanced activities have been obtained by substitution of the *N*-methyl by *N*-phenalkyl and *N*-phenacyl groups. A large number of such compounds have been prepared and tested in animals[86], and details of some of the more potent members are given in *Table 2.5.*

The effect of replacing *N*-methyl by *N*-phenethyl is similar in morphinan

Table 2.5. Relative analgesic activities of (−)-*N*-substituted 3-hydroxymorphinans in mice[86]



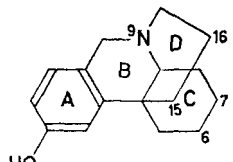
(XVII)

Derivative number	R	ED_{50} mg/kg	*Activity* (levorphanol = 1)
1	Me	0·3	1
2	$CH_2 \cdot CH_2$— 	0·01	30
3	$CH_2 \cdot CH_2$— 	0·018	17
4	$CH_2 \cdot CH_2$— 	0·019	16
5	$CH_2 \cdot CH_2$— 	0·034	9
6	$CH_2 \cdot CH_2$— 	0·04	7·5
7	$CH_2 \cdot CH_2$— 	0·045	6·7
8	$CH_2 \cdot CO$— 	0·046	6·5
9	$CH_2 \cdot CH_2$— 	0·063	5
10	$CH_2 \cdot CH_2$— 	0·113	3

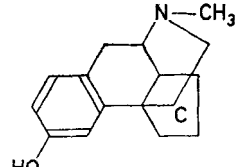
and morphine, but results with *N*-phenacyl markedly differ in the two series (*XII*–11 and *XVII*–8). In man, DeKornfeld[87] found 2 to 2·5 mg of levophenacylmorphan (*XVII*–8) to be the equivalent of 10 mg morphine for relief of post-operative pain. Withdrawal of levophenacylmorphiran after substitution for morphine or direct addiction studies with the compound produced morphine-like abstinence phenomena which were less severe than after morphine[37].

The observation that nalorphine is a potent, non-addicting analgesic in man prompted Keats[88] to study morphine antagonists derived from morphinan in the hope of finding a potent analgesic with less undesirable side-effects than nalorphine; (—)-3-hydroxy-*N*-propargylmorphinan (*XVII*; R = CH_2·C CH) proved to be at least as active as morphine in man, and although the disturbing mental changes seen after nalorphine were less frequent, it had similar respiratory depressant effects. The related *N*-(3,3-dimethylallyl-morphinan (*XVII*; R = CH_2·CH: CMe_2), however, was found to be a potent analgesic with mild respiratory depression. Its physical dependence capacity in monkeys is low but in tests in man at Lexington its addictive liability is equal to that of morphine. It is not a morphine antagonist.

Stereochemically, the fusion of rings B/C is *cis* in morphine and the morphinans, and *trans* in the isomeric isomorphinans. Formation of the B/C ring juncture by Gates's procedure gives a *trans* closure and has made available a number of isomorphinan derivatives[89]. In rats, (—)-3-hydroxy-*N*-methylisomorphinan (*XVIII*) is 8 to 10 times as active as morphine whereas its enantiomorph is inactive. The (—)-Δ^6-dehydro analogue is slightly less active than compound *XVIII*, and the racemic 6,7-dimethyl-Δ^6-dehydro analogue is about 6 times as active as morphine. The racemic 1- and 2-hydroxy-*N*-methyl compounds are inactive.



(*XIX*)



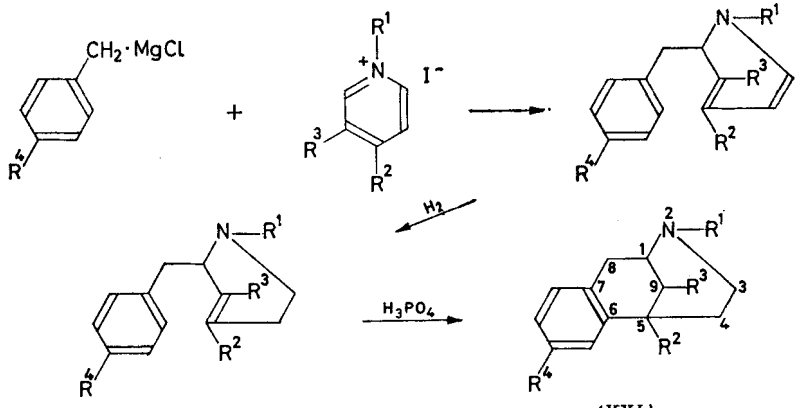
(*XX*)

Japanese workers, notably Sugimoto and Kugita[90], prepared isomers of morphinan in which the position of the nitrogen atom has been transposed *e.g.* 3-hydroxy-9-aza-*N*-morphinan (*XIX*), and this has an activity similar to that of morphine but it is much more toxic. In this compound, rings B/C and C/D are *cis* and *trans* fused respectively, as in morphine, and the skeletal structure is similar to that of some *Amaryllidaceae* alkaloids which are analgesics[91]. The 6-, 7-, 15- and 16-aza analogues are inactive[92], as also is the cyclopentane analogue (*XX*) of morphinan. The corresponding cycloheptane compound is as active as morphine but three times as toxic[93].

Benzomorphans

The high activity of some morphinan derivatives shows that the morphine molecule without both the 4,5-ether bridge and the structural features of

ring C retains its pharmacological properties. In 1955, May and Murphy[94] investigated the effect of reducing the morphine skeleton further by initiating the synthesis of a series of benzomorphan derivatives (*XXI*). In these, ring C of morphine and morphinan is replaced by methyl substituents at C–5 and C–9. Both the 5-methyl and 5,9-dimethyl compounds were obtained by an adaptation of Grewe's morphinan synthesis[95,96], outlined below, but the former were more conveniently made from 1-methyl-2-tetralone by Barltrop's method[97]. The stereochemistry of the benzomorphan molecule (*XXI*; $R^1{=}R^2{=}R^3{=}Me$) has not been established but the 5,9-dimethyl groups are tentatively assigned the *cis* configuration by analogy with morphinan[96]; an isomer, isolated in 1 per cent yield, is considered to be analogous sterically to isomorphinan (*i.e.* the 5,9-dimethyl groups are *trans*)[98]. In both isomers the ethanamine group that bridges the 1,5-positions is *cis* constrained.



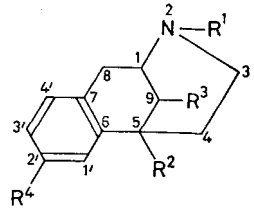
(*XXI*)

It is evident from *Table 2.6* that replacement of ring C of morphinan by methyl groups at C–5 and C–9 gives compounds whose activity is equal to, or greater than, that of morphine; with ring C represented by a single methyl group (at C–5) activity is, however, considerably reduced except in the *N*-phenethyl derivative (*XXI*–4). Thus compounds *XXI*–6 and *XXI*–3 (*Table 2.6*) possess about 70 and 20 per cent of the activity of morphine respectively. The effect of other structural variations in benzomorphans parallels, in most respects, alterations in the morphine and morphinan series. A hydroxyl group in the aromatic ring (at position 2′) is essential for high activity (*XXI*–5 and 6) while masking this group by conversion to the methyl ether reduces activity (*XXI*–6 and 10, and 11 and 14). Replacing *N*-methyl by longer alkyl chains, *e.g.* ethyl, n-propyl and n-butyl, reduces activity, although the n-pentyl compound is highly active (*XXI*–6 and 15 to 18). Although replacement of *N*-methyl by *N*-phenethyl also reduces activity (*XXI*–1 and 2) a similar replacement in the more active 5-methyl and 5,9-dimethyl members gives 20- and 12-fold increases in activity respectively (*XXI*–3 and 4, and 6 and 11). Compounds 5 and 6 are both less effective than their Mannich base counterparts (where *N*-methyl is replaced by N-$(CH_2)_2$·COPh) unlike the findings in the morphine and codeine series[82].

Finally, as with other types of asymmetric analgesics, activity resides largely in one enantiomorph (*XXI*–7 and 8, and 12 and 13).

(±)-2-Hydroxy-5,9-dimethyl-2-phenethyl-6,7-benzomorphan (phenazocine, Prinadol, Narphen, *XXI*–11) reported by May and Eddy[100] to be 9 times as potent as morphine in mice, was found in the hot plate test to be

Table 2.6. Analgesic activities of benzomorphan derivatives in mice



(XXI)
R^2 = Me

Derivative number	*Form*	R^1	R^3	R^4	ED_{50} (mg/kg) *oral*	*subcutaneously*	*Reference*
1	(±)	Me	H	H	42·1	22·1	95
2	(±)	$(CH_2)_2Ph$	H	H	—	55 approx.	99
3	(±)	Me	H	OH	—	10·4	95
4	(±)	$(CH_2)_2Ph$	H	OH	—	0·48	97
5	(±)	Me	Me	H	—	27·3	95
6	(±)	Me	Me	OH	23·9	3·0	95
7	(−)	Me	Me	OH	14·1	1·7	96
8	(+)	Me	Me	OH	—	Inactive	100
9*	(±)	Me	Me	OH	—	0·4	98
10	(±)	Me	Me	OMe	21·7	9·8	100
11	(±)	$(CH_2)_2Ph$	Me	OH	6·4	0·25	100
12	(−)	$(CH_2)_2Ph$	Me	OH	3·9	0·11	100
13	(+)	$(CH_2)_2Ph$	Me	OH	12·9	7·6	100
14	(±)	$(CH_2)_2Ph$	Me	OMe	10·6	6·5	100
15	(±)	Et	Me	OH	Inactive	Inactive	101
16	(±)	Pr	Me	OH	Inactive	Inactive	101
17	(±)	Bu	Me	OH	Inactive	Inactive	101
18	(±)	nC_5-H_{11}	Me	OH	—	2 approx.	101
N-Methylmorphinan					40·9	11·3	95
3-Hydroxy-*N*-methylmorphinan (racemorphan)					7·0	0·93	95
Morphine sulphate					3·7	2·1	100

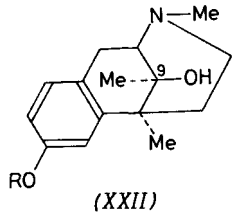
* Compound 9 is a stereoisomer of compound 6.

about 25 times as potent as morphine in rats and in the tail withdrawal test about 15 times as potent. However, orally phenazocine was only twice as potent as morphine in the latter test[102].

Clinical experience with phenazocine shows it to be effective against post-operative and chronic pain in doses of 1 to 1·5 mg (*i.e.* it is 7 to 10 times as potent as morphine) with a similar onset of action and duration of effect, and minimal circulatory and gastro-intestinal side-effects[103]. DeKornfeld and Lasagna[104], on the other hand, found phenazocine to be less potent in a test against post-operative pain (3 mg phenazocine≡10 mg morphine). Other workers[105,106] have reported that the respiratory depressant effects of 1 to 2 mg phenazocine were equivalent to those of 5 to 10 mg morphine in healthy subjects, while 1 mg phenazocine and 40 mg pethidine were equally effective in depressing alveolar ventilation[107]. However, the incidence of appreciable respiratory depression after phenazocine has been small (*e.g.*

Eckenhoff[103] observed it in two cases out of a total of 152 patients). Its use in obstetrics has also been favourably reported[108,109]. Doses of 1 to 4 mg, given intravenously, gave rapid analgesia in 202 patients, with no inhibition of uterine contraction[108]; lack of respiratory spontaneity was seen in 11 per cent of the newborn but many of these may have been due to other complications (*e.g.* breech delivery, prematurity). The earlier report of the low potency of phenazocine in suppressing abstinence from morphine (1 mg phenazocine in monkeys is equivalent to 0·18 mg morphine)[38] has not been confirmed in man; Fraser and Isbell[37] found 1 mg phenazocine to be equivalent to 8·15 mg morphine in addicts. Physical dependence on phenazocine tends to be milder than that caused by morphine and to develop more slowly.

In a further paper of the series, May and Kugita[110] report on 5,9-dimethyl-9-hydroxy derivatives that may be considered analogous to oxymorphone (*V*) and oxycodone (*XVI*) which are 14-hydroxy derivatives of hydromorphone and hydrocodone respectively and are more active than their precursors (see p. 53). In the benzomorphan series, however, a 9-hydroxy group (equivalent to the 14-hydroxy group of the morphine skeleton) is disadvantageous; compound *XXII* (R=H) and its C–9 diastereoisomer lie between morphine and pethidine in analgesic potency and are half as active as the analogue lacking the 9-hydroxy group (*XXI*–6). The methyl ether (*XXII*; R=Me) is comparable in activity with codeine by parenteral administration, but 3 times as potent orally. Substitution of *N*-methyl by *N*-phenethyl in compound *XXII* (R=H) gives a slightly less active compound, a result which emphasizes the variability of the effect of such replacement in benzomorphans.



(XXII)

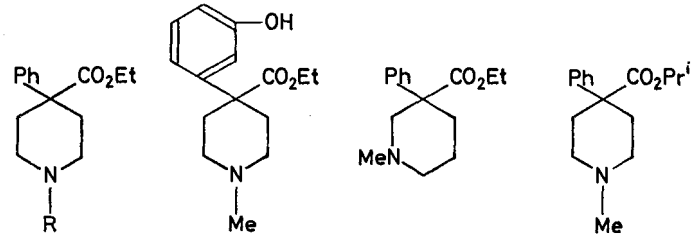
Pethidine and Derivatives

Ethyl 1-methyl-4-phenylpiperidine-4-carboxylate (pethidine, Meperidine, Dolantin, *XXIII*; R=Me), first introduced in 1939 by Eisleb and Schaumann[111], is now by far the most widely used synthetic analgesic[3]. In potency, pethidine is graded between codeine and morphine (50 to 100 mg equivalent to 10 mg morphine in man)[57] and is useful for the management of mild to moderate pain, especially in patients intolerant to opiates. Its toxicity is low and its action is somewhat shorter than that of morphine. At equivalent dosage, pethidine is at least as depressant as morphine upon respiration and while morphine-like side-effects such as nausea and vomiting frequently occur, it produces little disturbance of urinary function or bowel action. Although pethidine was originally designed as a spasmolytic, conflicting reports have been made with regard to its antispasmodic properties. It has

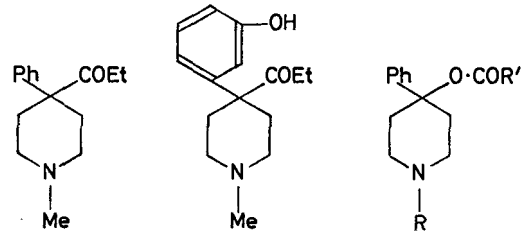
been reported effective, however, in controlling pain associated with smooth muscle spasm, *e.g.* biliary, renal and gastro-intestinal colic[57].

The generally accepted view that morphine is an unsatisfactory analgesic for obstetric use as it depresses both maternal and foetal respiration and tends to prolong labour, has promoted extensive study of the use of pethidine in this field. Most workers agree that pethidine, often in single doses of 100 mg and in combination with scopolamine, makes for a shorter labour by facilitating dilatation of the cervix and promoting relaxation. However, it increases the incidence of delay on the first breath and cry of the newborn infant[61].

Tolerance to the drug develops slowly and its addictive liability is judged to be lower than that of morphine (doses of more than 120 mg may substitute for 50 mg morphine in addicts)[36]. Nevertheless, the incidence of addiction



(XXIII) *(XXIV)* Bemidone *(XXV)* Isopethidine *(XXVI)*



(XXVII) *(XXVIII)* Ketobemidone *(XXIX)*

Table 2.7. Relative analgesic activities of pethidine and its analogues in mice[60]

Compound	*Activity* (Pethidine=1)
Pethidine (*XXIII*; R=Me)	1·0
Bemidone (*XXIV*)	1·5
Isopethidine (*XXV*)	0·5
XXVI	0·5
XXVII	0·5
Ketobemidone (*XXVIII*)	10·0
XXIX; R=Me, R′=Et	5–10

to pethidine exceeds that of other synthetic analgesics, although this fact is probably due to its wider use. Full accounts of the clinical use of pethidine have recently been given[50,61].

Earlier work on the structural modification of pethidine has been reviewed[60] and recent advances up to 1957 have been surveyed by Beckett and Casy[112].

Previous structure–action investigations may be summarized as follows (see also *Table* 2.7):

(*a*) Substitution in the 4-phenyl group generally reduces activity (*e.g.* with *o*- and *p*-hydroxyl and *p*-amino) although the *m*-hydroxyl (bemidone, *XXIV*) and *o*-methyl analogues are more active than pethidine.

(*b*) Shift in the position of the phenyl group leads to less active compounds (isopethidine, *XXV*).

(*c*) Changes in the ethyl ester group are disadvantageous (*e.g.* the isopropyl analogue (*XXVI*)) although here the results in man may be the reverse of those in animals[61].

(*d*) Replacement of the 4-ethoxycarbonyl group by amides and ketones produces less active compounds (*e.g. XXVII*) but in the bemidone series the ketone (ketobemidone, *XXVIII*) is more active than the analogous ester (*XXIV*). Reversal of the ethoxycarbonyl group (*e.g. XXIX*; R=Me, R′=Et) gives a more active compound.

Ketobemidone has been studied clinically and is still widely used in Germany and Scandinavia. Its potency is greater than that of morphine but its duration of action and side-effects are similar to those of morphine. Dependence on it develops rapidly and the abstinence syndrome may be severe[61].

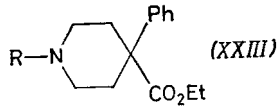
During the past 5 years a considerable number of new structural variations of pethidine have been reported; these may be conveniently dealt with in two subdivisions: (*a*) derivatives of ethyl 4-phenylpiperidine-4-carboxylate (norpethidine, *XXIII*; R=H) and (*b*) esters of 4-aryl-4-piperidinols (*XXIX*) the so-called reversed esters of pethidine.

Norpethidine Derivatives

The structural variation most thoroughly investigated is that of replacement of *N*-methyl by other groups, notably phenalkyl, brought about in most cases by alkylation of norpethidine with the appropriate alkyl or aralkyl halide (see *Table 2.8*). These studies probably stem from the observation made in 1956 by Perrine and Eddy[113] that *N*-phenethyl norpethidine (*XXIII*–2) is twice as active as pethidine in mice. Elpern, Gardner and Grumbach[114] examined the effect on activity in rats of lengthening the alkyl chain between the ring nitrogen atom and the aryl group, of chain branching and of the influence of substituents in the phenyl ring. In the unsubstituted phenalkyl series, activity increases as the chain is lengthened from one to three carbon atoms; however, increase to a four-carbon chain reduces activity (*XXIII*–1 to 4). In the *p*-amino and *p*-nitrophenalkyl series, maximum activity occurs with the 2-ethyl compounds (*XXIII*–5 to 8, and 9 to 12). Chain branching gives inactive or weakly active compounds. Substituents in the benzene ring, such as amino, nitro, methoxy and ring nitrogen (*i.e.* phenyl replaced by pyridyl) enhance activity in the phenethyl but not always in other series (*XXIII*–2 and 6, 10, 13, 14, and 3 and 7). *N*-*p*-Aminophenethyl norpethidine (anileridine, Leritine, *XXIII*–6) is 10 to 12 times more potent than pethidine in animals, with high oral activity and relatively mild side-reactions[115,123]. Its potency in man is 2 to 3 times that of pethidine[61].

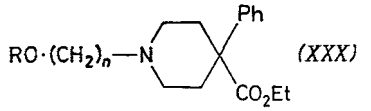
For the control of post-operative pain, 40 mg anileridine are said to be equivalent to 100 mg pethidine[35], while equi-analgesic doses of morphine and anileridine are 10 and 25 mg respectively[39]. Apart from its higher potency, anileridine possesses no significant advantage over pethidine; its respiratory

Table 2.8. Relative analgesic activities of *N*-substituted norpethidines in rats[115–122]



(XXIII)

Derivative number	R	*Activity* (pethidine=1)	*Derivative number*	R	*Activity* (pethidine=1)
1	$PhCH_2$	0·25	13	p-$MeO{\cdot}C_6H_4{\cdot}(CH_2)_2$	3
2	$Ph(CH_2)_2$	2	14	4-(C_5H_4N)*$(CH_2)_2$	9
3	$Ph(CH_2)_3$	13	15	$PhCH{:}CH{\cdot}CH_2$	29
4	$Ph(CH_2)_4$	2	16	$PhNH{\cdot}(CH_2)_2$	100
5	p-$NH_2{\cdot}C_6H_4{\cdot}CH_2$	1	17	$PhNH{\cdot}(CH_2)_3$	30
6	p-$NH_2{\cdot}C_6H_4{\cdot}(CH_2)_2$	11	18	C_6H_{13}	6·7
7	p-$NH_2{\cdot}C_6H_4{\cdot}(CH_2)_3$	6	19	C_7H_{15}	3·3
8	p-$NH_2{\cdot}C_6H_4{\cdot}(CH_2)_4$	2	20	C_8H_{17}	4·0
9	p-$NO_2{\cdot}C_6H_4{\cdot}CH_2$	0	21	C_9H_{19}	2·5
10	p-$NO_2{\cdot}C_6H_4{\cdot}(CH_2)_2$	6	22	$C_{10}H_{21}$	0
11	p-$NO_2{\cdot}C_6H_4{\cdot}(CH_2)_3$	5	23	BuCHMe	5·8
12	p-$NO_2{\cdot}C_6H_4{\cdot}(CH_2)_4$	0·5	24	BuCHEt	1·7



(XXX)

Derivative number	*n*	R	*Activity* (pethidine=1)
1	2	Et	5
2	4	Et	10
3	2	Ph	7
4	2	$PhCH_2$	7
5	2	p-$NO_2{\cdot}C_6H_4$	0·5
6	2	Fur.† CH_2	25
7	2	Pyr.‡ CH_2	10

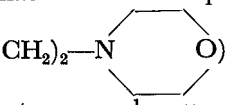
* 4-Pyridyl. † Tetrahydro-2-furyl. ‡ Tetrahydro-2-pyranyl.

depressant effect is similar but of shorter duration and the incidence of other side-reactions is similar[61]. It is used in obstetrics[124] and, more recently, as a supplement to nitrous oxide–oxygen anaesthesia[34]. The consumption of anileridine in the U.S.A. and Canada during 1959 exceeded all other synthetic analgesics, except pethidine[3]. It is a more satisfactory substitute for morphine in addicts than is pethidine, but its dose level is high in spite of its greater analgesic potency[61].

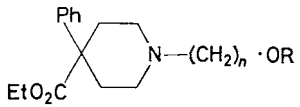
In the study of *N*-substituents mentioned above[114], activity was found to increase still further when the three-carbon chain contained a double bond (the *N*-cinnamyl analogue, *XXIII*–15) but this is lost when a triple bond is included[114]. A further series of compounds was prepared[116] in which an

imino group was placed between the aryl and alkyl portions of the aralkyl substituent; several of these compounds show very high activities (*XXIII*–16 and 17). Compound 17 (piminodine, Alvodine), recently marketed, is reported by DeKornfeld and Lasagna[125] to be somewhat more potent than morphine against post-operative pain (7·5 mg≡10 mg morphine). However, its addiction liability appears to be at least as great as that of morphine. A recent paper[117] relates to norpethidines substituted with long chain alkyl groups (*XXIII*–18 to 24); these were highly active in mice, and α-branching did not markedly depress activity.

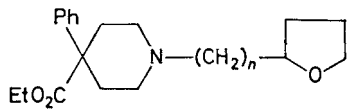
An examination of *N*-substituted norpethidines bearing alkyl groups terminated by various oxygen functions[118,119] derives from the observation that while 2-morpholinoethylnorpethidine (morpheridine, *XXIII*; R=



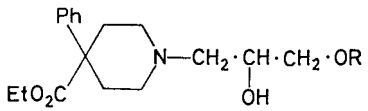
) and its sulphur analogue possess marked analgesic potency, replacement of oxygen (or sulphur) in the heterocyclic residue by carbon or nitrogen gives inactive compounds. This indicates that the presence of an oxygen or sulphur atom at some distance from the basic centre is desirable in this series of pethidine analogues[120,121]. The compounds prepared have the general formula *XXX* (R=alkyl, aryl or aralkyl). In the



(XXX)



(XXXI)

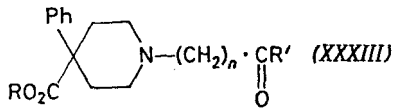


(XXXII)

alkoxy series (*XXX*; R=alkyl) the 2-ethoxyethyl and 4-ethoxybutyl compounds are the most potent, being 5 and 10 times as active as pethidine respectively (*cf. XXX*–1 and 2)[122]. The phenyl (*XXX*–3) and benzyl (benzethidine, *XXX*–4) analogues are about 7 times as active as pethidine. Substitution in the aromatic ring reduces potency (*cf. XXX*–3 and 5) and so does replacement of the alkoxy or aryloxy oxygen by sulphur. Inclusion of an additional ether group in the *N*-alkyl chain gives highly active compounds, *N*-2-(tetrahydrofurfuryloxy)ethylnorpethidine (furethidine, *XXX*–6) being reported as 25 times as active as pethidine. Activities of an even greater order are obtained by replacement of the open chain ether linkage with a methylene group giving two compounds (*XXXI*; *n*=3 and 4) which are 30 times as potent as pethidine. Lister[126], in a comparison of the pharmacology of benzethidine and furethidine with that of pethidine noted some differences in side-effects at equi-active analgesic dosage, in particular a reduction in histamine release. A similar series, including some previously known compounds[118,119], was prepared by Morren and Strubbe[127], who confirm that enhanced activity

may be achieved by inclusion of simple ether substituents in the *N*-alkyl chain. The 2-(2-hydroxyethoxy)ethyl compound (*XXX*; R=$CH_2 \cdot CH_2 \cdot OH$, n=2) is particularly potent[128] and is available commercially in Belgium under the name etoxeridine. Yet another related series was prepared by condensing 3-aryloxypropane-1,2-epoxides with norpethidine to give *N*-(3-aryloxy-2-hydroxypropyl)norpethidines (*XXXII*)[129]. The unique feature of this series is the 2-hydroxy group, which is shown to be important for analgesic action since the propanediol derivative (*XXXII*; R=Ph) is reported to be twice as active as the 3-phenoxypropyl analogue[130]. The propanediol (*XXXII*; R=Ph) is the most active member of the series (about 12 times as active as pethidine in mice) but activity is reduced by substitution in the aromatic ring, just as it is in the 2-phenoxyethyl compounds[122].

Table 2.9. Analgesic activities of basic ketones derived from norpethidine[131,132]

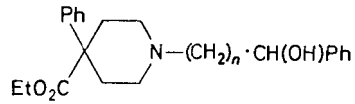


(XXXIII)

Derivative number	R	R′	*n*	ED_{50} (mg/kg) *Mice*	*Rats*
1	Et	Ph	2	0·44	0·21
2	Et	*m*-FC_6H_4	2	0·51	0·18
3	Et	*m*-MeC_6H_4	2	0·88	0·73
4	Et	*m*-$HO \cdot C_6H_4$	2	4·82	—
5	Et	*p*-FC_6H_4	2	1·10	0·44
6	Et	Ph	3	2·5	3·1
7	Et	*p*-FC_6H_4	3	1·9	3·1
8	Me	Ph	2	0·93	1·1
9	Pr	Ph	2	25	37
10	Pr^i	Ph	2	2·3	3·8
11	Bu	Ph	2	>80	—
Pethidine				25·3	41

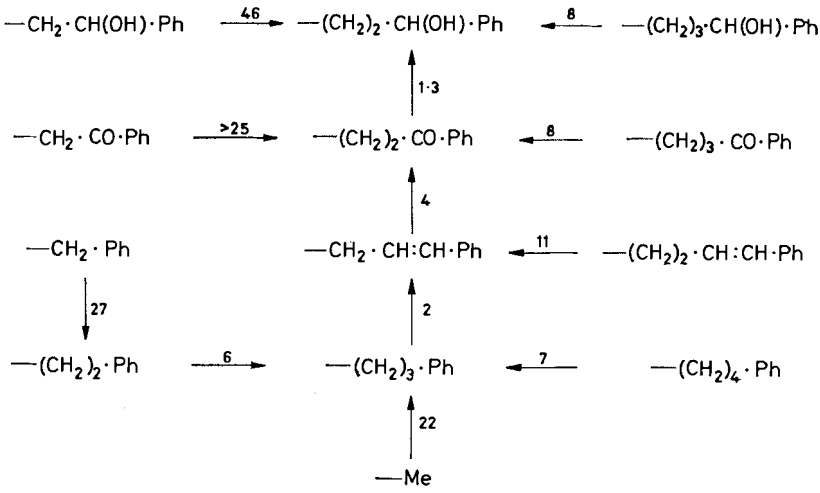
A series of aralkyl norpethidines (see *Table 2.9*) has been studied in which the alkyl chain is separated from the aryl residue by a carbonyl group[131,132]. The compounds (*XXXIII*; n=2) are Mannich bases and are prepared, in most cases, from norpethidine, formaldehyde and acetophenone, or substituted acetophenones; the homologues (*XXXIII*; n=3) are prepared by condensation between the appropriate haloalkyl aryl ketone and norpethidine. Highest activity was found in the 2-propiophenone compound (R 951, *XXXIII*–1) which is about 60 and 200 times more active than pethidine in mice and rats respectively[131]. The rate of development of tolerance to the analgesic action of compound R 951 in rats is reported to be lower than that with pethidine or morphine[133]. Increase of the alkyl chain length to three carbon atoms reduces potency (*cf. XXXIII*–1 and 6, and 5 and 7). Substitution in the aryl group (R′) has a similar effect although the decrease is small with the *m*-fluoro derivative (*cf. XXXIII*–1 to 5, and 6 and 7). The effect of varying the ester group (R in *XXXIII*) has also been studied; activity increases from methyl to ethyl, and then decreases rapidly with increase in

chain length to butyl[134]. The isopropyl ester, however, is nearly 10 times as active as its n-propyl analogue (*XXXIII*–9 and 10). The secondary alcohol (*XXXIV*; $n=2$) derived by reduction of the ketone, is somewhat more active than its precursor, but its acetyl derivative is less active than the ketone (*XXXIII*–1). In the butyrophenone series, the ketone and derived alcohol (*XXXIV*; $n=3$) have similar activities[135].



(XXXIV)

Janssen and Eddy[135] have given a semi-quantitative estimate of the influence of systematic chemical modifications on analgesic potency in mice and rats of a series of *N*-substituted norpethidines and reversed esters of pethidine. Their estimate, based upon results of the hot plate test obtained in three separate laboratories, is illustrated below.

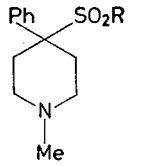


Potency relationships among a series of *N*-substituted norpethidines
(The formulae represent *N*-substituents. Arrows point to compounds of increased activity and the potency ratio of adjacent derivatives is given by the numeral next to the linking arrow, *i.e.* $A \xrightarrow{X} B$; $X=\frac{\text{P.R.B.}}{\text{P.R.A.}}$)

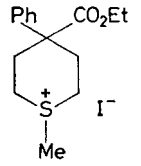
Attention has already been drawn to the large series of *N*-aralkylmorphinan derivatives that have been tested for analgesic properties (see p. 57). In general, the effect of a particular substituent group on the potency of the parent compound is similar in both the morphinan and pethidine series (*e.g.* a similar degree of enhancement with phenethyl, *p*-amino-, *p*-methoxy- and *p*-nitrophenethyl, and 2-(4-pyridyl)ethyl). However, some striking anomalies are apparent. While *N*-cinnamyl and *N*-3-phenylpropylnorpethidine are respectively 29 and 13 times as potent as pethidine, the corresponding morphinan counterparts are almost inactive. Conversely, while

N-phenacylnorpethidine has only one-tenth the activity of pethidine, (—)-*N*-phenacylnormorphinan is 6·5 times more active than levorphanol in mice and highly active in man.

A group of Swiss workers[136] in 1952 reported the preparation of a series of 4-alkylsulphone pethidine analogues (*XXXV*) and found a number of these (*e.g. XXXV*; R=Et, Pr, Bu^i) to be as effective as pethidine in mice in tests for analgesia. Some non-basic derivatives (*XXXV*; *N*-methyl replaced by *N*-tosyl) were also reported to have activities of the same order as that of pethidine[136]. In contrast to the isosteric replacement of carbon by sulphur described above, replacement of ring nitrogen by sulphur (*XXXVI*) results in loss of activity[137]. Bell and Archer[138] report that ethyl 3-α-phenyltropane-

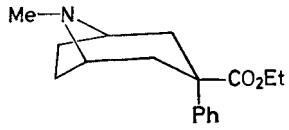


(XXXV)



(XXXVI)

3-β-carboxylate (*XXXVII*) is slightly more active than pethidine. It differs from pethidine in possessing an ethylene bridge across the 2,6-positions and probably also in the conformation of the 4-substituents.



(XXXVII)

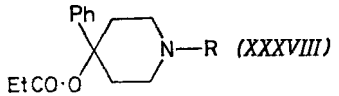
Reversed Esters of Pethidine

The original observation[139] that replacement of the ethoxycarbonyl group of pethidine by a propionoxy group is attended by an increase in potency has since been confirmed in numerous cases. Such a change usually produces a 20-fold increase in activity, regardless of the nature of the *N*-substituent[135]. In most cases, propionoxy esters are more active than acetoxy esters although Beckett, Casy and Kirk[140] found the reverse to be true with esters of 1-phenethyl-4-aryl-4-piperidinols.

The results of replacement of *N*-methyl by *N*-aralkyl groups in esters of 4-aryl-4-piperidinols are qualitatively similar to those obtained in the pethidine series (see *Table 2.10*). Compound *XXXVIII*–6 is one of the most active synthetic analgesics yet obtained[135]. A large number of esters of 4-aryl-1-phenethyl-4-piperidinol have been prepared and each compound was found to be more potent than the corresponding *N*-methyl analogue[140]. 4-Acetoxy-1-isopropyl-4-phenylpiperidine has significant analgesic activity in mice (ED_{50} of 15 mg/kg) but an *N*-dimethylamino analogue (a hydrazine derivative) is much weaker (ED_{50} of 40 mg/kg), in spite of the two *N*-substituents being of similar size[141].

Much information is now available regarding the effect of alkyl substitution in the piperidine ring. Methyl substitution in the 3-position is advantageous and leads to the potent compounds, alpha- and betaprodine (*XXXIX*; R=Me)[142]. The observed enhanced activity of alphaprodine over that of pethidine in animals has been confirmed in man (40 to 60 mg of alphaprodine is equivalent to 100 mg of pethidine)[143]. Alphaprodine has a brief duration of action, even shorter than that of morphine or pethidine,

Table 2.10. Analgesic activities of *N*-substituted-4-phenyl-4-propionoxy piperidines in mice[135]

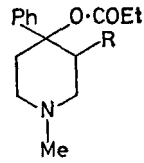


(XXXVIII)

Derivative number	R	ED_{50} μmoles/kg	*Molar potency ratio* (pethidine=1)
1	Me	11	7·4
2	$Ph(CH_2)_2$	3·2	25
3	$Ph(CH_2)_3$	0·5	162
4	$Ph(CH_2)_4$	0·65	54
5	$PhCH{:}CH{\cdot}CH_2$	0·31	261
6	$PhCH(O{\cdot}COEt){\cdot}(CH_2)_2$	0·054	1,500

and it has been used where such a length of effect is desirable, *e.g.* endoscopies, supplementation of analgesia and obstetrics. Side-effects are minimal, less than those of morphine, and possibly less than with pethidine; its addiction liability is similar to that of pethidine[61].

High activity is also associated with ethyl and n-propyl groups substituted in the 3-position but is lost if the substituent be large (*e.g.* benzyl)[142,144]. In a comparative series[140], the evidence indicated that, with lower alkyl groups, optimum activity is afforded by 3-methyl substitution. The 3-allyl analogue (allylprodine, *XXXIX*; $R=CH_2{\cdot}CH{:}CH_2$) has been reported to be 10 times as active as alphaprodine in rats but twice as toxic[145]. The only example of 2-methyl substitution available (*XL*) is one-fifth as active as the unsubstituted analogue; the latter (several times as potent as morphine in mice) is somewhat more active than the 2,6-dimethyl compound[146]. 3,5-Dimethyl analogues are inactive[147] while the 2,5-dimethyl compounds of Nazarov are highly active[148].



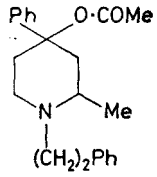
(XXXIX)

Alphaprodine; R = Me, 3-Me/4-Ph *trans*
Betaprodine ; R = Me, 3-Me/4-Ph *cis*

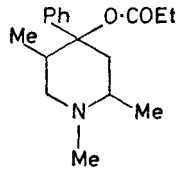
Trimeperidine (Promedol, *XLI*) is reported to be several times more active than pethidine in animals[149] and in the U.S.S.R. is used clinically[61] Apart from increased activity in man (10 to 20 mg subcutaneously is the

usual therapeutic dose) its duration of effect is longer than that of pethidine; side-reactions are similar although trimeperidine has a greater tendency to lower the blood-pressure. It has been used successfully in various spastic conditions such as biliary, renal and intestinal colic, and also in obstetrics. In spite of earlier reports of absence of tolerance and signs of physical dependence, addiction to trimeperidine has been reported. Zherebtsov[150] compared trimeperidine with a stereoisomer (Isopromedol) against pain associated with smooth muscle spasm, and considered the latter to be the superior analgesic both in potency and duration of action.

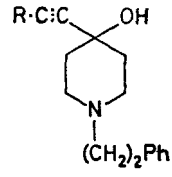
Structure–action relationships in substituted 4-piperidinols are not easy to establish since they are complicated by conformational differences among



(XL)



(XLI) Trimeperidine



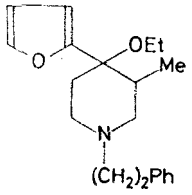
(XLII)

substituents. The influence of stereochemical factors in reversed esters is reflected in the wide potency differences found among geometric isomers such as trimeperidine and Isopromedol[151] and alpha- and betaprodine-type compounds[140,142]. A large number of esters of 4-piperidinols involving structural changes within the 4-aryl group has been prepared. The activities of mono- and dialkyl substituted 4-phenyl compounds exhibit no clear pattern in relation to position of substitution, rankings of isomers varying with changes in ester grouping and *N*-substituent[140,152,153]. Some of these compounds are highly active, *e.g.* the acetoxy esters of 1-phenethyl-4-(2,5-dimethylphenyl)-4-piperidinol and 3-methyl-1-phenethyl-4-*o*-tolyl-4-piperidinol are 2 and 13 times as active respectively as morphine in mice. The 3-methyl-4-*o*-tolyl-4-piperidinol derivative shows a marked separation of morphine-like effects in man. Keats, Telford and Kurosu[154] found it to be 3 to 4 times more potent than morphine against post-operative pain, with minimal nausea and absence of vomiting but severe respiratory depression.

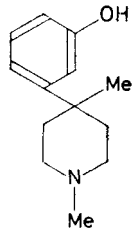
Fullerton[153] replaced 4-phenyl by other unsaturated groups capable of providing a π-electron cloud such as substituted acetylenes and ethylenes. Among those examined in mice, two compounds (*XLII*; R=Ph and Bu) showed significant activity in the hot plate test, but this activity is probably due to central nervous depression rather than analgesia. Separation of the phenyl from the piperidine ring by a methylene group (giving 4-benzyl compounds) leads to a loss of activity that is not recovered by fluorinating the ring[155,156]. Again some of these compounds are depressants of the central nervous system.

Isosteric replacement of phenyl by 2-furyl, 2-thienyl and 2-pyridyl is disadvantageous in 3-methyl-1-phenethyl-4-piperidinols and esters; this result is probably due to attendant changes in steric rather than electronic factors[155]. In the course of these syntheses, 4-alkoxy derivatives of significant activity were encountered. The 4-ethoxy compound (*XLIII*) is several times more

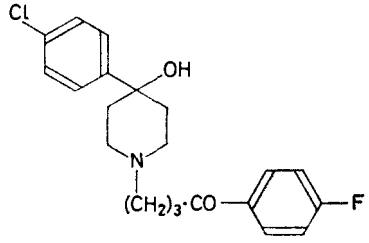
active than pethidine in mice and has a low toxicity. Higher and lower alkyl ethers are much less active, as also are analogues lacking the 3-methyl substituent[157]. McElvain and Clemens[158] have reported a series in which the 4-oxygen function is replaced by lower alkyl groups. The potency of these compounds is generally low although (*XLIV*) shows marked activity at high dose levels.



(XLIII)



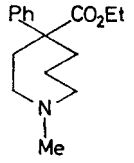
(XLIV)



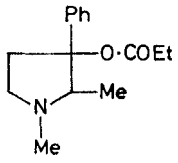
(XLV) Haloperidol

Some 4-aryl and 4-aralkylpiperidinols, in contrast to their esters, are central nervous depressants of the tranquillizing type (*e.g.* haloperidol, *XLV*)[8] rather than analgesics.

Seven- and eight-membered ring analogues of pethidine and its reversed esters are less active than corresponding six-membered compounds[159–161]. Ethyl 1-methyl-4-phenylazacycloheptane-4-carboxylate (ethoheptazine, Zactane, *XLVI*), for example, is only one-third as active as pethidine in rats[162]. It is effective orally against moderate pain (*e.g.* post-partum pain and chronic pain due to arthritis) in doses of 50 to 100 mg and is commonly



(XLVI) Ethoheptazine



(XLVII)

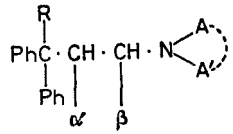
employed in combination with aspirin (Zactirin)[163,164]. Side-effects are minimal and its liability to cause addiction is low or non-existent[165]. Five-membered ring analogues of reversed esters of pethidine have been reported[166]; the most active member (Prodilidine, *XLVII*) has an analgesic activity in rats which is slightly less than that of codeine. In a triple blind cross-over study, 50 and 100 mg of the pyrrolidine derivative (*XLVII*) were found to be as effective respectively as 600 mg aspirin and 30 mg codeine[167].

Diphenylpropylamines

Compounds of general structure (*XLVIII*) have recently been extensively reviewed, particularly from a chemical aspect[168]. The best-known example of an analgesic of this class is methadone (*XLVIII*; R=COEt, α=H, β=Me, NAA′=NMe_2) which was introduced into medical practice on a

wide scale in 1946. It is a strong analgesic in man, being assessed as potent as morphine both by controlled clinical trial[54] and by ischaemic[26] and visceral[28] pain methods; its duration of effect is at least as long as that of morphine. Absence of appreciable sedative action renders it of little value in obstetrics and pre-anaesthetic analgesia. It is a powerful antitussive and its spasmolytic properties make it useful against bladder spasms and renal colic. Side-effects are similar to those of morphine, with less constipation; respiratory depression further militates against its use in obstetrics[61]. Tolerance to its therapeutic action develops after repeated administration and it sustains addiction at one-quarter of the required dose of morphine, with a longer-lasting effect. After withdrawal, physical dependence signs are slow to develop and are less severe than those after morphine withdrawal; methadone may thus be used for the withdrawal of patients from morphine and other drugs associated with severe abstinence phenomena[169,170].

Table 2.11. Analgesic activities of methadone and related compounds



(XLVIII)

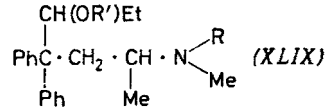
Compound	α	β	R	NAA'	ED_{50} *in mice* mg/kg[36]	*Activity in man*[54,61,171–174] (morphine=1)
Methadone	H	Me	COEt	NMe_2	1·6	1
Isomethadone	Me	H	COEt	NMe_2	2·5	2·6–3·0
Phenadoxone	H	Me	COEt	$N(CH_2)_4O$	1·1	0·6
Dipipanone	H	Me	COEt	NC_5H_{10}	2·0	2·0–2·5
Normethadone	H	H	COEt	NMe_2	2·5	—
Diethylthiambutene (*L*)				NEt_2	4·2	—
Ethylmethylthiambutene (*L*)				NMeEt	2·4	5·0–10·0

Variations in the basic group of methadone (see *Table 2.11*) have led to the morpholino (phenadoxone, Heptalgin, Heptazone) and piperidino (dipipanone, Pipadone) analogues, both of which are employed clinically. In controlled clinical trials, Nathan[171] reported 10 mg of phenadoxone to be equivalent to 16 mg of morphine against chronic pain, and Keats and Beecher[30] found 60 mg of phenadoxone to be equivalent to 10 mg of morphine against post-operative pain; these results represent an unusually high divergence. Side-effects at the higher dose level were more frequent than with morphine but effects upon respiration were similar for both drugs. The liability of phenadoxone causing addiction is probably less than that of morphine[61]. Dipipanone, at a dose level of 20 to 25 mg, is as effective as 10 mg of morphine against chronic and post-operative pain with minimal side-effects and a similar duration of action[61] and addiction liability[36]. Coleman, Levin and Jones[175] report its successful use as an adjunct to anaesthesia in obstetrics and surgery.

The α-methyl isomer, isomethadone (*XLVIII*; R=COEt, α=Me, β=H, NAA'=NMe_2) is less potent than methadone[54,172]. Its duration of effect is similar to that of morphine with fewer side-actions such as nausea and

vomiting, but with a similar degree of respiratory depression[61]. Its addictive properties have been established[176] and it suppresses the morphine abstinence syndrome (37 mg are equivalent to 50 mg morphine in this respect)[36]. The straight chain analogue (normethadone, *XLVIII*; R=COEt, α=β=H, NAA′=NMe_2) is significantly less active than methadone as an analgesic in mice and in man[177,178]. It is one of the components of an antitussive preparation Ticarda, which is widely used in Germany and neighbouring countries. The addiction liability of normethadone exceeds that of codeine[36], and several cases have been recorded of the use of Ticarda by addicts instead of other narcotics.

Table 2.12. Analgesic activities in mice of secondary and tertiary methadols[180, 181]



(XLIX)

Derivative number	*Isomer*	R	R′	ED_{50} (mg/kg)
1	α-(±)	Me	H	18·9
2	α-(±)	H	H	0·98
3	β-(±)	Me	H	7·3
4	α-(±)	Me	OCOMe	1·2
5	α-(±)	H	OCOMe	0·48
6	β-(±)	Me	OCOMe	0·8
7	β-(±)	H	OCOMe	1·2
8	α-(−)	Me	H	3·5
9	β-(+)	Me	H	63·7
10	α-(−)	Me	OCOMe	1·8
11	α-(−)	H	OCOMe	6·1
12	β-(+)	Me	OCOMe	4·1
13	α-(+)	Me	H	24·7
14	β-(−)	Me	H	7·6
15	α-(+)	Me	OCOMe	0·3
16	α-(+)	H	OCOMe	0·74
17	β-(−)	Me	OCOMe	0·4

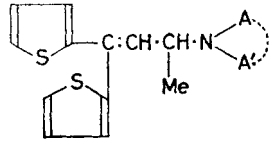
Compounds *XLIX*–8, 9, 10 and 12 are derived from (+)-methadone (ED50 of 25·7 mg/kg) and *XLIX*–13, 14, 15 and 17 are derived from (−)-methadone (ED50 of 0·8 mg/kg).

Reduction of methadone, either catalytically or with lithium aluminium hydride, gives only one of two possible diastereoisomers, while sodium–propanol reduction gives both forms (α- and β-methadol, *XLIX*–1 and 3). Reduction of (+)- and (—)-methadone has made available the four possible isomers of methadol[179]. In animals, α-(+) and β-(—)-methadol are far less active than their precursor, (—)-methadone, but acetylation considerably increases activity (see *Table 2.12*). The most active methadol, α-(—), derived from the weak analgesic (+)-methadone, is 7 times more active than its enantiomer, α-(+) from (—)-methadone, but acetylation reverses the potency ratio. In man, 5 to 10 mg of racemic α-acetylmethadol was effective orally against chronic pain, with a duration of effect similar to that of morphine and little evidence of respiratory depression[182]. Keats and Beecher[183], in a controlled clinical trial, estimated that 50 mg of α-(—)-acetylmethadol were equivalent to 10 mg of morphine against post-operative

pain (in animals the former was more active than morphine). Less work has been done on the (+)-isomer but results indicated it to be, as in animals, more potent than its enantiomer. The racemic and optical forms of α-acetylmethadol are addicting[184]. After parenteral administration to addicts, morphine-like effects of the α-(—)-isomer develop much more slowly than those of the racemate or (+)-form but are persistent; onset of action is more rapid with the (—)-isomer by mouth and addicts may be stabilized by 60 mg of the latter, given orally as infrequently as once in 72 hours.

The sulphone analogue (*XLVIII*; R=SO_2Et, α=H, β=Me, NAA′=NMe_2) is equal to methadone as an analgesic in animals[185] but the erratic clinical results obtained were traced to the instability of the compound on sterilization[30].

Isosteric replacement of phenyl is disadvantageous[60] but the related dithienylbutenylamines (*e.g.* dimethylthiambutene, *L*; NAA′=NMe_2) are potent analgesics, of a similar order of activity as morphine in animals[186]. In man, these compounds are less active, ethylmethylthiambutene (*L*; NAA′=NMeEt) the most potent member, having an optimal dose of more than 50 mg, equivalent to 10 mg of morphine, against post-operative pain[173,174]. Addiction liability[187], duration of action and side-effects are similar to those of morphine, except that less nausea and vomiting are produced. The pharmacology of the diethylamino derivative (*L*; NAA′=NEt_2) has been investigated in the dog and it is marketed as Themalon for use in veterinary practice[188].



(L)

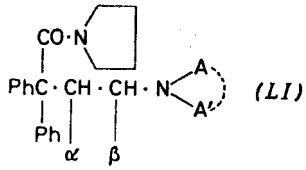
Methadone and related compounds provide a rich field of examples of stereochemical specificity in analgesics, analgesic potency and morphine-like side-reactions residing mainly in one isomer of each enantiomorphic pair[189,190]. For example, in a controlled clinical trial, 4 to 6 mg of (—)-methadone were found to be as effective as 7 to 9 mg of the racemate against post-operative pain[54]. In a similar trial (—)- and racemic isomethadone were respectively equivalent to, and one-third as potent as, morphine[54].

Recently, Janssen and Jageneau[17] investigated basic amides related to methadone, highest activity being found among *N*-pyrrolidino and dimethylamino-amides (see *Table 2.13*). An α-methyl group in the side chain considerably increases activity, and the β-methyl isomers are much less active (*cf. LI*–1 and 2, and 3 and 6, and 7 and 9); the reverse is true for corresponding methadone–isomethadone isomers. The stereochemical specificity of methadone type compounds, already referred to, is also evident in the amides with activity residing mainly in the (+)-isomer (*cf. LI*–3, 4, 5 and 7, 8).

Janssen and Jageneau[17] found dextromoramide (R-875, Palfium, *LI*–4) the most active member of the series, to be 18·5 and 40·5 times as active as morphine in mice and rats respectively; it has also been reported to be some

20 times more potent in both species[191]. Tolerance to the analgesic effects developed more slowly with dextromoramide than with morphine. Clinically it is an analgesic of high potency although not as active as in animals; a dose of 5 mg is reported to be equivalent to 10 mg of morphine for the treatment of post-operative pain as measured by peak effect, its duration of action being less than that of morphine[31]. Dextromoramide is equally effective by mouth

Table 2.13. Analgesic activities in mice of tertiary amides related to methadone[17]



(LI)

Derivative number	*a*	*β*	NAA′	*Isomer*	ED_{50} mg/kg	*Potency ratio* (methadone = 1)
1	Me	H	NMe_2	(±)	16·3	0·32
2	H	Me	NMe_2	(±)	>100	<0·05
3	Me	H	$N(CH_2)_4O$	(±)	1·7	3·6
4	Me	H	$N(CH_2)_4O$	(+)	0·12	13
5	Me	H	$N(CH_2)_4O$	(−)	85	0·07
6	H	Me	$N(CH_2)_4O$	(±)	57	0·09
7	Me	H	NC_5H_{10}	(±)	13·2	0·39
8	Me	H	NC_5H_{10}	(+)	7·8	0·66
9	H	Me	NC_5H_{10}	(±)	>50	<0·10

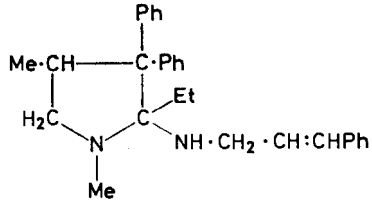
and by injection for the treatment of post-partum pain[192]. A low rate of tolerance development and minimal side-actions have been claimed for the drug but several workers have noted that a high incidence of nausea and vomiting accompanies its use[31,192,193]. It causes respiratory depression of a similar degree to morphine but severe cases are occasionally noted, possibly due to its rapid onset of action resulting in a relative overdose in patients who have been previously receiving narcotics[31]. Its use in tablet form only is permitted in France[40]. Fraser and Isbell[194] found the addiction liability of dextromoramide to be at least as great as that of morphine. (See La Barre[40] for a recent review of its clinical use.)

The influence of various basic groups upon activity has been further studied by preparation of piperazino analogues; 4,4-diphenyl-6-(4-methyl-1-piperazino)heptan-3-one (*XLVIII*; R=COEt, *a*=H, *β*=Me, NAA′=

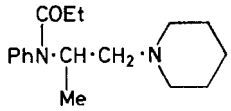
) is about half as active as methadone in rats[195]. A compound reputed to be the *N*-cinnamyl analogue of methadone (or isomethadone) has been reported[196] but Wilson[197] has advanced evidence for the alternative formula (*LII*); the compound was too toxic for its analgesic activity to be assessed. 3-(*N*-Cinnamyl-*N*-methylamino)-1,1-di(2-thienyl)-1-butene had little or no activity in rats[196]. Replacement of the basic group of methadone-type compounds by a sulphonium group abolishes activity[137]. Beckett, Casy, Harper and Phillips[198] prepared a series of thiambutenes bearing

substituted piperidino groups and larger ring analogues to obtain information of the optimal dimensions of the basic centre in analgesics; with the piperidino compound as standard, a seven-membered ring was advantageous while an eight-membered ring or an increase in the effective width of the basic group was not.

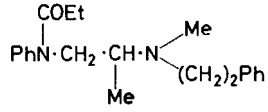
Wright, Brabander and Hardy[199] have modified the general structure (*XLVIII*) by replacing one phenyl group and its attached quaternary carbon



(LII)

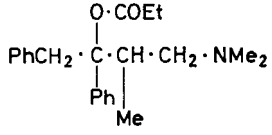


(LIII) Phenampromid



(LIV) Diampromid

atom with nitrogen. This replacement satisfies one of the structural requirements postulated for analgesics[200] in that it provides a flat aromatic ring which, furthermore, is still attached to a non-basic centre as the nitrogen atom is acylated. The analgesic potency of phenampromid (*LIII*) equals that of codeine in mice and pethidine in rats, while that of diampromid (*LIV*) approximates to the potency of pethidine and morphine in mice and rats respectively. In the treatment of post-operative pain, DeKornfeld and Lasagna[104] found phenampromid in doses of 25 and 50 mg to approach, but not to equal, the value of 10 mg morphine. Nalorphine antagonizes the analgesic and respiratory depressant actions of these compounds[201], both of which possess liability to addiction[202]. Phenampromid has been resolved and activity shown to reside mainly in the (—)-isomer.



(LV)

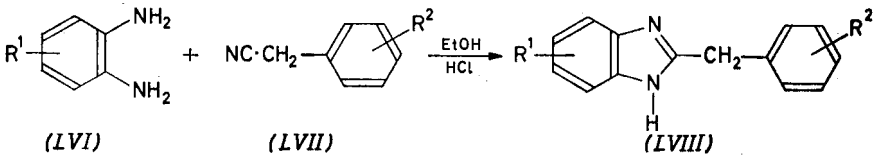
In a further modification of the general formula (*XLVIII*), Pohland and Sullivan[203] prepared a compound (*LV*) in which the two phenyl groups are on adjacent carbon atoms. The α-racemate, propoxyphene, in doses of 20 to 40 mg/kg raises the pain threshold of rats to a similar degree as does 2 mg/kg of methadone[204], the (+)-isomer, dextropropoxyphene (Darvon,

Doloxene) being twice as effective as the racemate. Replacement of dimethylamino by pyrrolidino in *LV* gives a compound of similar activity to propoxyphene in rats, while the corresponding 2-acetoxy-4-pyrrolidinobutane is twice as active[203]. In mice, Eddy[181] reports that the α-racemate and α-(+)-isomer of the latter compound have ED_{50} values of 4·32 and 2·15 mg/kg respectively (hot plate method) showing that morphine-like potencies are possible in this series. Clinically, dextropropoxyphene is equal in potency to codeine by oral administration and has less side-effects[205]. It is reported to be useful for the relief of mild to moderate pain as encountered, for example, in dentistry[206], rheumatic disorders[207] and post-partum care[208]. Van Bergen, North and Karp[209] found the analgesic effectiveness of dextropropoxyphene (100 mg), pethidine (100 mg) and codeine (65 mg) against post-operative pain to be indistinguishable. Fraser and Isbell[210] assess the addiction liabilities of both racemic and dextropropoxyphene to be less than that of codeine; the allyl test[39] applied to patients after 6 months of dextropropoxyphene therapy gave negative results[211].

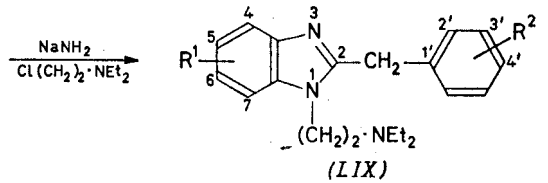
A series of compounds (*XLIX*; R=H) related to the methadols have also been prepared and are the first examples of potent analgesics that possess secondary, rather than tertiary, basic groups. Some members are very active in mice and, in two examples, more so than their tertiary analogues (see *Table 2.12*, *XLIX*–1 and 2, and 4 and 5); their clinical evaluation is in progress[212].

Benzimidazoles

In 1957, a new class of potent analgesics related to benzimidazole was described[213,214]. The compounds of general formula (*LIX*) were prepared by condensing substituted *o*-phenylenediamines (*LVI*) with a substituted phenylacetonitrile (or a related compound) (*LVII*) and alkylating the product (*LVIII*) with 2-diethylaminoethyl chloride.



(LVI) *(LVII)* *(LVIII)*

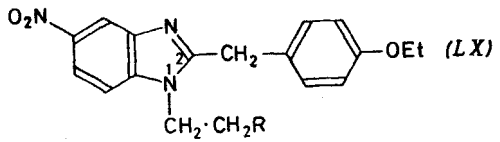


(LIX)

In subsequent papers[215], details of the synthesis and analgesic activities of the numerous compounds prepared have been reported. A large number of these are highly active in mice, some being many times more potent than morphine (see *Table 2.14*). Structure–action relationships that have been investigated are summarized as follows:

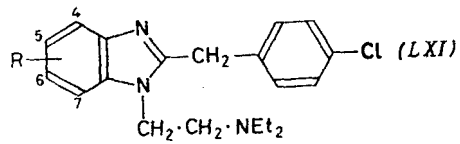
(*a*) The structure of the basic side chain at N^1 of the benzimidazole nucleus is 2-diethylaminoethyl for optimum activity, although high activities may be

Table 2.14. Analgesic activities in mice of benzimidazole derivatives[814,815] (relative to morphine = 1)



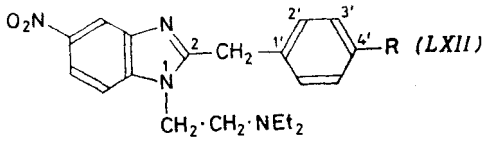
(*LX*)

R	NMe_2	NEt_2	NC_5H_{10}	$N(C_2H_4)_2O$	CH_2NEt_2
Activity	20	1,000	100	2	0·2



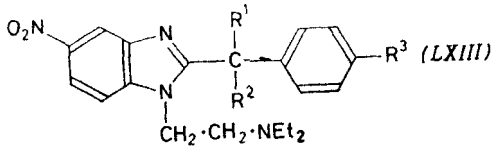
(*LXI*)

R	H	4-NO_2	5-NO_2	6-NO_2	7-NO_2
Activity	0·1	0	3	0·1	0



(*LXII*)

R	H	OMe	OEt	OPr^i	OPr	OBu	4′-Cl
Activity	2	100	1,000	500	200	5	3
R	H	SMe	SEt		SPr	SBu	4′-F
Activity	2	50	30		2	0·25	1
R	H	Me	Et		Pr	Bu	4′-OH
Activity	2	10	20		50	1	1



(*LXIII*)

R^1	H	Me	Et	Me	$CONH_2$	H	Me	$CONH_2$
R^2	H	H	H	Me	H	H	H	H
R^3	H	H	H	H	H	4′-OEt	4′-OEt	4′-OEt
Activity	2	2	0·5	0·7	2·5	1,000	50	200

obtained with other basic groups such as 2-dimethylamino and 2-piperidinoethyl (see *LX*). The ranking of the diethylamino and dimethylamino compounds is thus the reverse of that obtaining in methadone-type compounds.

(*b*) While the 4′-chloro derivative (*LIX*; R^1=H, R^2=4′-Cl) is only one-tenth as active as morphine, a 5-nitro group in the benzimidazole nucleus increases activity by a factor of 30 (see *LXI*); nitro groups in the 4-, 6- or

7-position are ineffective in this respect. This structural feature is unknown in other analgesic types.

(*c*) Substitution in the 4′-position of the benzyl fragment is advantageous, particularly with alkoxy groups (highest activity results with 4′-ethoxy) while thioethers and lower alkyl groups also give compounds of high activities. Chlorine is less effective (although *LIX*; R^1=5-NO_2, R^2=4′-Cl is 3 times as active as morphine) while fluorine and hydroxyl are disadvantageous (see *LXII*).

(*d*) The methylene group linking the benzimidazole and phenyl nuclei is best left unsubstituted, although the result of substitution upon activity appears to be dependent upon other substituents (*e.g.* the carbamoyl group, $CONH_2$, enhances the activity of the unsubstituted methylene derivative somewhat when R^3=H, and causes a five-fold reduction when R^3=OEt in *LXIII*).

While the 5-nitro and 4′-ethoxy groups both enhance activity separately, their effect together is additive and the compound (*LIX*; R^1=5-NO_2, R^2=4′-OEt) is the most powerful analgesic yet examined. Eddy[181] states it to be about 1,500 times as active as morphine in mice, with an ED_{50} value of just over 1 μg/kg. In man, Bromig[216] found the 4′-chloro derivative (*LIX*; R^1=5-NO_2, R^2=4′-Cl) to be 3 to 5 times as potent as morphine against post-operative pain, which is in close agreement with the activity found in mice. Its duration of action was shorter than that of morphine while undesirable side-reactions (*e.g.* respiratory depression and constipation) were noted in only a small percentage of cases. The compound possessed very little sedative action. In the same trial, the 4′-methoxy analogue (*LIX*; R^1=5-NO_2, R^2=4′-OMe) was 10 times as potent as morphine but produced greater incidence of side-reactions, especially severe respiratory depression. The action of orally administered benzimidazole analgesics, both in animal experiments and in man, is rather weak and irregular, while by injection the therapeutic ratio between analgesic and respiratory depression activity is rather narrow and does not offer any advantage over morphine. For these reasons, clinical trials have been discontinued[217]. In addicted monkeys, the 4′-chloro (*LIX*; R^1=5-NO_2, R^2=4′-Cl) and 4′-ethoxy (*LIX*; R^1=5-NO_2, R^2=4′-OEt) derivatives were twice and 1,500 times as potent as morphine in alleviating abstinence[38]. In addicts, the two derivatives have addiction potentials comparable to that of morphine[202].

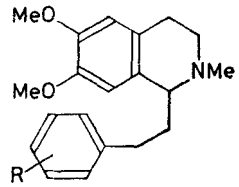
The highly potent compound (*LIX*; R^1=5-NO_2, R^2=4′-OEt) has found application in experiments designed to condition drug-seeking behaviour in rats[218]. Previous attempts failed because of aversive reactions induced by the pain of subcutaneous injection of morphine or the bitter taste of solutions of morphine or other opiates. The benzimidazole derivative, however, is effective at great dilutions (3 to 10 μg/l.) and water-deprived rats accept solutions containing these drug concentrations; morphine-like effects develop within 4 to 7 minutes after the rats begin to drink.

Reduced Isoquinolines

A series of halogen substituted 1-phenethyl-1,2,3,4-tetrahydroisoquinolines that have analgesic activities in mice of a similar order to, or greater than,

that of codeine were recently described (see *Table 2.15*)[219]. The 4′-fluoro derivative is about twice as active as codeine and activity decreases with increase in size of the halogen substituent; 3′,4′-dichloro-substitution gives a

Table 2.15. Analgesic activities in mice of halogen-substituted 1-phenethyl-1,2,3,4-tetrahydroisoquinolines[219]



(LXIV)

R	*Isomer*	ED_{50} (mg/kg)
4′-Chloro	(±)	18
4′-Chloro	(−)	8·4
4′-Chloro	(+)	Inactive
3′,4′-Dichloro	(±)	25
3′,4′-Dichloro	(−)	12
4′-Fluoro	(±)	13
4′-Bromo	(±)	31
Codeine phosphate		25

compound equal in potency to codeine. Stereochemical specificity in this series has been established in two cases (the 4′-chloro and 3′,4′-dichloro derivatives) activity residing in the (—)-enantiomers, while in contrast, spasmolytic properties are similar for both enantiomers. No substituent has yet been found which replaces the *N*-methyl and retains activity. Lengthening or shortening the phenalkyl side chain and aromatization of the nitrogen ring both result in loss of activity. Sadove, Ali and Schiffrin[220] studied the relative effectiveness of the racemic 4′-chloro compound and codeine by a double blind trial in 40 post-operative orthopaedic patients. They concluded that the analgesic potencies of the two drugs, given orally, are similar. In monkeys the physical dependence capacity of the 4′-chloro compound was found to be low. This new class of analgesics is particularly interesting as the reduced isoquinoline nucleus cannot be considered as a morphine fragment; such a nucleus, however, is not unique to the present series of analgesics and occurs both in 3-hydroxy-9-aza-*N*-morphinan and in some *Amaryllidaceae* alkaloids (see p. 58).

CONCLUSIONS

Pfeiffer[221] has listed the properties of the hypothetical ideal analgesic. He considers that it should: (*1*) not become ineffective through the development of tolerance and should not be habit-forming or addicting; (*2*) have a large therapeutic margin of safety; (*3*) be effective against all types of pain; (*4*) possess a short latent period and a long duration of action; in some instances (*e.g.* obstetrics) a short duration of action is advantageous; (*5*) not depress respiration or the cardiovascular system; (*6*) not alter sensory modalities other than pain; (*7*) not affect the gastro-intestinal tract; (*8*) be effective

both orally and parenterally; (*9*) not be an antidiuretic, and (*10*) be chemically stable and inexpensive.

As has been pointed out, morphine, a reliable, inexpensive analgesic of relatively low toxicity, possesses serious side-effects, and fails to fulfil many of the above requirements. The morphine modifications and synthetic analgesics described also possess, in lesser or greater degree, the undesirable properties of morphine.

It may therefore be argued that the multitude of synthetic analgesics available today represent no fundamental advance over morphine; synthetic compounds, with potencies equal to or greater than that of pethidine, are drugs of addiction (except nalorphine, which is a special case) and respiratory depressants. Some authorities[222] state that the introduction of further synthetic analgesics should be discouraged unless they represent an important progress in therapy by way of absence of side-effects. One reason for such a view is concerned with the international control of narcotics; increases in the number of synthetic drugs which are on a par therapeutically with natural products tend to impair the efficiency of such control. Eckenhoff and Oech[223] drew attention to the lack of unbiased comparative data on the analgesic effectiveness and side-reactions of the large number of analgesics in clinical use and considered that, if fewer narcotics had been produced, investigation of pharmacologic effects and mechanisms of action would have been more thorough.

While the ideal analgesic of Pfeiffer may, in fact, be unattainable, Robsom and Nissim[224] consider that a good alternative is to have a large number of analgesics differing in their side-actions, thus affording a wide choice in which the type of side-action determines the suitability of a product for a particular occasion. This alternative is now being established through the provision of a wide variety of analgesic agents with varying potency and a good deal of variation in their liability to side-actions[61]. Thus, several synthetic drugs are available which are superior to morphine in possessing less pronounced side-actions such as respiratory depression and gastrointestinal effects (*e.g.* pethidine, phenazocine), better oral effectiveness (*e.g.* levorphanol, methadone), greater duration of action (*e.g.* levorphanol), less tolerance development and primary addiction liability (*e.g.* methadone). Further, some synthetic compounds have found applications in special cases where morphine is unsuitable, the most important example being the use of pethidine, anileridine and phenazocine in obstetrics where morphine is considered a major risk for the infant. Other examples where synthetic analgesics have found successful application include pre-anaesthetic medication and the supplementation of general anaesthesia.

Examples of the quantitative separation of side-effects continue to grow while in former addicts there is now at least one instance of an analgesic, namely, nalorphine, in which qualitative separation in respect of addiction liability has been achieved. The results with *N*-(3,3-dimethylallyl)morphinan, a drug which combines potent analgesia with mild respiratory depression in man and which has a low physical dependence in monkeys, gives further encouragement to the view that a satisfactory clinical analgesic lacking in a major degree the undesirable side-effects of morphine may ultimately be discovered.

Another aspect that must be considered in assessing the value of the continued development of synthetic analgesics is the investigation into the mediation of analgesia and mechanisms of action of analgesics. The formulation of structure–action relationships requires data and the more there are available, the more securely may correlations be established and hypotheses rigorously tested.

In view of the above, the value of the continued search for analgesics seems to be established, subject to the proviso that new compounds should only be introduced into general practice after an objective assessment of the results of a rigidly planned and controlled clinical investigation.

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3

MECHANISMS OF NEUROMUSCULAR BLOCKADE

W. C. Bowman

INTRODUCTION

As long ago as the eighteenth century scientists were intrigued by the potency of curare preparations used as arrow poisons but scientific progress into the site of action of curare was not made until 1851 when Claude Bernard[1] published the results of his classical experiments on the frog. Later, the isolation of (+)-tubocurarine from tube-curare by King[2] in 1935 stimulated greater interest in the mode of action of neuromuscular blocking agents and resulted in major advances of knowledge concerning the physiological processes underlying the transmission of the excitation wave from somatic nerve-endings to voluntary muscle.

The clinical use of curare preparations began in 1879 when Hoffmann[3] attempted to control the convulsive seizures of tetanus. Some time later, Burman[4] used curare to treat various spastic states and Bennett[5] reported its value in preventing trauma in electroshock therapy. Muscle relaxants such as curare have also been of use in manipulative procedures by orthopaedic surgeons[6] and in ocular surgery[7], but their greatest value lies in their use as adjuvants in anaesthesia. Some twenty years ago, Griffith and Johnson[8] and Cullen[9] described the use of curare preparations for producing muscular relaxation during surgical anaesthesia, and since that time tubocurarine has been used in conjunction with most anaesthetic agents.

The difficulty of obtaining the plant source of tubocurarine and the occasional incidence of unwanted side-effects led investigators to search for synthetic compounds with a similar action. In the following pages the most important of these drugs useful in the clinic are described. The review first describes the physiology of neuromuscular transmission, since an understanding of this process is essential for appreciation of the complex mechanism of action of neuromuscular blocking agents. The primary action of these drugs is to prevent transmission across the junction between motor nerve and voluntary muscle; conduction of the nerve impulse along the motor axons and the contractile power of the muscle itself are unaffected. A description of the possible mechanisms of producing neuromuscular block is then given, after which the animal tests for screening potentially useful drugs are outlined. Finally, an account of the actions, uses and side-effects of the drugs used clinically is included.

RESTING POTENTIAL AND ACTION POTENTIAL IN NERVE AND MUSCLE

The membrane changes which occur during the passage of the wave of excitation in skeletal muscle and in non-myelinated nerve-fibres are essentially alike and it is convenient, therefore, to consider both together.

Nerve- and muscle-cells at rest are bounded by polarized membranes, the interiors of which are negative relative to the exteriors[10]. When such cells are stimulated, an excitation wave consisting of a self-propagated reversal in polarity passes rapidly along the membrane[11–13]. By inserting a micro-electrode through the membranes of squid giant nerve axons and frog sartorius muscle-cells and connecting it to a valve amplifier and cathode ray oscillograph, it has been shown that during activity the membrane potential of the nerve changes from about -50 to $+40$ mV, while that of the muscle changes from about -88 to $+30$ mV. This brief reversal of potential across the cell membrane is known as the action potential or spike potential (see *Figure 3.1*).

The minute and rapid changes in potential which occur in nerve- or muscle-cells during activity may also be recorded by means of two electrodes applied to the surface of the cells and connected through a valve amplifier to a cathode ray oscilloscope. The action potential recorded in this way is biphasic since each electrode in turn becomes negative with respect to the other, as the impulse travels along the cell membrane. If the membrane under the recording electrode farthest from the stimulating electrodes is destroyed, as for example by touching it with a hot wire, this recording electrode is then making contact with the negative interior of the cell and a steady current of injury (the demarcation potential) flows through the recording apparatus for a few hours. Action potentials recorded under these conditions are then monophasic since no variation in potential occurs at the second electrode. Such records are more easily interpreted.

Biochemical analysis[14] shows that the interstitial fluid surrounding the nerve- and muscle-cells contains high concentrations of sodium and chloride ions while the *intra*cellular fluid has high concentrations of potassium ions and organic anions (A^-) of unknown structure. One factor affecting the rate of diffusion of ions through the cell membrane is the potential difference across it. Cations entering the membrane are attracted by the negative charges on the inside of the membrane and the reverse is true for anions; thus cations tend to be driven into the cell and anions out of it. Another factor is the structure of the membrane itself since this has been shown to be freely permeable to potassium and chloride ions, much less permeable to sodium ions, and almost completely impermeable to A^-. Potassium ions tend to diffuse out of the cell as their internal concentration is high and this is partly offset by the tendency of the membrane potential to drive potassium ions back into the cell. The net result is that there is a slight tendency for potassium ions to diffuse out of the cell leaving A^- behind and thus the membrane is charged, giving it its resting potential. The tendency of chloride ions to diffuse into the cell along their concentration gradient is balanced by the tendency of the electric forces to keep the negatively charged chloride ions from entering the cell and therefore, there is no net diffusion of chloride ion through the membrane.

For sodium ions and A^-, the concentration gradient and the electric forces are acting in the same direction and there is, therefore, a strong tendency for A^- to diffuse out of the cell and for sodium ions to diffuse into it. However, the membrane is impermeable to A^- and much less permeable to sodium than to potassium ions. Experiments with radioactive tracers[15–18] have shown

that there is an appreciable steady leakage of sodium ions into the resting cell. However, the internal concentration remains low because a metabolic process known as 'the sodium pump' continually carries sodium ion out of the cell as fast as it enters, so that the net sodium flux is negligible. Little is known about this process requiring energy, but the extrusion of a sodium ion is usually accompanied by the uptake of a potassium ion. The potential differences across cell membranes have been calculated from a knowledge of

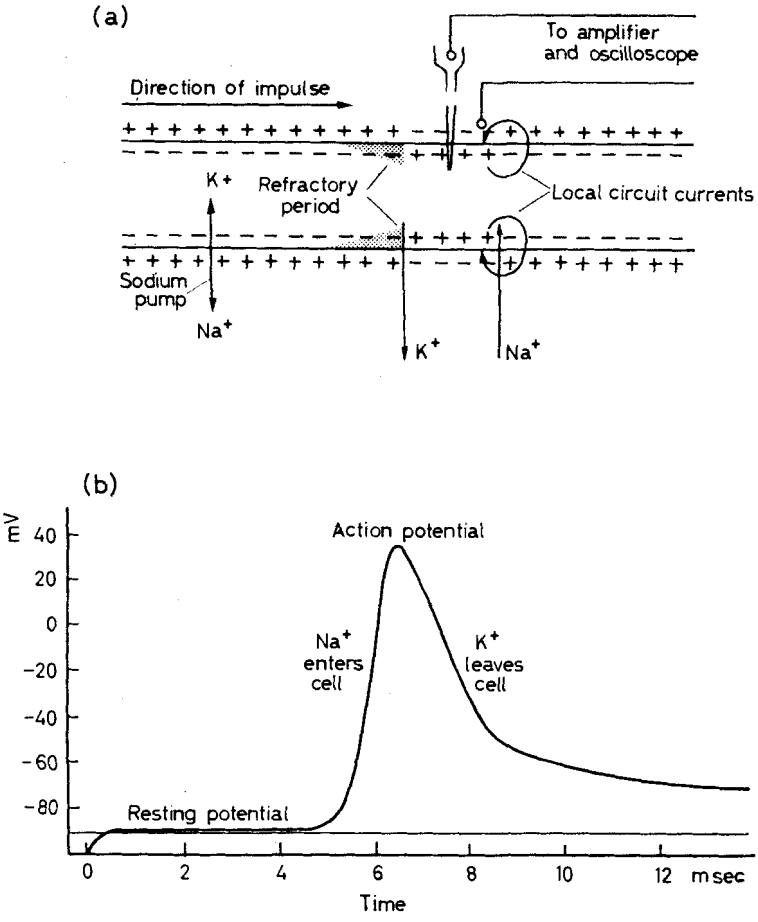


Figure 3.1

(*a*) Diagrammatic representation of the ionic exchanges taking place in muscle during and after a propagated action potential

(*b*) Resting potential and action potential in muscle, recorded by means of intracellular electrodes as represented in (*a*). Time 0 records the moment of stimulation. (After Hodgkin [13])

the concentrations of ions on either side of the membrane (*i.e.* from the Nernst equation) and the calculated values agree well with the potentials measured experimentally[19].

The brief reversal in membrane potential which occurs in an active nerve-

or muscle-cell is probably the result of the cell membrane becoming highly and selectively permeable to sodium ions[20–26]. These ions rapidly enter the fibre along their concentration gradient and reverse the potential. After the transient increase in sodium permeability, there is a delayed but maintained increase in potassium permeability which becomes appreciable near the peak of the action potential; the rate at which the potassium ions leave the cell is thereby increased and the potential of the membrane is brought back to its original resting value.

The reversed membrane potential caused by the rapid entry of sodium ions gives rise to local circuit currents which alter the permeability of the membrane immediately ahead of the active region. Sodium ions therefore enter this new region and the whole process is repeated, the excitation wave thereby being propagated along the cell membrane. At the end of the action potential, the permeability to potassium ions remains increased for a short time and there is a short-lived decrease in the permeability to sodium ions. These transient changes account for the refractory period during which the cell is inexcitable. Full excitability rapidly returns over a period which varies in different types of cell. These changes are illustrated diagrammatically in *Figure 3.1.*

During the period of recovery after the passage of a nerve impulse, the sodium–potassium ion interchange is reversed to its resting state through an increase in the activity of the sodium pump[27 28]. The recovery process in muscle may differ slightly from that in nerve but the difference has not yet been fully elucidated[29].

The motor nerve-fibres which arise in the anterior horn cells of the spinal cord and innervate skeletal muscles are not simple axis cylinders. They possess a myelin sheath consisting of lamellae of lecithin, with protein and water between the lamellae. This myelin sheath is broken up into short segments (about 0·7 mm long in a nerve-fibre of 10 μ diameter) by constrictions of the thin outer sheath or neurilemma. The constrictions are known as the nodes of Ranvier and here the axon is covered by neurilemma only (*Figure 3.2(a)*). Huxley and Stämpfli[30] have shown that in myelinated nerves the ionic interchange which occurs during activity is confined to the nodes of Ranvier. The reversed membrane potential at one node excites the next node by making current flow forwards in the axis cylinder and back in the fluid outside the myelin sheath (*Figure 3.2(b)*). The flow of current is carried out by conduction in an electrolyte and is a much more rapid process than the continuous ionic interchange which occurs in non-myelinated nerve- or muscle-fibres. Conduction in myelinated nerves is known as saltatory conduction (from the Latin *saltare*—to dance) as the impulse appears to jump from node to node. Myelin therefore acts as an insulator to increase the conduction velocity by forcing the currents produced during activity to act at a distance well ahead of the active region. The velocity of conduction in the fastest mammalian nerve-fibres, for example, is over 100 m/second.

NEUROMUSCULAR TRANSMISSION

The next important point to consider is the mechanism by which the nerve

impulse, arriving at the nerve terminals, gives rise to the wave of excitation in the muscle-cell membrane.

The Neuromuscular Junction

Figure 3.2(*a*) shows a diagrammatic representation of a single motor nerve-fibre arising in the anterior horn of the spinal cord and passing to a group of

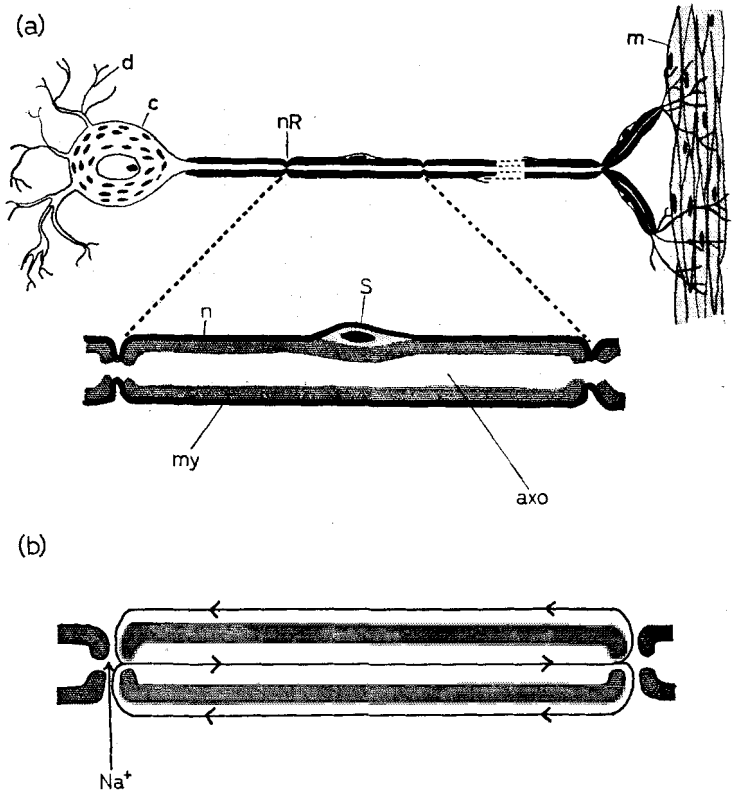


Figure 3.2
(*a*) Diagrammatic representation of a motor unit
axo—axoplasm; c—nerve cell body; d—dendrites; m—muscle-fibres; my—myelin; n—neurilemma or sheath of Schwann; nR—node of Ranvier; S—Schwann cell nucleus
(*b*) Diagram to illustrate the flow of current in a myelinated nerve fibre. Note that ionic interchange is confined to the nodes of Ranvier.
(After Huxley and Stämpfli[30])

striated muscle-fibres. A motor nerve-fibre branches extensively as it approaches its termination so that one nerve-fibre innervates usually more than 100 muscle-fibres. The individual motor nerve-fibre together with the muscle-fibres it innervates is called a motor-unit[31]. The nerve branch to a muscle-fibre loses its myelin sheath before it penetrates the sarcolemma or sheath of connective tissue bounding the muscle-fibre. The neurilemma merges with the sarcolemma and the axon spreads out under the sarcolemma where it terminates in a complex ending. There is no continuity between the

nerve terminal and the muscle-fibre but only a close contact over an extensive area, the nerve terminals often lying in grooves or synaptic gutters indenting the muscle-fibre surface. Histologically the cytoplasms of the nerve- and muscle-fibres are seen to be separated by continuous bounding membranes. This discontinuity is confirmed by electrical recording, for an electrode in the muscle-fibre fails to disclose, before the muscle response, even a trace of the nerve spike potential[32,33]. There is evidence of special structural features,

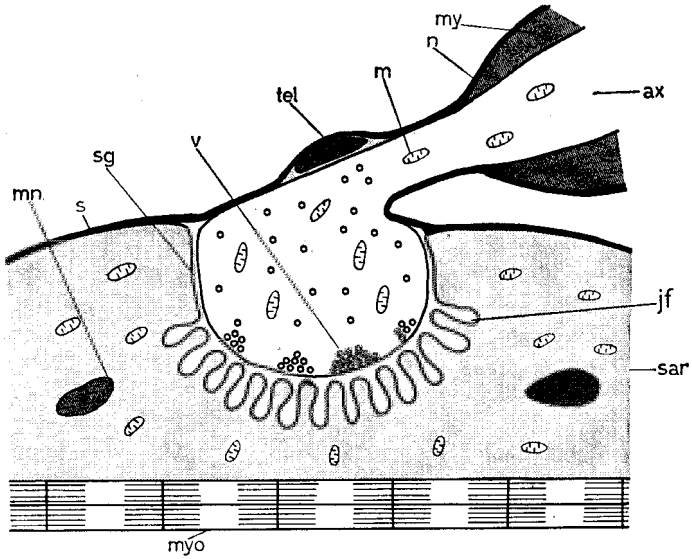


Figure 3.3. Diagrammatic representation of a neuromuscular junction as seen with the aid of the electron microscope
ax—axoplasm; jf—junctional folds; m—mitochondria; mn—muscle nucleus; my—myelin; myo—myofibrils; n—neurilemma; s—sarcolemma; sar—sarcoplasm; sg—synaptic gutter; tel—teloglia (terminal Schwann cell); v—vesicles

not only in the narrow cleft of the junction, but also immediately under the surface membrane of the muscle-fibre at the junctional region. This specialized junctional region of the muscle is called the motor end-plate, its receptive surface being expanded by an abundance of tubular folds (*Figure 3.3*). For a full description of the neuromuscular junction including electron microscope studies, a recent review by Couteaux[34] may be consulted.

Transmission of the wave of excitation from nerve terminals to motor end-plate is now generally accepted to be chemical. After the identification (as acetylcholine) of the chemical liberated from the vagus nerve (Loewi's Vagusstoffe) Dale, Feldberg and Vogt[35] and many other workers showed that the chemical concerned with neuromuscular transmission is also acetylcholine. The nerves innervating skeletal muscle are therefore classified as cholinergic nerves. Like the action of acetylcholine in autonomic ganglia but unlike its action on autonomic effector cells, the effect of acetylcholine at the neuromuscular junction is not blocked by atropine, nor is it mimicked by the

alkaloid muscarine. However, the alkaloid nicotine produces a similar response and according to Dale's[36] classification the action of acetylcholine at the neuromuscular junction is said to be nicotinic rather than muscarinic. Cholinergic nerve-endings also contain the two enzymes choline acetylase, which synthesizes acetylcholine by transferring acetyl groups from co-enzyme A to choline, and acetylcholinesterase, which is capable of rapidly hydrolysing and thereby inactivating acetylcholine. The two enzymes are probably formed in the nerve-cell body and are carried to the nerve-endings by the axoplasmic current streaming down each fibre.

Electron microscope studies[34, 37, 38] have shown the anatomical features of the neuromuscular junction and the more important of these are illustrated in *Figure 3.3*. The vesicles in the nerve terminals are thought to contain acetylcholine prior to its release[39–41] and there is evidence that choline acetylase is contained in other intracellular particles[42–44]. Acetylcholinesterase has three locations[45–51]: (*1*) inside the nerve-endings where it is possibly associated with the outer surface of the vesicles or the inner face of the pre-synaptic membrane, (*2*) the outer surface of the membrane of the nerve-endings, and (*3*) the surface of the motor end-plate where the largest part of the enzyme resides[34,52]. Koelle and Steiner[45] have suggested that the cholinesterase outside the nerve-endings (locations 2 and 3) is the functional enzyme and serves to hydrolyse the acetylcholine liberated during transmission, while the internal or 'reserve' cholinesterase (location 1) represents enzyme recently synthesized and in transit to functional external sites. However, internal cholinesterase may have a function of its own, 'mopping up' acetylcholine which spills over into the pre-synaptic axoplasm during the loading of the synaptic vesicles[44].

Synthesis of Acetylcholine

Choline is essential for the synthesis of acetylcholine and there is sufficient choline in plasma to support optimal synthesis no matter how heavy the traffic of nerve impulses may be. However, choline is unable to penetrate nerve axons along their length and evidence is accumulating to show that at the nerve terminals there is a specific choline transport mechanism, probably located in the pre-synaptic membrane or in intracellular particles within the nerve-endings. This transport mechanism carries extracellular choline to the intracellular sites where it is acetylated by choline acetylase[44]. Glucose and oxygen are necessary for optimal synthesis of acetylcholine[52,53] and sodium ions are also essential[44]. During activity, the depots of acetylcholine are maintained as the release of the transmitter accelerates its formation, but the mechanism whereby this occurs is not understood. MacIntosh[44] has suggested that sodium ions within nerve-endings stimulate vesicle formation, and when acetylcholine is removed from the vicinity of the synthesizing enzyme, synthesis is accelerated as fast as the supply of choline allows.

Release of Acetylcholine

Even in the absence of nerve impulses there is a spontaneous release of acetylcholine from the nerve-endings giving rise to minute voltage fluctuations in the region of the motor end-plate[33,54–57]. Each of these 'miniature

end-plate potentials' is the result of the discharge on to the motor end-plates of an amount of acetylcholine much too small to excite a propagated muscle excitation wave and this is released into the synaptic gap when a vesicle collides with or penetrates the pre-synaptic terminal membrane. The effective end-plate potential, or the response which triggers the muscle excitation wave, is the sum of a large number of these minute effects occurring simultaneously, immediately after the nerve impulse arrives at the nerve terminals[33,56]. Thus the nerve impulse leads to the synchronous liberation of the acetylcholine contained in many synaptic vesicles. The mechanism of release involves the passage of calcium ions into the pre-synaptic axoplasm[58–60], and the amount of acetylcholine released varies over a wide range with the calcium ion concentration[33,61]. Hodgkin and Keynes[60] have shown that at rest there is little calcium in the axoplasm, but during activity there is a sharp rise in calcium ion influx. During rest, the calcium ions that have entered are slowly pumped out. MacIntosh[44] has suggested that, when an impulse reaches the nerve-endings, the influx of calcium ions alters the properties of the vesicles or the axoplasm just inside the membrane so that the vesicles spill their contents into the synaptic gap. For optimal release of acetylcholine two other agents are necessary: carbon dioxide to increase the ionization of plasma calcium, and an unidentified plasma factor of low molecular weight[44].

Function of Acetylcholine

The generation of an end-plate potential is an essential intermediary between nerve and muscle action potentials. End-plate potential is a distinct local depolarization of the membrane of the motor end-plate brought about by the action of acetylcholine. When the muscle is at rest, the potential of the motor end-plate does not differ from that of the muscle membrane elsewhere[62]. Unlike the rest of the muscle membrane, however, the motor end-plate is extremely sensitive to the action of acetylcholine[63–65]. The brief jet of acetylcholine liberated from the nerve terminals on the arrival of a nerve impulse collides and momentarily reacts with special receptor molecules in the external surface of the motor end-plate[66–68]. This interaction between acetylcholine and its receptors makes the end-plate permeable to sodium and potassium as well as to other free anions and cations on either side of the membrane. The potential across the motor end-plate, therefore, tends towards an equilibrium level and is a simple depolarization rather than a reversal in potential resulting from a specific increase in the permeability to sodium, such as occurs in nerve- and muscle-fibres. This short circuit of the muscle membrane produces a high local current-density and provides for the amplification of ionic currents which is necessary when the impulse is transferred from the minute nerve-endings to the enormously greater surface of the muscle-fibre[56,62,69]. End-plate potential further differs from the action potential in that it is a graded, rather than an 'all or none', response. If it reaches a threshold level[70], it gives rise to local currents of sufficient intensity to increase the sodium permeability of surrounding portions of the membrane; thus, it initiates a propagating muscle action potential and this passes over the muscle membrane in the manner already described. The amount of

acetylcholine released from a nerve terminal is considerably in excess of that required to produce the threshold end-plate potential[71], yet it is destroyed within the space of a few milliseconds by the cholinesterase in the junctional region[72] and the end-plate has repolarized before the end of the refractory period of the muscle-fibre.

The function of the muscle membrane, therefore, is to receive the motor impulse at its specialized end-plate region, to distribute it over the whole length of the muscle-fibre, and to pass it on rapidly to the contractile material in the interior[73,74]. The means whereby the muscle excitation wave triggers a muscle contraction is not fully understood. For descriptions of the various theories concerned, the reader may consult articles by Spencer and Worthington[75], Mommaerts[76] and Weber and Portzehl[77].

According to Nachmansohn, acetylcholine plays an essential role not only in neuromuscular transmission but also in the generation of the action potential in both nerve- and muscle-cell membranes. He believes that the local circuit currents which occur in active cells release acetylcholine from an inactive bound form present in the cell membrane and that it is the acetylcholine rather than the local currents themselves which increases the permeability of the membrane to sodium ions. Nachmansohn rejects the idea that at the neuromuscular junction acetylcholine is released from pre-synaptic nerve-endings to act post-synaptically. He points out that electric currents are able to cross a non-conducting gap of more than 1 mm and suggests that it is unreasonable to assume that they would be unable to cross the distance of 500 to 700 Å between pre- and post-synaptic membranes. He postulates that the currents generated in the nerve terminals mobilize acetylcholine at the post-synaptic membrane and that this acetylcholine in turn generates the end-plate potential.

Despite a large number of biochemical and biophysical experiments (for a recent review see Nachmansohn[78]) this hypothesis is not generally accepted. The main arguments against it (see, for example, Feldberg[79]) are:

(*a*) Acetylcholine is not synthesized in appreciable amounts in some nerves although cholinesterase is universally distributed in nervous tissue[80].

(*b*) Lipid-soluble anticholinesterases block conduction but do not do so by depolarizing the membrane[81].

(*c*) The theory does not account for the presence of the synaptic vesicles which contain stored acetylcholine.

Space does not permit a discussion of the arguments for and against Nachmansohn's theory. However, the controversy does not seriously affect the following discussion of drugs which block neuromuscular transmission, for there is little disagreement concerning their site of action. The drugs to be described are mainly lipid-insoluble quaternary ammonium compounds and as such they are unable to penetrate axon or muscle-fibre membranes. Their site of action is therefore restricted to the external surface of the fine nerve terminals or the motor end-plates, parts of the system which, according to Nachmansohn, are not protected by diffusion barriers.

METHODS OF PRODUCING NEUROMUSCULAR BLOCK

Reduction of Transmitter Output from Nerve

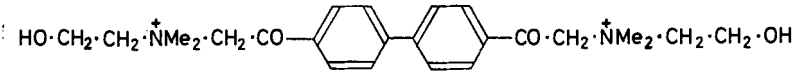
A reduction in the amount of acetylcholine released from the nerve-endings by a nerve impulse leads to neuromuscular block since each muscle-fibre

fails to contract as soon as the acetylcholine output falls below that required to produce the threshold of end-plate potential. There are two general mechanisms whereby the acetylcholine output may be reduced: (*a*) its synthesis in the nerve-endings may be depressed, and (*b*) its release from the nerve-endings may be prevented.

Depressed synthesis

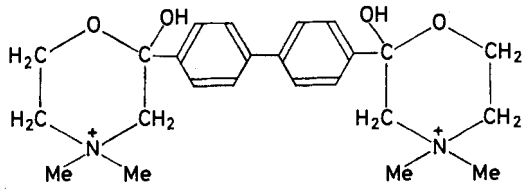
The important factors necessary for the synthesis of acetylcholine have already been mentioned and a lack of any of these decreases the ability of the nerve to manufacture its transmitter. No substance is known to inhibit specifically choline acetylase after systemic administration, although large amounts of many inhibitors of other enzymes may do so to some extent.

An important series of compounds known to reduce acetylcholine synthesis are the hemicholiniums. These bases were synthesized by Long and Schueler in 1954[82] and the formula of the most active member of the series, known as HC-3, is shown here. HC-3 is relatively specific in its actions although large doses of it react at other sites in the region of the neuromuscular junction[83].



HC - 3

Chemical and spectroscopic evidence[83] shows that HC-3 exists in the following form:



HC-3 is believed to act by combining with, and thereby temporarily inactivating, the choline-carrier mechanism which is responsible for transporting extra-cellular choline across the membranes of cholinergic nerve-endings to the intracellular sites of its acetylation. Thus, HC-3 renders the nerve terminals deficient in choline and therefore in acetylcholine. The block produced is characteristically slow in onset and is dependent on the frequency of stimulation of the nerve, for the acetylcholine already stored in the nerve terminals must be used up before any degree of transmission failure becomes evident. Full accounts of the evidence leading to the elucidation of the mechanism of action of the hemicholiniums have been published[44,83-88].

A striking characteristic of the transmission failure produced by HC-3 is the marked antagonistic ability of choline. Esters of choline are also effective, but only by virtue of their rapid hydrolysis by cholinesterase with the production of choline. If hydrolysis is prevented by means of an anticholinesterase, the antagonistic action of choline esters is markedly reduced[87]. Another

compound with effects similar to those of HC-3 is 3,6-bis(3-diethyl aminopropoxy)pyridazine bismethiodide (WIN 4981)[89].

In recent experiments[90], the triethyl analogue of choline (TEC, $[Et_3NCH_2CH_2OH]^+[Cl]^-$ has been shown to produce neuromuscular block which results from a reduced acetylcholine output from the nerve. *Figure 3.4* illustrates an experiment in the cat in which TEC produced a slowly developing paralysis of the tibialis anterior muscle rapidly stimulated through

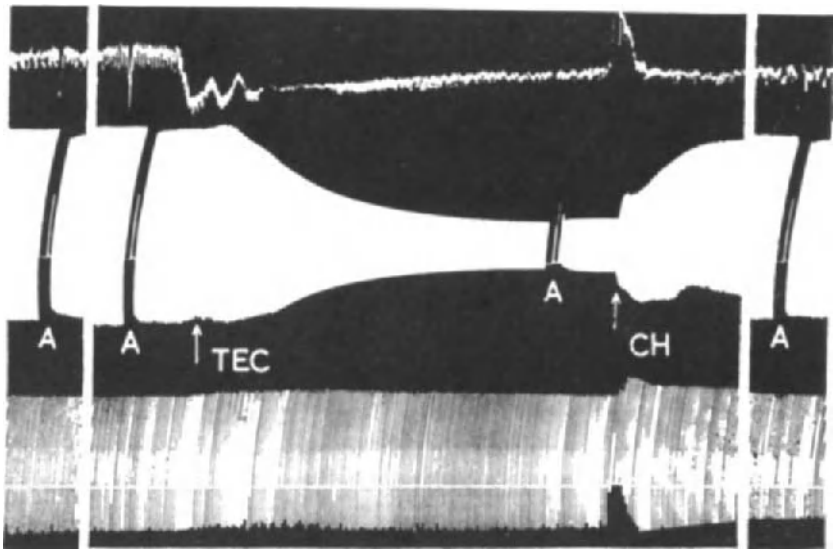


Figure 3.4. Cat, chloralose anaesthesia
Upper record: blood-pressure recorded from a common carotid artery.
Middle record: Maximal twitches of the right tibialis anterior muscle elicited indirectly at a frequency of 1/second
Lower record: Maximal twitches of the left tibialis anterior muscle elicited indirectly at a frequency of 1 in 10 seconds
At A, 5 μg of acetylcholine injected close-arterially into the right tibialis anterior muscle; during the acetylcholine injections, electrical stimulation was stopped. At TEC, 20 mg/kg of the triethyl analogue of choline and at CH, 5 mg/kg of choline were injected intravenously.

its nerve. During the paralysis, the response of the muscle to injected acetylcholine was unaltered, illustrating that the sensitivity of the motor end-plates as well as the contractile power of the muscle itself were not depressed; the paralysis was markedly antagonized by choline. It is possible that the mechanism of action of TEC, like that of HC-3, is inhibition of choline transport mechanisms. However, it has been shown *in vitro*[91] that TEC is readily acetylated by the enzyme, choline acetylase, and the nerve terminal, therefore, may accept TEC in place of choline and liberate an inactive transmitter. TEC also appears to cause an initial increase in the quantity of acetylcholine released by a nerve impulse and excessive release may lead to subsequent depletion of transmitter in the rapidly stimulated muscle. The effect, too, probably contributes to the transmission failure produced by TEC. The substance has already been shown to be effective in experimental tetanus[92], and is being tested in neurogenic spastic states in man.

Prevention of release

The agents to be discussed in this section are those which prevent acetylcholine release by an action on the fine motor nerve terminals. Substances with a more central action, thereby preventing acetylcholine release by abolishing conduction of the nerve impulse along the motor axons, are not included.

Ion changes—The dependence of acetylcholine release on the extracellular calcium ion concentration has already been discussed and lack of calcium produces neuromuscular block by interfering with the mechanism by which a nerve impulse causes release of the transmitter. Neuromuscular block is also produced by excess magnesium ions which interfere with the release of acetylcholine. Usually magnesium ions antagonize the action of calcium ions[55,58,61]. These effects are exerted both upon the acetylcholine release following nervous activity and upon the frequency of the spontaneously occurring miniature end-plate potentials. Large concentrations of both calcium and magnesium ions also have depressant effects on the direct excitability of the muscle-fibre membrane[93]. Excess phosphate ion also inhibits acetylcholine release[94].

Local anaesthetics—Harvey[95] was the first to show that intra-arterially injected procaine decreases the amount of acetylcholine released from the motor nerves, and probably other local anaesthetics have a similar action. Local anaesthetics prevent conduction along the axons (see Watson[96]) but their actions after close-arterial injection are first exerted on the fine non-myelinated nerve terminals[97]. Procaine probably acts by stabilizing the membrane and preventing the abrupt changes in permeability necessary for conduction along the terminals and release of the transmitter[98]. However, the action of procaine is not only pre-synaptic, since it also depresses the sensitivity of the motor end-plates to acetylcholine[95,99,100].

Botulinum toxin—In nerve–muscle preparations poisoned by botulinum toxin, nervous conduction is not affected and the muscle still responds to intra-arterially injected acetylcholine[101]. The paralysis is due to prevention of the release of acetylcholine from the nerve by the toxin[102–104]. Botulinum toxin also reduces the frequency, but not the amplitude, of spontaneously occurring miniature end-plate potentials. During paralysis produced by botulinum toxin, high-frequency stimulation of the nerve temporarily restores the mechanism releasing the transmitter, and miniature end-plate potentials reappear for a short time. Recently, Thesleff[105] has shown by electron microscope studies that paralysis occurs with no alteration in the ultra-structure of the motor nerve terminals, as the number and size of the pre-synaptic vesicles are unchanged in muscles poisoned by botulinum toxin. He has also demonstrated that the terminals contain multi-molecular quanta of acetylcholine, which shows that the synthesis of the transmitter has not been impaired. He therefore suggests that the toxin has a selective action on the mechanism responsible for the *release* of the transmitter in cholinergic nerves.

Motor End-plate Block

Two main mechanisms underly the effects of drugs which produce neu muscular block in this way:

(*a*) The threshold of the motor end-plates to acetylcholine may be raised so that a muscle contraction is only elicited by a quantity of acetylcholine greater than normal (curare-like drugs).

(*b*) An electrical inexcitability, arising as a result of prolonged depolarization, may be produced in the region of the motor end-plates (depolarizing drugs).

In the majority of animal species, the second mechanism changes in some way during the process of neuromuscular block and the threshold to acetylcholine is finally raised; Zaimis[106] has called this mechanism, a 'dual mode of action' block.

The terminology used to describe the different mechanisms whereby drugs produce block of the motor end-plates *in vivo* is somewhat confusing and is often misused. The more specific drugs act by combining with the same receptors on the motor end-plates as does acetylcholine itself, and therefore they may be said to compete with acetylcholine for its receptors. In this sense, the depolarizing blocking agents, as well as the curare-like drugs, compete with acetylcholine. The misuse arises with regard to the terms 'block by competition with acetylcholine' or 'competitive antagonism'; the curare-like drugs may produce block in this manner but the depolarizing drugs do not. An important criterion of competitive block is that the block is always overcome by increasing the concentration of stimulant drug, in this case acetylcholine. Furthermore, a similar degree of block is produced by using concentrations of agonist and antagonist which are always in the same ratio. Since the action of the depolarizing drugs resembles that of an excess of acetylcholine, an increase in the concentration of acetylcholine increases the degree of block. It seems inadvisable, therefore, to use the word 'competition' in connection with the drugs which act by depolarization. Different theories to explain the mechanism of action of antagonists have been published[107,108] and the reader may consult these references for further information.

Curare-like block

The classical experiments of Claude Bernard[109] localized the blocking action of curare in the frog at the junction between the motor nerve and skeletal muscle. Curare is a generic term for various crude extracts obtained from different species of *Strychnos* and *Chondodendron*, the most important of the alkaloids possessing neuromuscular blocking activity being (+)-tubocurarine. Tubocurarine prevents the response of skeletal muscle to motor nerve impulses and to injected acetylcholine; during the paralysis, nervous conduction continues and the muscle-fibres themselves (including their end-plate regions) retain their sensitivity to direct electrical stimulation and to the application of barium or potassium ions[62,65,100,110]. Under the influence of tubocurarine, the muscle end-plate potentials produced by successive shocks applied to the nerve rapidly diminish in size until they fail to reach the critical level necessary to initiate a propagated muscle action potential[111]. A similar effect on end-plate potentials may be produced by substances which decrease the amount of transmitter liberated from the nerve, but Dale, Feldberg and Vogt[112] showed that the output of acetylcholine at the height of paralysis by tubocurarine is not significantly affected. The paralysis is relieved by procedures which increase the local concentration of acetylcholine in the

region of the motor end-plates, and all these results suggest that tubocurarine blocks neuromuscular transmission by competition with acetylcholine.

In competitive block, a dynamic equilibrium exists between the blocking drug and acetylcholine, and molecules of both rapidly combine with, and dissociate from, the end-plate receptors. The degree of block therefore depends on the proportion of receptors occupied by the blocking drug, and this in turn depends upon the relative concentrations of the two substances in the fluid surrounding the motor end-plates. However, as a consequence of its destruction by cholinesterase, acetylcholine is only fleetingly present in the region of the motor end-plates, and del Castillo and Katz[100] have expressed the opinion that there may be no time for competition. These authors have shown that the release of tubocurarine by diffusion from its receptors is slower than was expected, and so it sets up a reversible equilibrium with the receptors, combination and release of the drug going on simultaneously with a fraction of the total receptors occupied and the remainder free. Acetylcholine suddenly released from the nerve-endings then occupies some of the free receptors and is released and destroyed before competition has set in[113]. Taylor[113] suggests that the term 'anti-depolarizing drugs' is a safer term than 'competitive'. However, while it may be safer it is perhaps too imprecise, for substances which prevent depolarization in a less specific way than by combining with acetylcholine receptors would then be included in this class. For example, ether reduces the ability of acetylcholine to depolarize the motor end-plates but its mechanism of action differs from that of tubocurarine. Similarly, the term 'non-depolarizing blocking agents' is insufficiently precise, for it must include those substances which act by preventing the release of acetylcholine from the nerve. Thus it is not unreasonable to use the terms 'competitive antagonism' or 'block by competition' to describe the effects of the curare-like drugs, since when anticholinesterases are present, released acetylcholine probably persists for a period long enough for competition to take place. Furthermore, full equilibrium between agonist and antagonist may be produced in *in vitro* experiments and in these circumstances tubocurarine has been shown to block acetylcholine competitively. However, it is impossible to determine *in vivo* if curare-like drugs fulfil all the criteria of block by competition. For example, gallamine is known to behave *in vivo* like tubocurarine, yet *in vitro* experiments on the frog's rectus abdominis muscle show that the block produced is not strictly competitive.

Many other alkaloids have been studied in the laboratory and as the paralysis they produce is antagonized by neostigmine, they probably resemble tubocurarine in their mechanism of action. Of the numerous alkaloids isolated from the curares, C-toxiferine I, next to tubocurarine itself, is perhaps the most interesting. This substance was isolated from the bark of *Strychnos toxifera* by King[2] and studied pharmacologically by Waser[114] and by Paton and Perry[115]. It is many times more potent than tubocurarine and is probably the most potent of all neuromuscular blocking agents. C-toxiferine I was at first believed to be monoquaternary but is now known to be bisquaternary, like most other blocking agents. It is unstable in solution, splitting up into two symmetrical monoquaternary compounds[116]. The substance has recently undergone clinical trials[117,118] and shows promise as a useful long-acting muscle relaxant. Another naturally-occurring alkaloid with curare-like

activity is β-erythroidine isolated from *Erythrina americana* by Folkers and Major[119]. This substance and its more potent dihydro derivative[120] are of interest in that their molecules contain only one nitrogen atom and this is tertiary. For a fuller description of the naturally-occurring neuromuscular blocking agents, reviews by McIntyre[121,122] may be consulted.

The expense and difficulty of obtaining pure alkaloids from natural sources stimulated efforts at synthesis and many hundreds of compounds have been screened for curare-like activity. The most important of these are: (*a*) gallamine triethiodide, synthesized by Bovet, Depierre and Lestrange[123], (*b*) benzoquinonium chloride, synthesized by Cavallito, Soria and Hoppe[124],

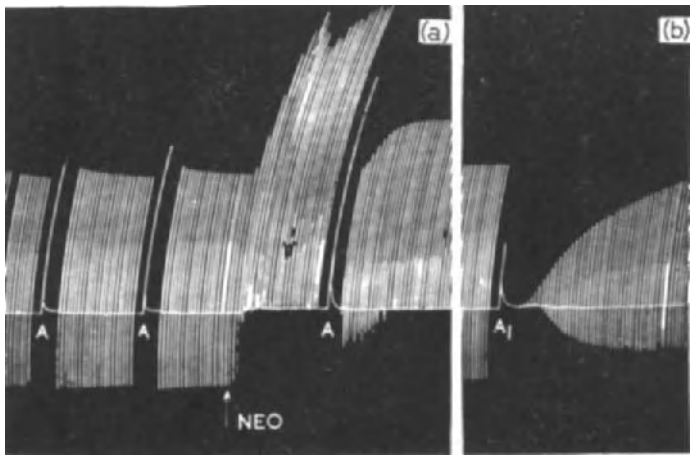


Figure 3.5. Cat, chloralose anaesthesia
Maximal twitches of a tibialis anterior muscle elicited indirectly at a frequency of 1 in 10 seconds. At A, 10 μg acetylcholine and at A_1, 20 μg acetylcholine injected close-arterially. During the acetylcholine injections, electrical stimulation was temporarily stopped. At NEO, 200 μg of neostigmine were administered intravenously. Between (*a*) and (*b*) a further 20 μg neostigmine were injected close-arterially.

and (*c*) laudexium methylsulphate, synthesized by Taylor and Collier[125]. These drugs will be discussed in detail in a later section. Their structural formulae are shown on p. 104.

Block by depolarization

Acetylcholine in high concentrations has long been known to produce neuromuscular block[126]. Owing to its rapid hydrolysis by cholinesterase in the blood and at the neuromuscular junction, it is usually necessary to administer an anticholinesterase before a blocking effect may be demonstrated. *Figure 3.5* illustrates both the stimulant and the blocking action of acetylcholine. However, acetylcholine is not used clinically for this blocking action.

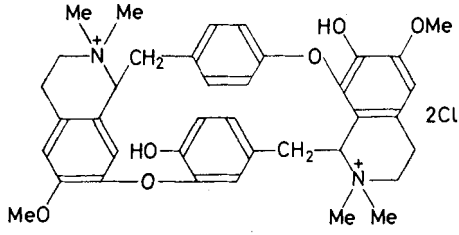
The development of the methonium compounds by Barlow and Ing[127] and simultaneously and independently by Paton and Zaimis[128–133] provided the first clinically useful neuromuscular blocking drug of the depolarizing

type. Decamethonium, the most active member of the series as far as skeletal muscle is concerned, is without effect on acetylcholine release; during complete paralysis of the indirectly-excited contractions, the muscle responds to direct stimulation, and thus the substance conforms to the definition of a neuromuscular blocking agent. Zaimis[134] then demonstrated that decamethonium resembled acetylcholine in its ability to stimulate skeletal muscle. Like acetylcholine, it produces a quick contraction on close-arterial injection and causes, after an initial contraction phase, a more sustained contracture accompanied by electrical silence in chronically denervated muscle and in some avian muscles. These effects of decamethonium and other depolarizing drugs are illustrated in *Figures 3.10 and 3.12*. Unlike acetylcholine, depolarizing blocking drugs are stable to cholinesterase and their effects, therefore, are considerably longer-lasting.

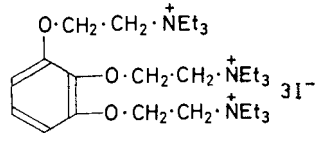
Using the tibialis anterior muscle of the cat, Paton and Zaimis[130–133] showed that the neuromuscular block produced by decamethonium differed in several respects from that produced by tubocurarine. For example, paralysis produced by decamethonium was preceded by fasciculations of the muscle and potentiation of the maximal twitch. This potentiation was shown to be the result of repetitive firing of the muscle-fibres, brought about mainly by summation of the effects of decamethonium and the acetylcholine released on stimulation and not by the weak anticholinesterase activity of the drug[130]. During partial decamethonium paralysis of the indirectly excited maximal twitches, procedures which readily antagonize block by tubocurarine are without effect or even deepen the paralysis. The pronounced anti-curare action of a tetanus or of neostigmine are outstanding examples of this. Furthermore, tubocurarine and decamethonium are themselves mutually antagonistic in the tibialis anterior muscle of the cat. A small dose of tubocurarine prevents the development of block by decamethonium[106] or if administered during the effect of decamethonium produces rapid recovery from the paralysis[135,136]. Similarly, a small dose of decamethonium administered during tubocurarine paralysis produces a striking anti-curare effect[137].

Such results led Paton and Zaimis to the conclusion that the blocking action of decamethonium was in some way a consequence of its depolarizing action. However, if depolarization of the motor end-plates were the only effect produced, then, providing it exceeded the threshold end-plate potential necessary to trigger off a propagated muscle excitation wave, the muscle should remain in tetanic spasm, the frequency of the tetanus being limited only by the refractory period of the surrounding muscle membrane. If the depolarization produced by decamethonium was less than the threshold end-plate potential, the effect of the drug would be to summate with the acetylcholine released from the nerve and so the only result in the nerve–muscle preparation would be potentiation of the maximal twitch. Small doses do produce this latter effect but with larger doses this phase of the action is transient and neuromuscular block rapidly follows. The direct cause of the block, therefore, must be another effect arising from prolonged depolarization of the end-plate.

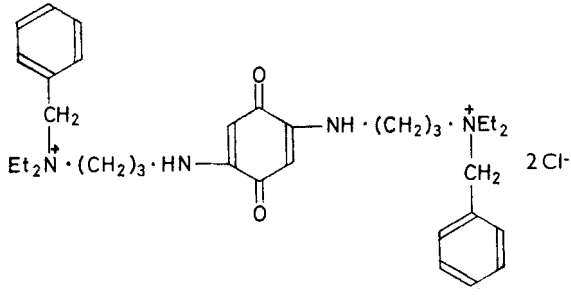
Burns and Paton[135] studied this problem in the gracilis muscle of the anaesthetized cat in which the end-plate region is conveniently localized.



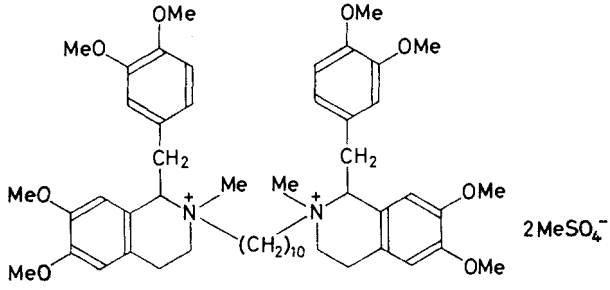
(+) Tubocurarine chloride



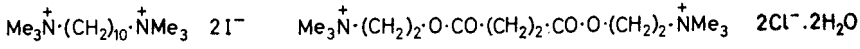
Gallamine triethiodide



Benzoquinonium chloride

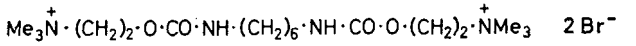


Laudexium methylsulphate



Decamethonium iodide

Suxamethonium chloride



Carbolonium bromide

Chemical formulae of clinically useful blocking agents

Using external recording electrodes, they showed that the depolarization produced by decamethonium was limited to the end-plate region of the muscle membrane, although it always extended slightly beyond the area in which it was possible to record end-plate potentials. After an initial transient increase in excitability (which was associated with random spontaneous fasciculations) the depolarized region became inexcitable to direct electrical stimulation although the rest of the muscle membrane remained excitable. Furthermore, a muscle action potential, produced by direct stimulation of one end of the muscle, travelled towards the end-plate region but did not pass it and so was not recorded on the side distal to the stimulating electrodes. After tubocurarine, on the other hand, the electrical excitability of the muscle remained unaltered. For a given degree of end-plate depolarization produced by decamethonium, the intensity of the neuromuscular block increased with time; the depolarization also spread slightly giving rise to a zone of inexcitability in the region of membrane immediately surrounding the end-plates. With doses of decamethonium producing complete paralysis, the end-plate depolarization was never complete and end-plate potentials were still initiated by stimulation of the nerve. However, these were set up in a region of inexcitability and were too distant from the excitable membrane to initiate a propagated muscle action potential. Thus, prolonged depolarization of the end-plates gave rise to an inexcitability at, and immediately surrounding, the end-plates. This inexcitability, and not the depolarization, is therefore the direct cause of the block. A similar type of block was produced by the intra-arterial injection of acetylcholine and by high-frequency stimulation of the motor nerve in the presence of an anticholinesterase. Removal of the depolarization by passing an anodal current at the end-plate region rapidly restored neuromuscular transmission, while cathodal current intensified the block. Furthermore, cathodal current applied to the end-plate region of a muscle not influenced by decamethonium produced a paralysis which was similar to that produced by decamethonium or acetylcholine. Therefore, Burns and Paton emphasized that the characteristics of the block were not specific for these drugs but were the properties of any process resulting in a persistent localized cathode; they also pointed out the similarity between such a motor end-plate block and the well-known cathodal block of peripheral nerve.

Dual block

Among the species of animals used for laboratory experiments, block due to long-lasting depolarization of the motor end-plates is the exception rather than the rule. Were it not for the fact that a similar type of paralysis is produced by these drugs in man[138,139] block by depolarization might be dismissed as a peculiarity of the cat.

Zaimis[106] showed that in the limb muscles of the monkey, rabbit, hare and dog depolarizing blocking agents exhibited a 'dual mode of action' (*i.e.* dual block). The paralysis was preceded by muscle fasciculations and by potentiation of the maximal twitch tension, indicating the depolarizing action of the drugs. However, the block itself exhibited the characteristics of that produced by tubocurarine, being antagonized by a tetanus and by neostigmine and increased by a small dose of tubocurarine. Similar results were obtained in

the guinea-pig by Hall and Parkes[140]. The two phases of the action are mutually antagonistic and muscles which exhibit this type of block are relatively insensitive to decamethonium and show a rapidly developing tachyphylaxis to it. Later, Jewell and Zaimis[141] found that decamethonium interrupted neuromuscular transmission in the soleus muscle of the cat by a dual mode of action (see *Figure 3.11(c)*) and Churchill-Davidson and Richardson[138] reported that the muscles of myasthenic patients were extremely resistant to decamethonium which produced a paralysis of the dual type. Differences in motor end-plate receptors may therefore exist not only between the muscles of different species but even among different muscles of the same species. *Figure 3.6* illustrates the block produced by decamethonium in the tibialis anterior muscle of the ferret in which the dual mode of action is particularly well marked. The differences between the characteristics of

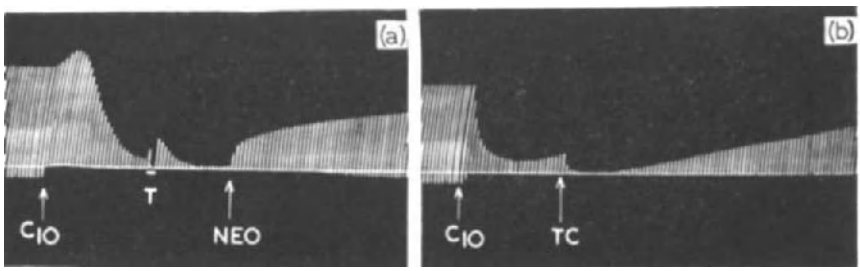


Figure 3.6. Ferret, chloralose anaesthesia
Maximal twitches of the tibialis anterior muscle elicited indirectly at a frequency of 1 in 10 seconds. At C_{10} in (*a*) 50 μg/kg and at C_{10} in (*b*) 150 μg/kg of decamethonium. At NEO, 75 μg/kg neostigmine and at TC, 100 μg/kg tubocurarine. All injections intravenously. At T, a motor nerve tetanus was applied for 5 seconds at a frequency of 50/second.

this block and of that produced by depolarizing substances in the tibialis anterior muscle of the cat are striking (compare *Figure 3.6* with *Figures 3.11 and 3.15*). Even in the tibialis anterior muscle of the cat, some drugs may block transmission by a dual mechanism. For example, Zaimis[106] has shown that tridecamethonium, having a chain of thirteen methylene groups between the two quaternary nitrogen atoms, produces a paralysis of this type, and recent experiments have indicated that the block produced by nicotine may also be due to such a mechanism.

Several reports[142–151] have suggested that prolonged administration of large amounts of depolarizing blocking agents may lead to dual block during anaesthesia in man. The conclusions have been based mainly upon the fact that neostigmine caused the return of spontaneous respiratory movements, but as Zaimis[152] points out, decreased respiration is not necessarily a consequence of peripheral block of the respiratory muscles. Effects on the respiratory centre due to other drugs or to other factors during anaesthesia may complicate the recordings, and in conscious volunteers, variations due to apprehension may impair the results. Christie, Wise and Churchill-Davidson[153] point out that neostigmine may stimulate respiration as a consequence of the unpleasant sequelae (*e.g.* severe colic and vomiting)

which follow its use even in the presence of atropine, and the respiratory stimulation may not be related to its ability to inhibit the cholinesterase of the respiratory muscles. Nevertheless, a depolarizing substance may produce dual block in some muscles of man, and it is necessary for the anaesthetist to decide the type of neuromuscular block which has been produced in each patient. From laboratory experiments, it is known that muscle action potentials are sustained at a constant level during an indirectly-elicited tetanus when partial block is produced by depolarization. During competitive block, on the other hand, the action potentials rapidly diminish. Churchill-Davidson and Christie[150] have recommended, as a means of determining the type of block in man, the application of high-frequency stimulation to a peripheral motor nerve while recording the action potentials of the muscle. However, the muscle chosen for study may not reflect the type of block prevailing in the majority of muscles.

It is worth mentioning an effect frequently obtained in animal experiments which has led to the erroneous conclusion that a dual block is present. In the tibialis anterior muscle of the cat, in which the block is due to depolarization, small doses of neostigmine administered at the onset of spontaneous recovery may sometimes produce an increase in the twitch tension. This effect is never as striking as the abrupt and rapid antagonism seen during tubocurarine paralysis, and electrical recording of the gross muscle action potential shows that it is not due to relief of the block. As the concentration of the blocking drug in the region of the motor end-plates begins to diminish through inactivation or excretion, any excess acetylcholine accumulating in the presence of neostigmine is probably insufficient to deepen the paralysis by summating with the blocking drug. However, it may cause repetitive firing in some of the muscle-fibres which are not blocked so that they contribute a greater tension to the gross effect. This is reflected in the repetitive action potential although no increase in peak voltage is seen. The greater twitch tension is not therefore a consequence of an increase in the number of muscle-fibres taking part and cannot be described as antagonism. Similar results are often obtained when small doses of neostigmine are injected during a constant degree of partial paralysis maintained by a continuous infusion of a depolarizing drug[154]. *Figure 3.7* illustrates a typical experiment in which carbolonium was the blocking agent used. The increase in twitch tension produced by neostigmine was caused by repetitive firing of the muscle-fibres as shown by the action potential recording; the peak voltage of the gross muscle action potential was slightly reduced. A second dose of neostigmine, however, produced a marked reduction in both twitch tension and action potential, and repetitive firing ceased.

A further possible explanation of the dual block reported in man is that after prolonged administration of large doses, the blocking agents cause a reduction in transmitter output from the nerve-endings, possibly by a hemicholinium-like action. Under light anaesthesia, there may be sufficient activity in the motor nerves to deplete the stores of preformed acetylcholine. Such an effect is consistent with the finding that prolonged administration of depolarizing drugs sensitizes the muscles to the blocking action of tubocurarine[149] and that neostigmine has some antagonistic action. The situation is therefore confusing and may give rise to difficulties for the anaesthetist

when faced with a case of prolonged apnoea. Neostigmine should not be administered unless there is proof that the block is dual in nature.

Thesleff[155] has criticized the use of external electrodes for recording end-plate depolarization on the grounds that it does not give quantitative values and therefore only allows a statement as to whether depolarization occurs or not. Using the extremely fine technique of ionophoretic micro-application of drugs to the end-plates of single muscle-fibres together with intracellular

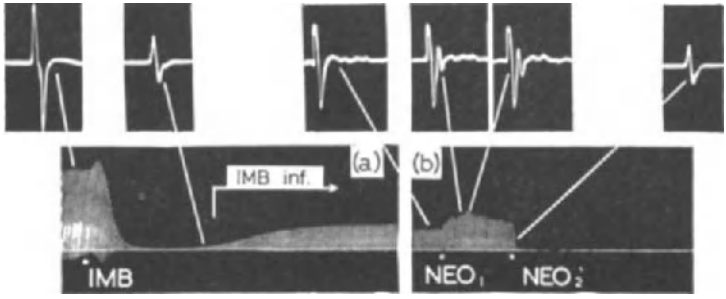


Figure 3.7. Cat, chloralose anaesthesia[154]
Upper records: gross muscle action potentials recorded from the tibialis anterior muscle. Lower records: maximal twitches of the same tibialis anterior muscle elicited indirectly once every 10 seconds. The oblique white lines indicate the twitches associated with the action potentials shows. At IMB, 10 μg/kg carbolonium bromide (Imbretil) injected intravenously. During the recovery period a continuous intravenous infusion of carbolonium bromide was started and maintained throughout the rest of the experiment. 3 hours and 10 minutes elapsed between (*a*) and (*b*). At NEO_1, 30 μg/kg and at NEO_2, 40 μg/kg neostigmine methylsulphate injected intravenously. At the time of the first injection of neostigmine the animal had received a total of 0·325 mg of carbolonium bromide in the form of a continuous infusion.

recording, Thesleff studied *in vitro* the interaction between neuromuscular blocking agents and acetylcholine in a number of isolated muscles from various animal species, including the tenuissimus muscle of the cat[156–160]. Thesleff's results differed from those of Burns and Paton[135] in two important respects:

(*a*) The period of depolarization produced by the depolarizing drugs was fleeting and the membrane was rapidly repolarized; neuromuscular block started during the period of depolarization but persisted after the membrane had repolarized.

(*b*) Mutual synergism occurred between the receptor desensitization produced by tubocurarine or gallamine and that produced by depolarizing agents.

Maclagan[161] later showed that in the tenuissimus muscle of the cat *in vivo* the effects of decamethonium were similar to those produced in the tibialis anterior muscle. Tubocurarine, administered even at a late stage of decamethonium paralysis, produced an abrupt antagonism of the block in both muscles—a result which is in striking contrast to those of Thesleff and which illustrates the fundamental difference in the mechanism of action of the two blocking drugs in these muscles. When studied *in vitro*, the results were variable, depending upon the anaesthetic under which the muscle was removed

from the animal and upon the composition of the bathing medium, particularly its bicarbonate content. Maclagan, therefore, emphasizes the dangers inherent in attempting to explain effects in the whole animal on the basis of results obtained *in vitro*.

The clinically useful neuromuscular blocking drugs with a mechanism of action similar to that of decamethonium are as follows: (*a*) suxamethonium chloride, first synthesized in 1906 by Hunt and Taveau[162] although its neuromuscular blocking action was not discovered until 1949 by Bovet and his colleagues[163], (*b*) suxethonium, synthesized in 1949 by Bovet and his colleagues[163] and differing from suxamethonium only by the substitution of an ethyl group for a methyl group on both quaternary nitrogens, and (*c*) carbolonium bromide, synthesized by Cheymol and his colleagues[164] in 1952. Their structural formulae are shown on p. 104, and their actions are discussed more fully in a later section.

SCREENING OF NEUROMUSCULAR BLOCKING AGENTS

An important factor in the screening of potentially useful blocking agents is the choice of the best animal species. Different species vary widely in their sensitivity to these drugs and one that resembles man as closely as possible is required. The cat, among the common laboratory animals, is the species in which the ratios of potency of the different blocking agents are similar to those in man. At the other extreme is the rat, which, although sensitive to tubocurarine, is markedly insensitive to the other clinically useful blocking agents. It has also been pointed out that the cat, unlike other species, resembles man in the nature of its response to depolarizing blocking agents. These two facts make the cat the most suitable animal for the preliminary screening tests, and the simultaneous recording of the contractions of the tibialis anterior and soleus muscles of the anaesthetized or decerebrate cat provides a simple and convenient preparation.

Once it has been shown that the drug under study blocks neuromuscular transmission in the cat, a study of its effects in some avian muscles provides a further indication of the mechanism of its blocking action. Drugs which reduce acetylcholine output or which raise the threshold of the motor endplates to acetylcholine produce a paralysis in avians which is indistinguishable from that produced in the cat. Drugs which possess a depolarizing action, on the other hand, produce an initial quick contraction followed by a more prolonged contracture of some avian muscles[165] (*Figure 3.12*). These effects may be studied by recording the contractions of the gastrocnemius muscle of the anaesthetized or decerebrate hen[165]. For a quick screening procedure, the effects of intravenous injection into the jugular vein of conscious young chicks provides useful information, curare-like drugs producing flaccid paralysis similar to that produced in other species, but depolarizing drugs producing a spastic paralysis in which the legs are rigidly extended and the head thrust back[166].

The choice of anaesthetic is another important factor in the initial screening tests in the cat, for some alter the responses to the blocking drugs and thereby render a determination of the mechanism of action more difficult.

For example, ether potentiates the action of curare-like drugs but reduces the effectiveness of depolarizing drugs. Furthermore, it prevents or reduces the initial potentiation of the maximal twitch and the muscle fasciculations characteristic of depolarizing drugs, and both of these effects are valuable indications of the mechanism of action. For the cat, intravenous chloralose (80 mg/kg) provides a suitable anaesthesia, the drug responses being similar to those obtained in the unanaesthetized decerebrate animal. In the hen, the anaesthetic is less important, similar responses to those after decerebration being obtained under anaesthesia with barbiturates or chloralose. In man, the blocking drugs may be used in conjunction with a variety of general anaesthetics, and once the mechanism of action has been determined, a study

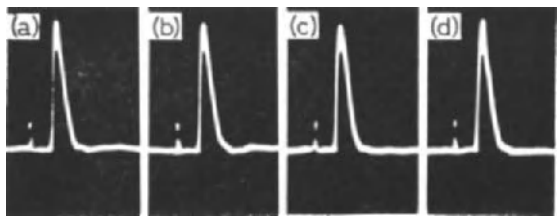


Figure 3.8. Cat, chloralose anaesthesia
Maximal monophasic action potentials recorded in the popliteal space from the peripheral end of the common peroneal nerve in response to stimulation of the motor roots at a frequency of 1/second and with a pulse width of 10 microseconds. (*a*) was recorded before tubocurarine; (*b*) and (*c*) were recorded at 3 and 10 minutes respectively after the intravenous injection of 1 mg/kg tubocurarine; (*d*) was recorded 35 minutes later when the muscle of the opposite leg had fully recovered from the paralysis.

of the effects of various anaesthetics on the responses to the drugs may provide useful information. In view of the marked effects of lowered muscle temperature on the responses to blocking drugs[167], great care should also be taken to maintain the body temperature of the experimental animal.

To classify a drug as a neuromuscular blocking agent, conduction of the nerve impulse along the motor axons must not be affected and, after the administration of paralysing doses, the muscle must still respond to direct electrical stimulation. *Figure 3.8* illustrates the first of these criteria. It shows that action potentials recorded from the peripheral end of the motor nerve in response to stimulation of the ventral roots are unaltered in shape and size in the presence of a concentration of tubocurarine three times greater than that necessary to cause complete neuromuscular block. In fact, few substances are known which are capable of affecting nervous conduction after intravenous injection, although the paralysis following the administration of large doses of the anticholinesterase, ethyl pyrophosphate, may be a consequence of such an action[168]. The second of the criteria is illustrated by *Figure 3.14*. During complete block of the indirectly elicited maximal twitches of the tibialis anterior muscle produced by γ-oxolaudonium, direct stimulation of the muscle itself produced maximal twitches showing that the paralysis was not a

consequence of an action of the drug on the contractile part of the muscle. γ-Oxolaudonium is an ultra-short-acting blocking drug of the curare type[169].

Having located the site of action of the drug at the neuromuscular junction, the mechanism of its blocking action may then be determined. The most direct evidence of an action on transmitter output is obtained from perfusion experiments in which the acetylcholine released from the nerve is collected and assayed (see, for example, the experiments of Dale, Feldberg and Vogt[35] showing the absence of effect of tubocurarine on acetylcholine release, or alternatively, the technique developed by Straughan[170] in isolated skeletal muscle). However, such experiments are beset with difficulties and suffer from the disadvantage that in order to obtain a measurable quantity of

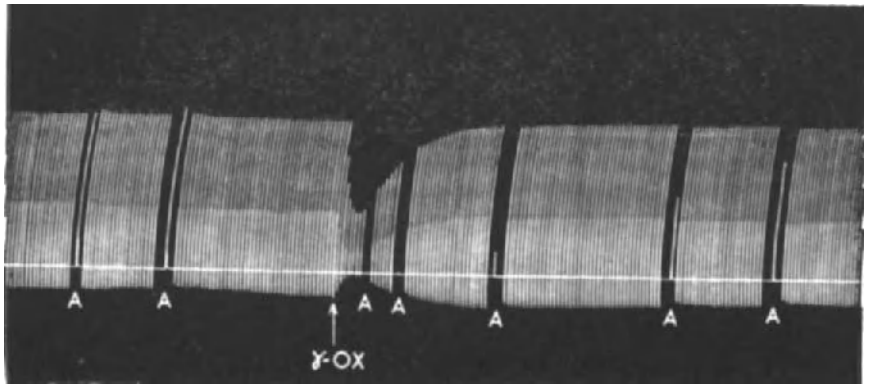


Figure 3.9. Cat, chloralose anaesthesia
Maximal twitches of a tibialis anterior muscle elicited indirectly at a frequency of 1 in 10 seconds. At A, 8 μg acetylcholine injected close-arterially. During the acetylcholine injections, electrical stimulation was temporarily stopped. At γ-OX, 5 mg/kg of γ-oxolaudonium bromide injected intravenously.

acetylcholine an anticholinesterase must be employed. Under these conditions the muscle often fails to contract even in the absence of the blocking drug. Indirect evidence on this point may be obtained by studying the effect of blocking doses of the drug on the response of the muscle to acetylcholine injected close-arterially. Substances which produce block by reducing the output of transmitter from the nerve are without effect on contractions of the muscle produced by injected acetylcholine, whereas drugs which block the motor end-plates abolish the response to injected acetylcholine even before the maximal twitches are completely blocked. This difference is illustrated by the block produced by the triethyl analogue of choline (*Figure 3.4*) and that produced by the curare-like drug, γ-oxolaudonium (*Figure 3.9*). Should the drug under study, like procaine, possess both types of action, then the results are more difficult to interpret and more precise information may only be obtained from perfusion experiments.

The clinically useful neuromuscular blocking drugs are at present confined to those which act on the motor end-plates, and the remainder of this section will be devoted to methods of distinguishing between the curare-like and the

depolarizing relaxants. However, these two mechanisms, or a combination of them, are not the only ones by which substances may produce motor end-plate block. For example, the neuromuscular blocking action of coniine and related hemlock alkaloids has recently been shown to differ in several respects from that of both depolarizing and curare-like blocking drugs[171]. The alkaloids on close-arterial injection produce an immediate acetylcholine-like contraction but the subsequent block of the indirectly excited maximal twitches is slow in onset and develops gradually over a period of 5 to 10 minutes. During the block the contractions produced by injected acetylcholine are abolished but the muscle still responds to direct stimulation. The

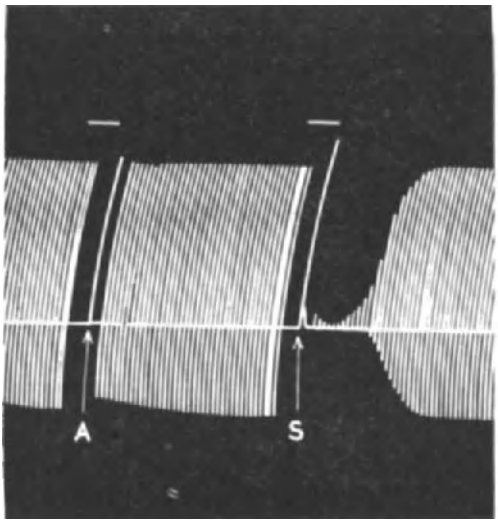


Figure 3.10.
Cat, chloralose anaesthesia
Maximal twitches of the tibialis anterior muscle elicited indirectly at a frequency of 1 in 10 seconds. During the periods marked by the horizontal bars, electrical stimulation was stopped. At A, 10 μg acetylcholine and at S, 10 μg suxamethonium injected close-arterially.

hemlock alkaloids are secondary and tertiary amines and probably penetrate cell membranes readily since they are well absorbed after oral administration and rapidly penetrate the blood–brain barrier to exert central actions in whole animal experiments. These observations, together with the finding that the peripheral neuromuscular blocking action is slow in onset, suggest that the alkaloids may depress the excitability of the end-plate membrane by an intracellular action similar to that which has been ascribed to mecamylamine in autonomic ganglia[172].

If the drug under study possesses a depolarizing action, it will produce the following effects:

(*a*) A quick acetylcholine-like contraction on close-arterial injection into the non-stimulated tibialis anterior muscle of the cat—This is illustrated in *Figure 3.10* where acetylcholine and suxamethonium in the absence of an anticholinesterase are seen to be roughly equipotent in causing contraction of the muscle but only suxamethonium causes subsequent block of the maximal twitches.

(*b*) A brief period of muscle fasciculations and potentiation of the indirectly excited maximal twitches before the block in the cat (see *Figure 3.11*)—

These potentiated 'twitches' are short-lasting tetani as the muscle action potential has become repetitive.

(*c*) A spastic rather than a flaccid paralysis on intravenous injection into the young chick—If the action of the drug is a consequence of depolarization, recovery with sublethal doses is abrupt and without an intervening phase of flaccid paralysis. If there is an intervening phase before recovery the block may also be of a dual type when tested in the cat. Such an effect is produced by tridecamethonium[108].

(*d*) A contractural response of the gastrocnemius muscle of the adult fowl—*Figure 3.12* shows both electrical and kymographic recordings of this

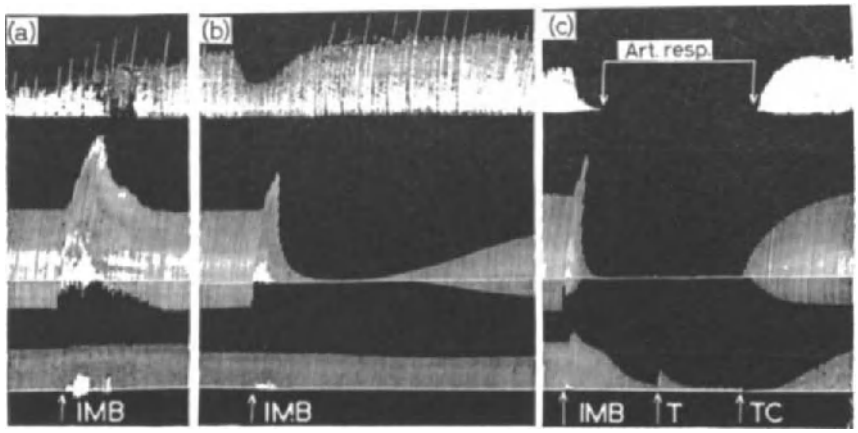


Figure 3.11. Cat, chloralose anaesthesia[154]
Upper record: Respiration. Middle and lower records: Maximal twitches of the tibialis anterior and soleus muscles respectively elicited indirectly at a frequency of 1 in 10 seconds. (*a*) and (*b*) are from the same experiment, (*c*) is from a different experiment. At IMB, 10, 15 and 30 μg/kg carbolonium bromide respectively injected intravensously. At TC, 100 μg/kg tubocurarine injected intravenously. At T, a motor nerve tetanus was applied for 5 seconds at 50/second. Note that in (*c*) tubocurarine antagonized both the respiratory paralysis and that of the tibialis anterior muscle. The soleus muscle however, exhibited the characteristics of dual block.

response to depolarizing drugs in the hen gastrocnemius muscle. A dual mode of action is indicated if the contracture is followed by a decrease in tension of the maximal twitches. However, great care must be exercised in the interpretation of a dual response, for the effect obtained may be a consequence of the parameters of the stimuli applied to the nerve. If brief tetanic responses are elicited instead of twitches, the contractions after the contracture are reduced in tension by depolarizing drugs[173]. Recent experiments[174] have shown that repetitive responses are often elicited in the hen gastrocnemius muscle by single shocks to the nerve if their duration is 0·5 millisecond or more. Such responses may also be reduced in tension by depolarizing drugs; to avoid the possibility of initiating repetitive responses, a pulse width of 50 microseconds is recommended. A decrease in twitch tension may also follow the contracture produced by very large doses of some depolarizing drugs, but this is not a dual mode of action. For example, decamethonium in doses from 5 to 300 μg/kg administered intravenously causes a marked

contracture without subsequent depression of the twitches. However, with larger doses the twitches after the contracture are often depressed (see *Figure 3.12(c) and (d)*). This secondary decrease in twitch tension, unlike that produced by tridecamethonium, does not exhibit the characteristics of block produced by tubocurarine and the effect is not therefore described as 'dual'. It is probably a consequence of membrane inexcitability persisting as the depolarization diminishes.

(*e*) A quick contraction followed by a more prolonged contracture in chronically denervated mammalian muscle[71,134].—In this muscle, the whole of the cell membrane, and not just the end-plate region, is sensitive to depolarizing substances[175]. However, adrenaline and other sympatho-

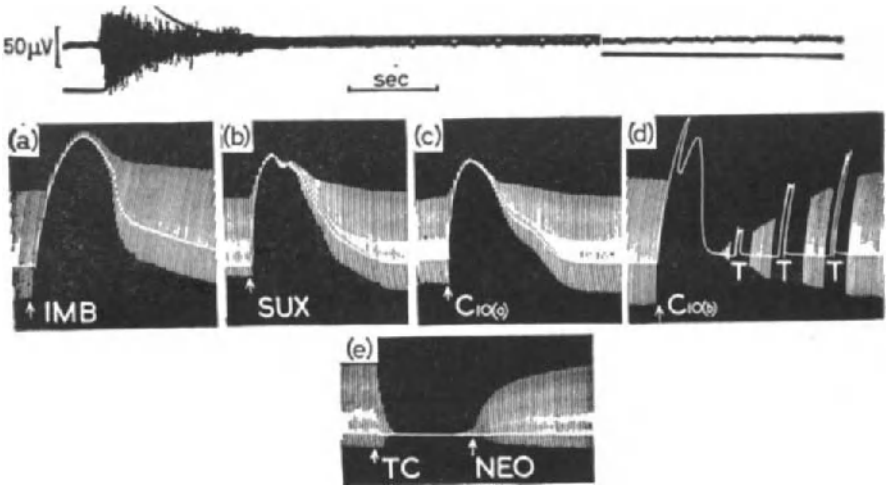


Figure 3.12. Hen, chloralose anaesthesia
Uppermost record: Electrical response (concentric needle electrode) and tension change (mechano-electric-transducer strain gauge) of the gastrocnemius muscle produced by the close-arterial injection of 5 μg acetylcholine. Note the 'electrical silence' during the sustained part of the tension curve. Middle and lower records: Maximal twitches of the gastrocnemius muscle elicited indirectly at a frequency of 1 in 10 seconds. At IMB, 2 μg/kg carbolonium; at SUX, 20 μg/kg suxamethonium chloride; at C_{10}(a), 10 μg/kg decamethonium iodide; at C_{10}(b). 0·4 mg/kg decamethonium iodide; at TC, 1 mg/kg tubocurarine and at NEO, 100 μg/kg neostigmine. All injections intravenously. At T, a motor nerve tetanus was applied for 10 seconds at a frequency of 100/second. All records are from different experiments except for (*c*) and (*d*) which are from the same experiment.

mimetic amines[176], and even tubocurarine[177] and gallamine[178], sometimes produce tension and so this preparation is not specific for depolarizing substances.

With the exception of the response to tubocurarine and gallamine in the denervated muscle, competitive blocking agents do not produce the above-mentioned effects. Their only action in both cat and hen is to block the indirectly excited maximal twitches (*Figures 3.12(e), 3.13, 3.14 and 3.16*).

The fact that a substance possesses a depolarizing action does not necessarily mean that its blocking action is a consequence of this effect. Therefore, it is necessary to determine the characteristics of the neuromuscular block produced. The method depends upon the fact that block

produced by curare-like drugs is antagonized by procedures which increase, in the region of the motor end-plates, the local concentration of acetylcholine or of substances with an acetylcholine-like action. However, these procedures are without effect or may even have the opposite action if the block is a consequence of depolarization. Thus, during a partial paralysis, the close-arterial injection of acetylcholine itself or the administration of an anti-cholinesterase such as neostigmine antagonizes the block by competition but increases that due to depolarization. *Figures 3.13, 3.14 and 3.21* illustrate these differences. In the absence of an anticholinesterase, the effects of injected acetylcholine are a consequence of the choline liberated through rapid hydrolysis of acetylcholine by cholinesterase.

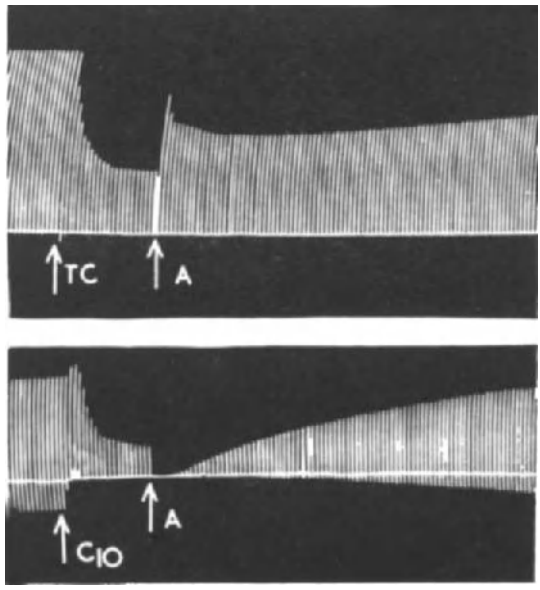


Figure 3.13.
Cat, chloralose anaesthesia
Maximal twitches of tibialis anterior muscles elicited indirectly at a frequency of 1 in 10 seconds. At TC, 0·3 mg/kg tubocurarine and at C_{10}, 40 μg/kg decamethonium iodide administered intravenously. At A, 0·3 mg acetylcholine administered close-arterially.

Among the curare-like blocking agents, an exception to the above generalization is the potent muscle relaxant benzoquinonium (Mytolon). Benzoquinonium resembles tubocurarine in the mechanism of its blocking action but its effects in most species are only weakly antagonized by anti-cholinesterases. This lack of antagonism is probably connected with the fact that benzoquinonium itself possesses some anticholinesterase action although this is not the full explanation[179].

Tetanic stimulation applied to the motor nerve during a partial paralysis of the maximal twitches provides further information concerning the mechanism of the blocking action. During block by competition, high-frequency stimulation of the nerve produces only a brief twitch-like response from the muscle and the post-tetanic twitches are markedly increased in tension (*Figure 3.14*). The waning tetanus may be explained on the assumption that during high-frequency stimulation the amount of acetylcholine released by each nerve impulse rapidly diminishes. In a muscle which is not paralysed, no effect on tension occurs until the transmitter output falls below

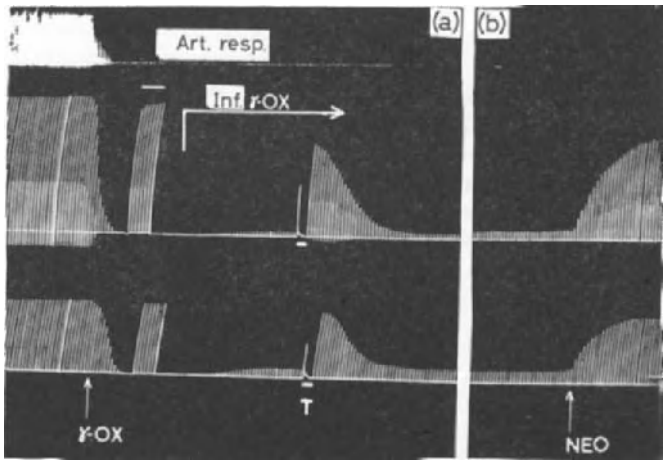


Figure 3.14. Cat, chloralose anaesthesia
Upper record: Respiration. Middle and lower records: Maximal twitches of the tibialis anterior and soleus muscles respectively elicited indirectly at a frequency of 1 in 10 seconds. At γ-OX, 10 mg/kg γ-oxolaudonium injected intravenously. During the period marked by the horizontal bar, the muscles themselves were stimulated directly. At the point shown, an intravenous infusion of γ-oxolaudonium (53 mg/kg/hour) was started and maintained throughout the rest of the experiment. At T, a motor nerve tetanus was applied for 10 seconds at a frequency of 50/second. At NEO, 50 μg/kg neostigmine were injected intravenously.

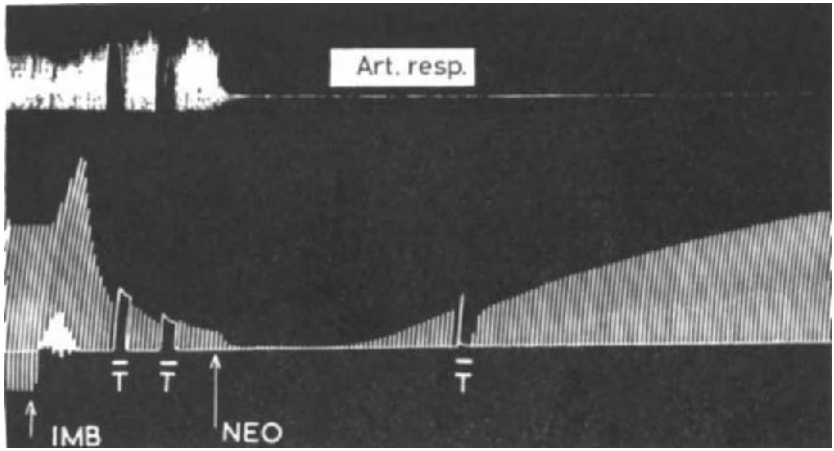


Figure 3.15. Cat, chloralose anaesthesia[154]
Upper record: Respiration. Lower record: Maximal twitches of the tibialis anterior muscle elicited indirectly at a frequency of 1 in 10 seconds. At IMB, 15 μg/kg carbolonium bromide and at NEO, 50 μg/kg neostigmine administered intravenously. At T, motor nerve tetani were applied for 5 seconds at a frequency of 50/second. During the tetani, the kymograph speed was increased. Note that spontaneous respiration continued until neostigmine was administered.

the critical level, but in a muscle partially blocked by a curare-like drug the threshold to acetylcholine is raised. Any change in transmitter output is therefore immediately reflected in the tension[180]. The increase in tension of the post-tetanic twitches is explained by the finding that after a tetanus the motor nerve releases a greater amount of transmitter in response to a single shock than it did formerly[181].

During partial block by depolarization, the tension developed during a tetanus is reduced but is sustained throughout the period of stimulation, and after the tetanus the twitches are unaltered in height. In the absence of an anticholinesterase, the excess transmitter released after the tetanus is probably insufficient to summate with the blocking drug. However, when cholinesterase is inhibited, acetylcholine accumulates both during and after the tetanus and increases the depth of paralysis. The tetanic tension therefore

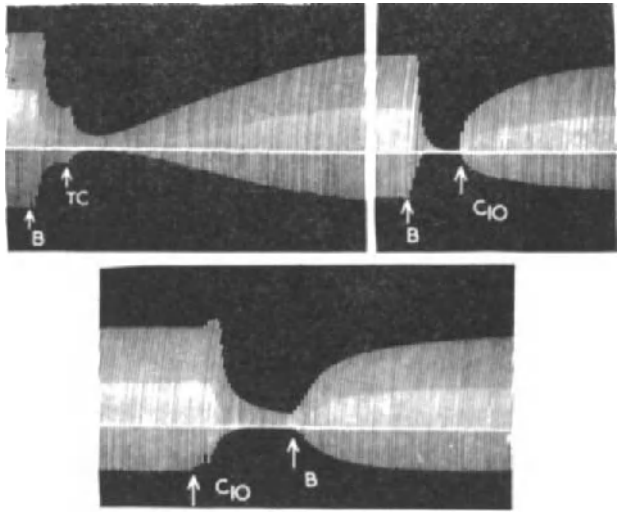


Figure 3.16. Cat, chloralose anaesthesia
Maximal twitches of tibialis anterior muscles elicited indirectly at a frequency of 1 in 10 seconds. At B, 0·2 mg/kg, 0·2 mg/kg and 0·07 mg/kg benzoquinonium respectively. At TC, 0·1 mg/kg tubocurarine and at C_1q, 35 μg/kg decamethonium. All injections intravenously.

rapidly wanes and the post-tetanic twitches are temporarily decreased. *Figure 3.15* shows the effect of tetanus before and after neostigmine during partial block produced by the depolarizing drug, carbolonium bromide (Imbretil).

The actions of competitive and depolarizing drugs are mutually antagonistic while the actions of drugs of the same type are additive. Experiments in which a small dose of tubocurarine or decamethonium is administered during partial block produced by the drug under study therefore provides further evidence of the mechanism of its action. *Figure 3.16* illustrates the additive effects of tubocurarine and benzoquinonium and the mutual antagonism which exists between the latter drug and decamethonium.

There are many more ways in which the response of a muscle partially paralysed by depolarization of the motor end-plates differs from one which

is paralysed by a competitive blocking agent. These differences have been thoroughly reviewed by Foldes[182] and it is sufficient to mention here one more distinguishing feature which is based on muscle differences. The respiratory muscles and the soleus muscle of the anaesthetized cat are relatively resistant to the blocking action of depolarizing drugs, and after the injection of a dose sufficient to cause complete paralysis of the maximal twitches of the tibialis anterior muscle, spontaneous breathing usually continues and the soleus muscle may remain apparently unaffected (*Figure 3.11(b)*). On the other hand, with the curare-like drugs there is little difference between the sensitivities of the different muscles (*Figure 3.14*) and with tubocurarine itself the respiratory muscles and the soleus are the first to be affected[132].

CLINICALLY USEFUL NEUROMUSCULAR BLOCKING AGENTS

A few generalizations concerning the side-effects of neuromuscular blocking agents and their interaction with other substances are listed below. These are considered before discussing the properties of individual agents.

Interaction with general anaesthetics and drugs used in premedication

Several general anaesthetics, for example, ether[183–189], chloroform[187,190], fluothane[187,191] and cyclopropane[186,187,192] intensify the action of curare-like blocking drugs, the effect of ether being by far the most powerful. The effect of anaesthetics on the depolarizing blocking drugs however is less striking. According to Foldes and his colleagues[186], ether does not influence the potency of decamethonium and suxamethonium in man although it reduces their effect in cats[133]. Nitrous oxide[190] and ethylene[193] in the concentrations used in clinical anaesthesia are said to be without effect on the activity of any of the blocking agents. Some barbiturates, particularly pentobarbitone, have been shown to potentiate the blocking action of both tubocurarine and decamethonium[184,194,195], and large doses of chlorpromazine, exceeding those used therapeutically, potentiate the action of tubocurarine and gallamine, but reduce that of decamethonium and suxamethonium[196]. Morphine and related analgesics have also been shown to potentiate the action of tubocurarine but to be without effect on that of decamethonium[193].

Hypothermia

A fall in muscle temperature increases the magnitude and the duration of the effect of depolarizing blocking agents in both animals[161,167] and man[197,198]. In contrast, the magnitude of a block produced by tubocurarine is reduced, although there is no change in its duration[161,167,197]. These changes may be due to a retardation of the process of repolarization at the motor end-plates. Consequently, the effectiveness of the depolarizing substances, including that of acetylcholine, is increased and the increased activity of the acetylcholine released from the motor nerve accounts for the reduced effect of tubocurarine[161,167]. These findings are important in view of the increasing number of operations carried out at present under hypothermia.

Histamine liberation

Many organic bases release histamine in the body and neuromuscular

blocking agents are no exception[199]. However, with the doses used to produce muscle relaxation in anaesthesia, this complication arises only with tubocurarine and possibly after prolonged infusions of suxamethonium. Suxamethonium is rapidly hydrolysed in the body to succinylmonocholine which, although relatively inactive as a neuromuscular blocking agent, is roughly equipotent as a histamine liberator[200]. Histamine release probably accounts for the numerous cases of bronchospasm which have followed the use of tubocurarine[199], and at least one case of bronchospasm was reported to have developed under continuous infusion of suxamethonium[201].

Actions at autonomic ganglia

Tubocurarine is the only neuromuscular blocking agent with a significant ganglionic blocking action, its potency being only a little less than that of hexamethonium[200]. It shows some specificity in this action in that parasympathetic ganglia are more affected than sympathetic ganglia[202], but it may cause a profound hypotension in anaesthetized animals in doses little greater than those necessary to block neuromuscular transmission[200,203]. The significance of the ganglionic blocking action of tubocurarine in clinical anaesthesia is not clear. Premedication with other drugs complicates any assessment and in the presence of atropine or scopolamine any block of parasympathetic ganglia by tubocurarine is unimportant.

In contrast to the action of tubocurarine, suxamethonium stimulates autonomic ganglia[199] and this may result in a mild hypertension during anaesthesia, particularly when it is administered in the form of a continuous infusion[200]. None of the other blocking agents has this effect in the usual paralysing doses.

Actions on the heart

In the dog large doses of tubocurarine block both the effects of vagal stimulation and those of acetylcholine and methacholine on the heart[205]. Gallamine possesses a similar but more potent action[206] and this occurs with doses given clinically. However, the resulting tachycardia occurring in the majority of patients receiving gallamine is relatively unimportant. This atropine-like activity of tubocurarine and gallamine is unusual in that it only affects the heart; other muscarinic actions of acetylcholine are not abolished.

Recently, a comparison has been made on the isolated guinea-pig heart of the ability of neuromuscular blocking agents to block the effects of preganglionic stimulation of the vagus[207]. The drugs shown to possess this property, arranged in order of decreasing potency, are as follows: hexafluorenium, gallamine, laudexium, decamethonium, tubocurarine, suxamethonium and succinyldisulphocholine. The effects were said to be due to a blocking action in cardiac vagal ganglia but it may be that they were caused by a specific atropine-like action such as that already described for tubocurarine and gallamine.

Both gallamine and tubocurarine prevent cardiac arrhythmias produced by adrenaline in the presence of cyclopropane anaesthesia[208]. The two drugs are roughly equipotent, depressing the sensitivity of the myocardium to adrenaline by a direct action. The effect is unrelated to their atropine-like action on the heart for atropine itself is inactive in this test.

Ionic Changes

Depolarization of the motor end-plates is associated with loss of potassium ions from the muscle cells, and in both animals and man under the influence of depolarizing blocking drugs this may be sufficient to raise the plasma potassium[200, 208]. This property probably accounts for the beneficial effects of intravenous infusions of potassium chloride occasionally seen in cases of prolonged apnoea after the administration of depolarizing relaxants in man[209]. With the doses usually used, the effect on plasma potassium is generally small but may be important in patients whose ionic balance is disturbed[200].

Recently, Ahmad and Lewis[210] have carried out experiments to determine the effects of decamethonium, suxamethonium and tubocurarine on the uptake or release of radioactive calcium, sodium and potassium in isolated sartorius muscles of the frog. The depolarizing drugs were associated with increased uptake of $^{47}Ca^{++}$ and $^{24}Na^{+}$ and increased release of $^{42}K^{+}$, whereas tubocurarine depressed uptake of $^{47}Ca^{++}$, caused no change in $^{24}Na^{+}$ uptake and did not release $^{42}K^{+}$.

Effects on muscle spindles

In mammals, a small motor-nerve system known as the gamma-efferents innervates the intrafusal fibres of the muscle spindles which are embedded between the larger muscle-fibres. When the intrafusal fibres shorten, afferent volleys are discharged along sensory fibres and initiate spinal reflexes which enhance skeletal muscle-tone. The gamma-efferents, like the large motor nerve-fibres, have been shown to be cholinergic[211]. Transmission at these sites is blocked by tubocurarine and probably by other curare-like agents, while the spindles are activated by acetylcholine, suxamethonium and decamethonium[212]. Paton[200] suggests that activation of the spindles by the depolarizing drugs may partly account for the muscle fasciculations which precede their blocking action. The fasciculations are synchronous responses of whole muscle units, rather than of single cells, and as such are difficult to account for on a basis of end-plate depolarization alone. However, another possibility suggested by Paton is that depolarization of one end-plate may retrogradely excite its associated nerve terminal so that an axon reflex is set up causing all the cells of the motor unit to react together. Probably both mechanisms contribute to the effect, for in animal experiments fasciculations produced by depolarizing drugs, although reduced, are not abolished after acute denervation. In acutely denervated muscles axon reflexes, but not spinal reflexes, may still take part.

The neuromuscular blocking agents which are, or have been, extensively used in surgical anaesthesia are those described below. For a fuller description of the effects and details of the technique of administration of most of the following drugs, the reader should consult the comprehensive monograph by Foldes[199].

Curare-like Blocking Agents

These are also referred to as competitive, non-depolarizing or anti-depolarizing blocking agents (see pp. 100 and 101).

(+) *Tubocurarine chloride*

In surgical anaesthesia, tubocurarine is usually injected intravenously after induction but before endotracheal intubation. The dose required depends upon the condition of the patient and upon the general anaesthetic being used. The synergism between ether and tubocurarine is particularly pronounced and, in the presence of ether, the usual dose of tubocurarine has often to be reduced by at least 60 per cent. This makes tubocurarine the blocking agent of choice with ether anaesthesia, for at the end of the operation the block wears off rapidly and spontaneous respiration quickly returns. On account of its histamine-releasing properties, the use of tubocurarine is contra-indicated in patients with a history of bronchial asthma or other allergic conditions. Except for diagnostic purposes, it is also contra-indicated in patients suffering from myasthenia gravis.

In man, about one-third of a dose administered by any route is excreted in the urine over a period of a few hours. The rest is degraded, probably in the liver. When a single moderate amount is administered intravenously, its action starts to decline in about 20 minutes yet when a second dose is administered even as late as 24 hours after the first, less drug is needed to produce an equivalent degree of paralysis. The brief duration of the paralysis produced by an initial dose is probably largely due to redistribution of the drug rather than to its elimination or destruction. For a full description of the distribution, destruction and elimination of tubocurarine and other blocking agents, an article by Kalow[213] may be consulted.

When necessary, neostigmine may be used to hasten the return of spontaneous respiration and of voluntary movements after block by tubocurarine. However, several cases have been reported in which neostigmine administered at the end of the operation was apparently without effect. This may occur in 'poor-risk' patients, who have a low intracellular potassium, and is now known as 'neostigmine-resistant curarization'[214]. The term is based on the fact that neostigmine does not cause the return of spontaneous respiration. However, there is as yet no evidence that the prolonged cessation of respiration in these cases is due to a peripheral block of the respiratory muscles by tubocurarine. Several other causes may contribute to the effect[215].

The dimethyl ether of tubocurarine in which the two hydroxyl groups are replaced by methoxyl groups is available as the chloride, iodide and bromide. It was first prepared from tubocurarine by King[2]. The drug was clinically evaluated by Stoelting and his colleagues[216,217] and by others[218,219]; it is 2 to 3 times more potent than tubocurarine[228] but the duration of its effect is slightly less[221]. Its histamine-liberating and autonomic effects are relatively less than those of tubocurarine[222,223]. Dimethyltubocurarine is excreted by the kidneys to a larger extent than tubocurarine itself; in man, for example, 55 per cent of an injected dose is recovered in the urine[224]. The blocking action of dimethyltubocurarine, like that of tubocurarine, is antagonized by neostigmine and by other anticholinesterases.

Gallamine triethiodide (*Flaxedil*)

Gallamine triethiodide was the first widely used synthetic neuromuscular blocking agent and is the only one of the curare-like type to withstand the test of time. It was first synthesized and studied pharmacologically by Bovet

and his colleagues[123,225] and later by others[178,206]. The use of gallamine in surgical anaesthesia in Great Britain was first reported by Mushin and co-workers in 1949[226]. The mode of administration of gallamine is similar to that of tubocurarine, and on a weight basis it is about one-fifth as potent as tubocurarine in man with a slightly shorter duration of action. It is the muscle relaxant of choice with cyclopropane anaesthesia as it counteracts the bradycardia frequently seen with this agent. It is not recommended in the presence of cardiovascular disorders and hyperthyroidism since it produces persistent elevation of blood-pressure and pulse-rate. Up to 100 per cent of an injected dose of gallamine is excreted unchanged in the urine[226]. Its blocking action is also antagonized by neostigmine.

Benzoquinonium chloride (Mytolon)

The pharmacological properties of benzoquinonium in animals were described by Hoppe[227,228] and others[179,229] and several authors have described its use as a muscle relaxant in surgical anaesthesia[189,230–233]. Benzoquinonium has a fairly pronounced anticholinesterase activity in mammals[228] (about one-tenth that of neostigmine on a weight basis) and causes bradycardia and a marked increase in bronchial and salivary secretions[231]. These side-effects are prevented by the administration of large doses of atropine or scopolamine. The block produced by benzoquinonium is curare-like[179] but its action is only weakly antagonized by neostigmine in mammals, including man[179,229,232]. An interesting species difference exists with regard to the blocking action of benzoquinonium, for in the fowl it is readily antagonized by neostigmine[179]. It is also of interest that benzoquinonium does not inhibit cholinesterase in the fowl[234]. Benzoquinonium is rapidly excreted by the kidneys in an active form, as much as 80 per cent of an injected dose appearing in the urine[227]. The occurrence of unwanted muscarinic side-effects and the lack of antagonistic ability of neostigmine have resulted in benzoquinonium being abandoned as an adjunct to surgical anaesthesia.

Laudexium methylsulphate (Laudolissin)

The pharmacological properties of laudexium were first investigated by Collier and Macauley[235] and it was first used in clinical anaesthesia by Bodman, Morton and Wylie[236] and later by others[237–240]. In anaesthetized patients it is about half as potent as tubocurarine. Its cumulative effect is greater than that of tubocurarine and it is more markedly potentiated by ether. Its blocking action is antagonized by neostigmine but recurarization may occur as it has a long duration of action.

Depolarizing Blocking Agents

Decamethonium iodide or bromide (Eulissin; Syncurine)

Decamethonium was first used in clinical anaesthesia by Organe, Paton and Zaimis[241]. In conscious volunteers it was found that the dose required to cause a 95 per cent depression of the grip strength caused a respiratory depression 2 to 4 times greater than that produced by curare-like blocking agents[242,243]. This is surprising since it is possible in the anaesthetized cat to

cause complete block of the maximal twitch s of the tibialis anterior muscle without seriously affecting respiration. Tubocurarine, on the other hand, abolishes spontaneous respiratory movements in doses slightly smaller than those which block the maximal twitches of the tibialis anterior muscle. The different results in the cat and man may be explained by the fact that spontaneous voluntary movements and respiratory movements do not resemble maximal twitches although they are similar to experimentally-produced tetani. To paralyse spontaneous movements or experimental tetani larger doses of decamethonium than those necessary to block maximal twitches are required. On the other hand, with tubocurarine this difference is much less marked and so respiratory movements and twitches are paralysed together.

In man, the dose and time relationships of decamethonium are independent of the general anaesthetic used[199]. With the exceptions of suxamethonium and suxethonium, it has the shortest duration of action of all the commonly used blocking agents. The muscle fasciculations which often precede the blocking action of decamethonium are weaker and occur less frequently than with suxamethonium[199]. Up to 80 to 90 per cent of an injected dose of decamethonium is excreted unchanged in the urine[133]. Unless administered in large amounts and for prolonged periods, the effects of decamethonium wear off abruptly. Anticholinesterases, such as neostigmine, are not antagonists of the action and usually deepen the paralysis. There is no antagonist to decamethonium and other depolarizing agents which is suitable for clinical use, although several substances have been shown experimentally to possess some effect[135,136,244–247]. Most of them may act through their weak curare-like activity thereby reducing the excessive depolarization and allowing repolarization of the end-plates to take place.

Suxamethonium (*succinyldicholine chloride or bromide; Anectine; Brevidil M; Scoline*)

The neuromuscular blocking action of suxamethonium was first described by Bovet and his colleagues[163] and by Phillips[248] in 1949, and the first clinical trials were reported in 1951[249–251]. Since its introduction, suxamethonium has been widely used as a muscle relaxant, both in short and long operations. In anaesthetized man, a single dose of 0·3 to 0·6 mg/kg produces muscular relaxation for up to 3 minutes. The smaller dose is suitable for endotracheal intubation and apnoea is usually absent or of less than 1 minute in duration[199]. For prolonged relaxation, continuous intravenous infusion or repeated injections are used. The short-acting relaxants possess the advantage over the long-acting ones in that the degree of relaxation is readily controlled throughout and spontaneous respiration rapidly returns on stopping the infusion.

Less than 3 per cent of the total dose of suxamethonium is excreted unchanged in the urine of anaesthetized patients[252]. Suxamethonium is rapidly hydrolysed by the butyrocholinesterase of the plasma, first to succinylmonocholine and choline and then, 6 to 7 times more slowly, to succinic acid and choline[253–255]. The primary breakdown product, succinylmonocholine, has a considerably weaker blocking action than suxamethonium itself[255] but it may nevertheless contribute to the effect, particularly when the parent compound is administered as a continuous infusion. In patients with low

plasma cholinesterase, for example, in liver disease or severe anaemia, the action of suxamethonium may be prolonged[199]. A similar prolongation of the action may be produced by the administration of anticholinesterases. However, the alkaline hydrolysis of suxamethonium is considerable and providing there is no acidosis present, this eventually terminates the effect of suxamethonium even in the absence of plasma cholinesterase[199].

Suxamethonium produces initial muscle fasciculations more readily than decamethonium and these are especially prominent following rapid injection. They are reduced in extent when the drug is injected slowly. They occur chiefly in the deeper muscles of the body and appear first in the muscles of the shoulder girdle, face and arm. They are then seen in the trunk and less strikingly in the legs. The use of suxamethonium is often followed by post-operative deep muscle ache which may be severe enough to necessitate pethidine for relief. The pain develops slowly and may be delayed as much as 24 hours after recovery from the anaesthetic. Its incidence is less in patients who rest in bed after operation[256,257]. Post-operative muscle pain constitutes one of the major disadvantages in the use of suxamethonium. Its occurrence is associated with depolarization and may be a consequence of the muscle fasciculations. Paton[200] points out that, during fasciculations, muscle-bundles contract without synchronous activity in other bundles and unsupported by tension development in the muscle as a whole. Such asynchronous contractions may produce fibre damage thereby causing pain.

The main contra-indication to the use of suxamethonium is in ocular surgery and possibly in patients with glaucoma. The extra-ocular muscles have been shown, both in the cat and in man, to react to suxamethonium by a contracture similar to that occurring in avian muscles, and this may lead to a sustained increase in intra-ocular pressure[258].

There have been many cases reported of unduly prolonged apnoea, particularly after the administration of large amounts of suxamethonium. One obvious cause is a low plasma cholinesterase activity but there are several other possibilities. The causes and treatment of prolonged apnoea have been fully discussed by Foldes[199] and by Churchill-Davidson[215].

Suxethonium bromide (Brevidil E)

Suxethonium was first used clinically by Valdoni in 1949[259]. Its action closely resembles that of suxamethonium but on a molar basis it is only about half as potent and the duration of its effect is shorter. It is hydrolysed by plasma cholinesterase about one and a half times faster than suxamethonium[199]. The incidence of muscle fasciculations is less than with suxamethonium, and according to Hale Enderby[260] post-operative pain occurs less frequently. Since its duration of action is even shorter than that of suxamethonium, it has been recommended for use in electroshock therapy[261,262].

Carbolonium bromide (Imbretil)

Carbolonium bromide was studied pharmacologically by Cheymol[164] and by Klupp[263] and their colleagues and is frequently employed in Europe as a muscle relaxant in surgical anaesthesia[198,264–269]. It has also been used in the treatment of tetanus[270]. There is unanimous agreement that the drug possesses a depolarizing action but opinions differ with regard to the type of

block produced. According to some of the earlier workers[267,268], neostigmine is capable of antagonizing the blocking action of carbolonium bromide in a number of species, including man. However, more recent reports[209,271] leave little doubt that the block in man is due to depolarization, although, as with other depolarizing blocking drugs, large amounts of carbolonium bromide administered over a long period may lead to a change in the characteristics of the block[209]. The drug has some anticholinesterase activity but muscarinic side-effects do not normally occur, probably because it also possesses an atropine-like action[164,263].

Carbolonium bromide and decamethonium are roughly equipotent in man[209] although the former is the more active in the cat[154]. The block develops more slowly and is longer-lasting than that produced by decamethonium[209]. Muscle fasciculations precede the blocking action[271] and in the cat the initial potentiation of the maximal twitch and the fasciculations are even more pronounced than those occurring with suxamethonium[154]. Carbolonium bromide is slowly eliminated by the kidneys, 67 per cent of an intravenous dose being excreted in the urine in the first hour[272]. The drug appears to possess no advantages over decamethonium as an adjunct to anaesthesia but it may be of more use than other relaxants in the control of convulsions in tetanus[269].

When first studied, suxamethonium was believed by many to have too short a duration of action to be of widespread clinical use as an adjunct to surgical anaesthesia. However, it now seems that the ideal muscle relaxant will be of ultra-short duration. For long operations adequate muscle relaxation will then be produced by continuous intravenous drip, thereby giving the anaesthetist full control of the degree of relaxation and allowing the rapid return of spontaneous movement and of full respiratory activity when the infusion is stopped. The two short-acting relaxants at present in use are of the depolarizing type and they have several disadvantages. The most important of these are post-operative muscle pains, the lack of a suitable antagonist, the possibility of prolonged apnoea, and the production of 'dual block', whatever its cause. These disadvantages have largely limited the use of depolarizing drugs, at least in Great Britain, to short-lasting operations. Foldes[199] summarizes his views on the ideal muscle relaxant as follows: 'A non-depolarizing muscle relaxant which will be as short acting and controllable as succinylcholine (suxamethonium), its fate in the organism being little affected by pathological changes, its breakdown products having no neuromuscular blocking effect and which will be easily reversible by a harmless antagonist, in the rare instances when an atypical response will make this necessary.' Attempts have been made to find such a short-acting curare-like blocking agent[169,273,274] and the search continues, providing a future goal for both the chemist and the pharmacologist.

A chapter on the chemical aspects of neuromuscular blockade will be included in Volume 3 of the present series.

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4

2-HALOGENOALKYLAMINES

J. D. P. GRAHAM

INTRODUCTION

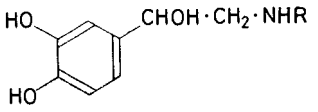
THE *autonomic or involuntary nervous system* is in continuity with the central nervous sytem and consists of nerves, nerve plexuses (or webs) and ganglia (or relay stations). The *afferent fibres* have their origin in most organs, and are found in quantity in the vagus, splanchnic and pelvic nerve-trunks. They run via the dorsal spinal roots into the central axis and connect with cells, fibres from which pass upward in the spinal cord to the medulla oblongata, the reticulum of the brain-stem and the hypothalamic nuclei. Other fibres run outwards to supply many structures of the body, especially the glands, vessels and smooth muscles. This *efferent outflow* is usually subdivided into the cranio-sacral or *parasympathetic* and the dorso-lumbar or *sympathetic*. Unlike the motor outflow to skeletal muscle, the fibres do not run uninterruptedly from their central place of origin to the tissues which they innervate but relay at ganglia. In the sympathetic system, the place of origin is regularly from the last cervical to the third lumbar segment of the spinal cord on either side of the midline, there being 22 symmetrically disposed pairs of ganglia arranged along the vertebrae of the spine of the skeleton. In addition, there are a number of less regularly disposed ganglia described as being prevertebral in situation; of these, the well-known coeliac ganglion (or solar plexus) is perhaps the most important.

Fibres which arise in the central nervous system and relay at a ganglion are called *preganglionic*. In the sympathetic nervous system, such fibres are usually short and form only a few well-marked nerves to which particular names have been applied by the anatomists, *e.g.* the splanchnic nerves. The passage of an impulse brings about the release of the transmitter substance, acetylcholine, at the nerve-ending in the ganglion and the fibres are described as *cholinergic*. Diffusion of this chemical across the synaptic space separating the ultimate terminals of the preganglionic fibre from the surface membrane of the next nerve-cell in the chain initiates depolarization of that membrane and activity in the cell. However, not all preganglionic fibres which enter a particular sympathetic ganglion have their synapse there; they may pass through and relay elsewhere. Postganglionic sympathetic fibres are generally *adrenergic* and release at the nerve-ending the catecholamine, noradrenaline (*I*; R =H) an amine derived from 3,4-dihydroxyphenylethylamine, dopamine (*II*). Adrenergic nerve-cells contain dopamine but there is no evidence that it is released.

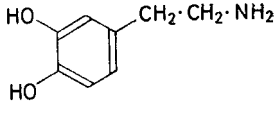
The activity of the sympathetic system is reinforced by the discharge from the medulla of the adrenal gland. The innervation of the adrenal medulla is by the greater splanchnic nerve, which is composed of preganglionic fibres. The medulla of the adrenal gland may be considered to be a modified ganglion

and the transmitter substance within the medulla stimulates the release of catecholamines into the blood-stream. However, only a small and variable proportion of the effluent is noradrenaline (*I*; R=H) and most of it is the *N*-methyl derivative, adrenaline (*I*; R=Me). Both amines are stored[1] in so-called chromaffin granules[2] in the cytoplasm of the adrenal medullary cell. The catecholamines, adrenaline and noradrenaline, have actions similar to the effects of stimulating the sympathetic nerves, and are therefore termed *sympathomimetic*.

When noradrenaline is released at or near the nerve-ending after the arrival of a postganglionic nerve impulse, it diffuses across the gap between the nerve and the surface membrane of the effector cell. The point of attachment of the amine to the membrane is envisaged as one especially apt by physical or chemical nature to receive it, an idea perhaps first postulated



(I)



(II) Dopamine

by Langley[3] in 1905. The concept of a precisely-formed and specifically-suited area on a cell for the attachment of a chemical molecule is embodied in the term *cell receptor*. This implies that an extraneous molecule has limited access to the semipermeable cell membrane. As the sympathomimetic amines released from the adrenal medulla and adrenergic nerves are adrenaline and noradrenaline, the adrenoceptive site must be such as to receive, attach to, and react with, these two catecholamines and possibly to others such as isoprenaline (*I*; R=Pr^i) an amine found in small quantities[4] in mammals.

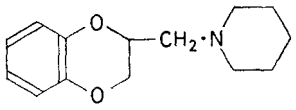
Table 4.1. Some effects of stimulating adrenergic nerves
(Those in the second column are prevented by 2-halogenoalkylamines; those in the fourth column are not)

Tissue or organ	*Effect*	*Tissue or organ*	*Effect*
Blood-vessels—skin	Constriction	Blood-vessels—heart	Dilatation
Blood-vessels—viscera	Constriction	Blood-vessels—skeletal muscles	Dilatation
Blood-vessels—lung	Constriction	Pupil	Dilatation
Spleen	Contraction	Heart	Acceleration
Sphincter muscles	Contraction	Gut—motility	Reduction
Pilomotor muscles	Contraction	Gut—secretion	Reduction
Salivary glands	Secretion	Bladder	Relaxation
Uterus (rabbit)	Contraction	Uterus (rat)	Relaxation

The adrenergic nerve-fibres innervate many structures and the effects of stimulating some of these fibres are listed in *Table 4.1*. The effect of stimulating the sympathetic nervous system is catabolic, preparing the animal in various ways for activity by promoting release and consumption of energy.

The effect of stimulating an adrenergic nerve may be interrupted by a drug such as guanethidine which depletes the store of noradrenaline and prevents its replacement so that the nerve impulse proves ineffective by

reason of lack of transmitter substance, although the effector cell remains reactive to extraneous catecholamines. Alternatively, the membrane of the reacting cell may be stabilized or rendered insensitive to catecholamines by a drug molecule which occupies the specific receptors. For example, piperoxan (*III*) competes in a reversible manner with the transmitter and its efficacy is regulated by the law of mass action. Other drugs such as dibenamine hydrochloride (*IV*), one of the *2-halogenoalkylamine series*, are bound more firmly to the active site and are not subject to the rules of substrate competition. They have been loosely referred to as 'adrenergic blocking drugs', a term which refers more accurately to guanethidine. Perhaps their most characteristic action is a powerful, long-lasting and unusual antagonism of some of the stimulant actions of catecholamines which has earned them the name of 'adrenomotor antagonists'.



(*III*) Piperoxan

$(PhCH_2)_2N \cdot CH_2 \cdot CH_2Cl, HCl$

(*IV*) Dibenamine hydrochloride

The Multiplicity of Receptors

The response of smooth muscle-fibres to adrenergic nerve stimulation or to administration of catecholamines varies in a remarkable way; some fibres, such as those of the uterus of the rabbit, contract, while others, such as those of the bronchial muscle of the rabbit, relax. The pattern of response of individual organs also varies from species to species; the response of at least one organ, the uterus, may alter under endocrine influence, the substance producing the alteration being extractable and transferable[5]; that of another organ varies with changes in the external environment[6].

The relative potency of the three amines, adrenaline, noradrenaline and isoprenaline, differs according to the tissue to which each is applied. Thus noradrenaline is usually the most potent of the three in stimulating smooth muscle to contract, and isoprenaline is the most potent in depressing tone and producing relaxation. The three amines have a capacity to induce both of these responses in the appropriate tissues. With cardiac muscle, the three stimulate and increase both the rate and the force, but isoprenaline is the most active. A comparison of these actions has recently been made and the first part of *Table 4.2* has been compiled from the results. Three other actions are recorded—glycogenolysis in liver cells where adrenaline is the most potent[8,11], the reduction in the potential difference across an isolated piece of frog skin where adrenaline is again the most potent[9]; and the darkening of the skin of *Xenopus* kept in the light on a white background[6] where isoprenaline is the most potent.

In 1906, Dale[12] described the phenomenon of adrenomotor antagonism after using an alkaloid of ergot. He showed that stimulation of the isolated uterus of a pregnant cat by adrenaline is abolished by the previous addition

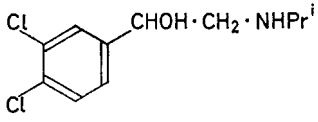
of ergot to the bathing fluid, whereas inhibition of the isolated uterus of a non-pregnant cat by adrenaline is unaltered by ergot.

Table 4.2. The effect and relative potencies of adrenaline, noradrenaline (Nor) and isoprenaline (Iso) on isolated smooth and cardiac muscle of rabbits, on mammalian liver and on batrachian skin before and after treatment with dibenamine or dichloroisoprenaline (DCI)[6–10]

Tissue	*Effect*	*Relative potency* (adrenaline = 1)		*Effect after treatment with:*	
		Nor	*Iso*	*Dibenamine*	*DCI*
Aorta	Contraction	1·0	0·01	Block	—
Gastric fundus	Contraction	1·0	0·001	Block	—
Uterus	Contraction	0·2	0·001	Block	—
Auricle	Stimulation	0·8	150.	—	Block
Gut	Relaxation	1·0	2·5	—*	Reduction*
Aorta (after dibenamine)	Relaxation	0·02	4·0	—	Block
Gastric fundus (after dibenamine)	Relaxation	0·2	30·0	—	Block
Liver	Glycogenolysis	0·1	0·01	Block	Block
Frog skin (potential difference)	Increase	0·01	0·1	Block	?
Xenopus (melanophore index)	Increase	2·0	4·0	—	Block

* Block if both dibenamine and DCI present.

The view is now held that there may be present in smooth muscle-fibres at least two types of adrenoceptive site. One, termed an *alpha*-[13] or *Ac*-receptor[14], initiates contraction when occupied, is best fitted by noradrenaline, and is blocked by 2-halogenoalkylamine compounds. The other,



(V) Dichloroisoprenaline

termed a *beta*-[13] or an *Ar*-[14] receptor, initiates depression or relaxation when occupied, is best fitted by isoprenaline, and is not blocked by 2-halogenoalkylamines, but by 3,4-dichloroisoprenaline (DCI, *V*). The beta-receptor is also found in cardiac muscle where it initiates stimulation when occupied. The cardiac stimulant receptor has been differentiated by one analyst[7] as a separate *Acr*-receptor. The receptor for activating glycogenolysis in liver cells differs from those in smooth muscle in two respects—it is best stimulated by adrenaline and it is blocked by 2-halogenoalkylamines and by DCI[10]. This receptor is intracellular in site whereas the others are possibly situated on or in the cell membrane. It has been termed a *gamma*-receptor while that for depression of *intestinal* smooth muscle is sometimes called a *delta*-receptor[7]. The idea that adrenoceptive sites, at least in muscle-fibres, are located on the surface of the cell is largely based on the finding that drugs which stimulate or depress these fibres produce dramatic alterations in the electro-stability of the membrane. Adrenaline stabilizes the membrane of smooth muscle-fibres which are inhibited by it[15] and depolarizes cardiac fibres which are stimulated[16]. These changes are accompanied by ionic

fluxes and the relation between the action of catecholamines and changes in potassium and sodium ions is close[8]. The amines are active when present as cations[17] and it is the ion of the 2-halogenoalkylamine which is the active blocking part of the molecule. Moreover, neither of these positively-charged particles is likely to penetrate the membrane of a muscle-fibre with any facility. Smooth muscle loses potassium ions on excitation[8] and this is prevented by treatment with 2-halogenoalkylamines.

Peripheral Antagonists of the Excitor Actions of Catecholamines

This heterogeneous group[18] is composed of plant alkaloids extracted from ergot and yohimbine and possessing indolic structures, of a variety of heterocyclic synthetic compounds of which piperoxan and phentolamine are probably the most effective, and of various aromatic synthetic compounds such as the 2-halogenoalkylamines (*Table 4.3*). The latter are congeners of dibenamine and exert a potent and long-lasting effect. The difficulties of solubility, stability, relative inactivity when taken orally and duration of action, however, restrict their use.

It is the aim of this chapter to review the actions, mode of action, chemical structures and clinical applications of the 2-halogenoalkylamines and to assess their present and future place in therapeutics.

Table 4.3. Peripheral antagonists of the excitor actions of catecholamines

Plant alkaloids	*Synthetic compounds*	
	Heterocyclic	*Aromatic*
Ergot Yohimbine	Benzazepines Benzodioxans Imidazolines Phenothiazines Phthalazines	Phenoxyethylamines 2-Halogenoalkylamines

DIBENAMINE

The prototype molecule of the series of which there are now about 1,500 known members[19] is *N,N*-dibenzyl-2-chloroethylamine, dibenamine (*IV*) which was characterized by Eisleb[20] in the American patent literature in 1934, incidental to the description of some chemical intermediates. This compound may be prepared from dibenzylamine and ethylene oxide which react to give *N,N*-dibenzylethanolamine. When this is treated in chloroform with a phosphorus halide, the desired *N,N*-dibenzylhalogenoalkylamine is formed and congeners of dibenamine may be synthesized by this route[21]. Some eleven years after the preparation of dibenamine was described, a brief report appeared[22] of its remarkable action of reversing the pressor response to injected adrenaline in the cat. This publication was followed rapidly by a series of papers which established the main points of its pharmacology[23,24]. Dibenamine, however, is only slightly soluble in water and produces local irritation, two properties possessed by 2-halogenoalkylamines of similar structure, irrespective of the substituents attached to the nitrogen atom and the halogen in the chain. In their classical paper[24], Nickerson and Goodman

showed that when the reversal of the pressor response to injected adrenaline was fully established, it was impossible to overcome the block by increasing the dose of adrenaline. This action has since been demonstrated in several mammals including the mouse[25] for other 2-halogenoalkylamines (*Figure 4.1*).

Nickerson and Goodman[24] stated: 'The reversal by dibenamine of the pressor response to epinephrine (adrenaline) probably respresents a blocking of the vasoconstrictor action and a consequent unmasking of the inhibitory vasodilator action of epinephrine.' These authors commented on the slow onset (30 minutes) and prolonged effect (3 to 4 days) of dibenamine and

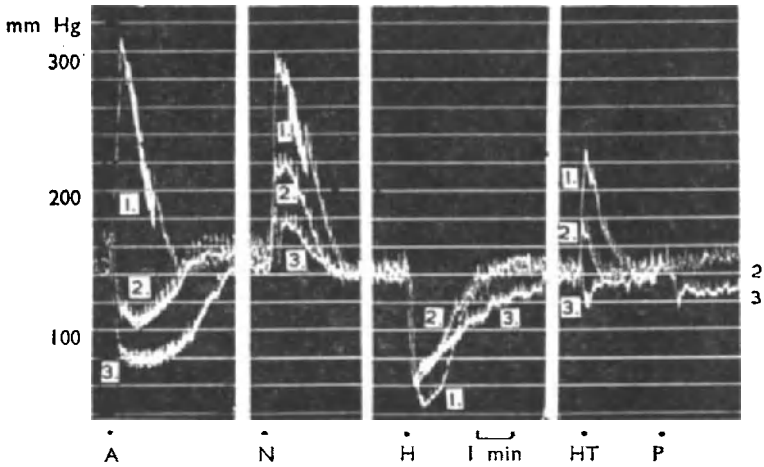


Figure 4.1. Record of the carotid blood-pressure in a male dog (11·3 kg) after pentobarbitone sodium (30 mg/kg intravenously) and atropine (3 mg subcutaneously)[26]

The panels record the effect of (−)-adrenaline, 4 μg/kg (A1), equimolar (−)-noradrenaline (N1), equimolar histamine (H1), and 5 times equimolar 5-hydroxytryptamine, 5-HT (HT1). Two injections of 0·30 mg/kg of *N*-ethyl-*N*-*o*-chlorobenzyl ethyleneiminium picrylsulphonate, *XXIX* (P) given at an interval of 30 minutes (P2 and 3) were allowed to act for 5 minutes and the standard injections repeated. The based pressure was slightly reduced (P3), the response to adrenaline reversed (A2 and 3), that to noradrenaline reduced (N2 and 3), that to histamine slightly reduced (H2 and 3), that to 5-HT reduced or reversed (HT2 and 3).

noted that the pressor response after stimulation of the splanchnic nerves was also reversed. However, the pressor effects resulting from stimulation of postganglionic adrenergic nerves was less easily prevented. The dilatation of the pupil, erection of hair, and contraction of the third eyelid which result from stimulation of the appropriate sympathetic nerve in cats were abolished. Contractions of the isolated uterus of the rabbit produced by treatment with adrenaline were prevented. Cardiac irregularities in dogs anaesthetized with cyclopropane and injected with adrenaline were suppressed, but the sinus tachycardia and increase in force of contraction of the heart were not prevented. It was also established that unlike other antagonists of adrenaline the actions of dibenamine were not influenced by the anaesthetic used in the experiment. It was shown that dibenamine antagonizes only the excitatory

actions of adrenaline (with the exception of cardiac stimulation) and the authors proposed that: 'The long duration of action of dibenamine suggests that some substance required for this reaction is destroyed or inactivated and that it is only slowly replaced or reactivated.' The pharmacological and possible clinical importance of a drug with these properties aroused so much interest that within two years Nickerson[27] in a review quoted more than a hundred papers referring to it.

Dibernamine, however, is not readily absorbed when given by mouth and is therefore irregular in its action. When injected intravenously, it is a cerebral stimulant of an unpleasant kind. There are, therefore, few reports of its clinical use since 1952, apart from two references[28,20] to the harmful effect which it may have on the heart. For some years experimentalists continued to use the compound as a tool of investigation of physiological mechanisms in which the activity of adrenergic nerves might be of importance but that use has now diminished and the last reports of pharmacological investigation of its properties were published[30,31] over five years ago. Topics investigated recently in which dibernamine played a decisive part include the adrenergic component in sweating in horses[32], the importance of constriction of the vessels of the glomerulus of the kidney under sympathetic influence during circulatory failure[33], and the component of the autonomic nervous system responsible for reducing heat loss in a cold environment[34]. Dibenamine has been superseded by more active and more reliable 2-halogenoalkylamines and is now largely of historical interest.

PHARMACOLOGICAL ACTIONS OF 2-HALOGENOALKYLAMINES

Antagonism of Injected Adrenaline and Noradrenaline and of the Effects of Stimulation of Adrenergic Nerves

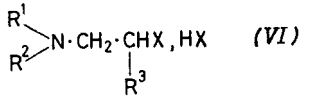
Stimulation of smooth muscle and glands

The 2-halogenoalkylamines block tissues which respond with an excitatory response to stimulation of adrenergic nerve or administration of catecholamines. This result has been repeatedly confirmed on a variety of preparations, which include spirally-cut strips from the aorta of the rabbit mounted in a bath[35], the isolated seminal vesicles of the guinea-pig or the rat[36], the radial fibres of the iris, the uterus of the rabbit or pregnant cat, the perfused vessels in the ear of the rabbit, the vessels of the hind-quarters of the guinea-pig, rat or other small animal, and the blood-pressure of rats, cats, dogs and rabbits. The systemic blood-pressure in mammals is a resultant of cardiac activity and peripheral resistance and both these factors may be greatly affected by central, peripheral and reflex control. It is important, therefore, to use animals which have been prepared by a standard procedure when comparing activity within a series of compounds. Graham[6,9,26] uses mature male rats injected with 3 mg/kg of atropine sulphate and anaesthetized with ether; the brain is then pithed and the rat is injected with heparin and 5 mg/kg of hexamethonium intravenously. This preparation has a steady blood-pressure of about 30 mm Hg and responds consistently to intravenous injections of adrenaline or of noradrenaline for 4 to 6 hours. It may be used to obtain an index of potency, the ED_{50}, or quantity of compound required

to reduce by 50 per cent the pressor response to a standard injection of amine. *Table 4.4* records some activities measured in this way.

The fact that 2-halogenoalkylamines antagonize the pressor effects of circulating catecholamines more easily than the stimulant effect of adrenergic nerves has been repeatedly demonstrated[39] and it has been established that equi-effective doses of pressor amines are equally antagonized by a given dose of 2-halogenoalkylamine. The clinical efficacy of piperoxan (*III*) as a diagnostic agent for phaeochromocytoma depends upon antagonism of the pressor effect of adrenaline and noradrenaline which may be circulating in excess in the blood after release from the secreting tumour tissue.

Table 4.4. The ED_{50} (μmoles/kg intravenously) of some 2-halogenoalkylamines, using (−)-adrenaline (Ad) and (−)-noradrenaline (Nor) as pressor agents. The approximate time during which an effective dose exerts its non-competitive effect against adrenaline and the delay in establishing optimal antagonism are also shown[9,37,38,223].



(*VI*)

Derivative number	R^1	R^2	R^3	X	ED_{50} *Ad*	ED_{50} *Nor*	*Duration* hours	*Onset* min
1	$PhCH_2$	$PhCH_2$	H	Cl	7·7	10·0	48	30
2	$PhCH_2$	$PhO{\cdot}CH_2{\cdot}CHMe$	H	Cl	0·35	0·52	24	10
3	Naphth-1-yl-methyl	Et	H	Br	0·75	0·98	6	5
4	Fluoren-9-yl	Et	H	Br	0·06	0·10	4	5
5	Me	Me	Ph	Br	0·03	0·04	2	<1
6	Me	Me	*p*-BrC_6H_4	Br	0·0003	0·0007	0	0

Most of the recent research work with 2-halogenoalkylamines has been carried out with phenoxybenzamine hydrochloride (dibenyline, dibenzyline, *VI*–2). This compound is about 20 times as potent as dibenamine. The pressor response to both amines is reversed by it in the rabbit[41]; in the vascular bed of the whole dog perfused with oxygenated blood from a pump there is sufficient tone for phenoxybenzamine to lower the blood-pressure[42] and to differentiate between antagonism of adrenaline and noradrenaline[43]. Similar studies have been carried out by measuring the flow of blood into the arteries and out from the veins of the spleen[44] and the hind paw of the dog[45]. Reversal of the constriction produced by noradrenaline is obtained with phenoxybenzamine in the splanchnic vascular bed, and vasoconstriction in the leg is abolished. The constrictor action on the pulmonary arterial bed is likewise prevented and the consequent pulmonary oedema reduced. In kidneys transplanted into the neck and therefore devoid of nervous but not of vascular connections, a rise in systemic pressure produces renal vasoconstriction[46]; phenoxybenzamine prevents this effect which may be due to a vasoconstrictor catecholamine. Phenoxybenzamine, however, potentiates the activity of bradykinin[47], a vasoactive polypeptide which dilates the arterioles.

The blocking action of 2-halogenoalkylamines was thought always to be of long duration, but recent work with more powerful compounds, such as *N*,*N*-dimethyl-2-bromo-2-phenylethylamine (*VI*-5) shows that they may be as brief in their action as the competitive antagonist, piperoxan. The clinically useful compounds, phenoxybenzamine and *N*-ethyl-*N*-naphth-1-ylmethyl-2-bromoethylamine hydrobromide (*VI*–3) have similar potencies and exert their actions for many hours. The rate of onset of action is largely dependent on chemical reactivity, the kinetics of which have been studied extensively[21,48,49]. Physical factors such as solubility in water and chemical factors such as changes in structure in buffered solutions at physiological pH values are also important. However, little work has been reported[50] on the factors which determine the activity of 2-halogenoalkylamines when given by different routes of administration. The difference between the potency of a compound given by mouth and by intravenous injection may be great, and the most potent compound by one route is not the most potent by the other. In a compound which is rapidly and completely cyclized (see p. 157), a large proportion of the product is inactivated by hydrolysis; in a compound which cyclizes slowly, there is time for absorption of the unreacted parent molecule.

Stimulation of cardiac muscle

Dibenamine and the other 2-halogenoalkylamines do not antagonize the stimulation of mammalian cardiac muscle by catecholamines[22,25], although one report[51] states that phenoxybenzamine prevents the increase in force of the intact heart in dogs injected with adrenaline. However, the stimulant effect of adrenaline on the heart of frogs is always antagonized and this observation has been extended to include the heart of the South African clawed toad, *Xenopus laevis*[6]. Thus it is possible that the heart muscle of mammals contains one type of receptor for catecholamines whereas the heart muscle of amphibia contains a different kind.

The cardiac irregularities produced by injections of adrenaline into dogs anaesthetized with various hydrocarbons are abolished by treatment with dibenamine or phenoxybenzamine[52] and use of this property has been made in clinical work[53]. As the arrhythmia which follows upon experimental coronary occlusion in dogs is not controlled by phenoxybenzamine[54] and as the histological picture of the area of infarct is not modified, catecholamines may not play a significant role in this syndrome. There is one report[55] of suppression in dogs of ventricular tachycardia caused by experimental infarct, but the extrasystoles which become so alarming during the conduct of hypothermic technique on animals or patients are not suppressed by these drugs[56]. Besides, the 2-halogenoalkylamines and their hydrolysis products exert a direct depressant action on heart muscle which may be severe[27].

Inhibition of smooth muscle

The 2-halogenoalkylamines do not generally antagonize the relaxation of smooth muscle produced by catecholamines[57]. However, the effect of adrenaline on the peristaltic reflex is prevented by these drugs[58]. Specific inhibition of vascular dilatation by adrenaline has also not been demonstrated, except when large amounts of the 2-halogenoalkylamine are used[59].

Metabolic effects

The more potent 2-halogenoalkylamines[10,60] such as phenoxybenzamine inhibit glycogenolysis in the liver and the use in blood fatty acid levels[226] induced by catecholamines. However, many substances devoid of antagonism to adrenaline are capable preventing the breakdown of glycogen, and this action may therefore be unrelated to blockade of any receptor for catecholamines. Recently, Sutherland and Rall[61] reviewed the role of catecholamines in the formation of the cyclic 3,5-phosphate of adenosince, since an increased rate of accumulation of this substance occurs in the liver and skeletal muscle when these amines are administered. At the same time, the liver tissue releases glucose whereas the skeletal muscle (which lacks glucose-6-phosphate) releases lactic acid. The 2-halogenoalkylamines inhibit the release of glucose from liver cells but not the release of lactic acid from muscle, and thus the site of their action may be related to the step in which glucose-6-phosphate is involved. It is tempting to relate the contraction or relaxation of smooth muscle to this biochemical process[62], as the metabolic pathways of carbohydrate utilization may be linked with the contractile activity of the muscle-fibre.

The injection of adrenaline also produces a transient rise in the blood level of potassium which is discharged from the liver. Dibenamine prevents this hyperkalaemia[63] but anti-adrenaline drugs which are not 2-halogenoalkylamines also possess this action. It has been suggested[64,65] that the underlying process is an increase in the permeability of cell membranes to extracellular potassium ions.

Endocrine glands

The thyroid hormone and the adrenal medullary hormones are in many ways synergistic in action but there have been few studies on the effect of the 2-halogenoalkylamines on thyroid function. According to one[66], phenoxybenzamine has little effect on the basal metabolic rate of hypothyroid rats but increases it in hyperthyroid rats. This finding, if confirmed, may be a manifestation of central stimulation.

The possibility of an adrenergic nerve stimulus to the pituitary gland has been investigated[67]. The 2-halogenoalkylamine drugs antagonize the stimulant effect of adrenaline which results in the release of anterior corticotrophin (ACTH). Dibenamine also interferes with post-coital ovulation[68] and other effects activated by release of hormones from the anterior pituitary in the rabbit. However, these anti-adrenaline drugs do not suppress the actions of the released ACTH in animals subjected to physical stress[69]. Thus the pressor action of adrenaline is antagonized but not the action of the released ACTH. A similar explanation probably applies to the milk let-down phenomenon in rats where dibenamine exerts blocking activity[70].

The central nervous system

Dibenamine is a drug which stimulates the central nervous system. It produces overbreathing and convulsions in mice, and when a solution is infused too quickly into the veins of a patient, unpleasant phenomena result —dizziness, disorientation, excitement. Phenoxybenzamine given to patients in smaller amounts has produced a feeling of sleepiness or of weakness[71].

However, it increases the central excitatory effect of morphine in cats[72].

Although the role of catecholamines in central activity remains obscure[73], their presence in the central nervous system has been demonstrated beyond doubt, and the 2-halogenoalkylamine drugs antagonize the stimulation of the central nervous system produced by large doses of catecholamines. For example, phenoxybenzamine suppresses the effect of adrenaline on rats

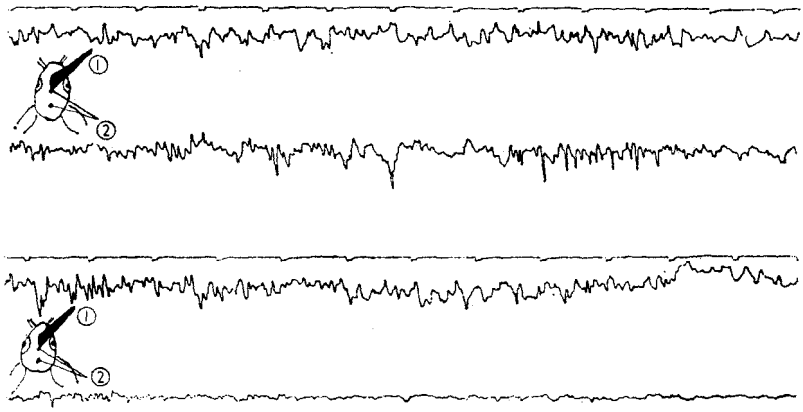
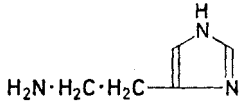
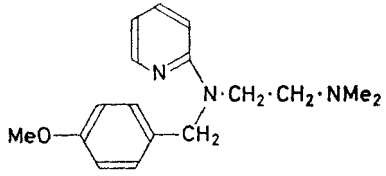


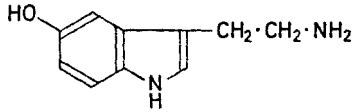
Figure 4.2. Electroencephalogram trace of a male rabbit (3·2 kg) on two channels from three pin electrodes inserted 0·5 cm into nasal passage, frontal and temporal lobes after 30 mg/kg of pentobarbitone sodium[9]
Upper record is control sleep pattern, lower record is depression in cortical activity 5 minutes after intravenous injection of 5 mg/kg *N*-ethyl-*N*-fluoren-9-yl-2-bromoethylamine hydrobromide (*VI*–4). Time interval is in seconds.



(VII) Histamine



(VIII) Mepyramine



(IX) 5-Hydroxytryptamine (5-HT)

whereby they no longer display a conditioned reflex to avoid an unpleasant electric shock[74]; however, this may not be a central effect but antagonism of the systemic toxic action of excess of adrenaline which had made the animals too weak to take avoiding action. A similar effect has been noted in pigeons trained to work on a fixed routine of pecking in response to light signals,

reinforced by a reward of food; the injection of adrenaline depresses the pecking response and phenoxybenzamine prevents this depression[75]. The emetic action of apomorphine, considered to be evidence of central stimulation, is also antagonized by dibenamine[76] in dogs. Phenoxybenzamine has a depressant effect on simple tendon reflexes repetitively elicited[77], and this is thought to be due to antagonism of the facilitatory effect of adrenaline[78]. One site of this complex activity of the 2-halogenoalkylamines may be in the ascending reticular formation. Adrenaline also causes an arousal response and activation of the cortical electroencephalogram (EEG), but phenoxybenzamine in doses sufficient to reverse the pressor action of the amine does not modify this central effect[79]. The position is therefore obscure, the nature of the effect depending somewhat on the dose and route of administration. Recently, an attempt has been made[9] to relate the potency of selected 2-halogenoalkylamines as peripheral antagonists of noradrenaline to their central activity. A battery of tests was employed, including antagonism of the lethal excitatory effect of amphetamine sulphate, synergism with barbiturate or reserpine (depressants), antagonism to leptazole (a stimulant), and the effect of injection of the compounds on the cortical EEG pattern in conscious and anaesthetized rabbits. An example of the latter test is shown in *Figure 4.2*. Various methods of injection of buffered solutions have been attempted, and one compound, *N*-ethyl-*N*-fluoren-9-yl-2-bromoethylamine hydrobromide (*VI*–4) gave consistent evidence of sedation.

Antagonism of Histamine and 5-Hydroxytryptamine

The methods commonly adopted to investigate antagonism to histamine are as follows:

(*a*) Estimation of the potency of the drug as an antagonist of the contraction of isolated guinea-pig ileum—this may be determined as the concentration required to produce a 50 per cent reduction of the response to a standard dose of histamine (the ED_{50}), or as the pA_x value[80].

(*b*) Estimation of the potency of the drug from a dose–response plot of the antagonism to the hypotensive effect of histamine injected intravenously in the cat or dog.

(*c*) Estimation of the potency of the drug from a dose–response plot of the antagonism to bronchospasm in guinea-pigs—animals are exposed singly

Table 4.5. The ED_5q (μmoles kg) of 2-halogenoalkylamines and mepyramine, using histamine aerosols in guinea-pigs and the pressor effect of 5-hydroxytryptamine in spinal rats[9,37,38] The compounds were given intraperitoneally to guinea-pigs and intravenously to rats.

Compound	ED_{50}	
	Histamine	*5-Hydroxytryptamine*
VI–1, dibenamine hydrochloride (*IV*)	>33	23·0
VI–2, phenoxybenzamine hydrochloride	0·1	1·4
VI–3	0·16	1·3
VI–4	12·5	3·5
VI–5	0·9	0·1
VIII, mepyramine	0·1	>10

to a spray of 0·5 per cent solution of histamine (base) in a closed box and the time taken for them to collapse from asphyxia is measured.

Table 4.5 shows the relative potencies of some 2-halogenoalkylamines. The parent compound of the series, dibenamine (*VI*–1) exerts a slight antagonism to histamine (*VII*) but the naphth-1-ylmethyl analogue (*VI*–3) is much more powerful[37,81]. Phenoxybenzamine (*VI*–2) and its congeners are also active[82], as are the bisphenylyloxy derivatives[81]. Dibenamine has no effect on the rate and extent of spreading of dyestuff when histamine is injected into the skin, whereas this property of histamine is prevented by antihistamines[83]. However, not all the demonstrable actions of histamine are antagonized by the specific antihistamine compounds[84] such as mepyramine (*VIII*). For example, the flow of gastric juice induced by injections of histamine, and the inhibition of the movements of the uterus of the rat by histamine, are not reduced. The local anaesthetic activity of many antihistamines is high whereas only the alcoholic degradation products of some 2-halogenoalkylamines exert this action[85].

The mode of action of the antihistamine effect of the 2-halogenoalkylamines, their structure–action relationships and the comparison of antihistamine and anti-adrenaline effects are discussed later, and it is possible that the same chemical species is responsible for both these activities in any one compound. The antihistamine action is not due to interference with the diamine oxidase (histaminase) which catalyses deaminative oxidation of the side chain of histamine, nor is it due to any modification of metabolic degradation or excretion of histamine.

The rate of onset of antagonism of the hypotensive action of histamine and of the hypertensive effect of adrenaline in anaesthetized mammals by the 2-halogenoalkylamines is similar, but the degree to which the effects may be antagonized is different. Generally, the effect of adrenaline is easily reversed, that of noradrenaline abolished with adequate dosage, whereas that of histamine is only reduced. Some derivatives, such as *N*-ethyl-*N*-naphth-1-ylmethyl-2-bromoethylamine (*VI*–3) however, have a strong antihistamine action and abolish the effect of histamine on the blood-pressure of a cat; others, such as *N*-ethyl-*N*-*o*-chlorobenzylethyleneiminium picrylsulphonate (*Figure 4.1*) may be used to differentiate between the antagonism to histamine and that to adrenaline–noradrenaline. The antagonism to histamine is short-lived and in isolated tissues is partially reversible by repeated washing.

Much less investigation of the relation between 2-halogenoalkylamines and 5-hydroxytryptamine (*IX*) has been atttempted. The most suitable tests are those on the contractile response of smooth muscle of the rat uterus or guinea-pig ileum or on the pressor response of the spinal atropinized rat under hexamethonium treatment. Dibenamine[86] exerts a long and complete block of the rat uterus, but if the time is short it may be ineffective[87–89]. The 2-halogenoalkylamines studied by Graham[90] antagonized the pressor effect of 5-hydroxytryptamine in rats, but they were more active against noradrenaline[91]. Some, such as *N*-ethyl-*N*-fluoren-9-yl-2-bromoethylamine (*VI*–4) are about 100 times more effective against noradrenaline than against 5-hydroxytryptamine; others, such as *N*-ethyl-*N*-naphth-1-ylmethyl-2-bromoethylamine (*VI*–3) are similar in effect against the two amines.

The effects of 5-hydroxytryptamine on the blood-pressure, however, are complex. Besides being a cardiac stimulant, this amine arouses cardio-inhibitory reflexes; it also contracts smooth muscle but opposing nervous influences are stimulated. The 2-halogenoalkylamines have no effect on the initial fall of blood-pressure which forms a part of the response to 5-hydroxytryptamine but readily antagonize the pressor component.

The antagonism exerted by dibenamine[88] on the constriction of the bronchi and vessels of the guinea-pig lung after injections of 5-hydroxytryptamine is weak, but other 2-halogenoalkylamines are more active. Some antihistamines antagonize the antidiuretic and oxytocic actions of 5-hydroxytryptamine[87], but dibenamine fails to antagonize the antidiuretic action of this amine[92] although it abolishes the oxytocic effect[87]. Given acutely, dibenamine may cause oliguria[93]; given repeatedly in lesser but still blocking doses, it does not do so[224].

Much work has been carried out on the relationship between 5-hydroxytryptamine and brain function, and the action of reserpine in dispersing this amine from tissue stores is well known. However, the 2-halogenoalkylamines which have central sedative action are not synergistic with reserpine although they are with barbiturates[9].

High concentrations of dibenamine antagonize the effect of acetylcholine on isolated strips of rabbit aorta[35]. However, concentrations of other 2-halogenoalkylamines such as *N*-naphth-1-ylmethyl- or *N*-fluoren-9-yl-*N*-ethyl-2-bromoethylamine, or phenoxybenzamine (*VI*–2) which antagonize the actions of adrenaline, noradrenaline, 5-hydroxytryptamine or histamine do not affect contractions of smooth muscle produced by acetylcholine, barium ions, pituitrin or potassium ions. The dimethyl-halogeno-phenylethylamines may even show powerful acetylcholine-like actions and no atropine-like activity[85].

Antagonism of Other Sympathomimetic Amines

The action of dibenamine in abolishing or reversing the pressor response to injected sympathomimetic amines is not confined to catecholamines[94]. In anaesthetized cats, for example, phenoxybenzamine reverses the pressor response to adrenaline, noradrenaline, 3,4-dihydroxynorephedrine, 4-(2-methylaminoethyl)catechol hydrochloride, adrenalone and catechol. Of the

(X) Amphetamine

(XI) Ephedrine

non-catechol pressor amines such as amphetamine (*X*), hydroxyamphetamine, ephedrine (*XI*), tyramine, phenylephrine and pholedrine, the pressor action is either abolished or reversed. This type of amine may exert its pressor action by displacing catecholamines from tissues into the blood-stream[95]. The reversal by phenoxybenzamine of the pressor response to such

an amine is therefore a further demonstration of the reversal of the pressor response to adrenaline and noradrenaline.

Other Actions of 2-Halogenoalkylamines

Toxicity

The 2-halogenoalkylamine compounds when dissolved in water release acid as a result of ionization and decomposition, and these solutions applied to the conjunctival sac exert an irritant action without producing local anaesthesia. Many of them are poorly absorbed when taken by mouth and the local irritant effect produces nausea or vomiting and diarrhoea. Damage to the viscera makes the drugs highly toxic when given intraperitoneally, although this may be limited by buffering the solution given. The 2-halogenoalkylamines, however, are less toxic than the comparable nitrogen mustards.

The oral activity of the 2-halogenoalkylamines is feeble and there is a wide variation in toxicity by other routes. Dibenamine and phenoxybenzamine are convulsants, whereas the *N*-ethyl-*N*-naphth-1-ylmethyl compounds produce a general depression in mice, as do the *N*-ethyl-*N*-fluoren-9-yl members of the series. Small animals remain still, quiet, with evident dragging of their hind legs and slow respiration. Superimposed on this picture of depression, there is a phase of jactitation which may be severe enough to cause convulsions. This stimulant effect is not related to potency as an adrenaline antagonist as it is seen with dibenamine, which is a feeble antagonist, and with *N,N*-dimethyl-2-bromo-2-phenylethylamine (*VI*–5) which is a potent agent. The naphthylmethyl compound (*VI*–3) may produce a characteristic pose with the mice raised on stiffened hind legs, the tail being held up from the ground and the forelegs performing running movements. However, the rate of onset of convulsions may be related to solubility; with the dimethyl derivative (*VI*–5), the onset is speedy, whereas with dibenamine it is delayed. Delayed deaths after 24 to 48 hours occur when some of the compounds are given by the intraperitoneal route. The fluoroethylamines[80], *e.g.* *N*-ethyl-*N*-naphth-1-ylmethyl-2-fluoroethylamine, are inactive as antagonists of adrenaline, unreactive chemically and do not produce an acid solution. They are less toxic by the intraperitoneal route than their bromo- or chloro-analogues but are convulsants and kill by asphyxiation.

Given subcutaneously, the 2-halogenoalkylamine compounds irritate the tissues, causing local inflammation, exudation, haemorrhage, round cell infiltration or necrosis in the skin. On inhalation the dry powders are sternutatory. By daily injection into cats and dogs, some depression, salivation or other disturbances have been noted but no loss of weight or apparent harmful effect on the formed elements of the blood[27]. Unlike bishaloalkylamines, these compounds do not depress bone marrow function; unlike ethyleneimines, they are not toxic to the renal tubules.

Local anaesthesia

Many of the active 2-halogenoalkylamines possess local anaesthetic action. Buffered solutions injected intradermally in guinea-pigs produce anaesthesia of a potency equal to or greater than that of procaine[9] (*e.g.* *N,N*-bis-2-phenoxyethyl-2-bromoethylamine). The discovery that the alcoholic

hydrolysis product of *N,N*-dimethyl-2-halogeno-2-phenylethylamine is some 20 to 30 times stronger than the parent compound in this respect[35,85] suggests that the activity previously noted in other series of analogues may have been due to the formation of the alcohols. Potency as a local anaesthetic thus depends on the rate and degree of cyclization and hydrolysis in aqueous solution.

Enzymes

Most interest in this field has been displayed in the relationship of the 2-halogenoalkylamines to amine oxidase, the *O*-methylating enzyme for catecholamines, histaminase and cholinesterase. Most of the compounds investigated[37] inhibit the action of amine oxidase, although the *N,N*-dimethyl-2-halogeno-2-phenylethylamines do not possess this action but inhibit histaminase[85]. The fluorenyl compound (*VI*–4) inhibits cholinesterase[9], while phenoxybenzamine has no effect on *O*-methyl transferase (see p. 165). These activities, however, do not bear any relation to the pharmacodynamic actions of the compounds.

Specificity

The specificity of an effective 2-halogenoalkylamine is high but not absolute, the dissociation constant for the alpha catecholamine receptor being lower than that of other receptors. It has been too generally assumed that the action of these drugs is specific, yet their antihistamine activity may be great[96], and their antagonism to 5-hydroxytryptamine considerable.

CHEMISTRY OF 2-HALOGENOALKYLAMINES

Synthesis of Amines and Intermediates

The general method is to prepare the *N,N*-disubstituted 2-amino-alcohol first and then replace the hydroxyl group with halogen, thus reversing the degradation process which occurs when the compounds are in aqueous solution. The early work on syntheses of these amines has been reviewed by Ullyot and Kerwin[19].

The amino-alcohols may be obtained by a variety of processes, according to the structure involved. These include: (*a*) Dialkylation of a primary 2-amino-alcohol by heating equimolar amounts of ethanolamine and aralkyl halide[20,97,98]. (*b*) Alkylation of a secondary 2-amino-alcohol which may itself be prepared from a primary amino-alcohol by reductive or other alkylation. Reaction of an alkylene oxide with a primary amine has also been successful[99]. Most of the organic halides used for alkylation, *e.g.* α-naphthylmethyl chloride or 9-bromofluorene are reactive enough for the condensation to proceed in refluxing ethanol, toluene or benzene. (*c*) Alkylation of a secondary amine with an aliphatic oxide. By heating with ethylene oxide the disubstituted ethanolamine may be obtained[100,101]. This method is preferred as an alternative route when alkylation by the previous methods fails or is accompanied by troublesome side-reactions. (*d*) Reduction with lithium aluminium hydride affords a convenient method for preparing 2-amino-alcohols from α-amino-esters; 2-*N,N*-dibenzylamino-1-propanol is prepared in this way by reduction from *N,N*-dibenzylalanine.

The 2-halogenoalkylamines may then be prepared from the amino-alcohols by a variety of processes: (*a*) the chloro-compounds are obtained by treatment of the alcohol as a free base or a salt, in chloroform or benzene with thionyl chloride; (*b*) the bromo-compounds are prepared similarly using thionyl bromide, or, alternatively, by heating the alcohol with hydrobromic acid[102]; (*c*) the iodo-compounds are prepared from the chloroethylamine by exchanging halogen with iodide in acetone; (*d*) these halogeno-amines are also made by reacting the amino-alcohol with phosphorus pentachloride, phosphorus tribromide or phosphorus tri-iodide in dry chloroform[21,49], and (*e*) the fluoro-compounds may be prepared by refluxing 1-bromo-2-fluoroethane and *N*-ethylnaphth-1-ylmethylamine in ethanol[21]:

$$R^1R^2NH + BrCH_2{\cdot}CH_2F \longrightarrow R^1R^2N{\cdot}CH_2{\cdot}CH_2F + HBr$$

The method using phosphorus halide has led to the isolation of the ethyleneiminium ions of some moderately reactive 2-halogenoalkylamines as picrylsulphonates[49]. This was the first successful isolation of the chemical species which is considered to be the pharmacologically active intermediate[48].

Chemical Structure and Pharmacological Activity

2-Halogenoalkylamines (*VI*; X=Cl, Br, I, F) contain a tertiary nitrogen atom which is usually separated from the halogen atom by two carbon atoms. The literature on structure and anti-adrenaline activity available up to 1952 has been reviewed by Ullyot and Kerwin[19]. The compounds studied since then have been specially selected for the investigation of structure–action theory[9,37,103,104].

Variations in the halogen

It has been found that with few exceptions[104] the order of anti-noradrenaline or anti-adrenaline potency for 2-halogenoalkylamines in which the only chemical variation is in the halogen is I≒Br>Cl>F[9,37,105,106].

Table 4.6. The effect of the halogen on potency[9,37,38]
The ED_{50} (μmoles/kg) of some 2-halogenoalkylamines using histamine aerosols (H) in guinea-pigs and the pressor effects of noradrenaline (Nor) and 5-hydroxyhyptamine (5-HT) in spinal rats are recorded. The LD_{50} (μmoles/kg in mice) is also shown. The compounds were given intraperitoneally to mice and guinea-pigs and intravenously to rats.

Compound	R^1	R^2	R^3	X	ED_{50} *Nor*	ED_{50} *H*	ED_{50} *5-HT*	LD_{50}
VI–7	Et	Naphth-1-ylmethyl	H	Cl	0·53	0·42		0·40
VI–8	Et	Naphth-1-ylmethyl	H	Br	0·48	0·20		0·08
VI–9	Et	Naphth-1-ylmethyl	H	I	0·95	0·17		0·07
VI–10	Et	Naphth-1-ylmethyl	H	F	—	—		1·21
VI–11	Me	Naphth-1-ylmethyl	H	Cl	70·0	0·66		0·50
VI–12	Me	Naphth-1-ylmethyl	H	Br	1·0	0·23		0·10
VI–13	Me	Naphth-1-ylmethyl	H	I	0·7	0·21		0·09
VI–14	Me	Naphth-1-ylmethyl	H	F	—	—		0·83
VI–15	Et	*p*-Chlorobenzyl	H	Cl	39·0	7·0	97·0	1·26
VI–16	Et	*p*-Chlorobenzyl	H	Br	5·0	1·0	10·0	0·17
VI–17	Et	*p*-Chlorobenzyl	H	I	4·0	0·3	6·0	0·18
VI–18	Me	Me	Ph	Cl	0·05	2·0	0·25	—
VI–5	Me	Me	Ph	Br	0·04	0·9	0·35	1·16
VI–19	Me	Me	Ph	I	0·03	2·0	0·05	—

This point is illustrated in *Table 4.6.* Fluoro-compounds are inactive as antagonists of adrenaline, noradrenaline or histamine[80] and they are toxic. The variation in activity with halogen results from the relative lability and reactivity of these atoms since specific biological activity in this series is related to chemical reactivity. If in (*VI*) X=sulphonic acid ester, activity is retained[19].

Variations in the N-*substituents*

Increasing the length of the carbon chain between the nitrogen and the halogen atoms decreases or abolishes activity[38,106]. *A chain of two carbon atoms is essential for activity in halogenoalkylamines.*

As may be seen from *Table 4.7*, it is essential that at least one of the *N*-substituents of a 2-chloroethylamine be a benzyl or a substituted benzyl group. With R^1 as benzyl, activity equal to that of dibenamine is shown by compounds in which R^2 is ethyl or ethoxyethyl, and if R^2 is 2-methoxybenzyl (*VI*–25) or 3,4-dimethoxybenzyl (*VI*–27) the compounds are more active than dibenamine. Introduction of one nuclear 3-trifluoromethyl group into the dibenamine molecule results in a marked loss of activity (*VI*–26). In the most active monobenzyl compound, phenoxybenzamine (*VI*–2), which has 20 times the potency of dibenamine[106], R^2 is 2-phenoxy-1-methylethyl. A halogen in the 3-position results in a more active compound than one in

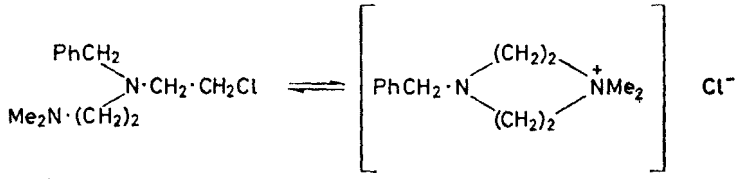
Table 4.7. Relative anti-noradrenaline activity of a series of simple *N*-alkyl and aralkyl-2-choroethylamines on intravenous injection[9,19,102,105,106] (*VI*; R^3=H, X=Cl)

Compound	R^1	R^2	*Relative activity* (dibenamine=1)
VI–20	Me	Me	Inactive
VI–21	Et	Et	Inactive
VI–22	Et	C_6H_{13}	Inactive
VI–2	$PhCH_2$	$PhO{\cdot}CH_2{\cdot}CHMe$	20·0
VI–23	$PhCH_2$	Et	1·0
VI–24	$PhCH_2$	$EtO{\cdot}CH_2{\cdot}CH_2$	1·0
VI–25	$PhCH_2$	$2\text{-}MeO{\cdot}C_6H_4{\cdot}CH_2$	4·0
VI–26	$PhCH_2$	$3\text{-}CF_3{\cdot}C_6H_4{\cdot}CH_2$	0·5
VI–27	$PhCH_2$	$3,4\text{-}(MeO)_2C_6H_3{\cdot}CH_2$	2·0
VI–28	$2\text{-}MeC_6H_4{\cdot}CH_2$	$2\text{-}MeC_6H_4{\cdot}CH_2$	1·0
VI–29	$3\text{-}MeC_6H_4{\cdot}CH_2$	$3\text{-}MeC_6H_4{\cdot}CH_2$	4·0
VI–30	$4\text{-}EtC_6H_4{\cdot}CH_2$	$4\text{-}EtC_6H_4{\cdot}CH_2$	Inactive
VI–31	$2\text{-}ClC_6H_4{\cdot}CH_2$	Et	15·0
VI–32	$3\text{-}ClC_6H_4{\cdot}CH_2$	Et	2·0
VI–33	$4\text{-}ClC_6H_4{\cdot}CH_2$	Et	0·25
VI–34	$4\text{-}Pr^iC_6H_4{\cdot}CH_2$	$4\text{-}Pr^iC_6H_4{\cdot}CH_2$	Inactive
VI–35	$2\text{-}MeO{\cdot}C_6H_4{\cdot}CH_2$	Et	1·0

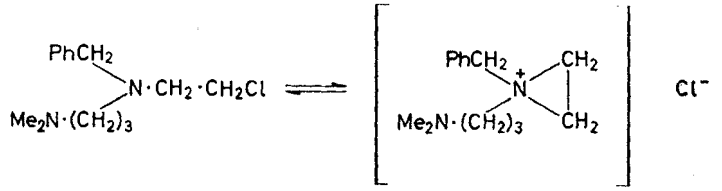
the 2- or the 4-position (*VI*–31, 32, 33). Introduction of one 4-methyl group into dibenamine has no effect but compounds with longer chains are inactive. A suggested explanation[47] is that these radicals lie off the plane of the ring and upset the fit of the molecule to the alpha-receptor.

When R^2 is a substituted aminoethyl group all activity is lost; if it is an

aminopropyl group activity is retained[19,106]. This observation is of interest because the first of these compounds reacts to form an ammonium compound, thus:



while the second one yields an ethyleneiminium ion thus:



A 2- or 4-methyl group in the rings of both benzyl groups has little effect, but a 3-methyl increases activity (*VI*–29); the corresponding di-3-trifluoromethyl compound is much less active and a larger 4-alkyl group gives an inactive compound. It has been suggested[106] that the ethyl and isopropyl groups do not lie in the plane of the ring and that this adversely affects the precision of fit to the receptor surface.

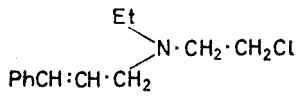
Replacing one of the benzyl groups of dibenamine with a phenethyl has little effect on potency but activity is doubled if the benzyl group is replaced by a 1-methyl-2-phenylethyl and increased fivefold by the introduction of a 3′,4′-dihydroxy-(or dimethoxy-)phenyl-1-methylethyl group. Replacing both benzyl groups by phenethyl causes loss of activity. However, when R^1 is benzyl and R^2 is cinnamyl, $PhCH{:}CH{\cdot}CH_2$, the resulting compound is slightly more active than dibenamine (*Table 4.8*).

Table 4.8. Relative anti-noradrenaline activity of a series of *N*-aralkyl-2-chloroethylamine derivatives on intravenous injection[19,106] (*VI*; R^3=H, X=Cl)

Compound	R^1	R^2	*Relative activity* (dibenamine=1)
VI–36	$PhCH_2$	$Ph(CH_2)_2$	1
VI–37	Et	$Ph(CH_2)_2$	<1
VI–38	$Ph(CH_2)_2$	$Ph(CH_2)_2$	<1
VI–39	$PhCH_2$	$PhCH_2{\cdot}CHMe$	2
VI–40	$PhCH_2$	$3,4\text{-}(OH)_2C_6H_3{\cdot}CH_2{\cdot}CHMe$	5
VI–41	$PhCH_2$	$PhCH{:}CH{\cdot}CH_2$	>1
VI–42	Et	$Ph(CH_2)_3$	Inactive

Removal of the phenyl group from the nitrogen atom by more than two carbons results in inactivity, *e.g.* *N*-ethyl-*N*-hydrocinnamyl-2-chloroethylamine. One exception to this rule has been known for some years. It is

N-cinnamyl-*N*-ethyl-2-chloroethylamine (*XII*) which has a potency similar to that of dibenamine. Phenoxyethylamines display a short, easily reversible antagonism of the pressor effects of adrenaline[107]. It was logical therefore to



(XII)

examine the effect of substituting R^1 in the 2-halogenoalkylamine compounds with a phenoxyethyl group and to extend the investigation to include substituents on the phenoxy ring[97,108] (*Table 4.9*). With R^2 as a phenoxyethyl

Table 4.9. The relative anti-noradrenaline activity of a series of *N*-argloxyalkyl-2-chloroethylamine derivatives on intravenous injection[19,97,106,109,110] (*VI*; R^3=H, X=Cl)

Compound	R^1	R^2	*Relative activity* (dibenamine=1)
VI–43	$PhO\cdot(CH_2)_2$	$PhO\cdot(CH_2)_2$	1
VI–44	$PhCH_2$	$PhO\cdot(CH_2)_2$	3
VI–45	$PhCH_2$	$3\text{-}EtC_6H_4\cdot O\cdot(CH_2)_2$	10
VI–46	$PhCH_2$	$2\text{-}CH_2{:}CH\cdot CH_2\cdot C_6H_4\cdot O\cdot(CH_2)_2$	< 1
VI–47	$PhCH_2$	$3,4\text{-}Me_2C_6H_3\cdot O\cdot(CH_2)_2$	< 1
VI–48	Et	$PhO\cdot(CH_2)_2$	< 1
VI–49	Et	$2\text{-}EtC_6H_4\cdot O\cdot(CH_2)_2$	1
VI–50	Et	$2\text{-}PhCH_2\cdot C_6H_4\cdot O\cdot(CH_2)_2$	5
VI–51	$CH_2{:}CH\cdot CH_2$	$2\text{-}PhCH_2\cdot C_6H_4\cdot O\cdot(CH_2)_2$	15
VI–52	Et	$PhO\cdot CH_2\cdot CHMe$	1
VI–53	$CH_2{:}CH\cdot CH_2$	$PhO\cdot CH_2\cdot CHMe$	2
VI–2	$PhCH_2$	$PhO\cdot CH_2\cdot CHMe$	20
VI–54	$PhO\cdot(CH_2)_2$	$PhO\cdot CH_2\cdot CHMe$	20
VI–55	$2\text{-}MeC_6H_4\cdot CH_2$	$PhO\cdot CH_2\cdot CHMe$	4
VI–56	$PhCH_2$	$2\text{-}EtC_6H_4\cdot O\cdot CH_2\cdot CHMe$	10

group, activity is considerably increased when R^1 is changed from ethyl to benzyl (*VI*–48 and 44, and *VI*–52 and 2). If the phenoxy-ring is substituted in the 2-position with a benzyl group, activity is greatly increased, and if at the same time R^1 is allyl activity is even higher (*VI*–51). When R^1 is benzyl, introduction of an ethyl group in the 2-position of the phenoxy-ring of R^2 enhances activity whereas the same group in the 4-position abolishes it[108]. If R^1 is ethyl and R^2 is phenoxyethyl, activity is weak (*VI*–48) but if the phenoxy-ring carries a 2-methyl, -ethyl, -isopropyl, -methoxyl, -ethoxyl, -propoxyl, -benzyl, or -phenyl activity is increased[97,108].

The bisphenoxyethyl compound (*VI*–43) is weak and in general, symmetrical bis-aryloxyalkyl compounds have little activity. Activity is further reduced if benzyl groups are carried in the 2-position of the phenoxy-rings. This contrasts with the observation that the activity of the *N*-ethyl-*N*-phenoxyethyl compound is lower than that of the *N*-ethyl-*N*-2-benzyl-phenoxy-derivative (*VI*–48 and 50). *N*-Phenoxyethyl-*N*-(1-methyl-2-phenoxyethyl)-2-chloroethylamine (*VI*–54) is 20 times stronger than dibenamine. *N*-Alky-*N*-(2-chloroethyl)benzhydrylamines have weak activity[38,111], as has *N*-(2-chloro-2-phenyl)-ethylmethylbenzylamine[112].

Monosubstituted 1-methyl-2-phenoxyethyl derivatives are most active

when the other substituent is benzyl (phenoxybenzamine[106]) and this activity is retained if the phenoxy-ring is substituted with a lower alkyl group in the 2-position.

Isosteric replacement of the oxygen atom of phenoxyalkyl compounds with a sulphur atom decreases activity[37] (*Table 4.10*) and so does replacement of the phenoxy-group with naphth-1-yloxy- or lengthening of the phenyl–nitrogen distance.

Table 4.10. The relative anti-adrenaline activity of compounds which contain isosteric substitution with S; compounds given intravenously[9,37,104]
(*VI*; R^3=H, X=Cl)

Compound	R^1	R^2	*Relative activity* (dibenamine=1)
VI–57	$PhCH_2$	2-Thienyl	0·1
VI–7	Et	Naphth-1-ylmethyl	10·0
VI–58	Et	3-Thionaphthenylmethyl	1·0
VI–59	Et	Naphth-2-ylmethyl	Inactive
VI–60	$PhCH_2$	Naphth-1-ylmethyl	0·1
VI–61	Et	Benzhydryl	1·0
VI–62	$PhCH_2$	Fluoren-9-yl	<1·0
VI–63	Et	Fluoren-9-yl	15·0
VI–64	$PhCH_2$	$2\text{-}MeC_6H_4\cdot O\cdot CH_2\cdot CH_2$	2·0
VI–65	$PhCH_2$	$2\text{-}MeC_6H_4\cdot S\cdot CH_2\cdot CH_2$	1·0
VI–66	Et	$2\text{-}MeC_6H_4\cdot O\cdot CH_2\cdot CH_2$	1·0
VI–67	Et	$2\text{-}MeC_6H_4\cdot S\cdot CH_2\cdot CH_2$	0·1

Aralkyl groups other than benzyl have been substituted on the nitrogen atom of 2-chloroethylamines (*Table 4.10*). Thus, when R^2 is benzhydryl[111], inden-1-yl, fluoren-9-yl, naphth-1-ylmethyl, acenaphthen-1-yl, or phenanthren-9-yl, activity, compared with that of dibenamine, is enhanced when R^1 is ethyl but usually depressed if R^1 is benzyl[9,19,37,46,81]. If R^2 is naphth-2-ylmethyl and R^1 is ethyl the compound is relatively inactive[37].

Table 4.11. The relative anti-adrenaline activity of compounds with polycyclic aralkyl substituents on intravenous injection[9,19,37,46,81]
(*VI*; R^3=H, X=Cl)

Compound	R^1	R^2	*Relative activity* (dibenamine=1)
VI–68	$PhCH_2$	Ph_2CH	<1·0
VI–69	Et	Ph_2CH	5·0
VI–23	Et	$PhCH_2$	1·0
VI–70	Et	Indan-1-yl	2·0
VI–63	Et	Fluoren-9-yl	15·0
VI–62	$PhCH_2$	Fluoren-9-yl	<1·0
VI–60	$PhCH_2$	Naphth-1-ylmethyl	0·1
VI–7	Et	Naphth-1-ylmethyl	10·0
VI–59	Et	Naphth-2-ylmethyl	Inactive
VI–71	Et	Acenaphthen-1-yl	2·0
VI–72	Et	Phenanthren-9-yl	2·0

Increased weighting of one substituent on the nitrogen atom from naphthyl to acenaphthenyl, phenanthenyl or fluorenyl (*Table 4.11*) increases activity, but only if the other group on the nitrogen is a simple alkyl, preferably ethyl, and not benzyl (*VI*–60 and 7)[37]. The naphthylmethyl group confers activity on the molecule only when the point of attachment is the C–1 (*VI*–7 and 59). Activity is diminished if the *N*-ethyl substituent is

replaced by either a methyl or a benzyl group but the extinguishing effect of this is less than was suggested by earlier workers[96,106] who examined only chloroethylamine derivatives. The bromo- and iodo-analogues are active[37]. Groups of a similar nature, acenaphthenyl and phenanthrenyl are not as effective as naphthyl.

Comparing the 3-thionaphthenylmethyl derivatives (*e.g. VI*–78, see *Table 4.12*) with the chlorobenzyl compounds[9] the effect of varying the halogen is abnormal. In the latter series as with most 2-halogenoalkylamines I≒Br>Cl, whereas in the former Cl>I>Br, and in *o*-tolyloxyethyl compounds Br>Cl>I. These compounds are not very active.

Table 4.12. The relative anti-noradrenaline activity of compounds with heterocyclic moieties on intravenous injection[9,19,106]
(*VI*; R^3=H, X=Cl)

Compound	R^1	R^2	*Relative activity* (dibenamine=1)
VI–73	$PhCH_2$	2-Furfuryl	<1
VI–74	2-Thenyl	2-Thenyl	<1
VI–75	$PhCH_2$·CHMe	2-Thenyl	10
VI–76	$PhCH_2$	2-Thenyl	2
VI–77	$PhCH_2$	1-Methyl-2-(2-pyridyloxy)	2
VI–78	Et	3-Thionaphthenylmethyl	1

The majority of compounds in which R^2 and/or R^1 is a heterocyclic group have the same order of activity as dibenamine (*Table 4.12*). But if R^2 is 2-thenyl and R^1 is a 1-methyl-2-phenoxyethyl group, the compound is about 10 times as active as dibenamine, whereas bis-*N*-2-thenyl-2-chloroethylamine is less active. The following are some heterocyclic *N*-substituents which have been incorporated in the 2-halogenoalkylamine molecule: 2-furfuryl, 2-thienyl, 3-benzthienylmethyl, 2-pyridylethyl, 1-methyl-2-(2-pyridyloxy)ethyl, 2-(1-benztriazolyl)ethyl, 3-thionaphthen-1-ylmethyl.

Table 4.13. The relative anti-noradrenaline activity of a series of compounds with substituents on the ethylamine chain[106]

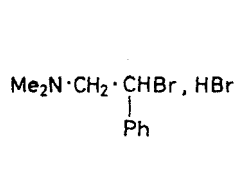
$(PhCH_2)_2N\cdot\underset{\alpha}{CH}\cdot\underset{\beta}{CHCl}$ *(XIII)*

Derivative number	α	β	*Relative activity* (dibenamine=1)
1	H	Me	1
2	Me	H	1
3	Me	Me	1
4	Et	H	<1

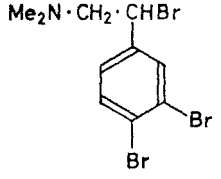
Variations in the α and β substituents in the ethylamine chain

There is no change in the potency of dibenamine as a result of methyl substitution on the α or the β carbon, or of α,β-dimethyl substitution[106] (see *Table 4.13*). In the phenoxybenzamine series, activity is lowered by the introduction of a β-methyl group and destroyed by an α-methyl group. If the

substituent on the β-carbon is a phenyl group, the derivative is *N,N*-dimethyl-2-halogeno-2-phenylethylamine (DMEA)[38,85]. Two members of this series are shown in formulae (*XIV*) and (*XV*) and others in *Table 4.14*. There are three structural requirements for anti-noradrenaline activity if there is a substituent on the β-carbon atom, *viz.*: (*a*) the substituent should have an aromatic ring structure; (*b*) the halogen should be on the β-carbon, and (*c*) the amino group should be secondary or tertiary. Optimal activity occurs if the β-substituent is a substituted phenyl or a naphthyl ring and the basic centre is the dimethylamino-group. If the approximation to the structure of



(*XIV*)



(*XV*)

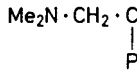
adrenaline gets close (*XV*) activity becomes as much as 10,000 times that of dibenamine, but the binding with the receptor is loose and the action is no longer non-competitive. Such a compound gives some 88 per cent of the theoretically maximum possible ethyleneiminium ion (E^+) within 1 minute of dissolving in water. Steric considerations imply that activity in this analogue may be accompanied by opening of the iminium ring to form an ester or a carbonium ion. The mode of action of these compounds may, therefore, differ from that of dibenamine, the E^+ blocking the receptor but further rapid degradation preventing alkylation or other cause of prolonged activity[113,223].

Table 4.14. The anti-noradrenaline activity of compounds in the DMEA series on intravenous injection[5,38]

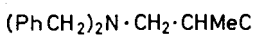
Compound	R^1	R^2	R^3	X	*Relative activity* (dibenamine = 1)
VI–18	Me	Me	Ph	Cl	100·0
VI–5	Me	Me	Ph	Br	200·0
VI–19	Me	Me	Ph	I	1,000·0
VI–79	Et	Et	Ph	Cl	7·0
VI–80	Pri	Pri	Ph	Br	0·2
VI–81	$PhCH_2$	$PhCH_2$	Ph	Cl	10·0
VI–82	Me	Me	3,4-$Br_2C_6H_3$	Br	10,000·0
VI–83	Me	Me	2-$C_{10}H_7$	Br	200·0

If the β-component is an unsubstituted phenyl group, the compound possesses some acetylcholine-like activity and a degree of relaxant activity on skeletal muscle. This is of the decamethonium rather than the curariform-type[38] and is dependent on the integrity of the dimethylamino-group. Combining the nitrogen atom, R^1 and R^2 in a heterocyclic ring such as morpholino does not significantly alter anti-adrenaline activity when compared with a weighted alkyl chain but if R^1 and R^2 are branched chains such as isopropyl, the compound is inactive[38]. The substituents on the nitrogen

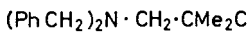
atom may be dialkyl, dibenzyl, a combination of alkyl or alkenyl and benzyl. These structural relations also hold if R^2 is a hydrogen atom but activity is reduced tenfold. If the hydrogen atom on the β-carbon of (*XIV*) is replaced with an alkyl residue (*XVI*) activity is lost. This contrasts with the retention of activity in β-methylated dibenamine (*XVII*) and in β,β-dimethyldibenamine (*XVIII*)[106].



(*XVI*)



(*XVII*)

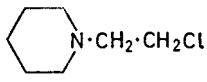


(*XVIII*)

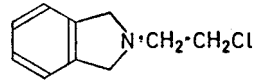
Some structures devoid of activity or possessing greatly reduced activity

Many compounds not mentioned in the above discussion are without activity; some of these come under the following headings:

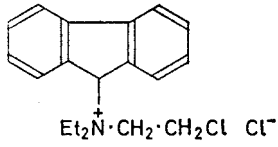
(*a*) When the nitrogen atom of the halogenoethylamine is part of a ring such as piperidine or isoindoline, the compounds are inactive (*XIX*) and (*XX*)[106].



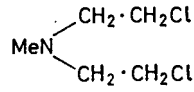
(*XIX*)



(*XX*)



(*XXI*)



(*XXII*) Mustine

(*b*) If one substituent on the nitrogen atom is an allyl group, the other being a benzyl group[106], activity resembles that of dibenamine, but if both substituents are allyl groups the compound is inactive[19].

(*c*) If one or both substituents on the nitrogen atom is an aryl group activity is very weak or absent[19], *e.g.* *N*-ethyl-*N*-phenyl-2-chloroethylamine hydrochloride[106].

(*d*) Early reports[106] that quaternary salts of active compounds were active have been found to be erroneous[19]. Compound (*XXI*) is inactive in a dosage of 20 mg/kg[19].

(*e*) Compounds of the nitrogen mustard type (*e.g.* mustine, *XXII*) are also generally inactive but some *N*-aryl mustards do lower the toxicity of adrenaline to mice[19]. An early report[106] of activity in *N*,*N*-bis(2-chloroethyl)benzylamine ($PhCH_2{\cdot}N(CH_2{\cdot}CH_2Cl)_2$, is the exception.

Oral activity

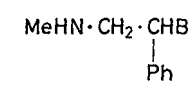
Compounds with the greatest activity when given intravenously (*e.g.* a 2-bromoalkylamine) are not as active orally as compounds with a greater stability (*e.g.* a 2-chloro-analogue)[50]. Of a small number of compounds examined, while those with R^1=phenoxyethyl were found to be orally active, the 1-methyl-2-phenoxyethyl analogue had increased activity. In dibenamine, substitution by a β-methyl group in the ethylamine chain increases oral activity, whereas in phenoxybenzamine it decreases it. More recent work on pairs of β-bromo- and β-chloroethylamines confirms that, orally, the bromo-compounds are almost inactive whereas the chloro analogues are active[114]. This divergence may be explained by the difference in the rapidity of the cyclization of the halogenoalkylamines[37] (see p. 157).

Structural Requirements for Optimal Activity

Nickerson[27], reviewing what was known in 1949 of these relationships, laid down a number of criteria for anti-adrenaline activity. These have been substantially confirmed by later experience.

(*1*) *The compound must have a 2-halogenoalkyl group* capable of forming an active intermediate. Such a compound may be active if X=chlorine, bromine, iodine or an alkyl- or arylsulphonate but would be inactive if X=fluorine, or if X=an alkyl, hydroxyl, carboxyl, ether, ester or nitrite group. In the dibenamine type of structure, any β-substituent which does not interfere with the formation of intermediates, gives an active compound whereas a substituent which prevents intermediate formation destroys activity (*XVII*, *XVIII*, *XIX*).

(*2*) *A tertiary amino group is essential*—A primary or secondary amine or a quaternary nitrogen is inactive. This statement has not been challenged for the dibenamine series but it does not apply to the *N*,*N*-dimethyl-2-halogeno-2-phenylethylamine (DMEA) series. The activity of the compound *N*,*N*-dimethyl-2-bromo-2-phenylethylamine (*XIV*) is 10 times greater than that of the compound *N*-methyl-2-bromo-2-phenylethylamine (*XXIII*). This relationship holds if the substituent on the nitrogen atom is ethyl, propyl or butyl.



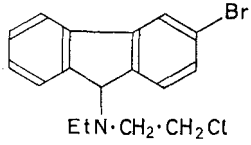
(XXIII)

(*3*) *It must include an unsaturated ring structure*, attached to the nitrogen atom in such a way that hyperconjugation stabilizes the intermediate formed. No compound in which both R^1 and R^2 are saturated is active. Ullyot and Kerwin[19] give details of the compound (*XXIV*) which is more active than dibenamine and in which a resonating hyperconjugated system is improbable. It does not apply to (*XVI*) and its congeners, and this rule must now be held to be of limited application only.

(*4*) *The unsaturated ring must satisfy steric requirements*[106]—Substitutions out of the plane of the benzyl ring inactivate the molecule (*e.g.* ethyl, isopropyl,

tertiary butyl) while substituents in the plane of the ring cause activity (*e.g.* methyl and methoxy). The ethyl group may be held out of the plane of the ring by steric hindrance with the adjacent hydrogen atom[106], whereas methoxy is held co-planar by a partial double bond character of the oxygen ring carbon bond. This rule has been subject to stringent examination[113] and is not fully established.

It has been stated[27] that: 'in the presence of one 2-haloalkyl and one suitable unsaturated grouping the nature of the third substituent on the nitrogen usually has only a minor influence on activity'. In the naphthylmethyl series[37] this is not true. If the third substituent is phenyl there is almost no

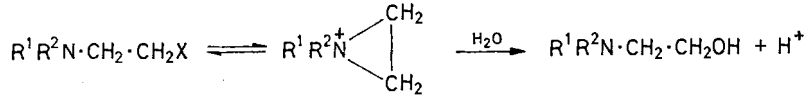


(XXIV)

activity. Where other structural factors favour activity, chlorine as halogen does not prevent activity but if other factors are unfavourable chlorine causes extinction of activity. Bromine and iodine enhance activity. Structures which favour the splitting off of the halogen also favour pharmacological activity. Attachment of the phenyl ring to the nitrogen atom prevents ionization because it prevents a movement of electrons from the nitrogen atom to the C–2.

PHARMACOLOGICAL ACTIVITY AND CHEMICAL REACTIVITY IN 2-HALOGENOALKYLAMINES

So far as is known, the 2-halogenoalkylamines are unique in that biological activity is dependent on chemical reactivity, the product of chemical and physical properties. The blocking action of 2-halogenoalkylamines on the alpha-receptor for catecholamines is not exerted by the parent compound but by the ethyleneiminium ion (E^+) which is formed from its reaction in aqueous solution:



In an early publication on the subject[24], the specific anti-adrenaline activity of the compounds was attributed to the action of the ethyleneiminium ion, by analogy with the similar reactions which had been closely investigated for the structurally related nitrogen mustard compounds[115]. This contention has not been seriously challenged although it has been criticized[116].

The Iminium Ion

The final proof that activity resides in the ethyleneiminium ion was produced by Graham[26] when activity was demonstrated in the ethyleneiminium ions

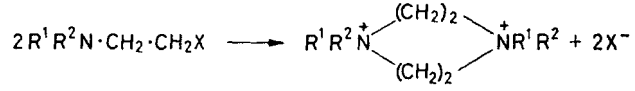
of three active 2-halogenoalkylamines which had been isolated as picrylsulphonates by Allen and Chapman[49]. Ethyleneimine itself has some activity[117] although it is such a toxic compound that the specificity of its action is doubtful. It also inhibits cholinesterase[118] (see p. 147).

The Ethanolamine Component

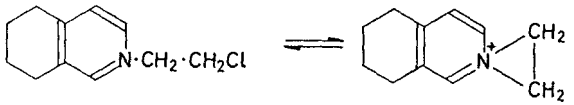
A neutralized solution of 2-halogenoalkylamine, given time, liberates all its acid, then its halogen, and loses its capacity to consume thiosulphate. In its final state it is inactive. A number of alcohols have also been tested directly[38,85,106] and found to be inactive.

The Piperazinium Form

The possibility that the piperazinium form (*XXV*) of the 2-halogenoalkylamine, which is undoubtedly formed to a variable degree when the parent body is dissolved in water, is the active species has been accepted by some workers. Nickerson and Gump[106], for example, tested several directly and found no activity. A less direct approach was made[9] by testing the activity of a solution of a compound which has been shown to form piperazinium ion in large amount[104].



(*XXV*)



(*XXVI*) (*XXVII*)

It has also been suggested that the piperazinium ion (*XXV*) or dimer is the active chemical species in the reaction mixture because it would give the same ultimate chemical analysis as the ion E^+. Simple piperazinium salts do not react with thiosulphate. Biologically active solutions do so; so also does the biologically active ethyleneiminium ion isolated as a picrylsulphonate[26], to an extent of 94 per cent of the theoretical maximum. The objection has been made[116] that the evidence of the relation between transformation of 2-halogenoalkylamines such as phenoxybenzamine and biological activity[119] is indirect. *N,N*-Dicyclohexyl-2-chloroethylamine is biologically inert but it produces chloride and hydrogen ions and consumes thiosulphate as do the biologically active compounds. 2-(2-Chloroethyl)-5,6,7,8-tetrahydroisoquinoline (*XXVI*) is only slightly active despite the fact that it forms a stable ion E^+ (*XXVII*) in buffered solutions. There are other examples of this kind[120]. The reason is in part the rigidity of the conformation of the spiro ions having the nitrogen in the ring so that they fail to fit the receptor surface, and inability to produce the ions in adequate amount. It is not impossible for an

intact 2-halogenoalkylamine to react with thiosulphate but there is no way of telling whether this is so or whether it is done via E^+ formation. The compounds containing fluorine consume no thiosulphate and are biologically inactive. A number of dimers (*XXV*) of active compounds have also been synthesized, tested directly and found to be inactive.

Vinylamine

It is chemically possible for hydrogen halide to be eliminated from the halogenoethyl group with formation of a vinylamine (*XXVIII*) but it is improbable that such intermediates are formed from active 2-halogenoethylamines when in buffered solution and there is some evidence to the contrary from known chemical kinetic studies. If the active 2-halogenoethylamine is dissolved in an aqueous medium the formation of acid can be followed

$$R^1R^2N\cdot CH_2\cdot CH_2X \longrightarrow R^1R^2N\cdot CH{:}CH_2 + HX$$
(XXVIII)

during the whole of the reaction. With a compound which liberates the whole of its halogen immediately and gives a corresponding amount of ethyleneiminium ion there is very little initial acidity. In the presence of the hydrolysis product (the ethanolamine) in the reaction mixture, the E^+ decays more quickly so that it may be that the following reaction takes place:

$$R^1R^2\overset{+}{N}\!\!<\!\!\begin{matrix}CH_2\\|\\CH_2\end{matrix} + R^1R^2N\cdot CH_2\cdot CH_2OH \longrightarrow R^1R^2N\cdot(CH_2)_2\cdot\overset{+}{N}R^1R^2\cdot CH_2\cdot CH_2OH$$

Whether this is of any significance in the body is not known. The basis of these suggestions[21,48] is illustrated in *Figure 4.3*. Such a vinylamine (*XXVIII*)

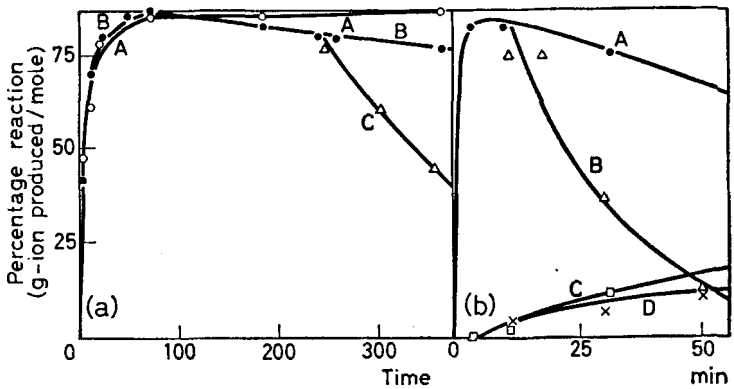


Figure 4.3. Decomposition of *N*-2-bromoethyl-*N*-methylnaphth-1-ylmethylamine in aqueous acetone[48]
(*a*) A, bromide ion formed. B, ethyleneiminium ion produced at 0_2C. decomposition of ethyleneiminium at 30_2C.
(*b*) Effect of *N*-2-hydroxyethyl-*N*-methylnaphth-1-ylmethylamine (curve B). A, normal decomposition. D and C, formation of hydrogen ion with and without respectively the added 2-hydroxyethyl compound.

may not react with sodium thiosulphate and this reaction is characteristic of the 2-halogenoalkylamines when in solution.

General Comments

The sulphur and nitrogen mustard derivatives to which the 2-halogenoalkylamines are chemically related form intermediate compounds which may react with constituents of biological systems in aqueous solution at physiological pH. The essential reaction is cyclization with formation of E^+ and halogen ions. The ethyleneiminium ion is very reactive, and it reverts to the parent compound in the presence of the halogen hydracid, while buffering may convert it to the dimer or the half-hydrolysis product. The latter may become E^+ again by cyclization of the remaining halogenoethylamine group. Ethyleneiminium ions react speedily and stoichiometrically with thiol (—SH) groups and use is made of this reaction to determine the amount of E^+ in a reaction mixture by measuring the consumption of added thiosulphate. If 0·0002 mole of the compound is added to 2·5 ml. of distilled water, 6 ml. acetone added, the solution brought rapidly to 30°C and 1 ml. of 0·2N sodium hydroxide added, the volume may then be made up to 10 ml. and the reaction mixture maintained at this temperature. At any desired interval of time aliquots may be taken, diluted, extracted with ether to remove unreacted parent compound and assayed biologically or titrated for consumption of sodium thiosulphate, pH estimated or free halogen measured. In this way curves relating the formation and loss of thiol-consuming power to time, and release of hydrogen and halide ions may be constructed and compared with curves derived by calculation on the basis of these presumed reactions, and with potency.

The evidence that the ethyleneiminium ion is the chemical species responsible for anti-adrenaline activity is fivefold:

(*1*) The production of ethyleneiminium ion from active 2-halogenoalkylamines[21,121] and the isolation and chemical characterization of one as an *insoluble picrylsulphonate*. This substance has not been tested biologically.

(*2*) The demonstration that fluorine-containing analogues of active naphthylmethyl-2-halogenoethylamines which are inactive, do not consume thiosulphate when in solution, do not produce acid, and are considered on chemical grounds to be unable to cyclize.

(*3*) The demonstration of a quantitative relation between the amount of thiosulphate-consuming species (E^+) in a neutralized solution of an active compound, the alteration of this property with time, and the anti-adrenaline activity[26,122]. This is shown in *Figure 4.4*. It will be noted that the relationship between production and decay of E^+ and the antagonism to adrenaline and noradrenaline is closer than that to antagonism of histamine, but that biological activity parallels the rise and fall of E^+ titre. Over a wide range of structures (three *N*-chlorobenzyl-2-halogenoethylamines, two *N*-fluoren-9-yl-2-halogenoethylamines and three *N*-naphth-1-ylmethyl-2-halogenoethylamines), there is a direct proportionality between the antagonism to adrenaline and the concentration of thiosulphate-consuming species in the mixture, and a limited relation to antihistamine and anti-5-hydroxytryptamine activity.

(*4*) The isolation and chemical characterization of the ethyleneiminium ions (*XXIX*) of several active 2-halogenoalkylamines[49] as *soluble picrylsulphonates* and the demonstration of their activity[9]. These were the *o*-, *m*- and *p*-*N*-chlorobenzyl-*N*-ethylethyleneiminium picrylsulphonates. The iminium ions of these compounds antagonized the pressor response to adrenaline,

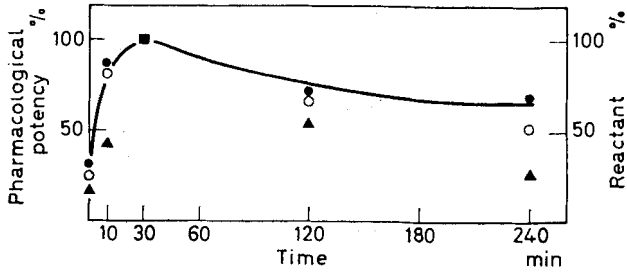
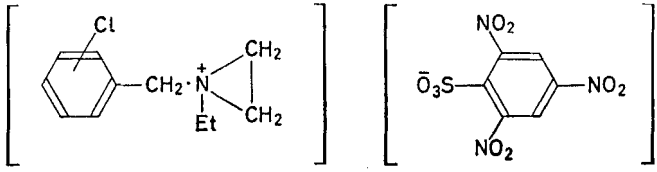


Figure 4.4. The relationship between chemical reactivity and pharmacological activity in *N*-ethyl-*N*-*p*-chlorobenzyl-2-chloroethylamine hydrochloride (*VI*–15)[26]
The line represents the variation with time of the production and decay of ethyleneiminium ion expressed as a percentage of the maximum found at the point of peak activity ■, (right-hand ordinate). The pharmacological potencies are expressed as percentage of the peak activities, measured as ED_{50} on the blood-pressure of dogs anaesthetized with pentobarbitone sodium, 30 mg/kg (left-hand ordinate)· ●, antagonism to (−)-adrenaline, 4 μg/kg. ○, antagonism to equimolar amounts of (−)-noradrenaline. ▲, antagonism to equimolar histamine.

noradrenaline and 5-hydroxytryptamine and the actions of histamine. The activity of one of these ions (*XXIX*) on the blood-pressure of a dog is shown in *Figure 4.1.*



(XXIX)

(*5*) It was reported in the original paper[24] and it has been confirmed since[37], that the anti-adrenaline action of these compounds is diminished or prevented if the animal under test is previously injected with sodium thiosulphate. The presumption is that the iminium ion, formed in the bloodstream, is in part inactivated by reaction with it and the predetermined potency of the compound is reduced. This has been found to be substantially correct for dibenamine, phenoxybenzamine, naphth-1-ylmethyl-, fluoren-9-yl and *N*,*N*-dimethyl-2-halogeno-2-phenylethylamine compounds.

It is not suggested that all structures of the ethyleneiminium type are active antagonists of adrenaline. *All active 2-halogenoalkylamines are alkylating agents but not all alkylating agents are anti-adrenaline compounds.* The injection of

the ethyleneiminium ion as a picrylsulphonate into the blood-stream proved more potent than the injection of the calculated quantity of the parent compound, from which it would be *derived* by cyclization. It follows that not all of the parent compound is necessarily successfully cyclized to the iminium ion *in vivo* but some may go to inactive dimer. The solubility and reactivity of the 2-halogenoalkylamine in the body may affect the result as well as the absolute potency of the ion. The superior activity of bromo- and iodo-compounds to chloroalkylamines was confirmed with these ions but the relative potency of bromo- and iodo-compounds may vary with species examined or tests made. The bromo- and iodoalkylamines release large amounts of ethyleneiminium ion at once, the chloro-compounds do so more slowly and to a lesser extent. The potency relation found for the picrylsulphonate to the ethyleneiminium ion E^+ in relation to the chlorobenzyl substituent was *ortho*>*meta*>*para* for the pure ions and *meta*>*ortho*>*para* for the parent compounds. The *o*-chloro-compound is more active than the *m*-chloro-compound in terms of potency once the drug has reached its biochemical site of action. The *o*-isomer is inferior to the *m*-compound in delivering the ion E^+ to the site (drug affinity). For drugs such as 2-halogenoalkylamines which form a biologically active and chemically unstable intermediate species, affinity is the result of a dual process—stability and access.

STABILITY OF 2-HALOGENOALKYLAMINES

In general, 2-halogenoalkylamine compounds are poorly soluble in water, although they are highly reactive once dissolved. They dissolve easily in acetone–water mixtures or in acid–alcohol and acidified propylene glycol in which they form a stable solution. Many of the *N*,*N*-dimethyl-2-halogeno-2-phenylethylamines are easily soluble in water. The addition of alkali increases the reactivity and makes all 2-halogenoalkylamines unstable. When they are administered orally absorption is poor and irregular. If given parenterally, the intravenous route should be used as the products of decomposition are acid. They have to be injected slowly, well diluted, in an attempt to avoid disturbance of the central nervous system.

MODE OF ACTION OF 2-HALOGENOALKYLAMINES

Two of the characteristics of dibenamine-like drugs are their prolonged duration of action and the completeness with which the selected responses are antagonized. Many studies have been made to determine the nature of the interaction between 2-halogenoalkylamines and the tissues, and to find out wherein these drugs differ from the better-known atropine, antihistamines or adrenaline antagonists such as piperoxan, in which there is a dynamic equilibrium between the agonist and the antagonist compounds in relation to occupany of receptors.

It was early demonstrated[24] that the activity of dibenamine is reduced or prevented if a high concentration of thiosulphate is maintained in the circulating blood of the animal under test. If this concentration is allowed to fall, the specific block then develops. It follows that, in the case of dibenamine at least, the drug is available for many hours in the body in active form. This

may result from the slow rate of cyclization of dibenamine, which is known to occur *in vitro* and so provide a prolonged supply of E^+. It has been suggested that dibenamine is stored in fat[123] and is slowly released to exert its effect[124].

Phenoxybenzamine injected into the artery supplying one leg of a dog produces adrenergic blockade in that leg but not in the rest of the body. Using cross-circulation experiments in cats and dogs, it has been shown that the block persists in the tissues of an animal long after the time when there is an effective concentration of the drug in the blood[122,125]. If tissue blockade is produced in a donor animal by dibenamine or its naphyth-1-ylmethyl analogue (*VI*–3) and then the animal's circulation is connected to that of another (with suitable precautions to prevent coagulation and ensure mixing of the blood-streams), the blood from the donor produces block in the recipient animal. About one-quarter of a large dose of dibenamine may be recovered unchanged from the tissues of dogs some 5 minutes after intravenous infusion, and another 16 per cent as dibenzylamine[123]; after 2 hours, about one-fifth of the total given may be recovered from the body fat. Moreover, there is a relationship between the dose of 2-halogenoalkylamine administered to an animal and the duration of an effective block, *e.g.* 1 mg/kg of the naphth-1-ylmethyl analogue of dibenamine lasts for 6 to 8 hours, 10 mg/kg for 48 hours, whereas with dibenamine 5 mg/kg lasts for 36 hours, 20 mg/kg for about 96 hours. It is unlikely that the continuous release of parent compound from such a depot as body fat or of active E^+ from the parent compound is an important factor in maintaining the block, but rather that it is prolonged inactivation of the cell receptor, as treatment with thiosulphate for up to 12 hours results in no reduction of blocking activity of the compound[122].

An attempt has been made to relate the persistence of action of these compounds to their presence in the body, by administering to mice phenoxybenzamine labelled at the methylene of the benzyl group with a ^{14}C atom[126] and following the distribution of radioactive emission[127]. Accumulation of large amounts, however, was not demonstrable in fat depots, radioactivity being found in the soft tissues, particularly the liver and kidneys, even after 24 hours. About half of the total activity was excreted in urine.

It has always been a notable feature of these compounds that the action, once established, is not removed from an isolated tissue by washing. This is in marked contrast to the activity of 'competitive' drug antagonists such as atropine and antihistamines. It has recently been shown by addition of ^{14}C-labelled *N*-ethyl-*N*-naphth-1-ylmethyl-2-bromoethylamine (*VI*–3) to plasma and subsequent electrophoresis[128] that this compound travels with the albumin. Further, the presence of thiosulphate in the fluid bathing a tissue exposed to this radioactive compound prevented its blocking action and reduced the attachment of radioactivity to the tissue. Radioactivity was present in other specimens not exposed to thiosulphate for as long as the blocking action persisted.

The blocking action may be reduced if a high concentration of adrenaline is present in the circulating blood or in the fluid bathing an isolated tissue at the time when the 2-halogenoalkylamine is injected. This suggests that there

is initially competition between the compound and the adrenaline for attachment to the receptor site. Another way of demonstrating that there is an initial competitive phase in the action of these compounds is by increasing the concentration of adrenaline injected or applied to the tissue subsequent to the drug. For a time which varies with the structure of the 2-halogenoalkylamine, there exists a situation wherein it is possible for the effect of a small dose of adrenaline to be abolished, while a large dose is still active. This relationship between drug and antagonist implies that not all the receptors on the tissue are occupied by the blocking agent and that there exists an equilibrium between the competing drugs. The law of mass action applies to this relationship. In due course, however, this relationship is lost, and adrenaline no longer elicits a response. There is then no equilibrium or dose–response relationship. An isolated tissue in a bath does not recover from this state as a result of repeated washing but an organ in a living animal may do so. The duration of the 'disequilibrium block' in an intact animal is related to the initial dosage of the compound. During the late stages of the recovery phase the condition is once more competitive.

The attachment of these compounds to the receptors for histamine (*VII*) is not so strong (see *Figure 4.4*). Structurally it is often found in a series that the most potent antagonist of adrenaline is not the most powerful antihistamine[9], and the nature of this antagonism has been the subject of intensive study[129]. These compounds antagonize the same actions of histamine as do the antihistamines. They do it quickly and effectively *in vivo* but in an isolated tissue the effect of mepyramine is reversible on washing, that of *N*-ethyl-*N*-naphth-1-ylmethyl-2-bromoethylamine (*VI*–3), for example, increasing for a time and being only in part reversible by washing. The same receptor is involved with the three substances, histamine, compound *VI*–3, and mepyramine, since pretreatment with one interferes with the action of the others.

Little attention has been paid until recently to an early report[80] that the pressor response to adrenaline in the initial stages of treatment of an animal with 2-halogenoalkylamine may be enhanced rather than antagonized. This effect suggests that a sensitization of the responding tissue to adrenaline occurs and this is of sufficient degree to obscure any blocking action of the antagonist. However, many 2-halogenoalkylamines themselves produce a rise in blood-pressure when injected into suitable preparations[130]. This effect is tachyphylactic in untreated animals, but absent in animals pretreated with reserpine. It is restored when such animals are given injections of adrenaline or noradrenaline. The 2-halogenoalkylamines may therefore displace or release catecholamine from tissue stores. This has been shown to be so[131,227] by direct measurement of the level of noradrenaline in the plasma of dogs before and after the administration of phenoxybenzamine. A rise occurs even after adrenalectomy and this results in an increased urinary excretion[132–134]. This displacement renders a tissue more sensitive to catecholamine applied to it, if the receptors are not occupied by the displacing drug[135]. If the adrenergic nerve to the spleen is stimulated, the transmitter substance noradrenaline may be detected in the venous effluent from the organ, and when phenoxybenzamine is administered to the animal the amount released is much increased[136,137]. It is likely that the initial action of 2-halogenoalkylamines

is to displace the amines from their store and to loosen their attachment to the cell receptors[138].

The nature of the biochemical process involved in the blocking action of the 2-halogenoalkylamines is still obscure, but it has been shown[24] that dibenamine does not modify the catabolism of adrenaline. The 2-halogenoalkylamines[139] have no effect on the activity of *O*-methyl transferase; and their mode of action differs from that of guanethidine, which releases catecholamine from its store at or near the nerve-ending and apparently prevents restoration. Passage of the adrenergic nerve impulse is not prevented, neither is transmission through autonomic ganglia[140]. It has always been a feature of these drugs that they antagonize the effects of injected catecholamine more easily than those of stimulating adrenergic nerves, and they may act by preventing the penetration of exogenous amine to an intracellular receptor.

The structural relationship of the 2-halogenoalkylamines to the nitrogen mustards has led to the idea that the two series of compounds act by alkylation of similar chemical groupings in cells. The mustards have been studied in greater detail[141,142] than the 2-halogenoalkylamines[120,143]. Phenoxybenzamine reacts with the —SH groups of thiosulphate, of dimercaprol (BAL) and of cysteine, but so do the nitrogen mustards and biologically inactive 2-halogenoalkylamines. All these compounds react freely with carboxyl groups to form esters, and with the amino groups of proteins and peptides. The biological potency of vasopressin and of insulin (two polypeptides) are reduced by exposure to dibenamine and the oxygen dissociation curve of haemoglobin is also modified. Mustards react readily with inorganic phosphate *in vitro* but phenoxybenzamine only does so to a slight extent. Belleau[113,144–147] has examined the nature of the structure of the E^+ best fitted for occupation of the alpha-receptor for catecholamines and has discussed in detail the structure–action relationship in a large number of 2-halogenoalkylamines of varying potency. He considers that active compounds of this class have a common phenylethylamine structural pattern (as has been suggested before[147]) and that the binding site or receptor for the active anionic group may be a carboxylate or a phosphate ion. Phosphate linkages with 2-halogenoalkylamines are the more stable of the two but can be dealkylated slowly. The availability of various ions, however, may differ in the body from that *in vitro*, *e.g.* denatured protein may have available —SH groups, while natural protein may not[148].

The potency of *N*-ethyl-*N*-fluoren-9-yl-2-iodoethylamine has been estimated after pretreating rats with effective amounts of various agents expected to interfere with specific biochemical mechanisms[149]. Pyrogallol, for example, which inhibits *O*-methyl transferase, increases the pressor effect of adrenaline but decreases the potency of the blocking agent. Bretylium tosylate which empties the store of catecholamine at the end of adrenergic nerves has no effect whereas cadmium chloride which chelates phosphate groups significantly reduces activity. This observation, although based on a single experiment, supports the suggestion that phosphate groups are involved.

A study has also been made[9] of the ion transfer across isolated frog skin[150]. Adrenaline stimulates the transfer of sodium and chloride ions, thereby

creating a change in trans-membrane potential, and the active 2-halogenoalkylamines prevent this action.

INTERACTION WITH AMINES

It has been known for many years that the presence of one pressor amine may interfere with the action of another. Tryptamine, for example, antagonizes the contraction by adrenaline of the rabbit uterus[151], whereas 5-hydroxytryptamine increases the pressor effect of adrenaline[152]. 2-Halogenoalkylamines also sensitize tissues to adrenaline for a brief period[153], suggesting that initially they are only loosely attached to the cell. Cocaine, ephedrine, piperoxan and amphetamine[154] restore the blood-pressure response to adrenaline when it has been abolished by dibenamine, and so also do ephedrine[155] and methoxamine[156], isoprenaline, ethylnoradrenaline, pilocarpine and tolazoline[157,158]. When the ganglia are blocked with hexamethonium, the block is reduced[159]. As mentioned earlier, the pressor response of injected adrenaline becomes tachyphylactic after small doses of dibenamine[160,161]. The depressor action of isoprenaline may be abolished by treatment with phenylephrine, whereas dibenamine restores it[162]. Adrenaline is said to stimulate the constrictor receptors of the vessels of a dog's hind leg, after phenoxybenzamine has been given in doses sufficient to reverse the constrictor response to injected adrenaline[163]; the constrictor component is merely masked by the concurrent intense stimulation of dilator receptors. According to another suggestion[164], dibenamine antagonizes the inhibition of smooth muscle which occurs in isolated guinea-pig ileum when this is stimulated electrically. These observations may be due to sensitization effects or to non-specific actions.

Isoprenaline is one of the amines most effective in 'unblocking' tissues from dibenamine. It is also a powerful and specific stimulant of beta-receptors for catecholamines (see p. 134). The dichloro-analogue of isoprenaline (DCI, *V*) antagonizes the actions of isoprenaline[165], and is a weak and long-lasting cardiac stimulant[166] which reduces the stimulant action of other catecholamines[167–169]. It is a weak glycogenolytic agent[14] and prolongs the pressor effect of adrenaline and noradrenaline[170]. Whereas the inhibitory action of phenylephrine on gut is abolished by phenoxybenzamine, that of isoprenaline is reduced by its dichloro-analogue[171]. DCI may therefore be a competitor for the catecholamine receptors, particularly the beta-receptors. It also reverses the action of phenoxybenzamine when this has abolished the pressor response to injected adrenaline in the rat[172]. The interaction of DCI with phenoxybenzamine is probably a competition for the receptor in the initial phase of access of the 2-halogenoalkylamine to the tissue.

THERAPEUTIC USES OF 2-HALOGENOALKYLAMINES

Dibenamine has proved to be of little use in medicine, and it is doubtful if it is active by mouth. However, success has been attained with phenoxybenzamine which exerts an effective antagonism of catecholamines in patients[173,174]. If given by intra-arterial injection, this compound increases blood-flow (as measured by occlusion plethysmography)[175] and reduces or

abolishes the vasoconstrictor action of noradrenaline and the effects of reflex stimulation of the sympathetic nerve-supply to the hand[176]. As a result, it produces a slow fall in diastolic pressure in normal subjects, accompanied by postural hypotension and reflex tachycardia[177]. The constrictor response to the cold pressor test and the rise in blood-pressure which occurs in the early stages of asphyxia are inhibited. These actions are probably due to antagonism to adrenergic vasoconstrictor function although patients tend to compensate so that tachyphylaxis is evident. A direct comparison in man has been made between phenoxybenzamine and other anti-adrenaline drugs such as phentolamine, piperoxan, benzazepine and tolazoline[178]. Phenoxybenzamine is the most effective[179], as it is in animals[180].

Side-effects

The clinical application of these potent drugs has been delayed and hindered because of the unpleasant side-effects which they tend to produce. Relaxation of peripheral arterioles causes orthostatic hypotension if the patient changes his posture too suddenly, and congestion of the nasal mucosa may cause annoying stuffiness. The adrenergic component of sweating may cease[181,182] while the loss of heat on exposure to cold may increase greatly, due to failure of the skin vessels to constrict[183].

Taken by mouth they are gastric irritants[28] and may cause vomiting, colic or diarrhoea. This gastric irritation may cause some reflex cardiac irritability although dibenamine is the only member of the series which has been proved to have an effect on the electrocardiogram in patients[29] and only then after large doses.

The effect of large doses of 2-halogenoalkylamines on the central nervous system is unpleasant. Dibenamine is a stimulant unless injected slowly[184] and usually causes dizziness, confusion and nausea. There are a number of reports which suggest that phenoxybenzamine may have a sedative effect in some type or phase of psychosis but they are vague and uncritical. In performing such trials, it is advisable to use the inactive ethanolamine derived from the parent compound as a control[185]. There is some experimental evidence[9] that the potent *N*-fluoren-9-yl-2-halogenoalkylamines exert a sedative action.

Peripheral vascular disorders

Oral administration of phenoxybenzamine produces a prolonged increase in blood-flow in patients suffering from disorders due to spasm of the arterioles[186–189]. A daily dose of 60 mg by mouth, for example, abolished whitening of the fingers on exposure to cold[189] and reduced the incidence of spontaneous attacks of 'dead fingers'[190]. As a preliminary test to evaluate the possibilities for sympathectomy, these drugs may be of value, or they may be useful in the relief of post-operative spasm of the vessels occurring after sympathectomy[191].

The improvement which may be expected if there is obliterative disease of the arteries is difficult to assess and it is always worth while to try these or similar drugs. If there is a modicum of spasm in the condition, subsequent to infarct, if the collateral circulation available is not functioning at full stretch, or if the occlusion is proximal to the affected area, good results may be obtained. Beurger's disease, other forms of obliterative endarteritis, the

syndrome of claudication, severe chilblains, frostbite, delayed healing of ulcers are suitable conditions for trial. Phenoxybenzamine by mouth in doses of 50 to 100 mg/day may cause dramatic improvement in acute ischaemia consequent upon lodgement of an embolus[190], in frostbite[192], and in immersion foot[193]. A higher dose may be needed to bring about healing of chronic ulcers on the lower leg. These trials have only been conducted on a small number of patients and the techniques employed have been uncritical. There are few indications in the available reports of planning, of the use of placebos, of the inactive product derived from the parent halogenoalkylamine by hydrolysis, of double blind technique or any refinement of evaluation.

Phaeochromocytoma

The diagnosis of this condition depends on the demonstration of an abnormally high output of urinary catecholamines in a patient who suffers from hypertensive attacks. A valuable adjunct to diagnosis is the response of a patient during such an attack to an anti-adrenaline drug. The preferred drug is benzodioxan since it is short-acting and fairly potent at antagonizing circulating adrenaline, but it has little potency against noradrenaline and none in blocking adrenergic nerves. A sharp fall in blood-pressure following its administration is, therefore, indicative of a high level of circulating adrenaline and a failure to elicit this response indicates that the hypertension is of other origin, *e.g.* neurogenic or renal[194]. Phenoxybenzamine antagonizes the effect of circulating adrenaline but it may be too potent to discriminate between the effects of circulating catecholamines and of over-activity of adrenergic nerves[195,196]. Once a diagnosis has been made, the pre-operative period is better managed with phenoxybenzamine than with any other drug.

Hypertensive disease

The neurogenic element in hypertension and the benefits of lowering the blood-pressure in hypertensive disease have been clarified by the use of ganglion-blocking drugs. The advantages of confining the blockade to the sympathetic side are many, and the efficacy of drugs such as guanethidine is presently being explored. Not enough has been made of the 2-halogenoalkylamines, which are effective in this condition[22]. In hypertensive disease, the effects of giving dibenamine have been reported as good, with relief of headaches, restoration of sight, and fall in blood-pressure[197,198]. Improvement is hastened if the patient is nursed as much as possible in a chair or a tilted bed in a posture which aids drainage of blood into the lower limbs. The chief drawback is the irregularity of absorption when taken by mouth and this makes it difficult to control the dose–effect relationship. The same argument applies to phenoxybenzamine[199–201] which may be ineffective by mouth but toxic by injection. For this reason its use in hypertension has been rejected by most physicians[202], but with the introduction of new and more potent congeners it may be time to reconsider the matter[228].

The rôle of the sympathetic nervous system in renal hypertension appears to be minimal. These drugs, therefore, have only a temporary effect in hypertension induced in rats or dogs by renal ischaemia[203,204] and are not very effective in human beings with renal hypertension[205,206]. In these

patients, water and sodium excretion are not altered by treatment with phenoxybenzamine, although plasma volume increases. The fall in blood-pressure produced by these drugs does not usually reduce the efficiency of the kidney which readily adjusts to the lowered filtration pressure, but they can do so in rats[224].

Toxaemia of pregnancy

The rôle of circulating amines in the hypertensive phase of pre-eclampsia is not clear but experience has shown that the ganglion-blocking drugs may be valuable in the management of these patients. It has been shown that in selected cases phenoxybenzamine given by mouth[207,208] also controls the hypertensive condition. The difficulty of ensuring regular absorption by the oral route and the awkwardness of giving the drug by infusion have limited the use of the drug for this purpose. It is worthy of note that an antagonism between the effects of ergometrine and dibenamine has been reported[209].

Peripheral failure of the circulation

The usual treatment for shock is to increase the available circulating volume by infusion of a plasma expander or whole blood. Another procedure is to inject a sympathomimetic drug such as methylamphetamine or to infuse noradrenaline in dextrose–water. These drugs stimulate the heart, which is already subject to reflex tachycardia, and further increase peripheral vascular spasm. The blood-pressure in the main arteries rises and the renal and central nervous functions improve as a result, but peripheral areas may suffer severely from ischaemia. It has been suggested that these agents do more harm than good and that low blood-pressure by itself does no harm if vasoconstriction is abolished by the use of a 2-halogenoalkylamine[210]. In animals experimentally affected by trauma and haemorrhage, the onset of irreversible shock is prevented[211] and stasis is removed[212] by treatment with 2-halogenoalkylamines. Renal function is only slightly depressed after haemorrhage when these drugs have been given[213] and survival time is increased[214]. Similar benefits follow the use of dibenamine to prevent the onset of the 'crush syndrome' in dogs[215]. The mode of action of 2-halogeno-alkylamines in preserving life under these circumstances has generally been attributed to prevention of vascular spasm due to adrenergic activity, but there may be an effect on the liver cells, protecting them from hypoxia[216] by an interaction with oxygen transporting systems rather than a circulatory action. Another suggestion is that phenoxybenzamine or its products (which are not blocking agents) modify the sensitivity of tissues to vaso-active materials in blood other than catecholamines. However, dibenamine may have an adverse effect on formation of oedema after burns[217].

Cardiac irregularity

The action of dibenamine in suppressing extra-systoles is well known and adequately documented[218]. This effect is in part due to the lowering of blood-pressure, and if the cardiac irregularity has been triggered by injection of catecholamines is, due to prevention of the pressor response to the amine. But it is also in part demonstrable as a direct depression of isolated heart muscle or as lowering of cortical EEG voltages. The increase in pulse-rate produced by catecholamines is not prevented, nor the increase in force, but

ectopic beating is depressed and irregularity prevented. More use may be made of this property during cardiac and intrathoracic surgery.

Pulmonary hypertension and pulmonary oedema

There is a brisk vasoconstriction in the pulmonary vascular bed when adrenaline is injected into the artery but it is generally believed that the sympathetic nervous system plays little part in regulating pulmonary arterial pressure. The hypertension due to adrenaline is easily prevented by phenoxybenzamine but it does not follow that 2-halogenoalkylamines are of much use in treating pulmonary hypertension in man[219].

Acute pulmonary oedema in rabbits resulting from gross pulmonary hypertension caused by injection of noradrenaline may be prevented by pretreatment with these drugs. This may not be due to suppression of the rise in pressure but may in part be due to a protective action on capillary membranes such as that noted with antihistamines such as mepyramine.

Glaucoma

There is a report of a marked fall in intra-ocular tension in glaucoma after giving dibenamine[220]. However, the effect may be due to miosis rather than antagonism of the vascular actions of noradrenaline.

Dermatology

Some skin diseases are improved by increased blood-flow after phenoxybenzamine. Acrocyanosis and chilblains[221] and excessive sweating are examples. The relief of hyperidrosis is probably due to the production of warm feet rather than a block of sweat glands. However, phenoxybenzamine may be applied as an ointment or in propylene glycol as a paint.

Psychoses

Early reports[184,222] of the value of dibenamine in the treatment of psychotic states are difficult to understand as this drug is a stimulant of the central nervous system.

Preparations and doses

Phenoxybenzamine may be given orally in the form of pills or by injection intravenously. The usual oral dose is 5 to 10 mg once in the evening. After a week, this may be doubled by adding a morning dose and a week later stepped up to three times per day after meals. The amount needed for any given patient is variable but usually 30 to 90 mg/day suffices. The dose may be reduced as soon as the drug begins to exert its action, or if digestion is upset. With less than 100 mg/day, the only side-effect may be a dizziness on first rising. Side-effects increase as the dosage increases although some patients have tolerated 500 mg in a day. Treatment is normally continued over months. If the effect fails, an interval of a week may be permitted every 2 to 3 months during which times other therapy may be given. In emergency, the intravenous injection of 10 to 30 mg is at once effective, but care has to be taken to give the injection slowly, preferably after a sedative. Dizziness, nausea, excitement and palpitations may disturb the patient. Intra-arterial injection of 1 to 5 mg cause an immediate, localized improvement in the

circulation and may be of value in cases of frostbite, gangrene of the limbs, or accidents such as the intra-arterial injection of solution of thiopentone. Reserpine may be used as an adjurant[228].

CONCLUSION

There appears to be a stabilizing action exerted by these compounds on the membrane of cells which react to catecholamines. This stabilization may follow the union of an ethyleneiminium ion of suitable type with a receptor of suitable shape (alpha). Subsequent alkylation, which may involve phosphate groups at some stage, damages the site so that recovery is delayed. There may be recovery of the receptor as a result of removal of the 2-halogenoalkylamine by hydrolysis and regeneration of receptors. The affinity of the ion for receptor sites for histamine and 5-hydroxytryptamine is less intense and the binding less permanent. More clinical work should be done with this group of drugs.

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5

ANAPHYLACTIC REACTIONS

G. E. DAVIES

INTRODUCTION

INJECTION of a small quantity of a foreign protein such as horse serum into a guinea-pig produces no observable effect, but dramatic changes follow a second similar injection three or four weeks later. The animal sneezes or coughs, scratches itself vigorously, has a laboured respiration, and finally convulses; it may die within a few minutes. Horse serum in this experiment has functioned as an *antigen*, its first dose stimulating the formation of substances which react with the second dose to produce *anaphylactic shock*. The importance of this laboratory phenomenon is its relationship to a wide group of human diseases which are known as *allergies* and which include asthma and hay fever.

The application of chemistry to the therapy of allergy has not been very fruitful in the past. A limited number of drugs has been used for the symptomatic treatment, the antihistamines in particular having a marked but limited success. However, a much fuller understanding of the basic chemical and biological processes involved in the anaphylactic and allergic reactions is needed before major advances in therapy may take place. Emphasis in this chapter has therefore been given to mechanisms of anaphylaxis since this is a field in which biochemistry is playing an ever-increasing rôle, providing a basis on which more effective synthetic anti-allergic drugs may be developed.

DEFINITIONS

Difficulties of terminology beset any discussion of immunological phenomena. Immunity is concerned with the defence of the organism against infection, partly through the production of substances (termed antibodies) which are harmful to the parasite. Anaphylactic and allergic reactions also depend upon antibodies but these reactions are in no way protective. They may, on the contrary, be very dangerous. Allergy in individuals of genetically-determined susceptibility may be regarded as an unwanted type of immunity.

Antigens and *antibodies* are only satisfactorily defined in terms of each other. *Antigens* are substances which stimulate the formation of antibodies and react specifically with such antibodies. *Antibodies* are globulins formed in response to, and able to react with, antigens. Both antigens and antibodies have extraordinary specificity: for example, antibodies formed in response to mouse serum do not react with rat serum, and a specific allergy confined to grass pollen does not react to other types of pollen.

Anaphylaxis is an artificially-induced state of heightened reactivity to antigenic substances, conditioned by the presence on tissues of globulin antibodies

specifically directed to these antigens and made manifest by the pharmacological effect of substances resident in various tissues and liberated therefrom by the effects of the antigen–antibody reaction. Anaphylaxis may be transferred passively with serum taken from an anaphylactic animal. Anaphylactic reactions characteristically appear very shortly after contact with antigen.

Allergy is a naturally-occurring state of increased reactivity to antigenic substances, and is generally dependent upon the presence of antibodies formed specifically against these substances.

The essential difference between these two states is in their method of production. Allergy is a naturally-occurring disease: anaphylaxis is an artificially-induced phenomenon. Both states depend for their manifestation upon the reaction of antigen with antibody.

Animals rendered anaphylactic by the possession of antibodies either engendered by the stimulation with antigen or received passively from another animal are said to be *sensitized.*

ANTIGEN–ANTIBODY COMBINATION

The essential step in the manifestation of an anaphylactic reaction is the combination of antigen with antibody. At least two special methods are available for the study of this combination, as follows:

Precipitation—Addition of a solution of antigen to a serum containing the specific antibodies may lead to the precipitation of a complex of antigen with antibody. This type of antibody is referred to as a *precipitating* antibody or *precipitin.* However, when increasing amounts of antigen are added to constant volumes of an antibody-containing serum, the amount of antigen–antibody complex precipitated increases to a maximum, and further increases in the amount of added antigen result in less precipitate as the complex is soluble in the presence of excess antigen. The tube containing the largest amount of precipitate is said to contain equivalent amounts of antigen and antibody. Examination of the supernatant fluid from this tube shows that it contains neither residual antigen nor residual antibody, the whole of both having been precipitated. Some antibodies fail to precipitate antigen and are termed *non-precipitating* or *univalent* antibodies[1]. A class of non-precipitating antibodies occurs in many types of human allergy. They have the property of being able to sensitize human skin and are known as *reagins.* They form the basis of the Prausnitz–Kustner reaction which is used as an aid to the diagnosis of some allergic diseases. In this test, a small amount of the patient's serum is injected at several sites into the skin of a normal person. The suspected allergen is then injected into one of the sites and also into an area adjacent to one of the sites. An inflammatory reaction developing rapidly at the site where the patient's serum was injected, but not at the control site, indicates that the appropriate antibody is present in the patient's serum.

Haemagglutination—Antibodies which are present in serum in insufficient amounts to produce visible precipitation of antigen may often be detected by their power to agglutinate red blood-cells which have been treated with tannic acid and then coated with antigen. Even more sensitive methods such as complement fixation or passive cutaneous anaphylaxis are also available. These methods have been adapted to determine the concentration of

antibodies, and the antibody content of serum is often expressed in terms of antibody nitrogen (AbN). This is the amount of protein nitrogen precipitated by an equivalent amount of antigen.

Although a wide range of substances may function as causal antigens for allergy or anaphylaxis, the subsequent reaction is not dependent on the nature of the antigen but is characteristic of the animal species or the method by which antigen is administered. Thus anaphylactic shock produced in the guinea-pig by egg albumin is similar to that produced by an azo-dye coupled to duck serum, and each acts as a specific antigen. This is an important consideration as it implies that an analysis of the sequence of reactions involved in anaphylaxis using one antigen is applicable to that using any other antigen.

DELAYED HYPERSENSITIVITY

Anaphylaxis is characterized by the contact of antigen with antibodies, the release of histamine and other substances, and the speed with which the shock becomes manifest. There is also another type of immuno-pathological reaction which characteristically becomes manifest after a longer time and hence is known as *delayed hypersensitivity*. This type is exemplified by the tuberculin reaction and is less understood than anaphylaxis. Delayed hypersensitivity may be transferred passively with leucocytes but not with serum[2].

TYPES OF ANAPHYLAXIS

The two essential stages of an anaphylactic reaction are *sensitization* which induces the formation of antibodies, and *challenge* or *provocation* in which these antibodies react with the antigen to produce the anaphylactic response. Anaphylactic reactions may be classified on the basis of the techniques used for these two stages. In *active anaphylaxis*, the antigen is given to an animal which actively produces antibodies so that anaphylactic shock results from

Table 5.1. Classification of anaphylactic reactions (Ag=antigen; Ab=antibody)

First injection	*State of sensitization*	*Second injection*	*Type of anaphylaxis*
Ag	Active	Ag	Active direct
Ab	Passive	Ag	Passive direct
Ag	–	Ab	Passive reversed

a second dose of antigen after an appropriate interval. Such an animal is said to be *actively sensitized*. In *passive anaphylaxis*, the serum from an actively sensitized animal is injected into a non-sensitized animal, and, if the antibody content of the donated serum is sufficiently high, anaphylactic shock results when an injection of antigen is given. The recipient animal is said to have been *passively sensitized*. Injection of antigen into an actively or passively sensitized animal results in *direct anaphylaxis*. Reversal of the order of injection so that the antibody is given after the injection of antigen results in *reversed anaphylaxis*. These various types of anaphylaxis are summarized in *Table 5.1*.

It will be appreciated that passive reversed anaphylaxis may also be induced by using the animal's own tissues as antigens. For example, if a rabbit is sensitized with guinea-pig serum, the anti-guinea-pig serum antibodies produce passive reversed anaphylaxis when injected into guinea-pigs. This type of anaphylaxis may also be produced by the injection of serum containing antibodies to the *Forssman antigen.* Forssman found that the injection of extracts of guinea-pig organs into rabbits produced antibodies which lysed sheep erythrocytes. This phenomenon at first sight appears to deny the specificity of antibodies but it has been shown to be due to the presence of similar antigenic substances in sheep cells and guinea-pig tissues. Such antigens are widely distributed in the plant and animal kingdoms, and among laboratory animals Forssman antigen is present in the sheep, guinea-pig, hamster, dog and cat, but not in the rabbit and rat[3].

Anaphylactic reactions may be further subdivided depending upon the method used for provocation. Administration of antigen may be by inhalation, by intravenous, intraperitoneal, subcutaneous or intramuscular injection, or by the oral route; *generalized* or *systemic* anaphylactic shock of varying

Table 5.2. Differences between the Arthus reaction and passive cutaneous anaphylaxis (P.C.A.)[4,6–10]

Characteristic	*Arthus reaction*	*P.C.A.*
Produced in rabbits	Yes	No
Minimal amount of Ab (μg nitrogen)		
guinea-pig	0·01	0·003
rat	0·01	0·5
Latent period	No	Yes
Time to maximum (minutes)	120–240	5–30
Precipitating Ab	Yes	No
Circulating polymorphs	Yes	No
Use of horse Ab	Yes	No
Chief effect observed	Necrosis	Increased vascular permeability
Antihistamine drugs	No effect	Partially inhibited

degrees of severity results. Small amounts of antigen, however, may be injected into restricted sites such as the skin[4] or joints[5]; *localized* anaphylaxis then results. For example, *cutaneous anaphylaxis* is produced by injecting the antigen into the skin of sensitized animals. Such localized reactions may be active or passive, direct or reversed. Anaphylactic reactions may also be shown *in vitro* using tissues taken from actively or passively sensitized animals, or tissues passively sensitized by incubating with antibody. Anaphylactic reactions depend upon antibodies being fixed to tissues with little or no circulating precipitating antibody. However, when such antibodies are circulating, the local injection of antigen results in a different type of reaction—the *Arthus reaction.* For example, when the antigen is injected into the skin of a highly sensitized guinea-pig (*i.e.* an animal with precipitins in its serum) the initial bleb at the injection site does not fade, as it does in cutaneous anaphylaxis, but rapidly becomes more evident; oedema increases and reaches its maximum in about 2 to 4 hours, during which time a haemorrhagic spot develops in the centre of the injection site, and this progresses to necrosis. The differences between the Arthus reaction and passive cutaneous

anaphylaxis are summarized in *Table 5.2* which is based on the review by Ovary[4].

ANAPHYLAXIS IN DIFFERENT SPECIES

Anaphylactic reactions result from the combination of antigen with cell-fixed antibody, and there are important differences in the responses of different animal species. The antigen–antibody combination results in the release of pharmacologically-active agents, and the manifestations of anaphylaxis are in part due to the species-specificity of these agents.

Guinea-pig—The guinea-pig exhibits two types of anaphylactic shock, depending upon the route of administration of antigen. Intravenous injection of antigen into sensitized animals produces within a few minutes an acute syndrome characterized by marked dyspnoea, and death is attributable to bronchospasm. The lungs taken from a guinea-pig immediately after death during anaphylactic shock remain firmly inflated as a result of broncho-constriction; lungs from non-sensitized animals collapse when the thorax is opened (*Figure 5.1*).

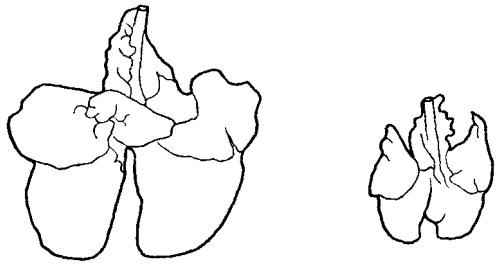


Figure 5.1. Lungs taken from a guinea-pig after fatal anaphylactic shock compared with normal lungs

There are four reasons why the respiratory system in guinea-pigs is involved in acute anaphylactic shock. Firstly, the antibodies appear to be localized around the bronchi, particularly in the collagenous tissue between the smooth muscle layer and the rings of cartilage[11]; secondly, histamine, the principal mediator in this form of death, is present in high concentration in guinea-pig lung, where it resides chiefly in the mast cells[12]; thirdly, the smooth muscle of guinea-pig lung is extremely sensitive to histamine, and lastly, the smooth muscle in the respiratory tree of the guinea-pig is arranged on the inner aspect of the cartilage, unlike that in most other species.

The subcutaneous or intraperitoneal injection of antigen into a sensitized guinea-pig, however, results in a more protracted type of anaphylactic shock, the main features being pruritus, dyspnoea, hypothermia and collapse[13]. Post-mortem examination reveals stasis of and/or haemorrhage in the stomach and intestine. The mediator of this type of shock is unknown, and anti-histamine drugs which abolish the lung involvement do not affect the protracted shock[14].

Dog—The intravenous injection of antigen into a sensitized dog produces

a sudden fall in blood-pressure. The heart beats with an accelerated rhythm and blood accumulates in the abdominal vessels. The liver is markedly enlarged and there is stagnation of blood in the portal region. Coagulability of the blood is decreased and there is a sharp fall in the total white cell count of the peripheral blood probably due to large numbers of polymorphs being trapped in the capillaries of the lungs. These symptoms are largely mediated by histamine and heparin liberated from the dog's liver, since removal of the liver lessens the shock.

Rabbit—Anaphylactic shock in the rabbit is accompanied by a fall in blood-pressure, death being due to circulatory collapse. The pulmonary artery is constricted and right-sided heart failure results. There are no marked changes in the liver.

Rat—In the rat, anaphylactic shock is characterized by progressive circulatory collapse. The earliest lesions appear in the small intestine resulting in oedema and haemorrhage with infiltration by eosinophils. Oedema rapidly spreads to the villi and is followed by desquamation of the mucosa[15].

METHODS OF PRODUCING ANAPHYLAXIS

Preparation of Antisera

Although any animal species may be used as a source of antibodies, the two species most commonly used for studies on passive anaphylaxis are the rabbit and the guinea-pig. Sensitization with simple solutions of protein or whole serum produce only low-titred antisera unless a prolonged course of immunization is undertaken. High-titred antisera may be produced by sensitization with alum-precipitated proteins or by using Freund's adjuvant[16]. Typical examples of each of these methods are given below. Methods used for the estimation of antibodies are beyond the scope of this review and may be referred to in a standard work on immunology[17].

Simple protein solutions or whole sera as antigens—Antibodies to whole serum may be prepared by intramuscular injections of 1 ml. of serum to rabbits weekly for 6 weeks, the animals being bled 1 week after the last injection. Instead of whole serum, solutions of protein (2 to 10 mg/ml.) such as egg albumin, bovine serum albumin, or human γ-globulin may be used.

Alum-precipitated protein—A 2·5 per cent solution of aluminium potassium sulphate in a 1 per cent solution of protein is first prepared, and precipitation is effected by the slow addition of 1·0N sodium hydroxide to pH 6·8. The precipitate is then suspended in saline, washed, and re-suspended in one-fifth of the original volume.

Freund's adjuvant—Many variants of Freund's original formula have been used in different laboratories, and the following has been found to be suitable:

Protein antigen	500 mg
1 per cent phenol	10 ml.
Arlacel A	10 ml.
Light liquid paraffin	20 ml.
Killed tubercle bacilli	2·5 mg

The dried tubercle bacilli are ground in a mortar and the liquid paraffin is added gradually to form an even suspension; the Arlacel A is then added and mixed thoroughly, followed by the antigen solution, with constant, vigorous stirring. The final mixture is a fairly thick emulsion which does not separate easily on standing. For sensitization, rabbits or guinea-pigs may be given 0·5 to 1 ml. intramuscularly at weekly intervals for 3 weeks and bled 7 to 10 days after the last injection. In place of tubercle bacilli, *Mycobacterium butyricum* may be used.

Rabbit anti-Forssman serum[18]—Boiled sheep cell stromata are prepared by repeated washings of erythrocytes with water saturated with carbon dioxide at 0°C until the supernatant is colourless. The stromata derived from 10 ml. of blood are then suspended in 2 ml. of saline, immersed in a boiling-water bath for 5 to 10 minutes, cooled and injected intraperitoneally into a rabbit. Two such injections are given, 1 week apart followed 1 week later by two intravenous injections of stromata derived from 1 ml. blood. Serum taken 4 days after the last injection lyse a 0·5 per cent suspension of sheep cells in the presence of complement sometimes giving a titre of 1:64,000.

Active Sensitization

Guinea-pig—A high degree of anaphylactic sensitivity appears in guinea-pigs 3 or 4 weeks after the intraperitoneal injection of 1 ml. of horse serum. Over 90 per cent of the animals so sensitized usually manifest a rapid lethal shock after intravenous injection of antigen. They are, however, less suitable for studies of *in vitro* anaphylaxis. For this purpose, intrahepatic injection of two doses of 0·05 ml. of horse serum in different sites has been recommended[19,20]. Isolated proteins may also be used as antigens either in Freund's adjuvant (2·5 mg protein) or alone (0·7 ml. of 5 per cent egg albumin).

Rabbit—Six daily intraperitoneal injections of 1 ml. of horse serum are given. The animal may be used 10 days to 6 weeks after the last injection[21].

Dog—One subcutaneous dose of 5 ml. of horse serum is given and this is followed 2 days later by 2 ml. intravenously. The animal may then be used 2 to 4 weeks later[22].

Rat—The intraperitoneal injection of 1 ml. horse serum and 1 ml. *Haemophilus pertussis* vaccine ($20{,}000 \times 10^6$ organisms) may be given; the animal is then used 2 weeks later.

For active cutaneous anaphylaxis, antigen (10 mg/ml. in 0·5 ml. Freund's adjuvant) is injected intramuscularly and a second similar injection given 1 week later. Five weeks later, 1 mg of antigen adsorbed on aluminium hydroxide is injected intravenously and a further similar injection is given 1 week later. The serum 10 to 12 days after the last intravenous injection contains about 4 mg of antibody nitrogen.

Cutaneous Anaphylaxis

General principles

Anaphylactic reactions in the skin produce an increase in capillary permeability at the site of the antigen–antibody interaction. An intravenously-injected dye accumulates at the site of this increased permeability, and the

reaction appears as a blue spot, the size and intensity of which is roughly proportional to the amount of reactant injected into the skin. These lesions may be measured on either the external or internal surfaces of the skin. For active cutaneous anaphylaxis, the dye is injected intravenously a few minutes before the intradermal injection of antigen. Trypan blue, pontamine sky blue, Evans Blue or Geigy Blue 6BX are suitable dyes. Ovary[4] recommends the use of Evans Blue (1·5 ml./kg of a 0·5 per cent solution) for guinea-pigs. Satisfactory results may also be obtained with pontamine sky blue (1·5 ml./kg of a 5 per cent solution). The antigen, when required, may also be mixed with the dye before injection.

Intravenous injections into rats may be made into a tail vein, a foot vein, or, in male animals, the dorsal vein of the penis. Satisfactory blueing may be also achieved by the intraperitoneal injection of Coomassie blue (5 ml./kg of a 1·5 per cent solution) 90 minutes before the intradermal injection. Tail veins may be used in mice.

Intradermal injections are usually made on the back. The fur is gently removed with electric clippers, and the volume injected should not exceed 0·1 ml. for guinea-pigs and rats, and 0·05 ml. for mice. In guinea-pigs, cutaneous anaphylaxis is visible in about 3 minutes, maximum intensity being reached in about 10 minutes. In rats and mice, the reaction develops more slowly and it is advisable to wait for 30 minutes before sacrificing the animals.

Passive cutaneous anaphylaxis[4,23]

Intravenous antibody—About 30 μg of antibody nitrogen maximally sensitizes a guinea-pig of 250 g body-weight, and a positive reaction results from the intradermal injection of 2·5 μg of egg albumin nitrogen some 48 hours later. As with passive anaphylactic shock, there is a latent period during which injection of antigen produces no response. The length of this period is proportional to the amount of antibody injected, and with 500 μg of antibody nitrogen it may be as short as 1 hour. Sensitization of the skin persists for between 8 and 12 days.

In rats 6 mg of antibody nitrogen is injected intravenously, and challenge is made with about 2 μg of antigen intradermally 24 hours later. In mice, 175μg of antibody nitrogen may be injected intravenously, and intradermal challenge may be made with 1 μg of antigen 48 hours later[4].

Intradermal antibody—Undiluted heterologous, and sometimes even homologous, serum may provoke increased capillary permeability. Serum is diluted at least 50 times to avoid non-specific reactions. With the egg albumin system, 0·003 μg of antibody nitrogen is sufficient for a positive reaction in guinea-pigs, provided the antigen in large excess (1 mg or more) is used and a latent period of from 3 to 6 hours is allowed. An inverse relationship exists between the amounts of injected antigen and antibody. Thus positive reactions result using either 1 μg of antibody nitrogen and 20 μg of antigen nitrogen or 0·01 μg of antibody nitrogen and 500 μg of antigen nitrogen.

In rats, intradermal injections of 10 to 80 μg of antibody nitrogen are made. Three and a half hours later, 0·5 ml. of 1·5 per cent solution of pontamine sky blue 6BX with 3 mg of antigen may be injected intravenously[4].

In mice, 0·25 ml. of dye mixed with 0·25 ml. saline containing 1·5 mg

antigen are injected intravenously. The minimal amount of antibody nitrogen to be injected intradermally is about 0·4 μg[4,24].

Anaphylactic Microshock[20]

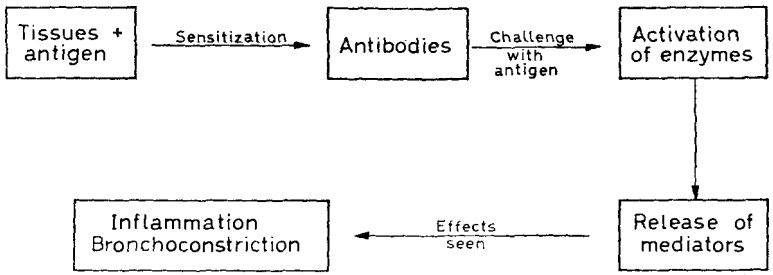
Guinea-pigs are sensitized by the intramuscular injection with a 5 per cent solution of crystalline egg albumin. Three weeks later, they are exposed to an aerosol of the same solution. The exposure is carried out in a glass case, measuring 24 × 12 × 12 inches. The case, which is open on one side, is placed on a smooth surface covered by rubber. Antigen contained in a nebulizer is sprayed into the chamber, and within 15 to 60 seconds a change in the respiration of the animal is apparent, the abdominal walls being tightly contracted. The animal may cough or sneeze, and respiration may then become either slower and deeper or more rapid and shallow; in the latter case, the head may move rapidly to and fro. The stage at which the respiratory distress and cyanosis is so great that convulsions may occur is called the 'convulsion point', and the duration of exposure to the aerosol is termed the 'pre-convulsion time'. If the animals are re-exposed at intervals of 2 to 7 days, an intermediate stage between desensitization and resensitization is reached. Some re-formation of antibodies has taken place, and the pre-convulsion time is approximately constant at this stage.

Lethal Shock after Intravenous Injection of Antigen

Intravenous injections may be made into a marginal ear vein of the guinea-pig. The animal, wrapped in a cloth, is held by an assistant and the ear veins transilluminated by applying a light to the end of a bent Perspex rod. Up to 1 ml. may be injected using a short-point needle, and no vasodilator or local anaesthetic is needed. Animals sensitized by the intraperitoneal injection of 1 ml. of horse serum 4 weeks previously die with acute anaphylactic shock 3 to 6 minutes after the intravenous injection of horse serum (0·2 ml./100 g body-weight).

MECHANISMS OF ANAPHYLAXIS

Anaphylactic reactions may be considered as a series of reactions according to the following scheme:



Sensitization

Sensitization depends on the presence of a sufficient quantity of antibody being produced by the animal or introduced by injection. Very small amounts

are sufficient for passive sensitization, and as little as 30 μg nitrogen of rabbit anti-egg albumin antibody sensitizes a guinea-pig so that fatal anaphylaxis results on injection of 1 mg of egg albumin[25]. Even smaller quantities sensitize the skin so that cutaneous anaphylaxis appears at the site of injectiou when antigen is given intravenously: for example, the minimal quantities for various species are: mice, 0·4 μg antibody nitrogen; guinea-pigs 0·003 μg, and rats, 0·5 μg. An isolated uterus of the guinea-pig may be sensitized by 0·01 μg[25]. These small quantities of antibody are not detected by serological procedures, and passive cutaneous anaphylaxis is one of the most sensitive tests for antibody.

After passive administration of antibody, there is a latent period during which the animal is refractory to anaphylaxis: the length of this period is a function of the amount of antibody used[4,26]. There is no satisfactory explanation for this latent period. Anaphylactic shock in actively sensitized animals begins within a few minutes of the injection of antigen, even when antigens of large molecular size are used. Passively-administered antibody takes longer to reach the sites to be sensitized suggesting that some time-consuming reaction takes place after antibody has reached these sites[26].

Passive sensitization *in vitro* has been studied with pieces of chopped guinea-pig lung[18]. The tissue is incubated with antibody and the degree of sensitization estimated by measuring the amount of histamine released upon the addition of antigen. Passive sensitization increases with time of incubation and at 38°C becomes maximal in 2 to 4 hours. It has a high temperature coefficient but is not completely abolished even at 0°C. Alterations in the ionic composition of the medium, including complete absence of calcium and magnesium ions, are without effect. Passive sensitization, however, may be inhibited by an excess of non-antibody γ-globulin[27].

There are species differences in passive sensitization. Humphrey and Mota[28] showed that guinea-pigs are sensitized with small amounts of guinea-pig or rabbit antibodies and rather larger amounts of monkey, dog or human antibodies, whereas antibodies from the goat, horse, rat, fowl and human auto-immune thyroid antiserum are ineffective. Using antibodies labelled with ^{131}I, and measuring uptake on guinea-pig mesentery *in vitro* and retention in the tissue after such uptake, no gross differences were found between those antibodies which sensitized and those which did not. Rat (non-sensitizing) or rabbit (sensitizing) antibody when adsorbed on to guinea-pig mesentery combined equally well with antigen. Reversed passive anaphylaxis occurred only when the antigen was a γ-globulin and when both antigen and antibody globulins originated from species whose antibodies were able to sensitize guinea-pigs for direct passive anaphylaxis. Passive sensitization of guinea-pig lung was inhibited by guinea-pig, rabbit and human sera, and by purified human γ-globulin but not by purified bovine γ-globulin[27]. The failure of antibodies from some species to sensitize guinea-pigs passively remains obscure; their inability to initiate activation of guinea-pig complement may be the governing factor[28].

The site of antibody fixation in the tissues is unknown. The fact that desensitization occurs on contact with antigen in the cold[29] and in the presence of metabolic inhibitors implies that active intracellular uptake of antigen is not necessary. Many antigens have a large molecular size yet

they are capable of inducing anaphylactic reactions rapidly after contact with sensitized cells and diffusion into the cell during such a short period is unlikely. Antigen releases histamine from intact sensitized cells but not from subcellular particles derived from these cells[30], and antibody may therefore be situated on or near the cell surface. Furthermore, non-precipitating antibodies are as effective as precipitating whole serum antibodies in producing passive anaphylactic sensitization in guinea-pigs[31].

Combination of Antigen with Antibody

While the combination of antigen with antibody is most commonly produced by the injection of antigen into an actively or passively sensitized animal, antigen–antibody complexes prepared *in vitro* may produce symptoms of anaphylaxis when injected into non-sensitized animals[32–36]. There is, however, no evidence that the symptoms of anaphylaxis result directly from the combination of antigen with antibody but rather that this is the first step in a series of reactions. Attempts have been made to demonstrate a relationship between antigen–antibody combination and proteolytic activity of blood, but as Paton[37] has pointed out, events in blood are not able to account for anaphylaxis in blood-free tissues, such as perfused lung or isolated uterus. Many of the steps in the development of an anaphylactic reaction are enzyme-initiated and it may be said that the antigen–antibody complex activates enzymes although direct evidence of such activation is meagre[38–41]. A group of substances, at least one component of which is an enzyme, is known as tissue complement.

Complement

Complement consists of at least four components, each of which is probably a protein present in fresh serum. Complement is not increased by the sensitization process but is characterized by its ability to participate in antigen–antibody reactions. One such reaction, widely used to estimate complement, is that between sheep red blood-cells and their antibodies; these antibodies are prepared by injecting animals (usually rabbits) repeatedly with washed sheep red blood-cells. Addition of this antiserum to a washed suspension of sheep red-cells sensitizes the cells, which haemolyse when a complement-containing serum is added. A freshly-collected antiserum may by itself contain enough complement to cause lysis and it is therefore necessary first to inactivate this complement by heating at 56 to 58°C for 20 minutes. When optimal amounts of red cells and inactivated antiserum are present, the amount of haemolysis is proportional to the concentration of added complement.

It is tempting to suggest that anaphylaxis depends on the availability of complement. Complement titres fall during acute anaphylactic shock in guinea-pigs[42,43], and there is a striking correlation in the rabbit between the time of disappearance of antigen from the circulation, the reduction in complement, and the appearance of characteristic lesions of hypersensitivity[44]. There is evidence also that complement is required for passive cutaneous

anaphylaxis in rats, the effects of anaphylatoxin on capillary permeability[45–47], and lysis of sensitized mast cells by antigen[48].

Mediators

The antigen–antibody interaction leads to the release or activation of physiologically active substances, which may be termed the mediators of anaphylaxis. A number of these mediators has been described, but others remain to be discovered. Proof that a given substance is a mediator of anaphylaxis depends upon the demonstration that: (*a*) it is present in the body in an inactive form, (*b*) it is released as a result of antigen–antibody combination, and (*c*) it is active in reproducing one or more of the symptoms of the anaphylactic reaction. Even when such proof has been obtained for a given substance in one species, it does not follow that the substance plays a similar, or indeed any role, in another species. One of the most studied mediators of anaphylaxis is histamine and the demonstration by Riley and West[49] of the presence of this base in the mast cells of the connective tissue, has led to a much fuller understanding of the mechanism of the anaphylactic release of histamine. Mast cells from sensitized animals may be lysed when brought into contact with antigen[50–52].

Histamine

Evidence for the release of histamine during anaphylaxis is adequately dealt with in many excellent reviews (for references, see Paton[53]). The following discussion is therefore restricted to the mechanism of its release and to a consideration of some of the short-comings of the histamine hypothesis.

Most of the present-day knowledge of the mechanism of the release of histamine during anaphylaxis in the guinea-pig follows from the work of Mongar and Schild and the following account is based on Schild's summaries[54,55]. When antigen is added to minced lung from a sensitized guinea-pig, histamine is released from the tissue. The histamine may be estimated on the isolated ileum of the guinea-pig where alone it is responsible for the contractions, for they are abolished by specific antagonists of histamine such as mepyramine. Histamine may also be released from both non-sensitized and sensitized guinea-pig tissues by a wide range of substances known collectively as histamine-releasers. Mongar and Schild used two of these, octylamine and compound 48/80, a low polymer (chiefly 2 to 4 units) of a substituted phenylethylamine[56,57], to show some fundamental differences between the releasing mechanism of these substances and that of antigen and sensitized tissue. The finding that low concentrations of iodoacetate and *p*-chlormercuri-benzoate, both of which react with —SH groups, inhibit the anaphylactic reaction indicates that the reaction involving free —SH groups forms an integral part of the reaction. The temperature–concentration curve of the histamine release reaction in anaphylaxis resembles an enzyme curve. Low temperatures produce a reversible slowing of the reaction whereas raised temperatures (about 42·5°C) produce an irreversible inactivation[29]. Further evidence that enzyme systems are involved is provided by the finding that the anaphylactic reaction depends on a heat-labile factor. Its nature has been investigated by passive sensitization of guinea-pig tissue *in vitro*.

Antibody which has been heated to 45°C is capable of sensitizing lung or intestine *in vitro* but unheated antibody does not sensitize tissue which has been similarly heated. The temperature coefficient of inactivation suggests that the heat-labile factor is probably a protein. The reaction also requires the presence of calcium and is dependent upon pH, maximum activity being found at pH 7·8 with little or no activity at pH 6·3. The effects of calcium and pH are interdependent: inhibition of histamine release by lowering the

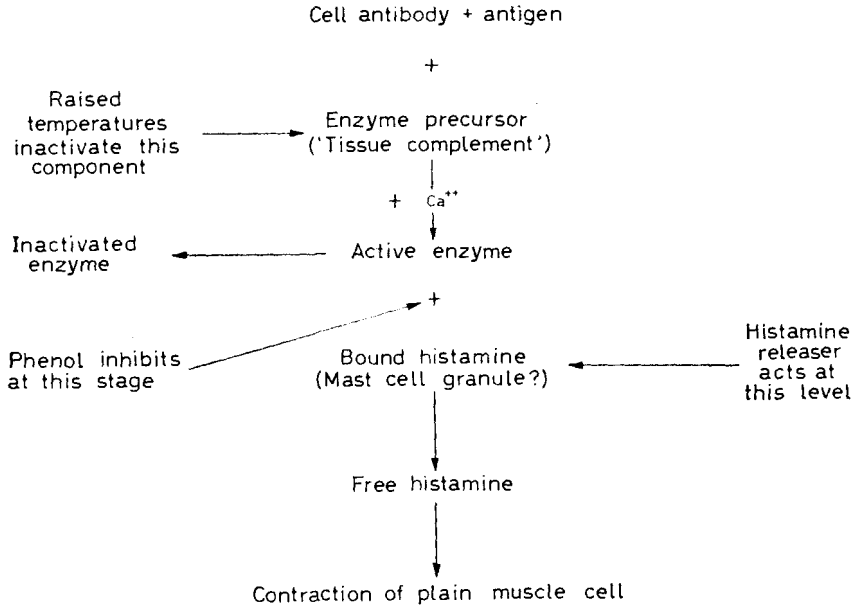


Figure 5.2. Scheme for the anaphylactic reaction[55]

pH may be counteracted by increasing the concentration of calcium. Conversely, a low concentration of calcium may be counteracted by raising the pH value.

Of interest is the observation that anaphylactic release of histamine is inhibited by phenol and antipyretic drugs[58]. Phenol does not interfere with the reaction of antigen with cell-fixed antibody, nor does it inactivate antibody. The combination of antigen with the cellular antibody results in the activation of an enzyme system which catalyses reactions leading to histamine release. An inhibitory substance such as phenol may block the actions of this enzyme but not its transformation to an inactive form, so that after removal of phenol no free enzyme is left to complete the reaction (see *Figure 5.2*).

Histamine-releasing organic bases act on suspensions of intracellular particles as well as on intact cells, although no appreciable histamine release is obtained by adding antigen to suspensions of intracellular particles from sensitized tissue. When the antigen is added to sensitized tissues in which the cell structures are intact, the intracellular fractions become depleted of

histamine. Thus, the integrity of cell structure may be necessary for histamine release in anaphylaxis[30].

There are some similarities between the effects of antigen in releasing histamine from sensitized tissues and its effect on the mast cells of the tissues. Addition of antigen to chopped lung or mesentery from sensitized guinea-pigs leads to a reduction in the number of mast cells and degranulation of the remaining mast cells[52]; this change parallels the histamine release from the tissue, is maximal at 40°C, and is inhibited by lowering the temperature to 15°C or by removing calcium. Mast cell changes in anaphylaxis *in vitro* are also partially inhibited by iodoacetate and phenol[12,50].

A dinucleotide of histamine has recently been isolated and synthesized[59]. Its structure is that of diphosphopyridine nucleotide (DPN) in which histamine replaces the nicotinamide moiety. Diphosphopyridine nucleotidase (DPNase) catalyses an exchange reaction between histamine and the nicotinamide moiety in DPN, and Alivisatos[60] has suggested that such an exchange is involved in the mechanism of anaphylaxis. An interesting recent observation shows that pyridine, nicotinamide, diethylnicotinamide, nicotinic acid, isonicotinic acid and isonicotinic acid hydrazide, all of which are inhibitors of DPNase, inhibit both histamine release and mast cell damage in anaphylaxis in both rats and guinea-pigs[61].

Antihistamine drugs antagonize the effects of histamine on smooth muscle more effectively than they antagonize the anaphylactic contraction of smooth muscle[62]. Since cells in guinea-pig lung probably constitute the main source of the released histamine and these are situated close to the smooth muscle-cells, the histamine is released in close proximity to the cells upon which it acts[12]. The antagonistic action of antihistamine drugs is proportional to the concentration of histamine present (*i.e.* they are competitive antagonists) but such drugs are thus less effective in the presence of high concentrations of histamine released locally from mast cells. Acute anaphylactic shock, characterized by bronchospasm, may be produced by intravenous injection of antigen into a sensitized animal, but pretreatment of these animals with antihistamine drugs prevents bronchospasm and protects many of the animals. A few animals, however, die some hours later with a protracted shock, and post-mortem examination reveals pulmonary oedema and stasis and/or haemorrhage of the gastro-intestinal tract[62]. Subcutaneous injection of antigen also leads to this protracted shock[13], and this is refractory to antihistamine treatment[14].

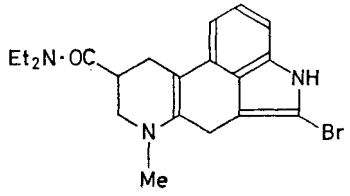
The role played by histamine in anaphylactic reactions in the skin is uncertain. Passive cutaneous anaphylaxis in rats is accompanied by a rapid fall in the histamine content of the skin, not only at the site of the injection of antibody but also at other skin sites not involved in the reaction[63,64]. The amount released, however, is not related to the dose of antibody used for sensitization and there is little or no relationship between the amount of histamine released and the degree of oedema[64]. Further, passive cutaneous anaphylactic reactions in rats are not inhibited by antihistamine drugs[65,66], and are equivalent in size and intensity to the intradermal injection of 1,000 times the quantity of histamine present in the corresponding area of skin[66]. Rocha e Silva and Rothschild[67] have shown that cutaneous anaphylaxis in rats is also obtained in animals from which histamine has been depleted by

dextran, egg-white or compound 48/80, so that histamine may not be an important mediator in the rat.

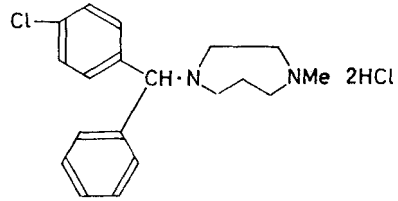
Serotonin (5-hydroxytryptamine, 5-HT)

Since the discovery of 5-HT, much experimentation has been devoted to a demonstration of its possible role in anaphylaxis. Several aspects of its pharmacology suggest this; for example, it has bronchoconstrictor activity in a variety of species[68,69], it increases capillary permeability in the rat[70], and it is contained in mast cells of the rat[71,72]. There is, however, a considerable species variation in the distribution of 5-HT. Guinea-pig lung contains only small quantities whereas the lungs of rats and rabbits contain appreciable quantities[73]; little or no 5-HT is found in the skins of species other than rats or mice[74].

The administration of 5-HT by aerosol produces a shock syndrome in guinea-pigs which is similar to that of anaphylaxis in this species. The condition is inhibited by the powerful anti-5-HT drugs, lysergic acid diethylamide



BOL-148



Homochlorcyclizine

(LSD), cyproheptadine[75] and methdilazine[76], but not by mepyramine. LSD, however, has little protective action against anaphylaxis in the guinea-pig[77], unless it is given intravenously[15]. Further, the blood level of 5-HT in guinea-pigs does not change during sensitization or anaphylactic shock[78,79]. The isolated uteri of guinea-pigs are at least 1,000 times more sensitive to histamine than to 5-HT, and the Schultz–Dale reaction of the sensitized guinea-pig uterus is blocked by specific antihistamine drugs (*e.g.* mepyramine) but not by specific antagonists of 5-HT (*e.g.* 2-bromolysergic acid diethylamide, BOL-148)[15]. The 5-HT antagonist, homochlorcyclizine, protects guinea-pigs against anaphylaxis, but this compound is also a potent antagonist of histamine[80]. Thus, there is little evidence that 5-HT is concerned in anaphylaxis in the guinea-pig. Pretreatment of sensitized rats with mepyramine or BOL-148, or with both antagonists, fails to alter the course of anaphylactic shock, and anaphylaxis is likewise unaffected by lowering tissue levels of 5-HT after treatment with reserpine[81]. On the other hand, the anaphylactic contractions of rat uteri *in vitro* are prevented by low concentrations of BOL-148[82]. The situation in mice is similar to that in rats, and there is no change in the 5-HT content of tissues or blood after anaphylaxis[83]. Fink[84] showed that LSD and reserpine inhibited the Schultz–Dale contraction of the sensitized mouse uterus, yet Udenfriend and Waalkes[85] were unable to demonstrate even the presence of 5-HT in the mouse uterus.

One of the earliest demonstrations of the release of 5-HT as a result of

antigen–antibody interaction was that of Humphrey and Jaques[86] who showed that the addition of antigen resulted in the release of 5-HT from the platelets of sensitized rabbit blood. The injection of antigen into the sensitized rabbit leads to the rapid appearance of both histamine and 5-HT in the plasma, chiefly from platelets[87,88]. During anaphylaxis, platelets disappear from the circulation and are trapped in the tissues, particularly the lungs[89].

Although Waalkes and Coburn[90] have shown that the changes in the platelet count and in the histamine and 5-HT levels found during anaphylaxis may be reproduced by the injection of glycogen, the bulk of evidence suggests that 5-HT is of little importance in anaphylaxis in rabbits.

Anaphylatoxin

When guinea-pig or rat serum is incubated with antigen–antibody precipitate or certain polysaccharides such as agar or zymosan, the supernatant fluid is capable of producing many of the features of anaphylaxis, in particular the release of histamine when it is injected into non-sensitized animals[91]. This histamine-releasing agent is known as anaphylatoxin. In the guinea-pig, anaphylatoxin produces mast cell damage similar to that produced by chemical histamine liberators, and the damage is inhibited by iodoacetate, *p*-chloromercuri-benzoate, phenol and low temperatures. However, unlike anaphylaxis, anaphylatoxin-induced damage is not inhibited by calcium deficiency or by previous heating of the tissue to 45°C. Mepyramine reduces, but does not abolish, the contraction of a guinea-pig ileum produced by anaphylatoxin. If the sensitized ileum is warmed to 45°C the anaphylactic response is abolished but the contraction induced by anaphylatoxin is maintained[92]. Desensitization to anaphylatoxin does not modify the anaphylactic contraction and ilea desensitized with antigen still respond to anaphylatoxin[93]. The activity of anaphylatoxin-containing serum is associated with the α_2-globulin fraction[94].

Anaphylatoxin formation using the serum of guinea-pigs is not influenced by previous sensitization, but heparin inhibits anaphylatoxin formation *in vitro*, without influencing anaphylactic shock *in vivo*[95]. Thus, the bulk of evidence suggests that anaphylatoxin is not an important mediator of direct anaphylaxis. It may, however, play a role in reversed anaphylaxis. Giertz, Hahn, Jurna, and Lange[95] found that much less anaphylatoxin was formed from guinea-pig serum after reversed Forssman anaphylaxis.

Slow Reacting Substance (SRS-A)

Kellaway and Trethewie[96] found that the effluent from perfused lungs of a sensitized guinea-pig injected with antigen produced a slow contraction of the guinea-pig ileum which was different from the rapid effect produced by histamine. The substance producing this contraction has been further studied by Brocklehurst[97,98], who showed that it is formed in the perfused tissue when the sensitized lung of man, monkey, guinea-pig or rabbit is challenged with the specific antigen; it appears with histamine in the effluent perfusion fluid. The release of SRS-A is slower in onset and more prolonged than that of histamine, and its effect on smooth muscle is not inhibited by

mepyramine. It may be formed in lung tissue as a result of antigen–antibody combination since it is not present in lung before challenge with antigen. Platelets and other constituents of blood are not necessary for its formation. SRS-A produces a contraction of human bronchioles *in vitro* but it has no effect on the isolated bronchial muscles of the cat, dog, rabbit or guinea-pig[99]. The absence of effect on the guinea-pig bronchial muscle suggests that it is unlikely to be a mediator of anaphylactic bronchoconstriction in the guinea-pig. It has been claimed, however, that ethanolamine and mepyramine inhibit anaphylaxis in guinea-pigs and also prevent the release of SRS-A during anaphylaxis *in vitro*[100].

The chemical nature of SRS-A is not yet known. It is soluble in 70 per cent alcohol[98] and is located in a single band when solutions of it are submitted to electrophoresis[101]. Its pharmacological properties differ from bradykinin, 5-HT and substance P[102].

Recent work in Scandinavia has shown that the mast cell is probably the source, not only of the histamine released during anaphylaxis, but also of the SRS-A. The temperature and pH curves for the release of both substances are similar, and the release of both is inhibited by anoxia, calcium deficiency, and enzyme inhibitors[103,104].

Bradykinin

This substance has several properties associated with mediators of anaphylaxis although, apart from one report[105], its release during anaphylaxis has not yet been demonstrated. It is a potent mediator of increased capillary permeability[106] and is a bronchoconstrictor in the guinea-pig[107,108]. However, aspirin, which inhibits the bronchoconstrictor effect of bradykinin in the guinea-pig does not inhibit anaphylactic shock in this species[109]. Bradykinin has recently been synthesized: it is a polypeptide and has the following structure[110,111]:

H·L-Arg·L-Pro·L-Pro·Gly·L-Phe·L-Ser·L-Pro·L-Phe·L-Arg·OH

(Arg=arginine; Pro=protine; Gly=glycine; Phe=phenylalanine; Ser=serine)

PF/P

Another possible mediator of anaphylactic inflammation has been studied by Davies and Lowe[112]. This is the permeability factor induced by precipitins (PF/P) which is formed when antigen–antibody precipitates are added to guinea-pig serum. It may be different from other previously described mediators.

INHIBITION OF ANAPHYLAXIS

The search for inhibitors of anaphylaxis has two purposes: firstly, it may provide a better understanding of the mechanisms of allergy and anaphylaxis, and secondly, it may stimulate the production of drugs useful for the treatment of allergic diseases.

The anaphylactic reaction may be divided into five stages, each of which is susceptible to inhibition:

Synthesis of antibody

Inhibition of antibody synthesis is possible but there is every likelihood that antibodies responsible for immunity to infectious disease would be similarly suppressed.

Fixation of antibody to cells

Under some conditions, the fixation of passively-administered antibody may be prevented by prior treatment of the animal or tissue with γ-globulins[113], since competition between antibody and other globulins may take place for sites on cells. This approach has not as yet provided any useful therapeutic measure.

Reaction of antibody with antigen

Although it has been shown that high concentrations of some chemical compounds, *e.g.* sodium salicylate[114], inhibit antigen–antibody precipitation *in vitro*, no useful drug has been found to have this effect in the whole animal. A special type of this inhibition was studied extensively by Landsteiner[115]. He found that when some reactive chemical compounds, such as acyl chlorides, were attached to protein, immunization of animals with the resulting conjugate stimulated the production of antibodies directed not only to the protein carrier but also to the combined grouping (called a 'hapten'). In guinea-pigs sensitized to the complete antigen (hapten+protein) administration of hapten alone prevented the anaphylactic reaction which otherwise developed when a dose of complete antigen was given. Haptens also inhibit precipitation of complete antigen by antibody *in vitro*. These inhibitory effects are caused by the combination of hapten with antibody, thus reducing the amount available for combination with complete antigen. These observations have not yet led to any therapeutic application.

If a sensitized animal is given small successive doses of specific antigen, it then becomes refractory to larger doses, which usually produce shock; the animal is said to be desensitized. It is presumed, but by no means proved, that the available antibody has been gradually used up by small doses of antigen, each of which produces a small anaphylactic response, so that none is left to react with the large dose of antigen. Desensitization is a temporary state which wanes when further antibody synthesis has taken place.

Release of mediators

Much work has been devoted to the inhibition of histamine release by antigen, especially by Mongar and Schild[58] but no useful inhibitor of anaphylaxis has yet emerged. Phenylbutazone, which is highly active *in vitro*[58], does not inhibit shock in the whole animal.

Antagonists of mediators

Successful anti-allergic and anti-anaphylactic drugs are usually antagonists of one or more of the mediators of anaphylaxis. By far the largest group consists of antagonists of histamine. There is abundant evidence that such drugs afford protection from acute anaphylactic shock in guinea-pigs. When sensitized animals inhale antigen, significant protection may be obtained by antihistamine drugs given orally, systemically[62,116–118], or by inhalation[119].

They are also active against shock induced by intravenous antigen challenge[120]. There is little doubt that these drugs exert their protective effect in anaphylactic shock in guinea-pigs by inhibiting the bronchoconstriction which otherwise results from the released histamine. Their activity, however, is limited, and the amounts required to exert an anti-anaphylactic effect are greater than those necessary to inhibit administered histamine[9,120]. Furthermore, animals protected from the acute bronchoconstriction frequently die later in protracted anaphylaxis, and antihistamine drugs are ineffective at this stage. In mice, large doses of antihistamine drugs produce only slight protection against anaphylactic shock[121].

Herxheimer[122] studied the effect of adrenaline-like compounds in the guinea-pig with his 'microshock' technique, and similar studies have been carried out by Swineford, Motley and Tull[119]. They found that both isoprenaline and adrenaline were effective when the animal was allowed to inhale relatively high concentrations (0·5 to 1·0 per cent) although ephedrine (3 per cent) was ineffective[119]. When the drugs were given intramuscularly 15 minutes before exposure of the animals to an aerosol of antigen, adrenaline was effective at about 0·2 mg/kg and isoprenaline at 0·005 mg/kg[122]. In the same study, ephedrine and methylephedrine were effective at a dose of 40 mg/kg when given intramuscularly 1 hour before exposure.

Coburn, Graham and Haninger[123] found that when whole egg-yolk was incorporated in the diet of baby guinea-pigs, some protection was afforded against anaphylactic 'arthritis'. The lipid, however, had little effect in the adult. The active material was present in the alcohol-soluble fraction and has been identified as *N*-(2-hydroxyethyl)palmitamide[124]. This substance and several closely related compounds have been found to be active in reducing the oedema produced by injecting antigen into the joints of passively sensitized guinea-pigs. The following substances produced inhibition in about one-half of the animals used (doses in brackets as μg/kg)[125]: soya-bean lecithin (0·3 to 30), egg-yolk (3-30), peanut oil (3), ethanolamine (3 to 3,000), *N*-(2-hydroxyethyl)palmitamide (0·3 to 3,000) and choline chloride (3 to 300).

In mice, *N*-(2-hydroxyethyl)palmitamide gave protection against fatal passive anaphylaxis[126], and activity was also shown by ethanolamine hydrochloride and by palmitic acid. *N*-(2-Hydroxyethyl)palmitamide and palmitic acid were also active against 5-HT in mice but ethanolamine hydrochloride was much less effective[126]. Ethanolamine and choline have recently been shown to have activity against anaphylactic shock in guinea-pigs partially protected with mepyramine[100]. Pretreatment with ethanolamine had no effect on the *in vitro* release of histamine when antigen was added to the lungs, but there was an inhibition of SRS-A release.

Interesting observations have been made with a series of tranquillizing drugs[118] in guinea-pigs passively sensitized with guinea-pig anti-ovalbumin serum 24 hours before exposure to an aerosol of antigen. The time period of spraying to produce dyspnoea was recorded ('pre-asthma time') and protective effects (doses in brackets as mg/kg) were obtained with: hydroxyzine (10), promazine (20), chlorpromazine (10), prochlorperazine (10) and benactyzine (2).

Ferguson, Greene and Wendel[127] recently administered various compounds to sensitized guinea-pigs before challenge with nebulized antigen to

determine their protective power. The most active in this series were 1-(3-hydroxyphenyl)-2-methyl-aminoethanol hydrochloride, 6,7-dihydroxy-1-isopropyl-1,2,3,4-tetrahydroisoquinoline, and a combination of this substance with trimeprazine. Herxheimer[128] also found some protective activity (doses in brackets as mg/kg) with: buscopan (0·1), caffeine (100), methanthelinium (5·0), pethidine (20) and sodium cyanate (50). Jaques[129] even obtained protection against anaphylactic shock in guinea-pigs with cotton oil, sesame oil, corn oil and bone oil.

Although outstandingly successful in the treatment of clinical allergies, cortisone and related compounds have little effect on anaphylaxis. If given during the sensitization period they may exert an indirect effect by depressing antibody synthesis. High doses, however, suppress generalized anaphylaxis in rats and mice[130–133] and reversed anaphylaxis in guinea-pigs[134,135], but have no effect on direct anaphylaxis[136,137] and passive cutaneous anaphylaxis in guinea-pigs[4].

ALLERGY IN MAN

Allergic diseases are the clinical counterparts of anaphylaxis. The range of causative antigens (or allergens) is wide and apparently inexhaustible, and includes animate material and inanimate substances such as metals (*e.g.* nickel or chromium) and many synthetic drugs. Allergic reactions in man are responsible for a variety of disease processes in a wide range of sites. The organs most commonly affected are lungs, nose, eyes, skin, blood-vessels, intestinal tract and joints.

Undoubtedly the most effective method of treating allergic diseases is by avoidance of the offending allergen. This may not always be possible, as the allergen may be so common that complete avoidance is impracticable, or the patient may have multiple sensitivities. However, when the allergen is identifiable, desensitization may be attempted. The patient is then given a course of injections of allergen in very small amounts. The number of types of effective drugs used in the symptomatic control of allergies is small although each type has many representatives. The most widely used are the antihistamine group of drugs, and these are mainly effective against those aspects of allergy concerned with the release of histamine. They are frequently and effectively used in the treatment of hay fever, although in this disease there exists a proportion of patients who do not respond to treatment. Tolerance to a particular antihistamine drug often develops and a change of treatment has to be made.

Bronchodilators are widely used in the treatment of asthma. Adrenaline and isoprenaline, administered by inhalation, are the most useful types. Ephedrine is active orally and parenterally, and although it has a longer duration of action, it is less effective than isoprenaline and adrenaline; it may give rise to serious cardiovascular and central nervous effects. Isoprenaline is active orally but it also sometimes gives rise to serious cardiovascular side-effects at effective dosage.

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