Advances in
PARASITOLOGY

VOLUME 14

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Edited by

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PREFACE

In the compilation of this book eight authors have written four important full contributions and two shorter updated reviews which bring earlier discussions up to date. Klaus Odening, who works in East Berlin, remarks in his Introduction that one problem of parasitology is that it has become regarded as a sub-area of ecology, although the term "host" and various synonyms such as "carrier" do not fit equally into these two subjects. Also, the term parasitology is usually restricted to certain organisms or to animals only, not including parasitism in microbiology and plant pathology. What is needed, he suggests, is to find a comprehensive definition which covers all aspects of the notion of "host", with identical definitions and terms for universal phenomena and with specific terms and notions for special cases. At present, zooparasitological terminology needs general clarification, and there is a need for a new theoretical foundation for the host notion, as well as clarification in respect of a steady growth of knowledge. This review is intended to clarify existing terms rather than introduce fresh terms. However, expanding knowledge calls for new terms as well as the casting out of obsolete terms. The level of association covers polyhospitalism (one parasite having several hosts) and monohospitalism (one parasite having but one host). Somatoxeny (body contact relation) involves both disseminating and transmissive hosts, respectively discharging parasites into open environment or by physical contact with the next host. It is unnecessary to discuss here or even to enumerate the parts of the entire scheme, and Klaus Odening himself requires 14 paragraphs to discuss his illuminating conclusions. Student and reader alike will need to put much into his reading of the whole review but will surely have a better knowledge hereafter than hitherto of the conception and terminology of hosts and parasites in the field of parasitology.

David W. T. Crompton and Mal C. Nesheim, of the Molteno Institute, Cambridge and Cornell University, Ithaca, N.Y., U.S.A. respectively, are also interested in ecological problems and in particular claim that the functional alimentary canal is an important habitat for a community of organisms living therein. The chosen hosts are notably the domestic duck, goose, fowl, turkey and pigeon, the nutrition of which involves more than 40 chemical compounds and inorganic elements which are abundantly expressed in a number of tables as required. Energy requirements are considered in some detail and the nature of diets is discussed. Diversity of meals taken may be exemplified by the mallard, which takes meals including masses of grass, seeds, insects and small molluscs, and the red grouse, which subsists largely on a diet of heather. The speed of food intake is correlated with the digestibility of foods taken. After considering in major sections the alimentary tract of domestic birds as a habitat for parasites (with a sub-section on the plasticity of the avian food canal) and the tract of germ-free domestic birds, parasites come largely into the picture. Major sections are concerned with the

observed distribution of parasites (with separate treatment of micro-organisms, protozoan organisms and helminths), the relationships between infective stages of parasites (Protozoa and helminths) and digestive physiology, and further relationships between parasites and digestive physiology (host effects on parasites and parasitic effects on hosts) with a final section on Conclusions (briefly) which brings out the fact that many parasites are dependent on the digestive physiology of their hosts and some inhabitants of the tract seem to be dependent on the activity of others. The main finding is that the alimentary tract as an environment is a subject needing further investigation, but here in this review there is plenty of groundwork for further studies of this kind, and much help of practical kinds.

Diana J. McLaren selected the sense organs of nematodes as her thesis, starting from the fact that round worms respond to a wide range of physical and chemical stimuli and must therefore have various sensory receptors and a related high degree of nervous coordination. Because of the limitations of the light microscope (LM) early studies of this kind were confined to larger nematodes, but the introduction of the electron microscope (EM) has revealed structures whose existence was assumed on the basis of comparison with *Ascaris* and can now be identified, and hitherto unrecognized sensory receptors have come to notice. In this review details of various types of nematode sense organs have been recognized and the functional significance of these organs has been assessed. Cephalic sense organs dealt with include labial and cephalic papillae, amphidial glands and sense organs, and cephalids. Cervical sense organs include deirids, hemizonids and hemizonions, lateral cervical sense organs in larval hookworms, sense organs in the bacillary bands of trichuroids and other sense organs associated with feeding, and photoreceptors. Caudal sense organs include caudal papillae, spicules, phasmidial sense organs and glands and a possible stretch receptor in *Heterakis gallinarum*. Non-regional sense organs include the setae and the sense organs associated with the "body pores" of dorylaimids. This is a formidable list, even in outline, and it is followed by further explanatory information. From a practical point of view the number of species of nematodes subjected to EM treatment is "virtually negligible". Yet, basic structural arrangement is emerging in some sense organs that have been studied. The arrangement includes three cell types, (i) a nerve cell terminating in one or more highly modified cilia, (ii) a non-nerve cell of secretory function intimately related to the nerve cell, and a second non-nerve cell surrounding the other two cell types and often having bundles of fibres. Much confusion has arisen in the literature due to varying names used, but in this review an attempt has been made to use a standard nomenclature, including the terms modified sensory cilium, gland cell and supporting cell respectively. The characteristics of these cell types are clearly defined.

Anya Oko Anya considers nematodes to be widely distributed invertebrates, essentially aquatic and with a remarkable "spread" into most ecological situations and he stresses that the most important factor in their ecological success has been the variety of reproductive strategies they display. He claims that the nematode egg shell has physical and chemical properties that deserve

special mention as the final development of a self-contained, self-maintaining and resistant environmental unit. In recent times observations relevant to our understanding of nematode reproduction have appeared in more regular fashion, so that this is an opportune time to deal with physiological aspects of reproduction in nematodes. The 12 subdivided main sections of Anya's review indicates major topics dealing with reproductive phenomena, the reproductive system, the male gamete and the female gamete, the physiology of fertilization, development, sex differentiation, nutrition and other factors in egg production, behavioural aspects of reproduction and reproductive phenomena and parasitism. Making some general observations, Anya indicates that in the sexes nematodes show differences in size and shape, special entities such as spicules and bursa, also telamon, but many plant parasitic and soil nematodes are hermaphrodite. All the main types of animal reproductive phenomena are known in nematodes, namely parthenogenesis, hermaphroditism, pseudogamy and syngamy. In his summary Anya stresses that all the main cytogenetic mechanisms seen in the animal kingdom are represented in the Nematoda. The reproductive system has been elaborated, especially in parasitic nematodes, so that greater histological and cytological differentiation has produced precise and even unique physiological mechanisms to ensure fertilization and embryonic development. He cites in particular physiological and biochemical mechanisms involved in egg-shell formation and resolves unique egg-shell properties that provide for the developing embryo a self-contained environment that is precisely regulated. Finally, Anya suggests that the success of nematode parasites emerges from their ability to maintain efficient reproductive procedures with only little morphological specialization and retained capability for biological variation in ontogeny.

The first of two short updated articles is by J. F. Michel, who deals with topics seen by him to contain new ideas or developments of special interest. At the time of writing of his full review the realization was forthcoming that in temperate regions nematodes of grazing animals complete few generations each year, some being monocyclic. Since that time appropriate methods of investigation have been more widely used and there has been good understanding of the epidemiology of various infections, and also progress in that control methods with deliberate aim now tend to replace haphazard use of anthelmintics and unsatisfactory practices. It is now being asked whether or not great progress in epidemiological studies have been due to modern approaches and new techniques which might also be applied to other problems. The review deals with methods of investigation, free-living and parasitic stages, with parasitic gastro-enteritis in sheep and in cattle, and with parasitic bronchitis in cattle. A final section of the review deals with opinions on the control of nematodes. In the earlier review, it was said that methods of control of nematodes should be based on factors which restrain the increase of parasitic populations in the field, and with specific objectives in view, small practices being regarded as irrelevant. Here are expressed various opinions which deal with the design and implementation of control measures and their integration into farming systems, and the final conclusion is reached that the opinion that measures for the control of helminthiasis must conflict with

agricultural objectives holds only where systems of management have been devised "without regard to the hazard of parasitism".

In the second short updated review on the immunology of schistosomiasis, S. R. Smithers and R. J. Terry state that some apparent anomalies have been cleared up and some questions have been answered since the publication of their earlier review. They deal here with acquired immunity, parasitic antigens and the schistosome granuloma, but the ultimate goal of effort in this area of research, successful vaccination against schistosomes, is still to be developed. The only recent developments on innate immunity refer to the death of cercariae in the skin. In respect of acquired immunity in schistosomiasis, the concept of concomitant immunity, mechanisms of immunity and host antigens are discussed. Concomitant immunity has been demonstrated in a number of experimental systems and may operate in human infections, but many more epidemiological studies must be carried out in various parts of the world before its existence can be accepted fully. Questions regarding parasitic antigens are discussed under the headings of surface and secretory antigens and circulating antigens respectively. Antigens of schistosome origin can be detected in urine or plasma of infected hosts, including man. The schistosome granuloma is considered on its own account, much work having been carried out on granuloma formation. It is concluded that immunological control of schistosomiasis is still to be achieved, but some approaches are under investigation, including the use of living cercariae and schistosomules, and the use of purified antigens. Some of these approaches may lead to success but the belief is expressed that success will depend on a greater understanding of the detailed mechanisms of immunity and devices by which the parasite may evade such immunity.

Once again it is time to say thank you for assistance given by friends and colleagues, who in turn have given expressions of thankfulness for help gladly given to them. It is good to know that all who may can help in this way for the benefit of all who hope for the success of this series of books. My thanks are due and graciously delivered to colleagues in the employ of Academic Press who have worked long and arduously to produce this book, sometimes in the face of setbacks of irritative kinds. I myself am grateful for the opportunity to work on this project, hoping that in pressing on towards the limit of existing knowledge we are laying down some wisdom as well as a fund of information and ideas that will assist research in the field of parasitology and help teachers and researchers along their tiresome terrain.

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Conception and Terminology of Hosts in Parasitology

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I. Introduction

The habit of defining the partners of a parasitic association as "host" and "parasite" gradually came to the fore around the middle of the last century. The term was used, for example, by Leuckart (1852, 1863, 1879), Küchenmeister (1855, cf. 1857) and van Beneden (1858, 1875). These obviously were the sources from which the term "host" took its way directly into English and other languages (cf. The Oxford English Dictionary Vol. V, 1933). Lankester made this comment on his translation of Küchenmeister (1857): "Host is a literal translation of the German 'Wirth', and although not perhaps previously used in the above sense in the English language, I have adopted it to prevent a somewhat tedious circumlocution." In earlier German and Latin literature, the use of the term had been preceded and accompanied by a number of different terms, such as "Wohnthier", "residence", "carrier" and "habitaculum". "Carrier" was still used by Leuckart (1863, 1879) as a synonym of host. Terms other than host were also used quite conspicuously in French texts. van Beneden (1858), for example, made reference to "hôte", "patron" and "île".

The growing use of the term "host" in helminthology and parasitology in the 19th century was accompanied by a trend to apply the self-same term

to one partner of a relationship (or association) different from that above, and indeed to apply it in a merely ecological relationship. (A differentiation should be made between association and biocenotic relationship. While association presupposes different partner species to draw near to each other for long-term symbiosis, this is not only biocenologic relationship, but autecologic community as well.) While the host–parasite partner system still lent itself to unambiguous definition, another couple of partners came in, loaded with much ambiguity and different possible applications, host–guest. The term of host consequently found its way into ecology, where its definition grew more difficult and its usage embraced different meanings. One of the problems facing us today, therefore, has something to do with the fact that parasitology, to some extent, can be considered a sub-area of ecology, but yet no common denominator can be found for the term of host and its applicability to both parasitology and ecology. The problem is further aggravated by the present notion generally given to parasitology, which is not identical with the theory of parasitism at all. Parasitology, in its present usage, usually is restricted to zooparasitology, which does not include parasitism in microbiology and phytopathology. Thus we see that the term of host has undergone differentiated development even within the one area of parasitism. Another, partially deviating usage of the term may be recorded from epidemiology.

After all, even within zooparasitology the host concept and its treatment have created additional problems and aspects, necessitating, from the middle of last century onwards, some differentiation between several categories of host. For these categories designations then had to be hammered out. Growing knowledge of developmental cycles, unknown before, and further progress in parasitology have led to the demand for adequate definitions and revision of conventional names, a process which is still in full swing.

What is needed now is to find a comprehensive definition which covers all aspects of the notion of host, with identical definitions and terms for universal phenomena and with specific terms and notions for special cases. The concerted demand for identical definition of universal phenomena is likely to come both from ecologists and from parasitologists, although it may be derived as well from the integrated position of the latter with the former. A critical analysis must be made of terms so far used homonymously (e.g. "transport host") or synonymously (e.g. in French publications, "hôtes intermédiaires", "hôtes transitoires", or in German texts, "zweiter Zwischenwirt", "Hilfswirt" ("second intermediate host", "auxiliary host")), and certain terms should be verified for their applicability. For example, separate developments in zooparasitology, microbiology and phytopathology have led to terminological separation, and sometimes wrongly so; while on the other hand the applicability of certain terms is too small even within one of these three fields. Analysis of the host notion, consequently, will imply both integrating and differentiating aspects.

There is now a growing urgency for quite general clarification of zooparasitological terminology, in addition to the general need for another

theoretical foundation for the host notion. The demand for such clarification is made with regard to the current steady growth in knowledge, against which background the theoretical ideas and terms of the last century cannot be considered sufficient any longer. The situation must also be changed whereby common terms quite often suffer from arbitrary interpretation and usage. In another paper, devoted to the same problems in the field of helminthology, I have tried to characterize the intricacy of the situation by calling it a "chaos of names" (Odening 1968–69), a chaos which may be attributed to several causes, among them conceptual confusion relating to certain terms, lack of care in using them, and sometimes even the absence of adequate terms at all. The following statement was made about the situation in the German-language area by Piekarski (1954): "The terms available are being used all the time with no clear-cut designation, and so equality is being established between intermediate and incidental, definitive and principal or intermediate and transport host. I feel, however, that certain specific characteristics might be coordinated to each of these categories." In English literature, for example, Noble and Noble (1964) have used one and the same term, "monoxenous", to label two different phenomena at two different points in the same book: 1. "Parasitic species employ either one host, and are thus called *monoxenous* (or monoecious), or two or more hosts and are called *heteroxenous*" (p. 34); 2. "The term *monoxenous* indicates limitation to a single host, as occurs with adult *Wuchereria bancrofti*. *Oligoxenous* is used to describe parasites, like adult *Echinococcus granulosus*, which have a small host range. *Polyxenous* refers to the condition involving many suitable hosts, or relatively little host specificity, as occurs with *Fasciola hepatica*" (p. 618). The meaning of "reservoir host" in Russian helminthological literature is found to differ from that (= euparatenic host) in English or German texts.

What is mostly needed is to check the translatability to other areas of terms originally used only in helminthology, such as "definitive host" and "intermediate host", and to unify the terms which are restricted to helminthology. These are, more or less, terminological problems which can be found in most languages. In certain cases, however, peculiarities should be brought to general attention, with a view to obtaining a terminology of widest possible universality. I shall limit my own reference, in this context, to English, French, Russian and German words.

This contribution is intended not primarily to introduce fresh terms but rather to clarify existing terms, with a view to facilitating communication on the subjects concerned and making them more easily understood. It is quite natural, on the other hand, that expansion of knowledge will necessitate expansion of terms; although there is just as much justification in dropping obsolete terms which have ceased to be meaningful. It goes without saying that no definite solution can be found to the problem as a whole merely by this attempted synopsis. What can be obtained at best is a reasonably comprehensive presentation of the problem, in the hope that more attention will be given to it by those professionally concerned in the disciplines involved.

II. Common Host Concept in Ecology and Parasitology

A. Polyhospitalism and Monohospitalism

In ecological literature, juxtaposition of "host" and "guest" (or, sometimes, "consort") may be found occasionally in the context of classification of heterospecific biocenotic relations and associations. The term host–guest relationship may be applied to label direct relations ("biosystems") between two dissimilar organisms, with three distinct stages being differentiated by degree of association:

(a) no companionship (i.e. no symbiosis of individuals);
(b) companionship with no permanent physical contact (parekia *sensu lato*);
(c) physical-contact association (somatoxeny).

Item (a) will include temporary parasitism and, partially, symbiosis *sensu lato.* Host–guest relations are somewhat incomplete in terms of successive polyhospitalism, in that one guest will successively or alternately call on several host individuals which, consequently, appear to be temporary or intermittent hosts. The "visit" of the guest usually will be restricted to contact with the surface of the host, contact from outside. (Hence, temporary parasitism will always be of ectoparasitic nature.) This host–guest relationship, in a way, is comparable to phytophagism of wild-living animals and predation (Fig. 1).

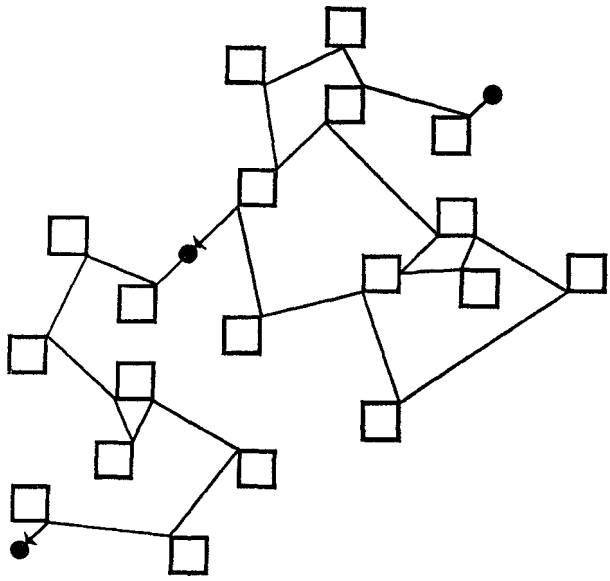

Fig. 1. Successive polyhospitalism. Each rhombic sign marks a temporary host, connecting lines represent host-to-host routes taken by individual temporary parasites, and black dots mark the occurrence of an individual temporary parasite. (Odening, 1974b.)

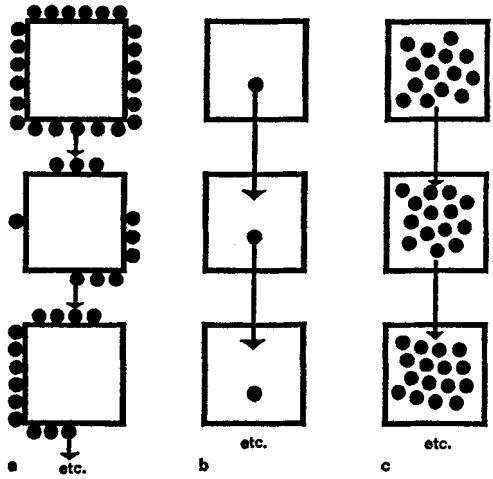

FIG. 2. Monohospitalism (somatoxenous, homoxenous). Each square represents one host of parasites. Arrows show routes taken by propagative forms of parasite to next host Black dots mark consecutive occurrence of individual parasites (each dot standing for one individual. (a) Ectoparasitic type of multiplicative reproduction (e.g. many arthropods with stationary parasitism, monogeneans); (b) entoparasitic type of propagative reproduction (frequent among helminths); (c) entoparasitic type of multiplicative reproduction (viruses as a rule, bacteria and protozoa quite often, sometimes fungi). (Odening, 1974b.)

Most of the host-guest relations quoted under (b) can be designated by the term simultaneous polyhospitalism, in that one guest has several partner hosts at a time (brood parasitism; social parasitism). Host, used in this context, always defines one link in a group of hosts. One lower type of such relations will be based on simultaneous polyhospitalism but accompanied by emerging monohospitalism (cohabitation in context of synekia).

All relations to which reference can be made under (c) are exclusively based on monohospitalism: one host organism will live in long-term physical contact with one guest species (referred to as stationary hosts or, rarely, transit hosts) (Fig. 2).

This kind of organization (Odening, 1974a) differs from other possible organizations of biocenotic relations (for example, the most common organization by advantage, disadvantage or indifference) in that it is based on development from lower to higher levels, from simple to involved integration stages: in other words, development which, generally, is irreversible. This may be seen clearly in the case of somatoxeny from which no way leads back to free life. However, probiotic and antibiotic relations will be changeable, at least with regard to indifferent relations, with different interpretations being possible. This is likely to demonstrate the wide gap that actually opens between these two partner systems, temporary parasitism and genuine (stationary) parasitism.

Physical-contact associations play a sizeable role in nature, since at least

one-fifth of all organisms are guests in this kind of association. By adding to them the hosts, one will find that almost every organism is partner, guest or host, to a physical-contact association. Hence, physical-contact systems are of universal occurrence in life, some of them being as old as life, others continuously originating even today from various levels of life development.

The following stages must be differentiated in physical-contact associations:

1. Ecological somatoxeny
   (a) Epoekia, entoekia, phoresy, zoochory
   (b) Commensalism
2. Ecophysiological somatoxeny
   (a) (Stationary) parasitism
   (b) Mutualism (symbiosis *sensu stricto*)

Utilization of the host body by the guest will be restricted to ecological use, in the first case, but include physiological use as well, in the second.

Such organization, again, reflects irreversible development from lower to higher levels (Fig. 3). Ecological somatoxeny may occur in two forms which are different in quality, one of these being a purely dimensional relationship (with the host serving as dwelling area, habitat or means of transport: epoekia, entoekia, phoresy, zoochory), the other one being both dimensional and nutritive (with the host providing not only housing, but the guest sharing the host's feed prior to intake and conversion: commensalism).

Ecophysiological somatoxeny is to describe ecological plus physiological connection between two organisms, which can mean one-sided metabolic dependence of the parasite on its host, in the case of parasitism, or metabolic interdependence of the two partners, in the case of mutualism (Baer, 1951; Cheng, 1967; Smyth, 1962; Šul'c and Gvozdev, 1970; Odening, 1974a).

Hence, certain direct biocenotic relations between two dissimilar organisms may as well be called host–guest relations by tradition in ecology. Yet such relations may be quite variegated or even differentiated. A common definition, therefore, will hardly be feasible.

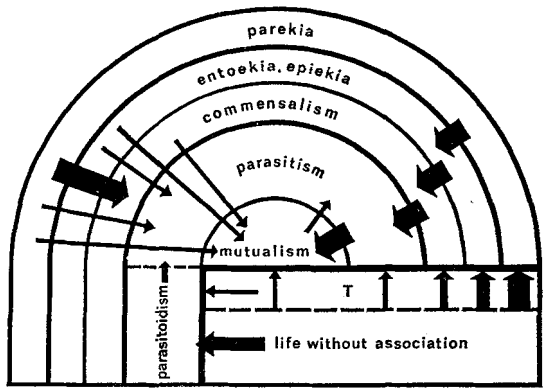

FIG. 3. Relations and possible transitions between interspecific levels of association. (T) is the area of transition between free life and association. (Odening, 1974a.)

Analysis of the host notion and its usage is likely to suggest that no polyhospitalism will be possible unless an animal (or micro-organism) species is involved as guest; it will not (or rarely) occur between higher plants which merely enable monohospitalism with physical-contact relationship. Unambiguous definition of monohospitalism is possible on the basis of physical-contact association. In other words, all the heterogeneity of, and problems relating to, a general definition of the host notion in ecological context are attributable to polyhospitalism. Due consideration of hospital conditions will depend, in the first place, on comparison between these and ahospital conditions, in the context of direct relationship between two organism species of which at least one usually will be an animal (or micro-organism). Generally, predation and most of phytophagism of free-living animals will be treated as an ahospital relationship without companionship. A distinct demarcation line between predation and any kind of hospitality is drawn by the very fact that the relationship as a whole may be readily materialized by immediate killing of the victim by the predatory partner, whereas problems of dubiosity are likely to crop up in the context of phytophagism, for example, when it comes to delimitation from temporary parasitism. A given relationship is identical with, or similar to, predation in cases in which phytophagous species eat up at a high rate a nutritive plant, to the point of destruction. One may suggest a parallel to temporary parasitism, if plant damage is slight enough for recovery (with plant suckers usually being defined as temporary parasites or, sometimes, even as stationary parasites). Then, if it comes to relations without companionship, prey parasitism, temporary parasitism, and a high percentage of conditions usually referred to as symbiosis *sensu lato*, may come in the category of (poly)hospital conditions. These will be based on occasional meetings between the two partners, and while in such cases the guest will draw something from the host (usually feed), this will not be accompanied by killing the latter. The host will be called on actively by the guest. If it is companionship without permanent physical contact, a group of hosts usually will be involved in one host–guest relationship, with the guest receiving something from the whole group—housing, usually feed as well, or services. The guest, again, will be the active partner. Quite different relations will result from cases in which living organisms are subjugated for service by another organism. "Slave keeping" of certain social Hymenoptera or domestication by man are some examples to this effect. These are "involuntary" guests under forced control.

The notion of host in the context of ecological polyhospitalism should be understood allegorically, because of the impossibility of unambiguous definition. It is to some extent comparable to notions such as brood parasitism, predatory or prey parasitism, etho-parasitism, social parasitism, storage and dimensional parasitism, and even temporary parasitism; none of these actually falls in the category of genuine—stationary—parasitism. All these "parasitisms" have only one point fully in common; they exist at the expense of other living organisms which usually are not killed. This very aspect has open (or flowing) boundaries to phytophagism and predation and can be

reversed to the opposite extent, violent death of the prey. Similarly, flowing borders exist between the hosts and prey of predatory species in (successive) polyhospitalism.

Temporary parasitism is not genuine parasitism, but temporary visit of a host for the purpose of feed intake (intermittent predation). Short periods of feed intake on the host will then usually alternate with much longer periods of free life. The hosts, accordingly, are intermittent temporary hosts.

Transition from temporary to stationary parasitism may be observed on ticks where the phase of feed intake is prolonged on the host. In the case of Ixodidae, this will imply inevitable restriction of the number of hosts. Ixodidae may have one, two or three hosts. However, typically, polyhospitalism tends to be linked up to an uncertain number of intermittent temporary hosts.

B. HOSTS IN SOMATOXENOUS ASSOCIATIONS

1. *The notion of host in somatoxeny*

The host will act as "carrier" of the other partner in monohospitalism on the basis of somatoxeny. The following levels may be involved:

(a) host = carrier (epoekia, entoekia, phoresy, zoochory);

(b) host = carrier + ecological feeder (commensalism);

(c) host = carrier + ecophysiological partner (parasitism, mutualism).

Therefore, in general ecology, somatoxenous monohospitalism will be accompanied by the following two basic types of host:

(i) "ecological hosts" or transport hosts (with functions according to (a) and (b) above);

(ii) "ecophysiological hosts" (with function according to (c) above).

Applied to parasitology, this will result in the occurrence of the following types:

Transport hosts (with function according to (a)).

Parasite hosts (with function according to (c)).

Parasitologically, somatoxeny necessarily implies stationary parasitism, that is genuine parasitism, lasting a long time and covering all or part of an individual parasite's life-span. Stationary parasitism will not end until the death of the host or parasite. It may end also by departure of a transitory parasite. Hence, parasite hosts (just as transport hosts) may occur on two models each, namely as stationary hosts and transitory hosts.

2. *Transport hosts*

Differentiation between ecological and ecophysiological somatoxeny implies basic qualitative differentiation of the hosts involved. Ecophysiological somatoxeny means integration at higher levels, but this will not rule out the temporary use of hosts (transport hosts) by many kinds of parasites at lower levels of integration (Fig. 4). After all, there is also a wide range of parasites with phases of free life. Most of the transport hosts are optional for the parasites (with Tabanidae being an example of a compulsory transport host for *Trypanosoma evansi*).

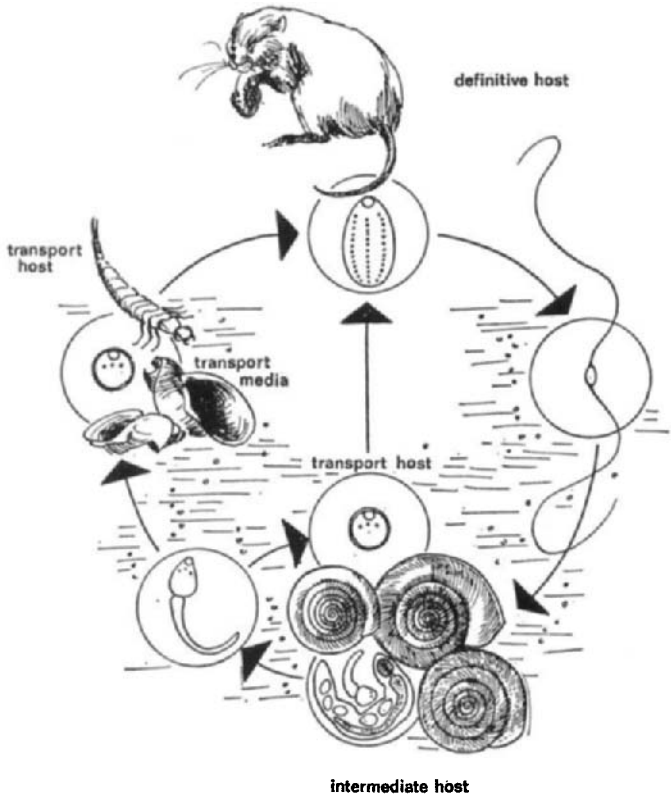

FIG. 4. Life-cycle of *Notocotylus noyeri* (trematode): a two-host cycle with transport host or transport media. (Odening, 1966.)

The term of transport host has often been used, unfortunately, in English and German literature as a synonym for paratenic host or, rarely, to describe certain kinds of intermediate hosts. (Both paratenic as well as intermediate hosts actually are parasite–hosts, because of the presence of a parasite–host relation; cf. p. 50). As a rare exception Sprent (1963b) may be cited: "The term transport host does not have quite the same meaning as paratenic host."

The treatment of host animals often differs essentially from that of host plants, which cannot be justified as an approach at all. Plants often are referred to as substrates or the like, with the word host being cautiously avoided. However, transport host is a phenomenon of fundamental and general relevance to ecology as a whole (shown also by manifestations such as epoekia, entoekia, phoresy and zoochory). The expression should thus also be used in the same sense in parasitology. (The cycle of *Fasciola hepatica*, accordingly, is a compulsory two-host cycle, with a plant being inserted as optional transport host.)

Transport host must be considered a category of universal relevance throughout parasitology, on account of its use by almost all big parasite

groups (viruses, bacteria, fungi, protozoa, helminths, arthropods). Greatest importance must be attributed to it in transmission from parasite host to parasite host of many parasite species, and in the propagation of parasite populations. In epidemiological context, transport hosts of pathogenic parasites are defined also as anadaptive transmitters (a pointer to the absence of parasite–host relation).

III. Ecological and Epidemiological Aspects Relating to the Host Concept in Parasitology

Transmission, quite generally, means transfer or conveyance of a parasite to a host (receptor, recipient) from a given position of the parasite (the donor, which may be another host or environment). The route taken by a parasite from one host to another of the same category is called transmission cycle. The contact chains, through which general contact is established between organisms in biocenosis, are specific chains of parasite transmission, in this context. A parasite population will depend urgently on the availability of transmission chains for survival. Continued transmission will ensure connection in terms of space and time between parasite and host populations. Transmission chains may be "horizontal" or "vertical" (transovarian, from mother to child). Any epidemiological classification by continuity of horizontal transmission chains will have to depend on a major criterion by which every host–parasite system can be assessed: host-to-host transmission of parasites by physical contact in whatever form (contaminative, phagous, inoculative), or free dissemination of parasites (into the open environment). The answer to this alternative question will be the yardstick by which all sorts of hosts in somatoxenous relations, transport and parasite hosts, can be grouped in the two basic ecologico-epidemiological categories of transmission, as transmitters or disseminators (Fig. 5).

Three groups of horizontal transmission chains are differentiated by continuity.

1. Continuous transmission chains with no exogenous phase will carry parasites directly and uninterruptedly from one host to another (direct and contact transmission). Parasites, as a rule, cannot survive or even exist outside a host. The pathogens of venereal diseases, for example, are transmitted by contact (between mucous membranes). The same applies to rabies virus (transmitted through bites), lice, Mallophaga, certain monogeneans, *Trichinella* (consumption), and virus of the mosaic disease.
2. Continuous transmission chains with exogenous forms of propagation have attached to them a (propagative) exogenous phase which, in their case, is needed for transmission. The parasite, by necessity, will be in a (more or less limited) different transmissive condition during this exogenous phase (for example, viruses and bacteria in aerosols, faeces, urine or other media; certain bacteria, protozoa, and fungi in spores or cysts particularly formed and generated for the exogenous phase of transmission; other forms of propagation, such as ova and larvae of helminths).
3. Discontinuous transmission chains describe routes on which life on a host

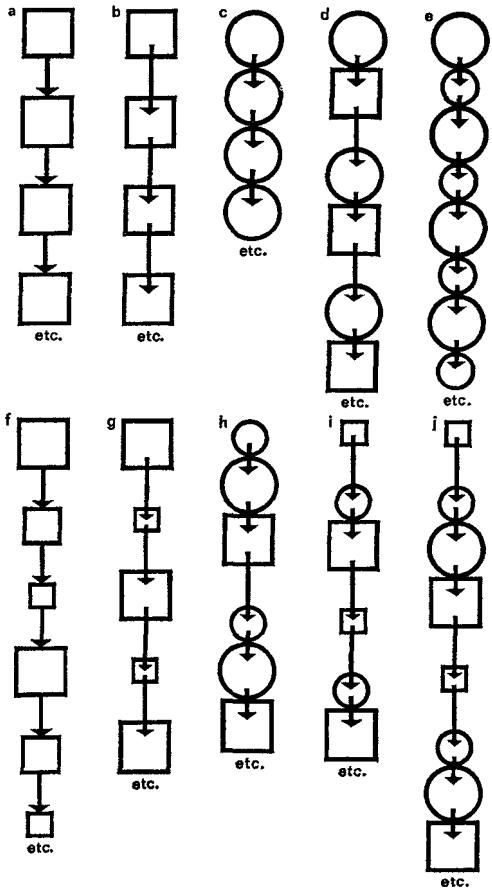

FIG. 5. Chains of parasitic transfer. Squares represent disseminating and circles represent transmissive hosts (different sizes representing different ontogenetic host categories). (a) Homoxenous–disseminating–ectoparasitic sequence of hosts; (b) homoxenous–disseminating–entoparasitic sequence of hosts; (c) homoxenous–transmissive–entoparasitic alternation of hosts; (d) diheteroxenous alternation of hosts, i.e. entoparasitic and transmissive combined with disseminating (e.g. *Taeniarhynchus saginatus*, *Taenia solium*); (e) diheteroxenous alternation of hosts, i.e. entoparasitic, transmissive (e.g. *Plasmodium malariae*); (f) triheteroxenous alternation of hosts, i.e. ectoparasitic, disseminating (ticks with three hosts); (g) diheteroxenous alternation of hosts, i.e. entoparasitic, depending on alternation of generations (original form represented by digenetic trematodes); (h) triheteroxenous alternation of hosts, i.e. entoparasitic, with one disseminator and two transmissive hosts (e.g. Pseudophyllidea); (i) triheteroxenous alternation of hosts, entoparasitic, with two disseminators and one transmissive host (many digenetic trematodes); (j) tetraheteroxenous alternation of hosts, entoparasitic, with two disseminators and two transmissive hosts (trematode genus *Strigea*). (Odening, 1974b.)

will be interrupted for an independent and separate hostless life period with no direct functionality for transmission. Such transmission chains then will be temporarily interrupted (free-living generations of certain helminths,

saprophytic phase of many fungi, certain bacteria). Facultative generation of permanent forms (e.g. permanent spores) by which to bridge the gap of a hostless period (e.g. hibernation of fungi with parasitic affinity for plants) is a process typically observed in the context of discontinuous transmission chains.

While, quite clearly, all hosts in the first group will be transmitters, disseminators will be involved in the other two groups. Certain viruses and bacteria may be transmitted through both dissemination (ground, water, atmosphere) and transmitters (bioreceptive route) from one and the same host.

Differentiation between active and passive transmitters, though quite common, is not as accurate as differentiation between contaminative, phagous (peroral) transmitters, on the one hand, and inoculative transmitters, on the other, with phagous transmitters being considered as passive and inoculative transmitters as active. Differentiation between "active" and "passive" will not always be practicable with satisfactory accuracy in cases of contaminative transmission, in which three additional types will be involved for distinction, these being tactile, excretory and lymphatic.

In addition to this very general ecological differentiation between disseminators and transmitters, the term "transmitter" is used with different meanings in a narrower context.

Every host, quite generally, can be called a transmitter, provided that it passes on a parasite to another host by any kind of contact whatsoever. The very nature of the parasite, homoxenous or heteroxenous, does not matter in this context (cf. p. 24). An unambiguous situation will result, if in a heteroxenous cycle a disseminator alternates with a transmitter or a transmitter with a facultatively inserted transport host in a homoxenous cycle. If, however, in a heteroxenous cycle or in a homoxenous cycle with a facultatively inserted transport host two transmitters are alternating, only one of these usually will be defined as "transmitter". Yet motivations for such an approach are quite diverse.

Hosts of malarial *Plasmodium* are transmitters in the widest sense, both in the context of man and mosquitos. Epidemiologically, however, only the mosquito will be defined as a transmitter, or, in a more specific way, as vector. Consequently, if in heteroxenous cycles there is alternation between two transmitters, the reference host in focus (man, domestic animals, crops) will never be labelled as transmitter in an epidemiological context (nor in epizootiology or epiphytology). It would doubtless be the most simple and comprehensible method and the most plausible approach to use the name vector for all transmitters involved in transmission to an epidemiological reference host. Yet vector has no generally consistent usage. Sometimes, the name vector has been used also to designate disseminating hosts from which an epidemiological reference host has been infested (e.g. molluscan hosts of *Schistosoma*, in which context the following conclusion was suggested by Noble and Noble (1964): "Normally, when two hosts are involved, the intermediate host—usually an invertebrate—is called the *vector*."). In this example, vector is not terminologically identical with transmitter. It would

be better, then, for the purpose of unambuigity, to distinguish "direct" from "indirect" vectors. Direct vectors would be transmitters which transmitted parasites to a reference host. Indirect vectors would be disseminators by which a reference host was preceded—for example, the molluscan hosts of a *Schistosoma* which must be distinguished from hosts that precede a direct vector, e.g. the first intermediate host of *Diphyllobothrium* or *Clonorchis* which are themselves transmitters (*Diphyllobothrium*) or disseminators (*Clonorchis*). The term vector, when used in a phytopathological context, usually refers to an organism which can trigger pathogenic infestation on account of its own activity ("locomotor capacity over distance"). Mere conveyance of a pathogen to a host, without causing infestation of the latter, will not be considered as vectorial capacity. More often than not the term vector will be even more narrowly used to refer only to arthropods or invertebrates.

Elkin (1973), in his comments on general epidemiology, has avoided the term host and made reference throughout merely to "carriers" and "transmitters". This usage of "transmitter", however, was meant to describe merely arthropods which were able to transmit pathogens to the "carrier". While in helminthological literature of the last century the German word Träger was often used as a synonym of host, English (and modern German) usage of "carrier" in an epidemiological context is often meant to describe something like a "quiescent carrier", one "who harbours the parasite without showing clinical evidence of infection and thus serves as a potential source of infection to others. In other words, the carrier represents the normal state of infection in which there is an equilibrium between the host and the parasite" (Belding, 1965).

Yet the term transmitter is not used only with regard to epidemiological viewpoints and in a limited sense. The transmitter concept will often be limited under phylogenetico-ecological viewpoints, when it comes to host-alternating parasites or parasites which make use of transport hosts. Alternation of host, after all, will often be based and motivated in the context of disseminating hosts in a certain sequence in which an additional "transmitter" (*sensu stricto*) will have as its only function to facilitate, promote and optimize transmission of a parasite onto the host which comes next in that sequence. This may be shown even by facultative participation of transport hosts in transmission of a parasite species. A "transmitter", in this context, will be determined both phylogenetically (secondary) and ecologically.

The original transmissive function may still be seen in cases in which, with the aid of a transmitting host, parasitic propagation forms are conveyed more easily, at higher rate and with lower loss to a representative of the category of which the source host (disseminating in this case) is part.

Most of those hosts that provide feed to source hosts are transmitters in this limited context. Yet such transmitters will no longer be clearly identifiable in all cases. If, for example, a disseminating host is lacking in a given cycle, it will not be easy to see immediately which of the hosts acts as

"transmitter" in the above sense. Such difficulty may occur mainly with regard to hosts which, in themselves, are parasites or temporary parasites of another host. Viruses, bacteria and protozoa which are transmitted through haematophagous articulates are examples to this effect (unless the articulates simply are transport hosts). While the arthropod host usually comes first in history for haemoflagellates, the vertebrate host will be the first one, if Haemosporidia are concerned.

The most general interpretation of the transmitter concept (as opposed to disseminator) seems to be the least ambiguous approach. Used with such comprehensive meaning, a transmitter can be parasite host or transport host, essential to a cycle or facultatively involved. Differentiation between transport hosts on the one hand, and parasite hosts on the other, has as its counterparts anadaptive and adaptive transmission.

An adaptive transmission, as a rule, will be facultative by its very nature and characterized by more or less accidental transport of parasites. Here, the relation between parasite and transmitter will be equal to epoekia, entoekia, zoochory and phoresy. In other words, there will be no mutual adaptation. (Yet unilateral adaptation of phoretes to a given transport host may be present in phoresy.) The transmitter, in such cases, will always be a transport host. Therefore, several different designations have been introduced to describe such transmission, which may often be called phoretic, accidental, unspecific, acyclic or mechanical. However, such relaxed forms of transmission should not be confused with facultative paratenic parasitism of helminths (pp. 50, 59), which is on a higher level (genuine penetration of the paratenic host as opposed to superficial external adhesion or intestinal passage). Transmission from contaminated matter to any other matter will always be of facultative nature. Anadaptive transmission implies that the parasites adhere to the outer surface of a transmitter (ectohaptic) or pass its intestine unchanged (entotransitive). On entotransitive transmission the parasites will be harboured by the transmitter for only a short period, and on ectohaptic transmission their adhesion will be limited: in such context, reference will be made to non-persistent (transitory) presence of parasites. This, actually, is the pattern of transmission and propagation for bacteria, fungi (spores), viruses and some protozoa. Anadaptive transmission is an infantile stage in the development of transmissive relations and has been found to overlap parasite spreading via transport hosts which convey parasites into open environment (phoresy, zoochory) rather than onto a destination host (the same applying to guests other than parasites). The notion of a transport host, therefore, is wider than that of a transmitter.

"Preparatory" hosts seem to be quite a distinct category. They are probably not genuine parasite hosts, but rather some sort of transport hosts, yet with some "preparatory" function (essential and compulsory to parasites?), a function to prepare for infectivity. The oocysts produced in the host *Lithobius* by the protozoon *Adelea ovata* are believed to become infective only when they have settled in the intestines of Isopoda (*Oniscus*) (cf. Dogel', 1962). Special transport hosts, in this context, are those which are able to serve additionally as "libérateur passif des oeufs" in the compulsory two-host

cycle of the trematode *Paralepoderma brumpti* (Buttner, 1950–51). Mention should be made also of a unique phoretic relation between the larvae of the nematode *Dictyocaulus filaria* and the sporangia of the fungus *Pilobolus*.

Adaptive transmission usually is compulsory (but paratenic parasitism of helminths is not obligatory, p. 50). It will take place more or less regularly, with parasite and transmitter being adapted to each other at different levels. This is a genuine host–parasite relation. In certain cases, the transmitter may be affected by disease. Development and/or reproduction of parasites in a transmitter is characteristic of this type of transmission, an adaptive transmission which is also called essential, biological, cyclic or specific. (An exception must be made for euparatenic parasitism which is not accompanied by development or reproduction of parasitic forms, cf. p. 59*.) The parasites will be recordable from the transmitter for a long time or even throughout their own life span (persistent or circulative parasitic forms). If it comes to parasites which, while being harboured in a transmitter, require for transmission development of distinct transmissive forms, transmission cannot yet begin on fresh arrival of parasitic forms in the given transmitter. (A certain time will have to elapse, celation or circulation time, before transmissive parasitic forms have developed.) On transition from anadaptive to adaptive transmission, prospects for actual occurrence of transmission will increase along with long-term infestation of transmitters as the source for infestation of the recipients.

A preceding and a succeeding host will be present in any host sequence (series of obligatory and facultative parasite hosts and transport hosts). The preceding host, if transmitting, will act as donor. Epidemiologically, any host will act as reservoir in a wider context, with the succeeding one being receptor. Any transmitting receptor host will be donor, provided that invasive parasitic forms are harboured by it. The epidemiological notion of reservoir is meant, in a wider sense, to include not only hosts, but also other sites and any object in the open environment where parasites can exist and from where they can be transmitted to receptors. The terms donor and reservoir, sometimes, are used as synonyms. In a limited epidemiological context the terms parasite reservoir or reservoir host, generally, are used to define hosts which may be a (transmitting or disseminating) source of infestation of a given epidemiological reference host. Hence, these may be hosts of different categories. "Animals that harbour the same species of parasites as man are known as reservoir hosts. Such hosts ensure the continuity of the parasite's life cycle and act as potential sources of human infection" (Belding, 1965). Meanwhile, in Soviet and Soviet-influenced helminthological literature, the term of reservoir host is used in the sense of paratenic host. While, epidemiologically, paratenic host is also a reservoir host, the epidemiological notion of reservoir host is much wider. Reservoir or reserve host is also used to mean "quiescent carrier".

Under certain circumstances, differentiation can be suggested between

* Euparatenic hosts do not really fit into this breakdown of transmitters, in that they may also be anadaptive transmitters with parasite–host relation.

primary and secondary reservoirs and, within each of these, first-level and second-level reservoirs, depending on the importance of parasite host species as reservoirs for infectious diseases or those caused by infestation. Primary reservoirs will then be those which always act as reservoirs in typical occurrence areas of a parasite. Secondary reservoirs will be those with additional and occasional reservoir function, mainly outside typical occurrence areas. Classification as first-level or second-level reservoirs will depend on the importance to continuous preservation of transmission chains. The epidemiological importance of individual host species is subject to possible modification by aspects of landscape, geography in a wider context, biocenosis, and time. Hence, primary reservoir areas are the primary centres (foci) of parasite occurrence from which parasites are spread to secondary reservoirs (usually through migration of animals). Wild-living game in free nature can be classical primary reservoirs (with the primary circulatory cycle between them). Hosts that represent certain geographical regions or landscapes should best be called vicarious hosts (a term which sometimes is chosen to replace paratenic host in English usage, which, however, cannot be recommended).

IV. Hosts as Categories of Suitability for Certain Parasites

Parasite hosts are characterized by a (stationary) host–parasite relation, as opposed to transport hosts or temporary or intermittent hosts. Such stationary relation, as a rule, involves ecophysiological use of the host by the parasite and response of the host. The host–parasite relation may be obligatory or facultative, because obligatory or optional parasites can be involved as partners.

However, some explanation will be necessary, if the above conditions are to be applied to the participating hosts, since in this context the definition obligatory or facultative host will not be as unambiguous as the definition obligatory or facultative parasite. It is true that a facultative parasite will have free host choice. An obligatory parasite, basically, will be bound to existence in a somatoxenous parasite–host relation. Yet an obligatory parasite may have hosts which do not fall in the category of "obligatory hosts". After all, the terms obligatory and facultative have two additional usages (in the context of obligatory parasites), and this may lead to confusion.

Cycles determined by obligatory parasites and, consequently, obligatory hosts may additionally also involve facultative parasite hosts (e.g. paratenic hosts, additional hosts of adults). Nevertheless, these hosts, as parasite hosts, will be essential to the survival of one individual parasite, but not to survival of the whole parasite species (which depends only on the hosts compulsory for the cycle); cf. p. 50.

Another usage of obligatory and facultative with reference to parasite hosts was introduced by Skrjabin and Šul'c (1937, 1940) and adopted by Šul'c and Gvozdev (1972) and, therefore, is quite common in Soviet literature. It is a usage of host categories by susceptibility to a certain parasite species (or helminth species). Skrjabin and Šul'c (1937, 1940) grouped host–parasite

relations by obligatory and facultative variants and, accordingly, a differentiation was made between obligatory and facultative hosts and parasites. This classification was later improved by Šul'c and Davtjan (1954) (cf. Table I and Fig. 6).

In obligatory host–parasite systems (Skrjabin and Šul'c) host and parasite are adapted to each other historically, ecologically, and physiologically, their coincidence being inevitable. In facultative host–parasite systems, however (Skrjabin and Šul'c), historical and ecophysiological links between the two partners are much weaker and more of an accidental nature. Mutual adaptation, accordingly, will be weak (or even absent). However, development of parasites will basically be ensured. In abortive host–parasite systems (Šul'c and Davtjan) the parasites (helminths) will succeed in collocation and in beginning some development but fail to bring the process to completion. In captive host–parasite systems (Šul'c and Davtjan) there will be only collocation of parasites (helminths), but development will not go beyond an initial phase.

In other words, both obligatory and facultative host–parasite systems provide possibilities for parasites to grow to infestivity or sexual maturity (more generally, to transmissivity or dissemination capacity) and, consequently, for their propagation and/or reproduction. The partners involved have been called genuine hosts and genuine parasites by Šul'c (1958).

The partners to abortive or captive host–parasite systems, however, have been called strange (or false) hosts and strange parasites by the same author. The parasites in the latter systems will not reach infestivity or sexual maturity, since biological deadlock, some sort of a blind alley, will be caused by the strange or alien hosts, and so further propagation of parasites usually will be impossible.

The following suggestion has been made by Šul'c and Gvozdev (1972): "These two categories of host–parasite relation (abortive and captive) might perhaps seem to be of no practical importance at all. Yet, it is, indeed. No pathological manifestations can be triggered by migration of helminth larvae in abortive or captive hosts. The parasites are likely to exhibit signs of life while harboured in the organism of a strange host. Host and parasite will come into interaction as a result of which immunological change in the host organism and a number of pathological responses will develop. It should be borne in mind as well that, with a certain host stock given, both abortive and captive hosts tend to act as reservoir hosts [= euparatenic hosts] and become available, in the process of evolution, as source material from which fresh reservoir and intermediate hosts will develop. In addition, the present reservoir hosts, on principle, may be considered as being captive host–parasite systems."

Abortive hosts may become intermediate hosts, and captive hosts may develop into euparatenic hosts, according to Šul'c (Fig. 6). Another distinction made by Šul'c (1958) was that of sporadic host–parasite systems. One may say that these are rare exceptions, but they nevertheless witness development to infestivity or sexual maturity. Experimental reproducibility

TABLE I

Subdivision of parasite hosts according to their aptitude for the parasite species and the frequency of the parasite

Designations of hosts					Authors
well adapted	badly or not adapted				
real	real and potential				
usual	unusual				Lapage (1956)
obligatory	facultative				Skrjabin and Šul'c (1937, 1940)
true	accidental or casual				Elkin (1973)
true			strange or false		Šul'c (1958)
normal			anomalous		various authors
normal	anomalous				various authors
principal, regular or preferential	incidental or by-	occasional, accidental or casual	unfit	blind alley	various authors
obligatory	facultative	sporadic	abortive	captive	Šul'c and Davtjan (1954)
specific	unspecific				various authors
specific			unspecific		various authors

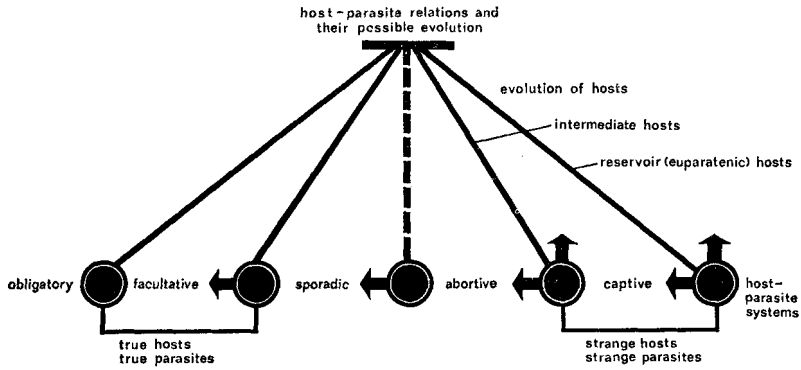

FIG. 6. Parasite–host systems at different levels of adaptation. (According to Šul'c, 1958; Šul'c and Gvozdev, 1972.)

is hardly possible. The dynamic nature of these categories has been underlined by Šul'c. Possibilities of transition and evolution are shown in Fig. 6. Transition between abortive and facultative hosts will be rendered feasible through the action of sporadic hosts.

The occasional host of Piekarski (1954) might perhaps be identical with the sporadic host of Šul'c, in certain cases: "A parasite sheltered in an *occasional host* . . . will not develop unless certain preconditions are satisfied. *Balantidium coli*, a parasite of swine, in the first place, for example, will be changed to a rabbit parasite by temporary one-track nutrition . . ."

The categories suggested by Šul'c and Skrjabin may be shown to be quite plausible. They are, first of all, categories of receptivity and adaptation, relating mainly to a given host–parasite system (rather than to the hosts proper). Yet translation to the hosts (and parasites) of the notions obligatory and facultative is not plausible in this context. What is actually meant by these notions is "properly adapted" or "less properly or not adapted" (cf. Table I). Similar pairs of notions, though not always fully identical, are principal host and incidental host (e.g. Belding, 1965) or, in German usage, Hauptwirt and Nebenwirt. These are used, primarily, for quantitative rather than qualitative assessment of a given host–parasite system (their usage, as a rule, depending on percentual host infestation). Lapage (1956) identified the principal host with the normal or usual host: "As a general rule, particular species of parasites will be found most often in hosts with which they have been associated for centuries, and these hosts are called the *normal* or *usual hosts* of the parasite concerned. Other hosts in which particular species of parasitic animals may be able to live, but in which they are not often found, are called the *abnormal* or *unusual hosts* of these species. Usually a normal host suffers less from the effects of the parasite, because it has, in the course of evolution, become adapted to the parasite, and the parasite has become adapted to the host's reactions against it. Host and parasite may thus slowly develop a mutual *tolerance* of each other and may eventually live together with a minimum of effect upon each other." The name of normal host has

been suggested for use also with different meaning (simply as a host in which normal development of the parasite is possible). Other terms are regular host (". . . host on which a parasite occurs regularly and which is best adapted to this parasite") and preferential host (". . . host which provides for a given parasite and its development the most favourable conditions and which, therefore, will be chosen by that parasite in the case of free choice") (Eichler, 1952).

Joyeux and Baer (1934), with reference to Sandground (1929), held the following concept of normal host: ". . . celui chez lequel le parasite se trouve dans les meilleures conditions pour assurer son existence pendant un temps suffisamment long, lui permettant d'achever sa croissance et d'assurer sa reproduction. La réaction de l'hôte normal vis-à-vis du parasite est, en général, insignifiante et de courte durée. Il est rare qu'elle mette la vie de l'hôte en danger, du moins dans des conditions naturelles."

Principal host (= Hauptwirt, host with highest rate of infestation in a given area), regular host, preferential host, and well adapted host, will usually all be identical—usually, but not necessarily always. After all, well founded assessment cannot be made on the basis of scattered cases; due consideration must be given to the evolution, population biology, and ecology of parasite and host species. Proper adaptation of a host species, therefore, must be measured not only by one given relation between one host and one parasite, but by testing several repetitive parasite passages.

Piekarski (1954) has been holding the concept that principal, regular, and preferential host are different names for one and the same host: "*Principal host* is the animal which (usually devoid of any primary defence capacity) provides optimum conditions for a parasite's life and intensive reproduction and (statistically) most common and reliable opportunity to reach sexual maturity, with no additional and extraordinary demands being involved (for example, demands on host nutrition) . . . An *incidental host*, however, with some capacity for 'defence action', will offer more sizeable resistance to the invading parasite, and, as a whole, the amount of parasites that undergo full development, even up to sexual maturity, on such a host will be smaller than that on a principal host . . ."

Incidental hosts, to which parasites are much less adapted and on which their growth and development are likely to be retarded, have been called second-level or auxiliary hosts by Michaiłow (1955).

The suitability of a given host for a given helminth parasite may be measured by the following criteria:

1. Optimum chance of meeting the parasite.
2. Settling rates of the parasite in a given host population (extensity) and in one individual host of that population (intensity).
3. Rate of parasite development on the host (measurable by the pre-patent period).
4. Longevity of the parasite on the host.
5. Reproductive capacity of the parasite on the host (depending on length of pre-patent period, longevity, accomplished body size, and efficiency of sexual organs).

6. Intensity of defensive response of the host.
7. Sensibility of the host to pathogenic effects, since immediate killing of the host will usually be unfavourable to the parasite.
8. Viability and transmissive potentiality of the parasite's reproductive forms.
9. Safe endurance and continuation of criteria 1 to 8, particularly 8, over numerous host passages. Item 1 will apply only to real hosts.

The above list of items is likely to suggest the complexity of the problem at hand. Positive or negative information on one or few criteria will not be sufficient to warrant full assessment.

The designations "true hosts and parasites" or "strange hosts and strange or false parasites" (Šul'c) are not unambiguous either (cf. Table I). The "true" nature of a host may be assessed by the criterion of "normal development" of the parasite which, in turn, is measurable by accomplishment of complete development to the extent to which completeness can be expected on the host category concerned (infestivity on intermediate hosts, sexual maturity on definitive hosts). An additional aspect which should be borne in mind is the existence of many parasites which have both real and potential hosts, with the former being found in nature and the latter being experimentally determined. At least some potential hosts can take action also in nature, provided certain changes occur. The name of potential host is meant to describe hosts on which normal parasite development is possible without host adjustment to the parasite concerned. Potential hosts, therefore, should be assumed to be representatives of "facultative hosts", according to Šul'c and Skrjabin. While potential hosts may occur in nature, they are not accessible as yet to the parasite species concerned, for geographical or ecological reasons, but such accessibility may become reality sometime in the future. Experimental hosts may be real or potential. There is a third group of experimental hosts with representatives which are neither real nor potential but artificial (since artificial modification, such as surface smoothening, must be applied to them to allow their infestation). Hence the "true hosts", according to Skrjabin and Šul'c, include both real and potential hosts. Yet there might be some justification in considering a potential host also as a "strange host", since a potential host, too, is alien to the parasite concerned; there is no mutual adaptation at all.

There is some correspondence, in a way, between certain parallel variants of hosts, such as Šul'c's "true hosts" and normal hosts, "obligatory hosts" and regular hosts or preferential hosts or principal hosts, as well as "facultative hosts" and incidental hosts or "hosts in case of need" (Notwirt), the latter being "a host actively called on by a parasite, if no really suitable host is available" (Eichler, 1952).

Epidemiologists tend to differentiate hosts also by evolutionary aspects of adaptation. The differentiation of hosts and parasites, as proposed by Elkin and Jaškul' (in Elkin, 1973), included "true" or "principal" as well as "casual" or "incidental". In this context as well "true hosts" are hosts in an evolutionarily adapted host–parasite system, whereas "casual hosts" are unadapted. "Casual hosts" are likely to lead and trap the parasite in a

blind alley, in almost all cases. Yet under certain circumstances they can be helpful in parasite propagation and transmission. The "true" or "principal hosts" proposed by Elkin, therefore, would perhaps be comparable with the "obligatory hosts" proposed by Skrjabin and Šul'c, while some of the "casual" or incidental" hosts would correspond to "facultative" hosts, and others to abortive and captive hosts (as far as they capture the parasite in a blind alley) (cf. Table I).

General suitability of a host species for a given parasite species will depend on a number of factors, evolutionary as well as ecologico-epidemiological. Subdivision of hosts by receptivity, resistance or sensibility would cover only one sub-aspect of the problem. Problems relating to the complex of adaptation within a given host–parasite system are correlated, last but not least, with the duration of historic links between host and parasite. And against the background of the history of relations between host and parasite, a differentiation will be possible between primary and secondary hosts.

Mutual adaptation between parasite and host is of great significance to any assessment of a host–parasite relation. Accidental parasites are not adapted to a specific host or host group, and facultative parasites only partially. Developments are assumed to lead, for the most part, from weaker and more general orientation of parasite to host to stronger and more specific forms of adaptation. This, however, is but one trend which must be looked at in the context of others. While evolution is assumed to lead from facultative to obligatory parasitism, similarly, the parasite in an infantile phase of a host–parasite relation is believed to have, quite often, an affinity for several or many hosts which will be gradually restricted to few or even one host in the course of phylogenesis. Yet this trend may be reversible, in that restricted host specificity may be re-converted to a wider range of possible hosts.

The concept of narrowing host specificity is accompanied by the assumption that the damage inflicted upon a host in historically more recent host–parasite relations and with undeveloped specific defence capacity, will be of higher severity than that inflicted upon a host in a system well established in the course of history. Such evolution may lead eventually to mutualism. Other trends, however, are possible, for example, relocation of the parasite on one and the same host, say, from intestinal to tissue parasitism, which should aggravate the risk to the host.

Host specificity is the term to define restriction of a parasite species to one host group or host species. In reality, host specificity can be measured by the range of hosts naturally occupied by one and the same parasite species, with potential host specificity being identical with or broader than real host specificity. Host specificity may be broad, narrow, variable or rigid, with flowing boundaries between these variants. It will result always from interaction of evolutionary, ecological and physiological factors. The evolutionary factor, in this context, need not necessarily be mutual adaptation between parasite and host, but it may be based merely on host phylogenesis (related organisms as potential hosts). Three extreme forms of host specificity are

conceivable in cases in which one parasite is capable of attacking several hosts, and one of the three above host specificity factors will play the major role in each of these extreme forms:

1. The hosts involved (which will be restricted, more or less, to a small group) are closely related to each other ("phylogenetic host specificity").
2. All the hosts involved are not closely related to each other, in terms of family links, but have ecological aspects in common, which may explain infestation by one and the same parasite ("ecological host specificity").
3. The hosts involved are neither related to each other nor do they have any common ecological aspects ("physiological host specificity").

The host specificity of the parasite has as its counterpart on the host side receptivity or species-based non-receptivity (axeny).

Host specificity is materialized not always by non-receptivity, but sometimes even by "choice" by the parasite of a specific host (e.g. active visit of host by parasite). Host specificity and receptivity may be graded by adaptation of the parasite or by active resistance offered by the host. Active and part of passive resistance are triggered from a physiologico-biochemical mechanism. Parasites will have no chance to establish themselves unless they are able to neutralize defence.

Terms relating to real host specificity are monoxeny, oligoxeny and polyxeny. Monoxeny (which should not be confused with homoxeny or monohospitalism) stands for a situation in which the parasite has only one host species (at least in a certain phase). Oligoxeny describes relations in which only few hosts are acceptable to one parasite. Polyxeny (which should not be confused with heteroxeny or polyhospitalism) defines a situation in which one parasite has many hosts. These parasitological terms are complementary to terminology used in the field of ecology, including monophagous (or stenophagous), polyphagous, and stenotope/eurytope (relating to nutrition or habitat, respectively), and stenoecious/euryecious (relating to general demands on life). However, their complementary nature to ecological terms does not mean complete congruence with the latter, since their notions and meanings may be larger or smaller, depending on context. The following proposal to link up the terms for host specificity to quantitative use has been made by Zmoray (1968):

(a) stenoxenous parasites—those with few hosts
  (aa) monoxenous parasites—those with one single host
  (bb) stenoligoxenous parasites—those with two or three hosts
(b) euryxenous parasites—those with a larger number of hosts
  (aa) euroligoxenous parasites—those with four or five hosts
  (bb) polyxenous parasites—those with more than six hosts

These expressions should remain limited in usage to guest–host relations (xeny = hostdom, relatedness to host), since they cannot always express the ecological valence of a guest in its totality (which would be possible only in the case of absolutely permanent entoparasites), but describe merely the host-related aspect of that valence.

V. Host Categories of the Parasite Life-cycle

A. Homoxeny, Heteroxeny, Ontogenetic Types of Parasite Life-cycles

1. *Homoxeny*

Parasites, purposefully, are called homoxenous, if they need only one and the same host category for their entire life-cycle (cf. Osche,1959; Odening, 1968–69, 1974a,b). The term of "monoxenous" has been quite common (from Neveu-Lemaire, 1908, 1912, and Brumpt, 1910), but it should not be used in this context because of the risk of being confused with the corresponding term applied to definition of host specificity (cf. Schuurmans-Stekhoven, 1959, Zmoray, 1968; Ryžikov, 1973). Homoxeny defines parasite transmission via a sequence of hosts of one and the same ontogenetico-cyclic category. These hosts, identical in category, should not necessarily be one and the same species. A homoxenous parasite, therefore, can be monoxenous, oligoxenous or polyxenous. The category of hosts for homoxenous parasites is generally characterized by including solitary hosts, in terms of ontogenesis and cycle. Included may be stationary and transit hosts, depending on the given form of parasitism. Transit hosts are hosts which will be abandoned by the parasite after some time of residence or when the parasite has reached a certain level in its development (e.g. Nematomorpha, Mermithoidea). Hosts of homoxenous obligatory parasites are characterized also by their stadiogenous nature, which implies that they will ensure individual parasite development from one stage to another ("upward development"). Also there are reproductive and non-reproductive hosts in the solitary host group, in other words, hosts which enable reproduction of the parasite and others which do not. Solitary stationary hosts are usually reproductive as far as the parasite is concerned. Again, differentiation is made between propagative and multiplicative reproduction. In propagative reproduction, parasites will not be propagated on the host, but there is production of propagative forms (dispersion phase) which usually will leave the host permanently. Primarily, such propagative-reproductive hosts are disseminators. Multiplicative reproduction of the parasite will take place on the host (overflow reproduction, agglomeration phase). Multiplicative reproduction either will take place in conjunction with its propagative counterpart or include propagative action in itself.

Hosts with multiplicative reproduction of the parasite may be disseminating or transmissive. Every obligatory reproductive host is a stadiogenous host by virtue of its reproductive function. Non-reproductive solitary hosts are transit hosts. Distinction should be made in terms of origin between primary and secondary (cf. p. 80) homoxeny.

2. *Heteroxeny*

Parasites are called heteroxenous, if they need different host categories for their life cycle. The term of heteroxeny has long been internationally

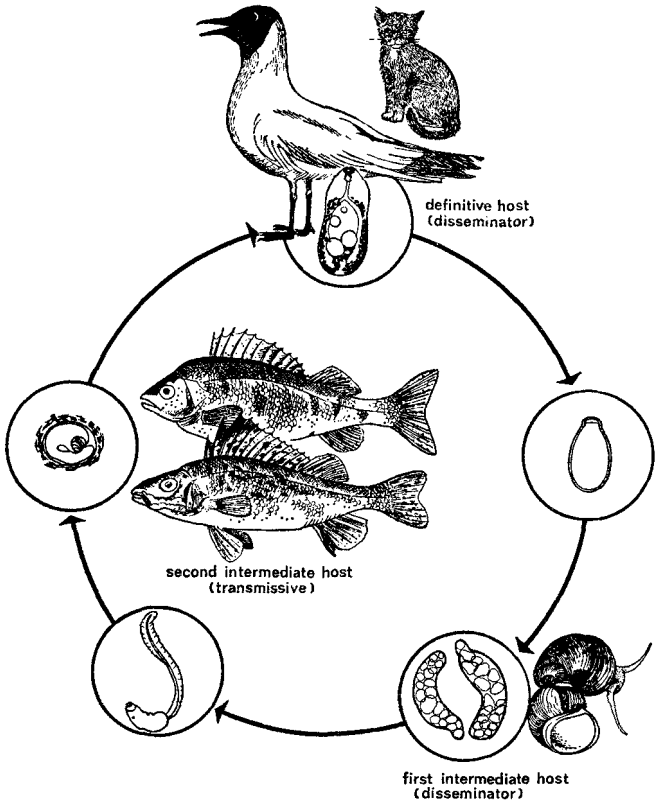

FIG. 7. Three-host cycle of *Apophallus donicus* (trematode).

accepted.* It has proved to be unambiguous both as a term and semantically. Unambiguous sub-terms are diheteroxeny, triheteroxeny and tetraheteroxeny, rather than dixeny and polyxeny. Neveu-Lemaire (1921, 1936) distinguished diheteroxeny and "polyheteroxeny". Sul'c and Gvozdev (1972, p. 11) used polyxeny even as a synonym of heteroxeny.

The decision to use monoxeny as opposed to heteroxeny proved to be another unfortunate approach. The linguistic and logical antonym to heteroxeny is homoxeny (rather than monoxeny).

Differentiated meanings of compound terms are likely to arise from the very fact that different interpretations may be given to "xenos", a Greek word with the two meanings "host" and "stranger". Hence, if it comes to *in vitro* cultivation (of one organism species), the expressions "monoxenic" or "polyxenic" are used to mean "in the presence of one or many different species". In the epidemiological approach to heteroxeny, differentiation is sometimes made between epidemiological reference host and "xenorganism".

* From Neveu-Lemaire (1908, 1912) and Brumpt (1910). The term metaxeny was used in the same context by Ross (1910) who followed De Bary.

Diseases caused by heteroxenous pathogens (which actually deserved the name heteroxenosis) accordingly are called metaxenosis (a "xenorganism" being required). Diseases caused by homoxenous pathogens (which actually deserved the name homoxenosis), on the other hand, are called axenosis (no "xenorganism" being required; Moškovskij, 1950). Diseases caused by polyxenous pathogens (which actually deserved the name polyxenosis) have been called paraxenosis by Moškovskij (1950).

Heteroxeny requires for its own implementation parasite transmission via alternation of hosts of different ontogenetico-cyclic categories. Diheteroxeny will usually be the predominant condition (two-host cycle). Triheteroxeny (three-host cycle) will be present in connection with certain ticks (a situation to which actually no true heteroxeny is applicable), to some extent also in connection with the protozoon *Karyolysus*, numerous trematodes, some

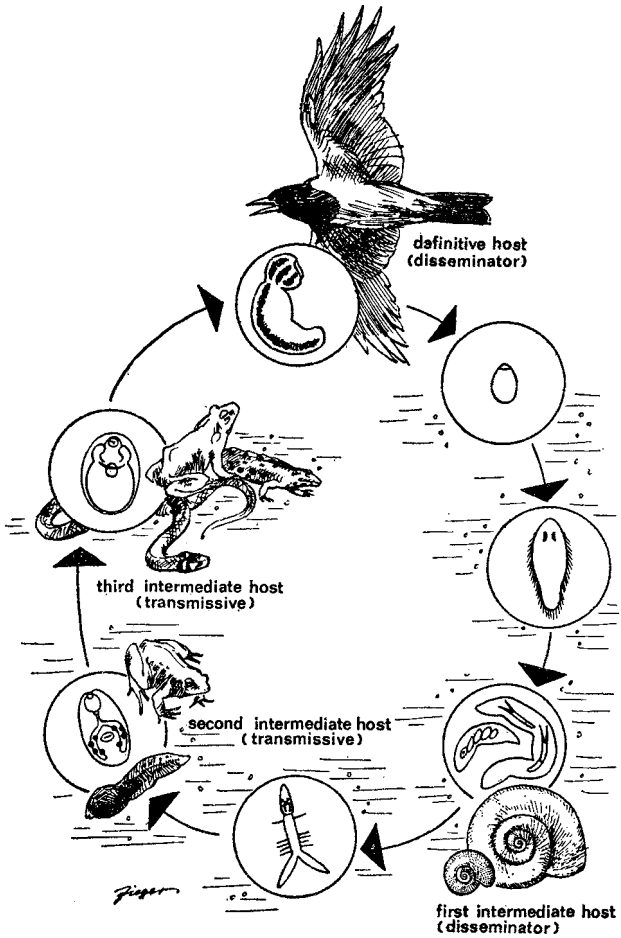

FIG. 8. Four-host cycle of *Strigea sphaerula* (trematode). (Odening, 1967.)

cestodes, and a few nematodes (Fig. 7). Tetraheteroxeny (four-host cycle) will be recordable only from a few cestodes(?) and highly advanced trematodes (Fig. 8). These terms, with no additional indication attached to them, always stand for a compulsory situation in which one parasite species requires inevitably two, three or even four different hosts for its own complete development.

A highly sophisticated evolutionary process is needed to develop life cycles with more than one host compulsorily needed to complete ontogenetico-cyclic development of a parasite. A parasite species can have different motivations for alternation of hosts. Such alternation, basically, may have evolved on disseminating or transmissive foundations.

The development from (original) homoxeny to heteroxeny marks an important step in evolution, a "rise" of the parasite (towards specialization and adaptation). Heteroxeny means progression and thus is comparable with fertility adaptation to offset potential loss on the way to a host. Ecologically and in evolution, it means growing intricacy of the, originally, two-pole parasite–host relation which now is likely to be changed to a three-pole or even four-pole relation. Secondary developments may include reduction of diheteroxeny to homoxeny (with certain helminths, including *Ascaris*), or triheteroxeny to diheteroxeny (cestodes, certain trematodes), or tetraheteroxeny to triheteroxeny (trematode genus *Alaria*). Such reductions are considered favourable and show progress, in that the intricate cycle will be rendered facultative, and in certain cases it will come to adapt to changing nutritive habits of hosts.

One variant of host alternation is disseminative and determined primarily by "replacement" of one host by another. This is not apparently caused by removal of obstacles on the way to a host which thus far has been the only host, but it has something to do with the developmental rhythm of the parasite and its demands on life. "Demand for transmission", say, as a motivation for genesis, cannot be derived from present conditions. One host is likely to act as a source of infestation to the other rather than as a transmissive host. (Succession of hosts does not depend on physical coincidence, and, more often than not, the latter will not even be possible.) Differentiation is suggested between two types:

(i) The first type of host alternation depends on alternation of generations from which it has actually emerged. Such alternation of generations is coupled with alternation between a host of parthenitae (molluscan host) and a host of adults (vertebrate host) of trematodes, as well as with alternation between aecidial host and host of teleutospores of rust fungi.

Such alternation of host is backed by a long history in terms of phylogenesis, and for some rust fungi it can be traced back as far as the Mesozoic period. (Its genesis was connected with advance from tropical to moderate climates and transition from forest to grassland.) Change for fresh hosts as a whole obviously has played a certain role in this context (tropical ferns in more recent Paleozoic, Gymnospermae in Mesozoic, Angiospermae as of Upper Cretaceous). Genesis of host alternation in dependence on alternation of generations was quite complicated with regard to trematodes (no

demand for transmission, but simply incidental or occasional or demand for dispersion).

Host alternation of digeneans in dependence on alternation of generations has originated roughly in parallel with the transition from Triassic to Jurassic, according to Ginecinskaja (1968).

Hosts that alternate in the context of alternation of generations are always reproductive hosts, and carriers of a distinct parasite generation which differs from the parasite generation on the other participating host.

(ii) Ectoparasitic alternation of hosts is neither transmissive nor caused by alternation of generations. It is found to apply to ectoparasitic isopods (Epicaridae) and ticks (Ixodidae) (if the latter can be related at all to heteroxeny). Larvae and nymphs parasitize on a host different to that called on by adults. This type of host alternation has some links with temporary parasitism in polyhospitalism. Ixodidae, in particular, represent a boundary case between temporary and transit parasitism, and hence also between polyhospitalism and monohospitalism. Under these conditions the complete parasite rather than propagative phases will be disseminated by the hosts.

Basically, one or two hosts of larvae and one host of adults may be distinguished as two different categories in ectoparasitic host alternation. All hosts involved are transit hosts. The hosts of larvae and adults are stadiogenous hosts only in connection with isopods. Yet most of tick ontogenesis will take place in free environment. A host of adult ticks does not have the reproductive function it has for isopods, since oviposition of Ixodidae females takes place in the open, i.e. outside the host. This is another indicator to similarity between tick parasitism and polyhospitalism of temporary parasites.

Transmissive alternation of hosts is an important device for the parasite to change hosts, as it implies acquisition by the parasite of "transmissive hosts" to facilitate the journey to the original, disseminating, and exclusive host. A transmissive host will come in as a mediator to enable infestation of the only host so far. Such alternation of hosts came into being along with the emergence of certain transmissive relations.

The original transmissive function (i.e. physical meeting between transmissive host and receiving host, recipient) will be quite obvious in situations in which parasitic propagative forms are returned more easily, at higher rate, and with less loss to their original host through the mediation of an animal (rarely a plant). Therefore, all transport hosts and those parasite hosts which are part of an original host's nutrition (cf. Božkov, 1970b) can be considered as being transmissive hosts through which infestation of the original host is facilitated.

Another form of transmissive relation has resulted from changes in originally solitary hosts to the effect that these became haematophagous or suckers of body fluid or plant juice, in other words, from change towards temporary or even stationary parasitism. This seems to suggest that all transmissive hosts cannot be hosts secondarily added in the course of history. The arthropod host usually will be primary, in terms of history, for haemoflagellates, heteroxenous viruses, rickettsiae and bacteria, while the

same role will be played by the vertebrate host when it comes to Haemosporidia. Invertebrates are assumed to have been the primary hosts to haemoresident coccidia (*Haemogregarina*, *Hepatozoon*, *Karyolysus*). In these cases, alternation of hosts may have been caused by peroral intake of the invertebrates by vertebrates in whose bodies the parasites continued their own development. Such route of alternation, however, would not have been feasible unless the invertebrates concerned were blood-suckers of the vertebrates (cf. Doflein and Reichenow, 1949).

Sporozoa do have alternation of generations, but their alternation of hosts is not triggered by it. The cause, here, is transmissive. Coccidia mark transition from all-homoxenous species to those which may be linked first to facultative and later to obligate transport hosts (with entotransitive transmission, e.g. in the case of *Cyclospora*). From this point, the route will lead to adaptive transmission, first by phagous transmissive hosts (e.g. in case of *Aggregata*). Particular transmissive relations have emerged in connection with protozoal haemoparasites. The sporozoites of *Schellackia*, for example, needed for invasion of vertebrate hosts do occur in the latters' erythrocytes. The cycle of this parasite can be completed even after the carrying vertebrate hosts are eaten up by vertebrates of the same or of other species. Additional reference to acarids may offer a clue to the way in which facultative insertion of some

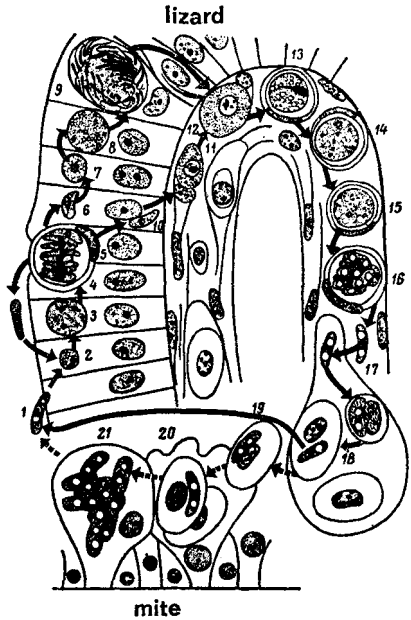

FIG. 9. Life-cycle of *Schellackia bolivari* (sporozoan). Schizogony (1–5), gametogony (6–13), sporogony (14–16) in obligate schizosporogonial host, (17–18) entry of sporozoites into erythrocytes (19–21) residence of sporozoites in additional host of sporozoites (intake of erythrocytes and release of sporozoites into intestinal cells), (Adapted from Reichenow and modified by Odening, 1974a.)

sort of euparatenic host (Fig. 9) has perhaps encouraged emergence of obligate heteroxeny. The cycles of both *Schellackia* and *Lankesterella* are likely to reveal to some extent the approach taken by coccidia in changing from environmental phase, with disseminating host, to conditions devoid of environmental phase but, consequently, with transmissive hosts. The cycle of *Schellackia* is still facultatively heteroxenous, the transmissive hosts involved still being phagous. Further transition to alternation of hosts between vertebrates, on the one hand, and haematophagous arthropods, on the other, is likely to enable these two host species to exchange parasites between them, and survival of either transmissive host involved becomes possible.

Trypanosomatidae, by virtue of their original nature, are intestinal parasites of arthropods and other invertebrates. Change for plant juice, blood, other body fluids, and cells of vertebrates was a process which accompanied change relating to primary invertebrate hosts which turned haematophagous or began to suck plant juice. Alternation of hosts was another accompanying phenomenon. Many of the articulate hosts of Trypanosomatidae, known today, can be related to the original host. Development and reproduction on the articulate host were found to take place in a characteristic manner. Trypanosomes, the original parasites of arthropods, more recently had added to them vertebrates as transmissive hosts. The representatives of the *vivax*, *congolense* and *brucei* groups, however, lost their original arthropod host for another one (tsetse fly). Conditions may undergo quite a reversal in the course of evolution.

Heteroxeny, historically, developed from (primary) homoxeny. The same course of development is believed to apply to transmission and its most probable emergence from primary dissemination. Yet this fundamental evolutionary relationship must not lead us to the conclusion that any present homoxeny is primary in nature. Nor is any present disseminator of primary nature, since transmissive hosts, too, may become disseminators (e.g. in certain cestodes and trematodes, if their cycles are shortened due to neoteny).

Transmissive hosts of heteroxenous helminths were added historically to one original disseminator and in trematodes to two. These have been the course of development of (primary) diheteroxeny of nematodes and acanthocephalans or triheteroxeny of trematodes. Triheteroxeny of certain cestodes and nematodes and the very rare case of tetraheteroxeny of certain trematodes (*Strigea*, Fig. 8) are developmental cycles with two transmissive hosts involved. Alternation of hosts caused by alternation of generations is combined with transmissive alternation of hosts in triheteroxeny of trematodes (Fig. 7). Helminths are assumed to have changed for transmissive alternation of hosts not only via insertion of facultative transport hosts, but perhaps also via hosts which are comparable to certain paratenic hosts known today.

3. *Ontogenetic types of parasite life-cycles*

I. Different levels of development are defined by the term of life-cycle, with the following principal forms being known (Fig. 10):

(a) Simple development (without alternation of generations) is one

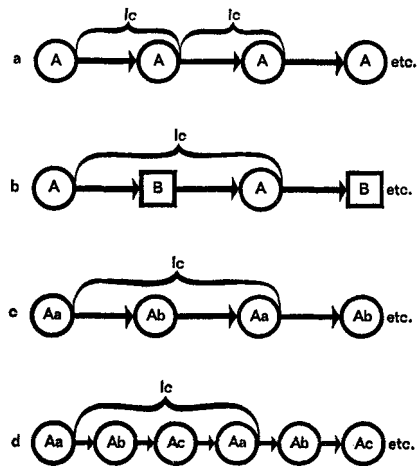

FIG. 10. Several life-cycles (lc): (a) simple development circuit, (b) cyclogenerational development circuit, (c), (d) form-cyclic heterogeneous consecutions of simple development circuits.

between equal levels of successive generations. It is characterized by simple succession of identical generations (homogeneous sequence of generations), including species with asexual and those with sexual reproduction.

(b) Development against the background of generational cycles is one in the presence of alternation of generations. It is a succession of generations for which the generational facets are heterogeneous. Development here will range from one level in a given generation to the same level in an equal generation (which can at best be the second next rather than the next). The simple and most common case of alternation of generations will imply that two generations alternate with regard to their ways of reproduction or karyophase to form one continuous cycle of development. Facultative alternation of generations (e.g. of many protozoa) is one of the routes to proceed to such obligate and regular alternation of generations. An obligate generational alternation cycle quite often may be accompanied by simple facets of development in fixed number and sequence or in facultative manner, depending on environmental factors.

Differentiation must be made between primary alternation of generations (sporozoa and fungi) and secondary alternation (certain metazoic parasites). Secondary alternation of generations is a derived variant: metagenesis is alternation of asexual reproduction through polycellular phases with bisexual reproduction, heterogony is alternation of degenerated bisexual reproduction (parthenogenesis) with normal bisexual reproduction.

Metagenesis is a rare development. Reproduction of the cestodes *Multiceps*, *Echinococcus* and *Alveococcus*, and others, takes place vegetatively in the larval stage (*Coenurus*, hydatid cysts etc.). Mother sporocyst of certain trematodes is known to undergo simple (twofold or threefold) division.

Heterogony is typical of the development of digenetic trematodes. Parthenogenetic generations in molluscan hosts will alternate with bisexual generations in vertebrate hosts. There are certain cases of trematodes with a combination of heterogony with metagenesis due to vegetative division of the mother sporocyst.

Parasitism of the majority of these groups of organisms is believed to have been preceded by alternation of generations which actually might have been a triggering factor.

Obligate primary alternation of generations among sporozoa is based on facultative or irregular alternation between bisexual reproduction and agamic cytogony, which had been recorded repeatedly from other sporozoa. Such alternation of generations is as old as sporozoan parasitism and has been adapted, in the course of time, to the specific requirements of parasitic life. Similar developments have taken place among fungi.

Ginecinskaja (1968) assumed that the "protrematodes" originally had been facultative larval parasites and, more recently, became parasites in adult condition of molluscs on which they underwent normal bisexual reproduction. The formation of propagative larvae was thought to have entailed alternation between free-living and parasitic generations (yet not as an "alternation of generations", in the first place, but as a facultative succession of bisexual parasitic and free-living generations). Evolution then became divergent, with the parasitic forms being further adapted to parasitism and free-living forms following different lines. The parasitic protrematodes improved their fertility by modification of their sexual organs.

Proterandry has been assumed by Ginecinskaja to be some sort of pre-adaptation leading to parthenogenesis, with reference having been made to observations on turbellarians. Favouring factors might have included affluence of nutrients offered by the host and acceleration of reproduction. (Parthenogenesis or proterandry was found to occur also in certain nematodes that are parasitic on vertebrates and alternate with free-living bisexual generations, and similarly in certain insects that are parasitic on plants, e.g. gall wasps and plant-lice.)

(c) Simple development by form-cyclic heterogeneous succession may be caused by morphology and/or physiology (rather than by modes of reproduction or karyophase). "Asexual alternation of generations" has been another term to label this phenomenon but applied only to part of it. Therefore, heterogeneous form cycles may be differentiated for the following situations:

(i) Asexually reproducing organisms, including heteroxenous flagellates (morphological), viruses, rickettsiae and Spirochaetae (physiological?).

(ii) Metazoa, including parthenitae of trematodes (morphological).

Individual development will be identical with the life-cycle only for simple development facets. Development facets based on generation cycles and form cycles are supra-individual life-cycles.

There may be combinations between simple development and development on the basis of generation cycles (Fig. 11c-d), between simple development and development on the basis of form cycles (Fig. 11a-b), and between all three.

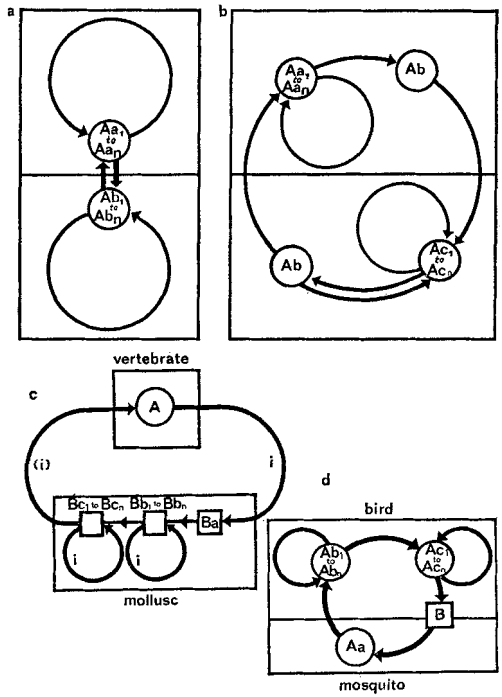

FIG. 11. (a, b) Consecution of form-cyclic heterogeneous generations: (a) heteroxenous viruses, rickettsiae, spirochaetae (difference between Aa and Ab detectable only by host), (b) *Trypanosoma cruzi* (Aa epimastigote form, Ab trypomastigote form, Ac mastigote form). (c, d) Combination of form-cyclic heterogeneous consecution and alternation of generations: (c) digenetic trematodes (A bisexual type of generation, B parthenogenetic type of generation, e.g. Ba mother sporocyst, Bb mother rediae, Bc daughter rediae), (d) *Plasmodium praecox* (Aa sporozoites, Ab macroschizozoites, Ac microschizozoites, B gametes). (Odening, 1974a.)

The different forms of life-cycles of parasites may be interconnected in various ways with alternation of hosts. Evolution has been responsible for progress from simple development to heterogenous form cycles and generation cycles, (primary) asexual to bisexual reproduction, and (primary) homoxeny to heteroxeny. The facultative state in which the road is paved for more complicated developments can be considered a transitional field in-between simple development and homoxeny, on the one hand, and the obligate condition of complicated supra-individual life-cycles and heteroxeny, on the other.

Separate developments may include secondary simplification or secondary conversion to facultativeness of certain life-cycle phases in connection with certain hosts. Such hosts then should be called alternative rather than facultative.

II. Variable life-cycles may include alternative and parallel cycles. These are cycles of parasites which will form occasionally (alternating) or permanently and regularly (parallel) forms of different type (different in dimensions,

morphological structure and/or demands on life and ontogenesis). Such formation may be triggered by an inherent rhythm (obligate) or under the impact of environment (facultative). Developments in such cycles may be alternative or parallel, on two levels in quality.

(a) *Alternative cycles* (Cameron, 1956) are facultative in nature and predominate among rust fungi. Generational reproduction of Uredinales may

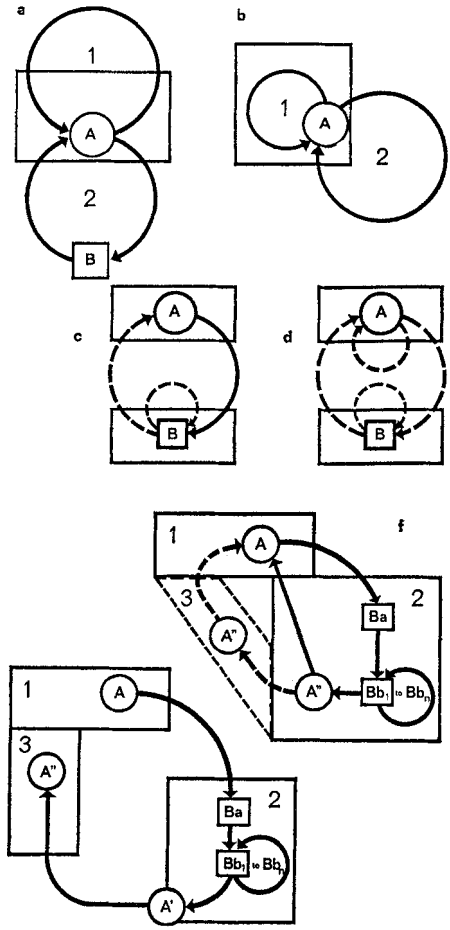

FIG. 12. (a) Alternative cycles without alternation of generations (1), with alternation o generations (2), between parasitic (A) and free-living (B) types of generations. (b) Parallel cycles with multiplicative reproduction (1), with propagative reproduction (2). (c) Alternative cycles of *Trypanosoma cruzi*, certain rickettsiae, rust fungi (A–B obligate levels, B–A facultative or alternative levels). (d) Relatively independent alternative cycles of *Toxoplasma*. (e)–(f) Life-cycle of *Diplostomum flexicaudum*, examples of alternative cycles (f), a facultative heterochronous polyvalent host (f; 2), and optional involvement of a euparatenic host (f; 3); (e) normal three-host cycle (A adults, A″ metacercariae, A′ free-swimming cercariae, B parthenita). ((a)–(b) according to Odening, 1974a; (c)–(f) original.)

be homogeneous-propagative (through uredospores) or heterogeneous-propagative (through teleutospores), i.e. by two possible modes of reproduction (Fig. 12). Only one of them will apply at any time. Teleutosporulation in Uredinales seems to be induced by the end of the vegetational period of their "summer hosts". Similar modes of reproduction seem to apply to certain nematodes (e.g. *Strongyloides*) where a free-living bisexual generation may undergo either homogeneous-propagative reproduction (to bring forth its own type) or heterogeneous-propagative reproduction to produce a parasitic generation which will be different in type (Fig. 12).

In some such cases, environmental factors may be inducive to one of these modes. Alternative cycles will only to some extent be linked to alternation of

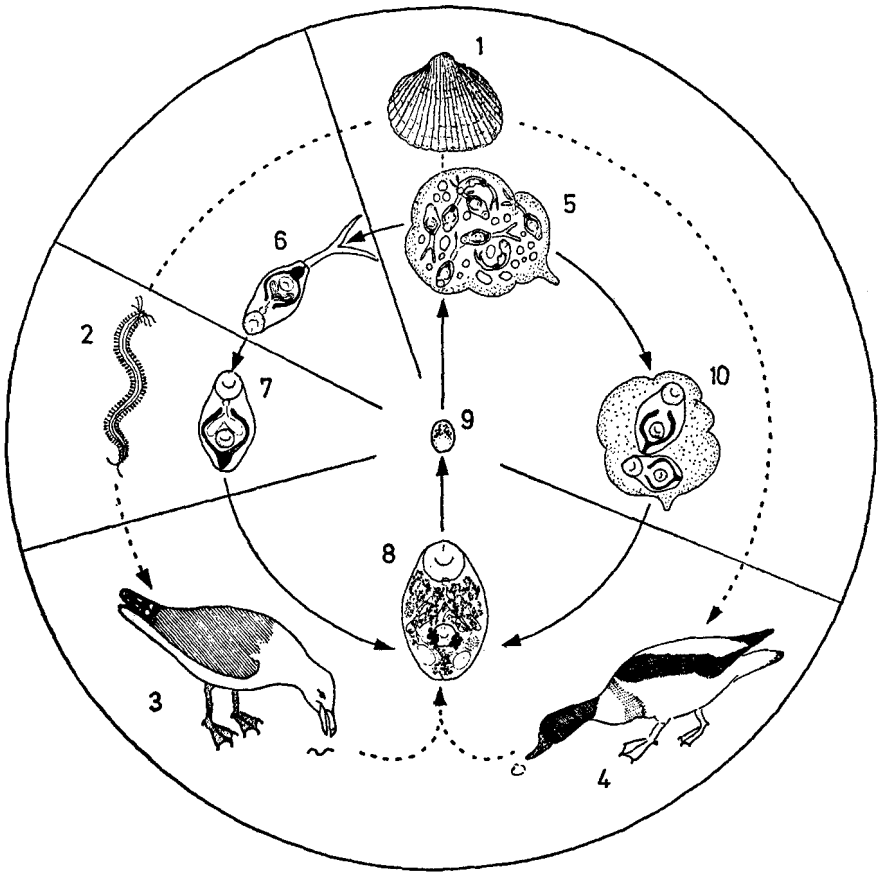

FIG. 13. Alternative life-cycles of *Gymnophallus choledochus* (trematode): 1–3. Three-host cycle (spring and summer; 6. free-swimming cercariae, 7. metacercariae in second intermediate host); 1–4. Two-host cycle (autumn and winter; 10. sporocyst with metacercariae in polyvalent intermediate host); 1. Partheno-intermediate host or combined partheno-intermediate and larvo-intermediate hosts; 2. Larvo-intermediate host; 3–4. Different definitive hosts. (Loos-Frank, 1969.)

generations. There may be alternation of parasite generations which cannot be equalled with alternative cycles (this being possible only in cases of facultative alternation of generations). However, it should be sufficient to suggest, in this context, that there are relations between alternation of generations, alternative and parallel cycles, as well as the problem of polymorphous species and "cyclic sequence of development facets".

Several examples of alternative cycles may be found among trematodes. For example, the three-host cycle of *Diplostomum flexicaudum* (snail–fish–gull) may be alternative (Fig. 12). "Precocious" metacercariae may develop, with no free-swimming cercarial stage being involved, even in the snails under certain circumstances. Such cycles then can be completed as a two-host cycle (snail–bird) (cf. Olivier, 1940). *Metagonimoides oregonensis* appears to have three strains of different cycles which are linked up with the three following different types of molluscan hosts:

(i) Normal three-host cycle with free-swimming cercariae.

(ii) Cycle reduced to two hosts by coincidence of first and second intermediate hosts.

(iii) Alternative or parallel cycles (successive or simultaneous combination of (i) and (ii); cf. Lang and Gleason, 1967).

The shortened alternative "winter cycle" of *Gymnophallus choledochus* is secondary by nature and helps to broaden the host range (Fig. 13; cf. Loos-Frank, 1969). Another possible alternative development may be caused by neoteny in certain helminths (e.g. in the monogenean *Polystoma* and in certain cestodes and trematodes).

The cestode *Biacetabulum* constitutes a boundary case between alternative cycles caused by neoteny and a cycle with additional host (paradefinitive host in this case) (Fig. 14).

Toxoplasma develops in the form of alternative cycles, too (Fig. 12), which are controlled by the given host specificity. They are in lawful interconnection, however, with no determined order. The two components of these alternative cycles are highly independent of each other, and, theoretically, complete separation might be possible in the case of isolation. Designation of Felidae as "definitive hosts" and the label "intermediate host" for all the other hosts would by-pass reality just as much as differentiation between "cyclic" and "acyclic" development or "specific" and "unspecific" hosts. The two alternative cycles of *Toxoplasma* might be adequately defined by differentiation between eucyclic and paracyclic development and between schizosporogonial (felid) hosts, on the one hand, and cystical (non-felid) hosts, on the other.

Alternation of hosts in the context of heteroxenous viruses, rickettsiae and spirochaetae, implying hosts of highly differentiated nature, such as invertebrates, vertebrates and plants, might be assumed to be accompanied by modification of (non-morphological?) properties of the parasite and thus give a cyclic series of development facets (Fig. 11). This series, however, has been found to be not always obligate for the developmental rhythm of parasite generations involved, which may be seen, for example, from trans-ovarian transmission of rickettsiae and *Anaplasma* between non-vertebrate

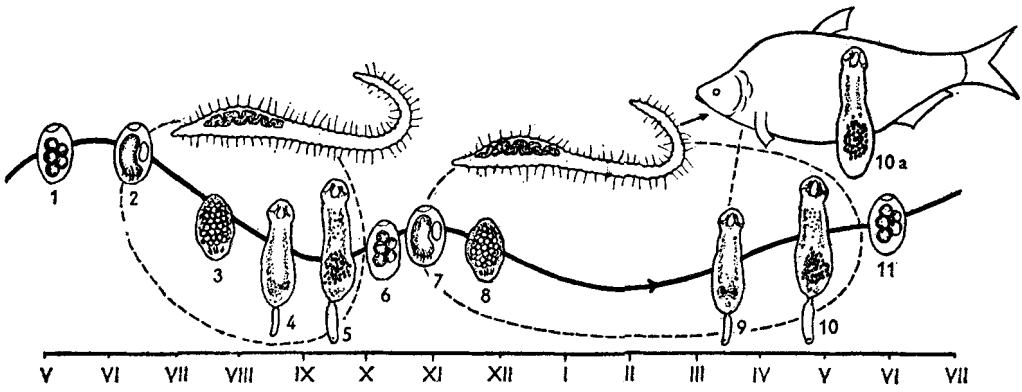

FIG. 14. Alternative life-cycles of *Biacetabulum* (Cestoidea) and their seasonal dependence: 1–5 and 6–10. Development in one-host cycle (neoteny of plerocercoid); 6–10a. Two-host cycle. (According to Kulakovskaja, 1964.)

hosts. This might suggest the presence of facultative alternative cycles in this context. Yet knowledge so far accumulated about life-cycles of viruses and bacteria is still insufficient. It is a frequent assumption that only individual development and simple development facets are involved. Phage and bacterial populations are living together, after all, which seems to indicate that the development facets of bacteriophages are arranged as a cyclic series (with a lytic parasitoid series of generations being replaced by a moderately parasitic series).

Alternative development may also be observed throughout complete phases of certain life-cycles (without basic change to the complete cycle). Alternative production of daughter parthenitae or cercariae by the parthenitae of certain trematodes, for example, will depend on interaction of several factors.

Cycles with possible intercalation of metaparatenic hosts (cf. p. 69) should not be included in this context, according to the above definition of alternative cycles as an alternative to development, different in quality. Yet there are many parasitologists who actually treat cycles with possible intercalation of such metaparatenic hosts (which might be considered as "phylogenetic reminiscence hosts") as alternative cycles, in that they do refer to a cycle with or without intermediate host, for example, in the context of *Hymenolepis nana*, or to a cycle with two or three intermediate hosts in the context of subgenus *Paralaria* (Fig. 23). Yet these cases do not represent different developments of parasites; it is rather one and the same development, distributed over different numbers of hosts. This manifestation is typical of parasitic life-cycles with additional hosts intercalated (p. 50); they are not alternative cycles in terms of the above description. Nevertheless, metaparatenic hosts do hold a distinguished position among the additional hosts, since they are the only additional hosts that are able to accomplish all functions of an obligate host (intermediate host). For that matter, they may

be considered as forming a transitional area between alternative cycles, on the one hand, and cycles with additional hosts, on the other.

An example of actual transition to alternative cycles is given by the trematode *Haplometra cylindracea*. The cycle is two-host (snail–frog), the frog being a polyvalent host in this case (cf. p. 77) which has originated from unification of second intermediate host and definitive host. Development really is alternative, since in some cases encystation of metacercariae will take place in the frog, and in others it will not, which means skipping over the metacercarial stage (Grabda-Kazubska, 1974).

(b) *In parallel cycles,* various possible developments are usually implemented at one time and in an obligate manner (sometimes facultatively). In the nematode *Tachygonetria vivipara* two forms of females, differing by ovogenesis, parasitize simultaneously on one common host. One of them is oviparous, with the positioned ova leaving the host (propagative reproduction). The other one is larviparous, with the larvae remaining placed in the host (multiplicative reproduction). Both propagative and multiplicative oviforms are produced by one and the same females of the nematode *Thelandros tba* (Fig. 11b). Coexistence of propagative with multiplicative reproduction may be observed from sporozoa, too (Fig. 10d). Such parallel cycles have originated from a genetically fixed rhythm of development. However, (delayed) propagative reproduction of sporozoa may be triggered by action of the host.

The "parallel cycle" of sporozoa is related to the entire micropopulation of parasites rather than to one generation, as used to be the case with the "alternative cycle" of parthenitae of trematodes. The difference is attributable to the monocellular or polycellular nature of the parasites involved rather than to the nature of the cycle. This shows that alternative and parallel cycles are somewhat similar to each other, even overlapping in certain areas.

Certain facultative alternative cycles play a role in transition to parasitic life by offering conditions for development in different environments. Parallel cycles, however, might be secondary adaptations of parasites. Variability of life-cycles seems to offer a good foundation on which to distribute development among different hosts, once parasitism has become established.

B. SPECIAL HOST CATEGORIES IN DIFFERENT GROUPS OF PARASITES

The phenomenon of heteroxeny, which, after all, is based on host categories different by ontogenesis or at least cycle, was first discovered in helminths (trematodes, cestodes) towards the middle of last century. In helminthology the prefix "hetero" is used preferably to suggest ontogenetic difference between host categories. The terms definitive host and intermediate host, now accepted quite generally on an international scale, emerged about the same time, just after the disclosure of the phenomenon of heteroxeny. More recent attempts to apply the same terms to other groups, such as protozoa, viruses and fungi, proved to be somewhat problematical. Any explanation of the problems involved, therefore, should begin with some reference to the common helminthological definitions for hosts of heteroxenous helminths,

especially as the host relations of helminths are more intricate than those of any other kind of parasite.

1. *The hosts of helminths*

The terminology to describe hosts of heteroxenous helminths was first initiated by van Beneden and Leuckart. van Beneden (1858), with reference to trematodes and cestodes, used the terms first host (premier patron, première île) and definitive or last host (hôte définitif, autre île, dernier hôte), which would follow each other in cases of heteroxeny (which he called "transmigration"): "Les premiers patrons servent au développement de leur jeune âge, c'est leur gangue de jeunesse; les derniers seuls leur fournissent une gangue qui leur permette d'atteindre leur développement sexuel complet" (loc. cit., p. 311). To van Beneden the "first host" primarily was a transmissive host (patron de passage, hôte comestible). This marks the actual origin of the present terms of definitive host and intermediate host. Leuckart (1863) also used the term definitive host (definitiver Wirth), but sometimes he would use "definitive carrier" (definitiver Träger). The names used by him for the other host category were intermediate host (Zwischenwirth) or intermediate carrier (Zwischenträger) or, less often, first host. In making reference to trematodes Leuckart would speak of the duplicity of intermediate hosts (zwiefache Zwischenwirthe). Leuckart's choice of "intermediate host" was motivated by his own conception of certain larval stages (rest and waiting stages), which he considered to be intermediate stages in ontogenesis, an explanation which may be derived from this literal quotation "... *vertheilen sich die charakteristischen Momente des Lebens auf drei, meist auch formell voneinander verschiedene Entwicklungszustände,* auf den Embryo, das geschlechtsreife Thier und einen Zwischenzustand ...". This intermediate stage would be compared by him with a "pupa", yet always with reference to juvenile or larval forms. The host of the intermediate stage will be a transmissive host in almost all circumstances (rarely a transit host). Yet a somewhat sophisticated element will be introduced by the possible occurrence of intermediate generations instead of an intermediate stage (the parthenitae or altrices of trematodes). The hosts of these generations will be (primarily) disseminators. For this and other reasons, they do not really fit into the picture of the intermediate host as painted by van Beneden and Leuckart. In the context of trematodes Leuckart would differentiate two principal forms (altrices and sexual animals) and two intermediate forms (fibrillating embryo and cercaria), but his own classification of altrices as intermediate generations meant their dismissal as "principal forms" and the downgrading of their hosts to the level of intermediate host. Here is his own literal explanation for his choice of the reference point in his host classification (loc. cit., p. 45): "Dass es die Zeit der Geschlechtsreife ist, an die wir unsere Darstellung anknüpfen, bedarf kaum der weiteren Begründung. Sehen wir dieselbe doch überall bei den Thieren den Beginn eines neuen Entwicklungscyclus einleiten."

Leuckart thus seems to indicate that there are two different basic categories of intermediate hosts, viz. larvo-intermediate and partheno-intermediate

hosts. Nevertheless, Leuckart's (and van Beneden's) concept of intermediate host is based entirely on larvo-intermediate hosts (which are transmissive hosts in almost all cases). Partheno-intermediate hosts are considered an exception: "Nach unseren bisherigen Erfahrungen gilt es also als Regel, *dass die Lebensgeschichte der Parasiten über zwei* (auch wohl mehr) *Träger vertheilt, von denen der eine den Jugendzustand, der andere das geschlechtsreife Thier beherbergt*" (Leuckart, loc. it., p. 81).

Leuckart, in fact, did make reference to "parasites", although the above conclusions had been derived from helminths only. More recent confusion could be imputed to Leuckart's conclusion, according to which changes in the usually encapsulated intermediate stages in larvo-intermediate hosts were confined, as a rule, to growth in size. Leuckart's propositions in a way were identical with those defended by all helminthologists of the last century who failed to differentiate between trematode cercaria and metacercaria as two stages of larval life, but spoke merely of "encapsulated cercariae" (instead of metacercariae). van Beneden (1875) called the intermediate host an animal by which the helminths are temporarily harboured. Such formulation suffers from inaccuracy, since the very same thing applies to the definitive host which by no means serves its helminths as a life-time harbour. In addition, the intermediate stages of larvo-intermediate hosts quite often will be connected with the host throughout host life unless that host is eaten up by the definitive host.

van Beneden (1875) defined the parasite in the definitive host as nostosite (derived from "nostos", the Greek word for return; in this case, return into the host in which the "transmigration" comes to its close). The parasite in the intermediate host was named by him xenosite (derived from "xenos", a Greek expression used in this context to mean stranger rather than host).

Reference to these earliest definitions has been made in some detail to show how difficult it is to derive from them any high-accuracy definition, and besides, these early expressions are in fact the sources available for modern definition.

(a) *Definitive host.* van Beneden and Leuckart have held concepts from which it may be derived that the definitive host will enable helminths to undergo full sexual development, after which the fully mature animal will be harboured by the same host that will eventually introduce another cycle of development. It should be borne in mind, in this context, that reproduction of the altrices of trematodes was believed about the middle of last century to be asexual. Yet the definition should be completed in the following way, since the parthenitae of trematodes are parthenogenetic females. The definitive host is the host in which bisexual reproduction takes place, according to the original definition which is incomplete. A complete definition would read as follows: *The definitive host will enable the heteroxenous helminths to undergo development from a certain stage* (*invasive larva*) *via the pre-adult phase to full sexual maturity; it will harbour the adult; there will be bisexual reproduction* (*usually fertilization*) *resulting in propagative forms* (*ova, larvae*) *which depart from the host* (*or, less often, are provided in the host for departure*).

Almost all definitive hosts of helminths are disseminators, and helminth reproduction on them will be propagative, but only rarely multiplicative as well.

In an abstract way, definitive hosts differ from intermediate hosts quite obviously, and even without the above definition, on account of their interconnection in a cycle, an expression of heteroxeny. The two categories are likely to manifest the differentiated nature of developmental stages or generations in one heteroxenous helminth cycle. The designations of definitive and intermediate hosts, therefore, are used to describe a sequence in a heteroxenous life cycle in which start and finish are set by defining the definitive host. (Hence, intermediate hosts must be enumerated, if more than one are involved in a cycle.)

The functions of a definitive host, sometimes, may be distributed among two different hosts (e.g. the hosts of certain nematodes; Fig. 15). Juxtaposition of definitive and intermediate hosts would not give much sense in such cases, as it would give rise to the question of the one decisive function out of all those quoted in the definitive host definition. Such a question, however, cannot be answered at all, since the definitive host in helminthology actually can be characterized only by the totality of all functions quoted above. The nematode genus *Fergusobia* is characterized by alternation between a bisexual generation and a generation with parthenogenetic reproduction. The female of the bisexual generation will parasitize on a fly. The larvae produced will abandon the host fly. They will gather in the sexual organs of the host fly (that will be infested only with female animals) and leave together with the fly's ova, which will be deposited in young buds of eucalyptus trees. The larvae will then continue to grow as parasites on the plant tissue in which they will produce gall. Grubs will hatch from the fly ova after six weeks and penetrate the gall tissue. The nematode larvae will develop into parthenogenetic, plant-parasitizing females that may follow each other in several consecutive generations. The bisexual generation will eventually emerge in autumn. After copulation and immediately before pupation the nematode females, so far plant parasites, will penetrate the female fly grubs, which completes the cycle. The males of the bisexual generation thus are plant parasites only, while the females will stay on the host plant until copulation takes place and in the host fly thereafter. In this case and in the case of *Howardula* (Fig. 15) coupled hosts of adults will be the only appropriate definition.

Principal host was sometimes used in place of definitive host in earlier German literature (Hauptwirt) and can be found occasionally even today. Yet, in the meantime, the expression principal host has been accepted almost universally with restricted use to describe a category of host specificity. Ambiguous use of the term may be quite confusing and should be avoided at all costs. The expression definitive host, on the other hand, is used unambiguously; it has become established and is understood on an international scale.

The limited applicability of the terms definitive and intermediate hosts to heteroxenous helminths actually may be inferred from both their history and present definition. Yet sometimes the same terms can be found describing

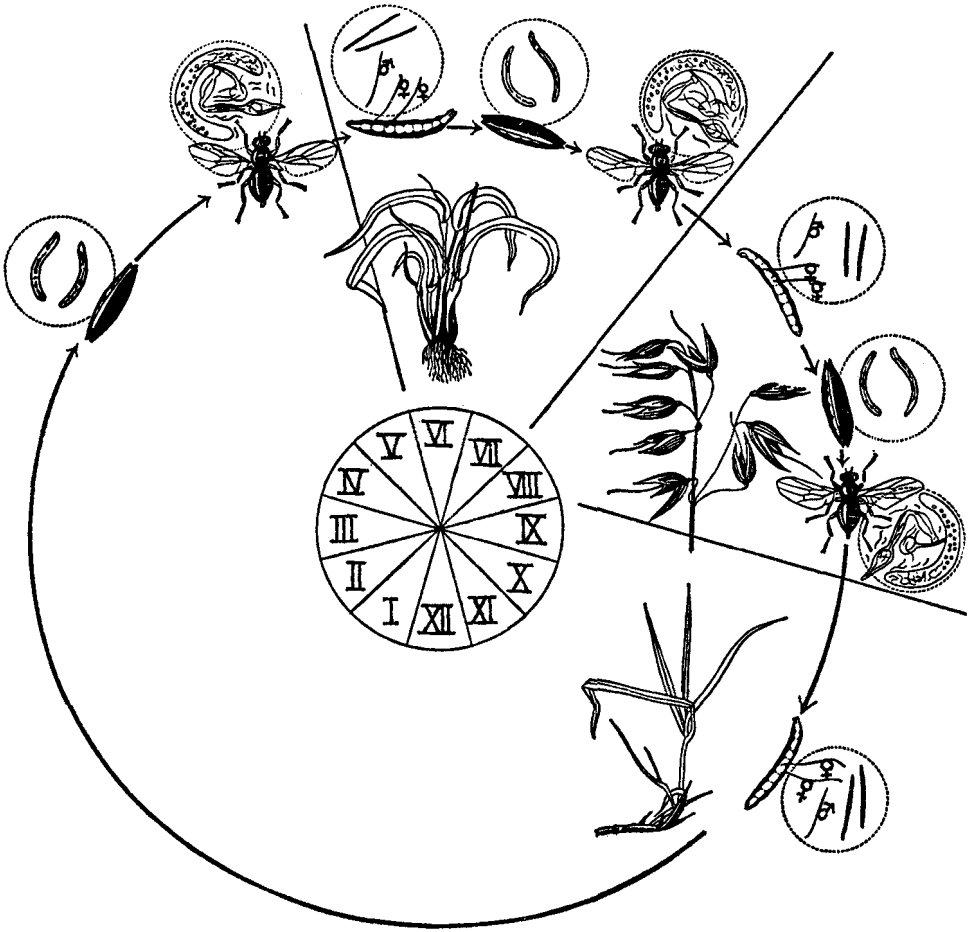

FIG. 15. Life-cycle of *Howardula oscinellae* (nematode) with coupled hosts of adults (fly and oats plant); one to eight viviparous females parasitizing in abdominal cavity of host flies, larval development beginning in fly, departure of larvae from fly and penetration of oats plant tissue where maggot development takes place; site where nematodes reach sexual maturity; females, after mating, penetrate fly maggots. (According to Goodey, 1933; adapted from Odening, 1969.)

hosts of homoxenous helminths. Such procedure cannot be recommended. Although every definitive host is a host of adults, there are hosts of adults that are not definitive hosts (cf. Section Vc).

(b) *Intermediate host.* The term of intermediate host is internationally common and understood in helminthology. This heterogeneous group of hosts may be defined more conveniently by making reference to the fact that all obligate hosts of a cycle are involved unless they are definitive hosts (or coupled hosts of adults) (a precise ontogenetic reference point being established only to define the definitive host). The name hôtes transitoires is

common in some of the French literature (e.g. Brumpt, 1910; Neveu-Lemaire, 1912). This might be mixed up with transit host from which the parasite will part after some time.

Also in French literature first and second host has often been used instead of first and second intermediate host (e.g. Combes, 1968), names which are readily understood.

(i) Partheno-intermediate hosts. These (primarily) disseminate forms of propagation (cercariae). By ecological function they are somewhat similar to definitive hosts on account of primary release of the descendants into the environment. Both of them have propagative helminth reproduction in common which is not present in the context of larvo-intermediate hosts. Hence, similarity of partheno-intermediate hosts with definitive hosts may be seen also from the standpoint of helminth ontogenesis. This similarity is further underlined and renders differentiation more difficult by occurrence of parthenogenesis in the hermaphrodite generation, for example, in the case of neoteny (cf. Buttner, 1950–51, 1955). Exclusive comparison between bisexual reproduction (in definitive hosts) and non-reproduction or reproduction other than bisexual (in intermediate hosts), therefore, can be considered a drawback of traditional subdivision into intermediate and definitive hosts. The functions of a partheno-intermediate host are quite analogous to those of a definitive host, except for parthenogenetic instead of bisexual reproduction and, as a rule, multiplicative parthenogenetic reproduction prior to and concomitant with propagative reproduction (or, less often, additional vegetative reproduction).

Heyneman (1960) defined the partheno-intermediate hosts as initial hosts and thus proposed their explicit differentiation from intermediate hosts.

(ii) Larvo-intermediate hosts. In almost all cases, these will be transmissive hosts of the larval and juvenile stages of heteroxenous helminths.

A number of special names has been invented mainly in the German literature (some of them then being translated into Russian) for some of these hosts. Most of them were derived from erroneous concepts but have continued to crop up in publications, with confusion and especial risk when translated into different languages. "Auxiliary host" or "supplementary (additive) host" are examples to this effect (dopolnitel'nyj chozjain). The German term for "auxiliary host" dates back to Looss (1894), by whom it used to be related to the situation in the triheteroxenous trematode cycle, to define the second intermediate host. His usage, after all, was based on the distinction between partheno-intermediate and larvo-intermediate hosts, although the larvo-intermediate hosts were incorrectly interpreted. The role of the auxiliary host was allegedly restricted to providing merely for the helminths' accommodation "in welcher die Schmarotzer längere Zeit, aber normalerweise *ohne Weiterentwicklung*, auszuharren vermögen. Aus diesem Grunde erscheint mir die Bezeichnung "Hilfswirth" für den letzteren praktischer als die Bezeichnung "zweiter Zwischenwirth", die eine *Gleichstellung* mit dem ersten und eigentlichen Zwischenwirth in sich einschliesst" (loc. cit., p. 237). Looss also used the term auxiliary host to

include mere transport hosts (e.g. the plants in the cycle of *Fasciola*). By presenting the partheno-intermediate host of the trematodes as the actual intermediate host, Looss brought himself in opposition to Leuckart and van Beneden, the inventors of the intermediate host term who explicitly considered the larvo-intermediate host as the real intermediate host (with partheno-intermediate hosts being considered as an exception by Leuckart). The difference between partheno-intermediate hosts on the one hand, and larvo-intermediate hosts on the other, has nothing to do, by the way, with what had been proposed by Looss, namely, development of helminths in the former and absence of such development in the latter. Both intermediate host categories are "developmental hosts" (and differ essentially from one another by the very fact that partheno-intermediate hosts are "reproductive hosts" and as such usually disseminators, whereas larvo-intermediate hosts usually are "non-reproductive hosts" and, nearly always, transmissive hosts).

This erroneous assessment of an alleged lack of helminth development in the auxiliary host has more recently caused much confusion in connection with the term transport host and paratenic (euparatenic) host. The following statement, for example, has been made by Fülleborn (1923): "I have used the term '*transport host*' instead of 'intermediate host' in cases where (as in many cercarial carriers) the intercalated host did not promote any development of the parasite but merely was to 'move' the parasite onto the *definitive* host (which then would eat up the transport host)." However, quite generally, there is no "cercarial carrier" at all, and the second intermediate host of trematodes does promote the development of penetrated cercariae into metacercariae. Terms used as synonyms by Nöller and Ullrich (1927) were second intermediate host, auxiliary host and transport host. And Sprehn, accordingly, made the following proposition (1932): "The creature that harbours a parasite is called *host*. The host in which or on which mature forms of the parasite live is called *definitive host* or principal host. If, however, the parasite cannot reach sexual maturity until it leaves for another host, the definitive host, the original host is an *intermediate host*. Two sub-variants are included in the group of intermediate hosts, the *actual intermediate hosts* in or on which development takes place of the juvenile parasite forms, and the *auxiliary hosts* which merely provide accommodation for the parasite until the latter will have moved on to its definitive host. The word *transport host* has been coined by Fülleborn as well to suggest that the intermediate or auxiliary host bearer of this name has one major task to accomplish, that is to transport the parasite to its definitive host. Such transport usually will be effected through consumption of the transport host by the definitive host concerned." However, the sequence of hosts within a given cycle cannot in fact be used as yet for conclusions about an equivalence of hosts that occur on a certain site. Such conclusions will not be permissible unless an analysis has been made of the individual case, with information having been obtained on what is contained in the host concerned, what processes are taking place in it, the functions it has, its phylogenetic entry into the cycle, and other phenomena. The expression intermediate host as such does not yield any information on any of these points. It simply implies that it is the counterpart

or counterparts to the definitive host in a cycle of heteroxenous helminths. In which case, enumeration of intermediate hosts, if there are more than one, will hardly reveal more than a sequence.

Even the first intermediate hosts in compared three-host cycles may be quite different in nature (e.g. those of triheteroxenous trematodes and pseudophyllideans), against the background of the fundamental difference that exists between partheno-intermediate hosts on the one hand, and larvo-intermediate hosts on the other. Such difference in nature may exist even between those intermediate hosts that are intercalated immediately prior to the definitive hosts of different cycles. They may be partheno-intermediate or larvo-intermediate hosts. They may be single, second or, rarely, even third intermediate hosts (*Strigea*, Fig. 8; eventually *Diphyllobothrium norvegicum*,* cf. Vik, 1957; Savinov, 1969b).

The new term "intercalary host" (vstavočnyj chozjain) and the sequence, intermediate host–intercalary host–supplementary (additive) host–definitive host (Skrjabin and Šul'c, 1937) were introduced in the Russian literature with reference to the obligate four-host cycle. More recently, the sequence was modified into intermediate host–supplementary host–intercalary host–definitive host (Skrjabin and Šul'c, 1940; cf. Savinov, 1964b, 1969b; Sudarikov, 1959). Skrjabin's and Šul'c's invention of the term intercalary host still coincided with the general assumption that a four-host cycle was obligate for the subgenus *Paralaria* (to which the only species belongs to whose cycle the term was originally applied). The term, in other words, was coined for one definite intermediate host in an obligate four-host cycle. However, *Paralaria* then proved to be linked not to an obligate but at best to an alternative four-host cycle (Fig. 23). The third intermediate host that may occur alternatively in such a cycle was defined as intercalary host by Savinov (1954), but in a more recent paper (1964b) it was mentioned as metaparatenic host (metarezervuarnyj chozjain) (cf. p. 69). The only obligate four-host cycle so far known of trematodes is that of *Strigea* (Pearson, 1959; Odening, 1967; Fig. 8). Sudarikov (1959) as well as Šul'c and Gvozdev (1972; p. 228) have made another reference to the original position of the intercalary host, according to Skrjabin and Šul'c (1937), by describing it as a host intercalated (in the cases of *Strigea* and *Alaria*) between the first and second intermediate hosts or between intermediate host and supplementary host. However, in another chapter of the same book (1972; p. 13) Šul'c and Gvozdev declared the intercalary host to be identical with Savinov's metaparatenic host. Hence, in Russian literature "intercalary host" has been used as a synonym for second intermediate host (in the cycles of *Alaria* and *Strigea*), third intermediate host (in the cycles of *Alaria*, *Strigea*, and *Diphyllobothrium norvegicum*, cf. Savinov, 1969b), metaparatenic host (in the cycle of *Paralaria*), and metaparatenic host in general. In the English literature, there has been occasional usage of "intercalary host" for paratenic

* It may be that in this case the "third intermediate host" is actually a para-paratenic host, since this species has recently been declared a synonym of *D. dendriticum*, a species with a 3-host cycle, by some Finnish workers.

host. The metaparatenic host in the cycle of *Paralaria* was referred to as auxiliary host by Johnson (1968) (in distinction from the paratenic hosts in the same cycle).

It appears to be quite obvious that there is also a phylogenetic aspect behind the above special names for intermediate hosts in German and Russian papers. Yet, particularly in this area, remarkable differences can be found between various groups of helminths. Also, it should be borne in mind that no phylogenetic aspects were involved in the original definitions of definitive and intermediate hosts. The ontogenetico-cyclic host terminology should be kept strictly clear of any phylogenetic aspect in view of the possible risk involved in differentiated individual assessment and because of the disparity by which heteroxeny of the various groups of helminths has evolved (cf. Heyneman, 1960; Ginecinskaja, 1968; Pearson, 1972, for trematodes; Joyeux and Baer in Grassé, 1961; Llewellyn, 1965, for cestodes; Sprent, 1954, 1962; Osche, 1957, 1959; Chabaud, Nigon, Ritter, Théodoridès in Grassé, 1965; Skrjabin and Ivaškin, 1968; Šul'c and Gvozdev, 1970–72, for nematodes; Petročenko, 1956, 1958; Golvan, 1958; Baer in Grassé, 1961, for acanthocephalans).

It must be stressed, in this context, that the intermediate hosts involved are obligate, which means that for ontogenetico-cyclic reasons (rather than for ecological reasons only) the typical heteroxenous helminth cycle cannot come into effect without their involvement. However, the German and Russian special names chosen for certain intermediate hosts are likely to give the impression that they are facultative or, at best, hosts of minor importance in the cycle. This erroneous impression may be supported by the very names formulated, their original (wrong) interpretation (Looss, Fülleborn), or their factually wrong reference (to *Paralaria*). This is likely to lead to confusion, since the absence of any one obligate host in the cycle would lead to the latter's disruption. All ontogenetico-cyclic hosts, consequently, are of equal importance to the parasite.

The German and Russian words coined for "auxiliary host", "supplementary host" and "intercalary host" to describe certain intermediate hosts, after all, have a phylogenetic reference, albeit quite indeterminate. In addition, they are likely to simulate the presence of a certain ontogenetico-cyclic reference. However, such reference is quite impossible in the context of intermediate hosts, since comparison between partheno-intermediate and larvo-intermediate hosts is the only real criterion for differentiation. Further differentiation within the group of larvo-intermediate hosts is feasible only by their sequence in the cycle (by enumeration) rather than by ontogenetic characteristics, since there is quite a bit of difference between the various helminth classes with regard to larval development, and the latter, therefore, cannot be used as a coherent basis of judgement.

As to the particular nature of intermediate hosts and their specific affinity for each of the existing classes of helminths, it may be possible and advisable to find unambiguous and unique names by which to give clear-cut expression to the situation in each of the helminth groups, for example, partheno-intermediate hosts or hosts of parthenitae or altrices of trematodes, often

called merely molluscan hosts, or (obligate) hosts of mesocercariae or (obligate) hosts of metacercariae.

2. *The hosts of other heteroxenous parasite groups*

In spite of certain original shortcomings, the ontogenetico-cyclic classification of helminth hosts actually does reflect both the ontogenesis and the life-cycle of the helminths. This, however, will be the major problem, whenever an attempt is made to translate the terms definitive and intermediate hosts to parasites other than helminths. Throughout the ontogenesis of helminths, there is a characteristic sequence in the occurrence of periods of development ("ascending development" to the next higher stage), no-development periods (periods of alternation with no "ascending development") and reproduction periods (with no "ascending development" either). The periods of alternation are usually paralleled by typically circumscribed stages of development, while the periods of development are transitional phases between two consecutive stages. A period of alternation (according to Savinov, 1964a) is flanked by two "turnover moments" with a period of stabilization between them (Fig. 16). The first turnover moment marks the turnover point from "ascending development" to non-development ("moment of emergence of new demands"). The second turnover moment terminates the no-development period and marks the turnover point to another period of development ("moment of satisfaction of emerged demands"). In hosts obligate to helminth cycles a period of development will usually be followed by a period of reproduction or a period of stabilization.

Another substantial factor which should be borne in mind in the context of helminths is that, quite generally, only segments of individual development will take place in both the larvo-intermediate and definitive hosts (whereas

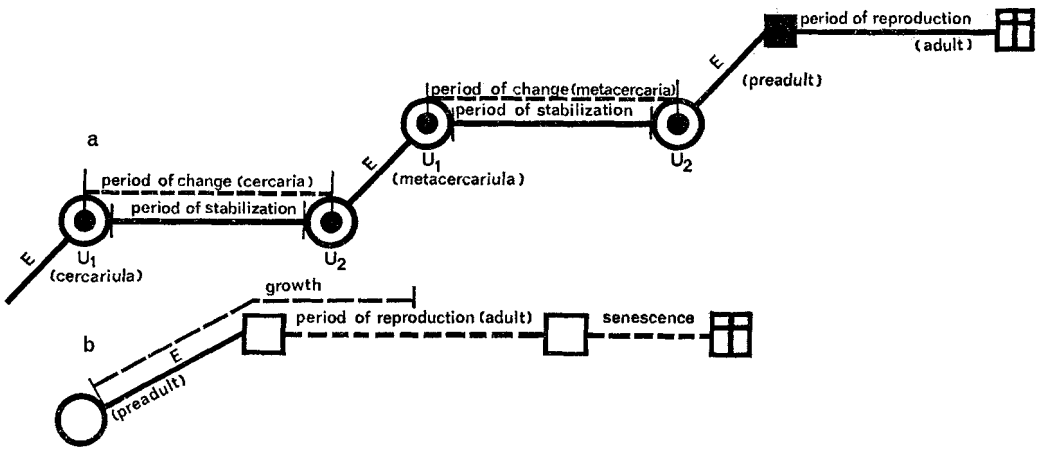

FIG. 16. Phased-out development of helminths. (a) Individual development of bisexual type of trematode generation (E—period of development, U_1 and U_2—turnover moments); (b) postlarval development. (Odening, 1974c.)

supra-individual development will take place to some extent only in partheno-intermediate hosts). The conditions of helminths (including those of pentastomids) may at best be compared with those of heteroxenous isopods to which the terms definitive and intermediate hosts are then applicable as well. Host categorization by ontogenetic aspects seems to be feasible only for heteroxenous parasitic metazoa (helminths, isopods) and for heteroxenous rust fungi, sporozoa, and perhaps flagellates (Table II).

Any attempt to apply the terms definitive or intermediate hosts to heteroxenous flagellates (Trypanosomatidae, Bodonidae) will be doomed to failure, from the very outset, because of complete absence of sexual forms. While

TABLE II

Ontogenetic-cyclical host categories of heteroxenous parasites

Parasite group	Example	Host categories obligatorily or optionally (additionally or alternatively) connected in a cycle	
Flagellata (also piroplasmids and others?)	*Trypanosoma brucei*	1 oblig. host of the forms in avertebrates (avertebrate host)	1 oblig. host of the forms in vertebrates (vertebrate host)
	Trypanosoma cruzi	1 to several oblig. hosts of the forms in avertebrates (avertebrate hosts)	0 to 1 altern. host of the forms in vertebrates (vertebrate host)
	Trypanosoma rangeli	As for *T. cruzi*	0 to 1 addit. vertebrate host
Sporozoa	*Plasmodium*	1 oblig. schizogonial host	1 oblig. sporogonial host
	Schellackia	1 to several oblig. schizosporogonial hosts	0 to 1 addit. host of sporozoites
	Toxoplasma	0 to several altern. schizosporogonial hosts	0 to several altern. cystical hosts
Uredinales	*Puccinia graminis*	1 oblig. aecidial host	0 to several altern. hosts of uredospores, 1 oblig. host of teleutospores
helminths (incl. pentastomids), isopods	*Taenia solium*, epicarids, *Clonorchis*, *Strigea*	1 to 3 (oblig.) intermediate hosts	1 (oblig.) definitive host
	(cf. pp. 62–69)	as above, plus 0 to several addit. hosts of larvae	as above, plus 0 to several addit. hosts of adults
	Toxocara	0 to several addit. hosts of larvae	1 oblig. host of adults
	Howardula	2 coupled oblig. hosts of adults	

bisexual reproduction does occur in the context of heteroxenous sporozoa, this can hardly be compared with reproduction of helminths. Which stage in the gametogony phase of sporozoa will correspond to the adult of helminth? If the adult is assumed to disseminate propagative forms, these would be identical with the gametocytes or gametes of sporozoa. The gamont should correspond to the adult under such circumstances. However, certain protozoologists obviously interpret the gametes as the very forms that correspond to adult helminths, since in the context of malarial plasmodia they would consider the *Anopheles* as definitive host and man as intermediate host. Fertilization (rather than gametogenesis) is their deciding criterion for a definitive host. As a whole, asexual reproduction will take place both in man (schizogony) and in *Anopheles* (sporogony), which in the context of helminths would be the criterion of processes in the intermediate host. The phase of gametogony will begin in man and end in *Anopheles*. Both hosts, consequently, show certain features of a definitive host of helminths. *Anopheles*, by strict judgement, merely is a "fertilization host" and, therefore, not fully comparable with the definitive host as conceived in helminthology, The processes which really are strictly distinct between the two hosts are schizogony on the one hand and sporogony on the other. Therefore, a proposal has been made to call such hosts schizogonial or sporogonial hosts (Odening, 1974a). Other approaches have been oriented to definition by groups of host organisms ("vertebrate host" and "avertebrate host" or "host plant"). Such an approach will undoubtedly satisfy the demand for unambiguity in all cases in which there is actually a wide systematic gap between the two host categories under review (also in cases of heteroxenous viruses and bacteria). It will fail, however, in other cases, for example *Aggregata*, in which two invertebrate hosts follow each other with nothing in between. Yet classification by groups of host organisms is no longer ontogenetic classification.

Host names directly related to ontogenesis and cycle of the parasites have continued to exist only for the heteroxenous rust fungi. They are aecidial host (host of aecidiospores), host of uredospores, and host of teleutospores (cf. Gäumann, 1964). The aecidial host is the carrier of both the haploid phase (the gametophyte) and of a sexually differentiated generation. It is the site of copulation and "asexual" formation of aecidiospores. The genuine host of uredospores (carrier of the dikaryophase or sporophyte) is the site of asexual production of uredospores, and karyogamy eventually will take place on the host of teleutospores (carrier of the dikaryophase). "Gametes" (basidiospores) will be formed in the open environment. A host of uredospores may temporarily occur as an independent element, but this will happen only in summer, and it will become a host of teleutospores towards the end of summer. Such deviation of reproduction modes from those of metazoa (separation of cell copulation from fertilization, expansion of sexual processes over two generations, additional asexual reproduction) is the reason why the terms definitive and intermediate hosts cannot be applied to fungi. Characteristics of the definitive host do apply to both fundamental host types of Uredinales.

The host categories involved in heteroxenous protozoa and fungi are based, indeed, on developmental differences between the parasites concerned, but such differences so far are unknown in the context of heteroxenous viruses and bacteria. All knowledge so far accumulated seems to suggest that the differences between host categories of heteroxenous viruses and bacteria have something to do merely with the hosts themselves and parasite action upon these. Piroplasmida seem to take some transitional position between the groups which exhibit different developments in the two host categories, on the one hand, and those which do not on the other.

Certain authors have resorted to fairly arbitrary usage of the terms definitive and intermediate hosts by applying them to protozoa (cf. Chandler and Read, 1967), and even to heteroxenous viruses and bacteria. They have obviously mixed up the terms intermediate host and vector. Reichenow (cf. Doflein and Reichenow, 1949) with regard to protozoa defined each historically acquired secondary host as intermediate host, a procedure which deviates entirely from common helminthological usage of the term.

C. ADDITIONAL HOSTS

Additional hosts are facultative parasite hosts of obligate parasites. Typically (helminths), they are "partially substituting hosts of environment and function". Hence, they are dispensable in an ontogenetic cycle, but they can be added to a cycle and take the place of environment or of another host.

The name additional host seems to be unambiguous (Odening, 1968–69, 1969, 1974a,c). However, the concomitant German designation in the sense of substituting host (Ersatzwirt) needs some explanation. What is taking place, as a matter of fact, is nothing but facultative substitution for something which has already started to function (as host or as environment). The expressions substitution or replacement are not used to describe vicarious action (in terms of geography, host, history or ecology). Obligate substitution will take place inevitably in any parasite cycle (sequence and alternation of hosts). However, additional substitution (and only this is meant by the term "substituting host") will take place facultatively. Viewed from the environmental angle, substitution is sudden change, but to the parasite it means relocation. A cycle will not be affected in terms of ontogenesis or cyclic development of the parasite by appearance of additional hosts. The development or non-development life periods of the parasites will be distributed among additional hosts, and this is all that happens. This is basically the reason why additional hosts will not be able to prolong a given cycle, since the time span needed for the development of the parasite and the life span of its non-development stages essentially are established in the parasite and do not depend on any variable number of hosts involved. This concept applies to all cases other than those in which the potential life span of a parasite is longer than that of its obligate hosts. In the latter cases, however, a cycle might be really prolonged through involvement of long-life additional hosts. Under natural conditions, therefore, additional hosts are important for

"saving" parasite forms from destruction rather than for "prolongation" of a cycle. Such a role may be associated with accumulation effects and fresh possibilities of transmission and even help to prolong parasite life, provided that the life span of an additional host was longer than that of a substituted obligate host.

Occurrence of additional hosts has so far been observed primarily in connection with helminths. Their identification and delimitation from indispensable hosts as well as differentiation between various categories have been the subject of effort from the 1920s to the present day. There is much discrepancy, uncertainty, and individualism of views regarding this problem, a state of confusion which, after all, has had terminological repercussions and must be imputed to a very slow growth rate of knowledge on the subject. A more general account was possible until recent years and was entirely lacking in the twenties or thirties. Phenomena of "re-encapsulation" or "re-fixation" were first reported by Seurat (1912) for nematodes and by Okumura (1919) for *Diphyllobothrium* (= *Spirometra*) *mansoni*. The first comprehensive account of known additional hosts of larvae of helminths was given by Joyeux and Baer (1934), who made this statement: "Enfin, pour un certain nombre d'helminthes, existe une troisième catégorie d'hôtes, que nous allons étudier au cours de ce travail, et qui sont intercalés entre l'intermédiaire et le définitif. Parfois, ils ne jouent aucun rôle dans le développement du ver et le cycle s'accomplit parfaitement sans eux; cependant, ils peuvent contribuer à perpétuer l'espèce parasitaire en accumulant les larves par divers procédés et en favorisant, par cela même, les infestations intenses de l'hôte définitif dont ils sont la proie. Dans d'autres cas, ils sont indispensables; la larve subit chez eux une maturation plus au moins complète, nécessaire avant de pénétrer dans l'hôte définitif." The hosts in question were called by them "hôtes d'attente" (in German translation: Wartewirte). The confusion of facultative with allegedly or actually obligate hosts, as well as of stadiogenic with non-stadiogenic hosts, introduces a contradictory element to this first definition of additional hosts. The term "hôte d'attente", which was untranslatable into English, was replaced by paratenic host more recently by Baer (1951). This term, in the meantime, has met with growing international acceptance. We should like to recommend this term for general usage, since all other expressions so far chosen and arbitrarily used have proved to be ambiguous (reservoir host, reserve host, transport host, vicarious host, transfer host, carrier host, auxiliary host, intercalary host) or not applicable internationally (hôte d'attente, Wartewirt, Stapelwirt). Baer (1951) assumed the paratenic hosts to be "potential intermediate hosts" which were dispensable to the cycle, since no development of helminthic stages took place in them. The paratenic hosts were defined as "potential intermediate hosts" by other authors as well (among them Pavlovskij and Gnesdilov, 1939; Noble and Noble, 1964). Savinov undertook to deal with this erroneous interpretation (1964b). An explanation as to the only possible nature of potential hosts is given on p. 21. Paratenic hosts cannot be potential intermediate hosts, since they fail to meet the totality of demands made on the functionality of an obligate host (cf. Table III; an exception might be conceded

TABLE III

Characteristics of an ontogenetic-cyclical host classification of the parasite hosts of helminths

Term	Helminth form characteristic for the host category	Characteristics								Main ontogenetic periods
		obligatory	additional	stadiogenous	non-stadiogenous	cyclic	postcyclic	non-reproductive or multiplicative–reproductive	propagative–reproductive	
definitive host	adult ♀, ♂, ☿	+		+		+			+	period of development, then of reproduction
larvo-intermediate host	larva (or juvenile form)	+		+		+		+		period of development, then of stabilization
partheno-intermediate host	parthenogenetic ♀	+		+		+			+	period of development, then of reproduction
euparatenic host	larva		+		+	+		+		(interrupted) period of stabilization
metaparatenic host	larva or juvenile stage		+	+		+		+		period of development, then of stabilization
paraparatenic host	larva		+	+		+		+		(interrupted) period of development, then period of stabilization
paradefinitive host	adult		+	+		+			+	(interrupted) period of development, then period of reproduction
postcyclic host	adult		+		+		+		+	(interrupted) period of reproduction

to the metaparatenic host, but even this might at best be called an alternative rather than a potential intermediate host). If a person decides to walk from Paris to Brussels but is replaced by another one a few miles from the finish or represented by the latter only at the prize ceremony, the one who took over for almost nothing can hardly be called a potential Paris–Brussels walker! The attributes "facultative" or "functional" will not hold water either in this context. Neveu-Lemaire (1936), for example, called the paratenic hosts "hôtes intermédiaires facultatifs". Baer (1951) used the terms "obligate" and "facultative" not only with regard to ontogenesis, that is the cycle, but also in terms of ecology. This usage led him to formulations such as "facultative intermediate host" and "obligate paratenic host". The latter was meant to be a paratenic host which had become indispensable in nature for purely ecological reasons, although it proved to be dispensable to an experimental cycle. This formulation, however, remains contradictory and confusing, even if there were such a case (which, certainly, would be a rare exception). It appears to express some uncertainty which actually does exist on whether the host concerned (above all, hosts of nematodes and acanthocephalans and, to some extent, even cestodes) is an additional host of larvae or an obligate host (intermediate host).

Another definition was proposed by Skrjabin and Šul'c (1940) with regard to the phenomenon of re-encapsulation or re-fixation. They held that in all cases their "rezervuarnyj chozjain" was nothing but a facultative and non-stadiogenic host of larvae. They underlined, in this context, the morphologically unchanged re-location of helminth larvae. Great attention has been given more recently to the phenomenon of "reservoir parasitism" (paratenic parasitism) by Soviet helminthologists (Ryžikov, 1954, 1966; Šumakovič and Ryžikov, 1954; Ivaškin, 1961a,b; Šul'c and Davtjan, 1955; Savinov, 1953, 1954, 1958, 1964a,b, 1969a,b,c, 1970; see also Ryšavý, 1961, 1964, 1968; Ryšavý and Baruš, 1965; Czapliński, 1963; Odening, 1961, 1963, 1965a,b, 1967, 1968a,b, 1968–69, 1969, 1974a,c). The most comprehensive and detailed analysis and classification of additional hosts of larvae of helminths so far has been offered by Savinov (1964a,b, 1969a,b). Much of his work has been utilized as a basis for the views presented in this review. Substantial value should be attributed to his comparison between stadiogenic and non-stadiogenic hosts and the delimitation of euparatenic, paraparatenic and metaparatenic hosts. The phenomenon of additional hosts of adults was studied or taken into consideration by Božkov (1969b, 1974a,b, 1975) and Odening (1968–69, 1974a,c,d).

A comparative account of additional hosts versus (obligate) hosts indispensable to ontogenesis and cycle, seems to be of primary interest. Obligate hosts, according to Savinov, constitute the inevitable minimum of stadiogenic hosts. Basically, additional hosts of helminths may be found to occur at two different levels of development:

(i) level of larval development or non-development existence of larvae;

(ii) level of adult development (pre-adult) and non-development existence of the adult (reproduction period) (cf. Table III).

Another conclusion from such comparison is that additional hosts

may occur either during periods of development (stadiogenic additional hosts) or during non-development periods (non-stadiogenic additional hosts). Individual development of helminths will be characterized by consecution of "development phases" (development segments) and "development stages" (clearly distinguished levels of development which often will become springboards from which to trigger the next higher development phase only by total change of environment). A development stage which depends on alternation of hosts or host–environment or environment–host alternation for rise to another phase of development will be called hold-on or holding stage (with development being suspended in it and the stage "waiting" for total change of environment). Such hold-on stages which mark static periods of development, for example, include all invasive larvae. These may be passive (all encysted larvae) or active (majority of cercariae and all larvae with percutaneous route of penetration). The duration of passive hold-on stages usually will be longer than that of active variants. "Static state" and "waiting" in this context do not stand for passivity or immobility but for absence of development (usually with no feed intake).

A phase of development (especially one in the environment) may be similar to a hold-on stage, in that there can be temporary external stagnation (for example, in embryogenesis within the egg capsules). These "static phases" differ from real hold-on stages, in that no total (qualitative) change of environment is required for continuation but merely modification of certain environmental factors (particularly quantitative factors, such as temperature).

Stages which are not explicit hold-on stages may be reached in hosts or environment where development will then go on "automatically" (with no total change of environment). These stages too are characterized by stagnation, though often of short duration (except for the stage of the adult or the parthenogenetic form). The point at which a host is substituted may be important under certain circumstances, during a phase of development or in a parasite stage.

A thorough analysis of the "phased-out development" of helminths was undertaken by Savinov (1964a,b). This has in fact proved to be a field which provides an important clue to more understanding of the differences which exist between the parasitic activities of helminths, protozoa, bacteria, fungi and viruses. Helminth development is of very special interest for its conspicuous alternation of periods of development (phases) and "non-development" periods (with these terms coinciding to a great degree with insect field, where all development takes place on distinct morphological levels, which is slightly indicated by the larval development of nematodes). The term "development" is taken to mean the processes of morphophysiological differentiation and integration (ascending development of an individual up to a terminal stage) rather than processes relating to ageing and change in viability and invasiveness or rejection and regeneration of body parts or reproduction.

In Savinov's terminology, "phased-out" helminth development, typically, means consecutive occurrence of periods of development (also referred to as "ecologico-ontogenetic" stages by Savinov and as phases by the author)

and periods of alternation (related to the author's stages of development, i.e. to points where they clearly come to the fore) (Fig. 16). A period of development will take place depending on certain environmental conditions. It will usually end when changing demands of the helminth phase can no longer be met by the given environment. This will mark the onset of a first-category turnover moment ("moment of emergence of new demands"), and development will stop. A period of stabilization will follow (which we will call stage because of its affinity with certain morpho-ontogenetic "terminal stages"). This stage will not end until the emerged demands have been met, generally following change of environment. This will trigger a second-category turnover moment ("moment of satisfaction of emerged demands"), a starting point of transition from non-development to development. The periods of alternation will each be flanked by one turnover moment of first category and one of second category.

In addition to development in stages, clearly defined and organized by total change of environment, there are periods of alternation (or "moments of alternation", if these periods are short). In these periods or moments of alternation, the turnover moments of first and second categories will occur in one and the same environment or localization of the given helminth forms. Savinov, therefore, proposed subdivision into monolocal and bilocal periods or moments of alternation. Delimitation of different periods of development (by detection of periods of alternation between them) may be quite difficult due to close neighbourhood between environments or organs in which turnover moments may occur. Moments of alternation can be short enough to go unnoticed. Also, there may be cases in which subsistence conditions for two or more periods of development are different by nature but identical by location. Such differences between prerequisites for existence can be visualized by testing the demands on existence, and such difference can result from differentiated host specificity of the two "masked" periods of development (e.g. metaparatenic hosts, cf. p. 69, against which the hosts of adults require closer and narrower specificity). The conditions needed for two periods of development may differ from each other even in situations in which both of them are based on one and the same environment or localization. Termination of a phase of development will not always coincide with the completion of morphological changes which are characteristic of certain morpho-ontogenetic stages.

Momentary disagreement between change of environment and number of periods of development may be caused by and depend on phylogenesis. Displacements in consecutive occurrence of periods of alternation, stabilization and reproduction may also be caused by neoteny (cf. Buttner, 1955). The following possible developments in this context were mentioned by Savinov (1964a):

1. A less organized ontogenesis may become more or less strongly articulated;
2. in a strongly organized ontogenesis, certain periods of alternation may fail to occur due to merging of phases;
3. an unchanged number of periods of development may be accompanied by change in their demands on environment;

4. an unchanged number of phases with no change in their demands on environment may be accompanied by dislocation within two adjacent phases of certain differentiation and integration processes;
5. bilocal periods (or moments) of alternation may become monolocal.

One period of development (phase) will be limited against another one not so much by morphological change but rather by (sometimes very short) interruption of such change. The end of one development phase will then hardly stand out against the beginning of the next. While helminths, sometimes, may fail to undergo any development against the background of changing environment (e.g. through migration in a host's body), two or more periods of developments may on the other hand be passed in one and the same environment. The morphological and biochemical quality of a development period at its beginning will differ from that at its end, a change usually demonstrated externally. Such development periods are somewhat "incomplete", and therefore most of them remain unnamed; usually only their final levels ("stages of development") are given names: miracidium, coracidium, acanthor, procercoid, coenurus, cysticercoid, oncosphere, adult etc. The only names of real phases of development are "acanthellae" and "pre-adult". Such lack of names for periods of development must be deplored as a terminological shortcoming which may lead to non-differentiation or confusion of development phases and complete development stages. Diminutives to describe periods of development, such as procercoidulus, cysticerculus, cercariula, and others, have been proposed by Šul'c and Dikov (1964) in a bid to remedy the situation.

The periods of alternation (most of them being morpho-ontogenetic stages) are "static periods" which contain, often concealed, the two turnover moments (first and second categories) and the stabilization period. Different parts of one and the same period of alternation may have different expressions in the form of reactions. Sometimes, much can depend on the part of the stabilization period which has come to the fore. Such dependence will be more clearly recordable from active forms which will draw energy from stored matter during the period of stabilization (e.g. coracidium, cercariae). Primarily passive forms may also exhibit manifestations of ageing and changes in viability and invasiveness during their period of stabilization (which may last years in their case). Stages of development with euparatenic, plurirefixatory powers (p. 62) may have alternating passive and active stabilization period components during each re-fixation.

Savinov (1964a) proposed differentiation between periods of stabilization with delayed course of ageing processes, on the one hand, and periods with accelerated decline in viability on the other. Further differentiation will be possible between various stabilization modes, in hosts, in environment, in eggs or egg capsules and in cysts. There are some more subperiods which may be differentiated within a stabilization period: one course in the first (stadiogenic) host which follows the first-category turnover moment and one course in the second (stadiogenic) host which lasts up to the second-category turnover moment (a course often characterized by migration).

Another course of a period of stabilization may be found in non-

stadiogenic, euparatenic hosts (p. 62). Those two courses of a period of stabilization which take place in stadiogenic hosts have been named metaphase and prophase courses, with some reference having been made to Savinov's proposals. Metaphase and prophase stabilization may develop in a stadiogenic host and (for partimal* helminths) in the environment. Metaphase stabilization will be characterized by less activity. "Less activity" is a relative assessment, since there are absolute differences between helminth forms with passive behaviour in metaphase stabilization (e.g. encysted invasive larvae) and other forms with active behaviour under the same circumstances (e.g. free-swimming miracidia, cercariae, and invasive larvae with free movement in the environment).

In a euparatenic stationary host, metaphase stabilization will cover the largest part of the stabilization period (the full range of a stabilization period in the euparatenic host being called euparatenic static period by the author, but "reservation" by Savinov, the latter name being chosen because reservoir host is the most common name for euparatenic host in the Russian terminology). Prophase stabilization will be characterized by more activity. Corresponding to it is another subperiod in the euparatenic stationary host (from invasion to definite re-fixation, in other words, the process of re-fixation as opposed to a static state). These two courses have been called prerefixatory and postrefixatory periods (Savinov's "period of migration" and "period of passive stationary stabilization"). Another course of a stabilization period may occur in one and the same stadiogenic host, between two consecutive phases of development. This subperiod (often characterized by migration) has been named diaphase stabilization period, with reference to Savinov.

The difference between first-category and second-category turnover moments is primarily one of changed environment. This should support conclusions as to biochemical change in the helminth forms, according to Savinov.

The demands made by helminths on their environment in the period of stabilization should be considered less assuming than those made during a period of development. Change during the periods of stabilization and their differentiated lengths may have repercussions to the effect that a maximum period of alternation need not be identical with the maximum life span of helminth forms during the period of stabilization. The helminth form may lose its invasiveness after some stabilization (thus bringing the period of alternation to its maximum) without suffering death. In other words, maximum and minimum durations should be distinguished, when it comes to periods of alternation.

The notion of a period of stabilization is only to some extent congruent with the (heterogeneous) notion of diapause (entomology). There are inevitable diapauses and exogenously induced, facultative diapauses. A diapause will not be congruent with a period of stabilization in helminths unless it is inevitable by the law of nature and without any development whatsoever

* Partimal parasites (Skrjabin and Šul'c, 1940) have a period of development in the environment.

(complete standstill rather than retardation). Such congruence, also, will develop only in a passive period of stabilization characterized by static condition on the surface (for example, in encysted larvae), because diapause also means suspension of external manifestations of life (latency), and such external stoppage does not occur in active stabilization periods of helminths (miracidia, cercariae etc.).

Accidental interruption of a coherent development period (say, by artificial impact) will be followed by temporary stagnation. Such "static phase" can be considered a facultative diapause (triggered by ways not endogenous).

Savinov's criterion by which to differentiate between various individual host–parasite relations is the existence of a stabilization or a development period, to which I should like to add the presence or absence of a reproduction period. Yet even within the stabilization period, there may be host–parasite relations quite different in nature.

I should like to add to Savinov's periods of development and alternation the period of reproduction, during which the adults or parthenogenetic females (including parthenitae of trematodes) will produce reproductive and/or propagative forms. In typical helminth development, the reproduction period will be preceded by the development period of the pre-adult. This is a phase on its own, because its demands on environment are somewhat different (more specific or less specific) from those of the adult stage. This may be seen, for example, from the "unfit or abortive hosts" in which no sexual maturity will be reached. Hence, there must be a moment of alternation between the pre-adult phase and the period of reproduction. It is the pre-adult phase to which the pre-patent period (equal or greater in duration) must be attributed (time which elapses before first propagative forms are detectable). The imaginal stage of helminths is generally clearly demarcated in terms of morphogenesis too. It usually starts with the production of reproductive and/or propagative forms and will end by definite cessation of such production. The processes of morphogenesis are usually completed when the stage of reproduction has been reached. Sometimes, growth may continue, and the onset of production of propagative or reproductive forms will not in every case coincide with termination of growth. The period of reproduction will be ended by death of the individual or by transition to senescence which will end itself in death. There are flowing boundaries between the development period of the pre-adult, reproduction period and period of senescence. Both the periods of reproduction and senescence will exhibit some superficial similarity with the periods of development and stabilization, identity with the latter resulting mainly from the absence of morphophysiological differentiation. The difference that remains between the two former periods and the two latter is, of course, reproduction proper, but has something to do also with the position and limitation of the period of reproduction (and of senescence). Unlike the period of development, there is no flanking by two periods of alternation, and unlike the period of stabilization, there is no flanking by two different turnover moments. New demands on environment are not satisfied in the period of stabilization, but in the period of reproduction the demands made by the adult stage on environment will be met.

Savinov (1964b) found two typical processes to take place in the development (*sensu lato*) of helminths:

(i) "stopping" (interruptive) processes (morphophysiological differentiation and integration, in the first place);

(ii) continuous (non-interruptive) processes (above all, ageing).

The first type will apply to the period of reproduction, which is an interruptive development. Two different types of development should be considered in this context: (a) morphophysiological differentiation and integration of the individual in terms of "ascending development" up to a "final stage"; (b) processes relating to the production of propagative and/or reproductive forms. Such processes of reproduction can be interrupted as well. For example, temporary interruption of a reproduction period will take place whenever a host of adults is substituted by a postcyclic host. A period of reproduction may be disrupted even with no change of host (e.g. by change in disposition, by the action of medicaments, or due to an inherent reproductive rhythm of the parasite).

Evolution of cycles of bioreceptive additional hosts may in itself be remarkably differentiated with regard to systematic grouping. These hosts may fall in a group identical to that of the interruptively substituted obligate original category, but they may also be part of another group. A differentiation on an individual basis, i.e. within a given cycle, was proposed by Božkov (1969b) for postcyclic hosts, and he distinguished in this context "eupostcyclic hosts" from "parapostcyclic hosts". The same differentiation may indeed be found at three levels in connection with oligoxenous and polyxenous parasites:

(i) additional host and interruptively substituted obligate host (intermediate and definitive hosts) are of one and the same species;

(ii) additional host is not of the same species but of a species which is part of the real host range of the obligate original category;

(iii) additional host is neither of the same species nor of a species which is part of the real host range of the obligate original category.

1. *Additional hosts of larvae* (*paratenic hosts* sensu lato)

(a) *Non-stadiogenous additional hosts of helminth larvae.* These contain larvae with no period of development, and consequently in a period of stabilization (hold-on or static stage). They are paratenic hosts *sensu stricto* or euparatenic hosts (synonyms for hôte d'attente *s.s.*, Wartewirt *s.s.*, Stapelwirt *s.s.*, Ruhewirt, rezervuarnyj chozjain). Helminths thus accommodated will not undergo phased-out development. They are substituting hosts for a complete stage of development (invasive larva) just as it is. They will undertake the terminal function of an intermediate host (carriership) or of the environment by accommodating completely developed larval stages from the environment as well as from living or dead organisms.

Savinov's assessment of euparatenic parasitism of helminths was based on a reference to the level of morphophysiological differentiation of larvae with euparatenic potentiality. According to him, euparatenic parasitism of cestodes occurred on a fairly low level of morphogenesis, whereas somewhat higher

standards might be recorded from nematodes and trematodes. The highest level of morphophysiological differentiation was observed from acanthocephalans with capacity for euparatenic parasitism. Such assessment, however, seems to lack hard evidence, since Savinov himself also held that euparatenic parasitism might occur at quite differentiated levels even within the classes of cestodes, nematodes and trematodes.

Savinov (1969b) also classified euparatenic parasitism of helminth larvae according to the positions of hosts in a given life-cycle. He used characters of the Greek alphabet for continuous labelling of the consecutive hosts in a cycle (ending with the definitive host) and Arabic numerals to designate the positions of possible euparatenic hosts in each cycle (counting back from the definitive host). This approach enabled precise localization of each host, although, in cases of euparatenic hosts, additional data were required on the nature of the preceding and following host categories. Hence, euparatenic parasitism of helminths may develop on the following positions in a cycle (cf. Figs. 17, 18, 19):

1. Alpha 2 (georeceptive, preceding host of adults)
2. Alpha 3 (georeceptive, preceding intermediate host)
3. Beta 2 (between intermediate and definitive hosts)
4. Beta 3 (between first and second intermediate hosts)
5. Beta 3 (between intermediate host and metaparatenic host)
6. Gamma 2 (between second intermediate host and definitive host)
7. Gamma 3 (between second intermediate host and metaparatenic host).

The following forms can be distinguished:

(i) *Euparatenic transit hosts.* Harbouring, different in length of time, and perhaps associated with migration, is followed by "excretion" from the host of the morphologically unchanged larva which thus will part from the host.

Ancylostoma caninum is an example to this effect (cf. Savinov, 1964b).

(ii) *Euparatenic stationary hosts.* Helminth larvae, once harboured, will stay in these hosts.

Georeceptive euparatenic hosts. External environment is facultatively replaced (through active/percutaneous or passive/peroral geoinvasion) by these hosts (geosubstitution). Taking position Alpha 2, these hosts are identical with the "syngamoidal type of georeceptive reservoir hosts", as described by Šumakovič and Ryžikov (1954) and by Ryžikov (1966). A differentiation has been proposed in this context by Savinov (1969b) between two variants of occurrence of such hosts, one in connection with homoxenous nematodes (Fig. 17a) ("geonematodes") and one in connection with heteroxenous nematodes ("bionematodes") (Fig. 17d). Euparatenic parasitism of nematodes may occur georeceptively in positions Alpha 2 or Alpha 3. Some examples of occurrence in position Alpha 2 include *Rhabdias bufonis* (according to Leuckart), *Syngamus trachea* (Fig. 20), *Ancylostoma caninum* (cf. Little, 1961), *Uncinaria stenocephala* (cf. Savinov, 1963), *Capillaria putorii*, *Toxocara cati* (cf. Sprent, 1956). Euparatenic parasitism of these species will develop either before first larval moulting (*Capillaria putorii*) or after the second moulting (*Syngamus trachea*). Euparatenic parasitism

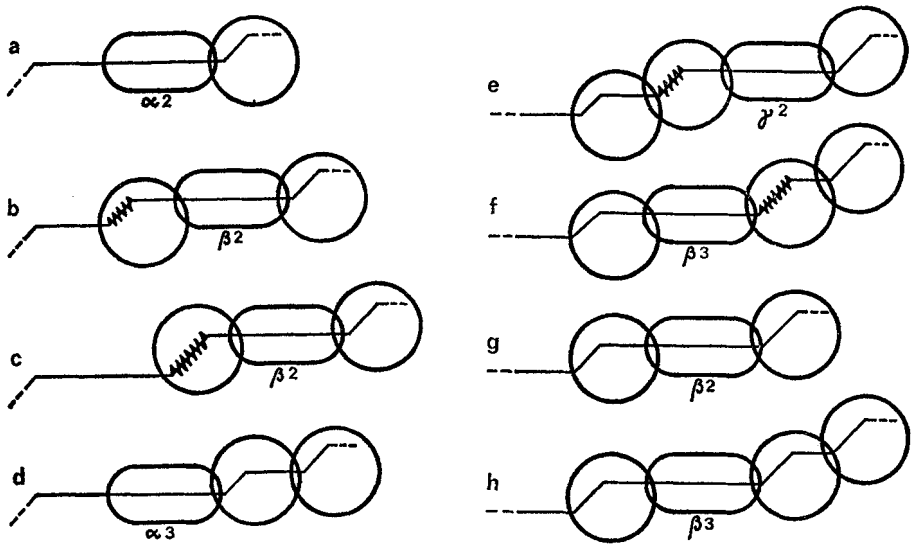

FIG. 17. Position of euparatenic hosts in life-cycle of nematodes; oval signs represent euparatenic hosts, horizontal lines mark no-development periods, and diagonal lines mark periods of development (development in metaparatenic host featured as zigzag line), cf. text. (According to Savinov, 1969b.)

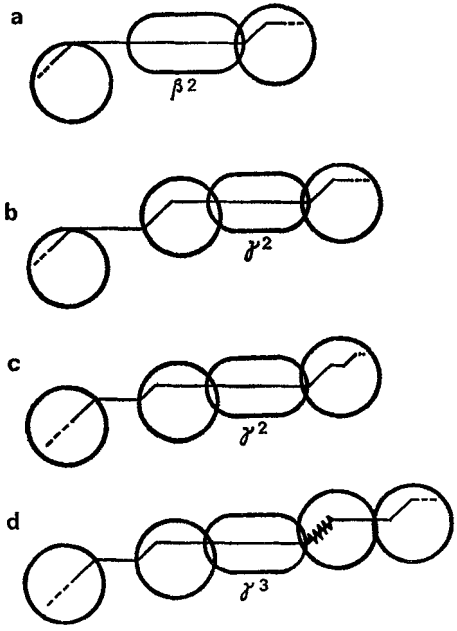

FIG. 18. Position of euparatenic hosts in life-cycle of trematodes; (a) level of cercaria, (b) level of metacercaria, (c) and (d) level of mesocercaria; for more explanation see Fig. 17 and text. (According to Savinov, 1969b)

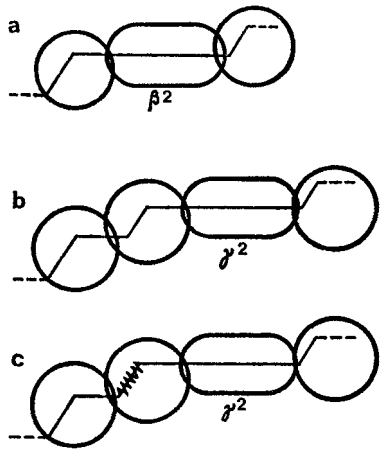

FIG. 19. Position of euparatenic hosts in life-cycle of cestodes; for more explanation see Fig. 17 and text. (According to Savinov, 1969b.)

of *Amplicaecum robertsi* may occur in position Alpha 3 (cf. Sprent, 1963a).

Bioreceptive euparatenic hosts. A host of a certain larval stage is facultatively substituted by another one (bioinvasion), and the helminth larvae are relocated without any change of stage. Substitution will apply only to hosts of complete larval or juvenile stages (biosubstitution). The hold-on stages will (theoretically) retain unlimited potentiality of relocation or re-fixation (plurirefixatory power). The process of facultative biosubstitution will be highly repetitive, and so possible substitution will not be limited to intermediate hosts but will apply as well to other paratenic hosts, above all to euparatenic hosts. The term "paratenesis" has been proposed by Beaver (1969) to describe the transport of unchanged invasive larvae between two and more euparatenic hosts. This group of paratenic hosts is identical with some of the "alarioidal type" and the "spirocercoidal type of bioreceptive reservoir hosts", as described by Šumakovič and Ryžikov (1954) and Ryžikov (1966). Examples may be quoted in connection with nematodes, acanthocephalans, trematodes and cestodes.

Nematodes (Figs 17b,c,e,f,g,h, 21). Bioreceptive euparatenic parasitism of this species may develop in position Beta 2 (between metaparatenic and definitive hosts) (after first moulting of *Toxocara canis*, after second moulting of *Toxacaris leonina;* cf. Fülleborn, 1922; Savinov, 1964b). Also affected by this position are *Parascaris equorum* (cf. Hobmaier, 1927), *Ascarops strongylina* (Fig. 21, after second moulting, though this one and all the following have bioreceptive euparatenic parasitism in position Beta 2 as well as between intermediate and definitive hosts), *Streptocara crassicauda* (in third larval stage), *Spirocerca lupi, Physocephalus sexalatus, Rictularia coloradensis, Tetrameres fissicauda, Aelurostrongylus abstrusus, Crenosoma vulpes, C. mephitidis, Skrjabingylus chitwoodorum.*

Position Gamma 2 (between metaparatenic and definitive hosts) applies

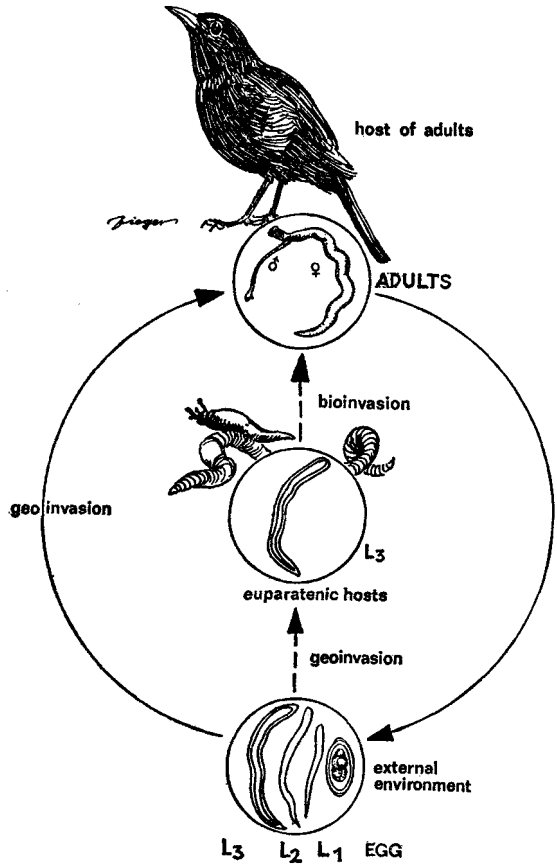

FIG. 20. Life-cycle of *Syngamus trachea* (nematode). (Odening, 1969.)

to *Procamallanus cearensis*, *Gnathostoma hispidum*, *G. spinigerum* (cf. Savinov, 1969b). Position Gamma 3 (between intermediate and metaparatenic hosts) may be occupied by *Camallanus sweeti* (cf. Savinov, 1969b) as well as by *Contracaecum microcephalum* (after first moulting), but between first and second intermediate hosts in the latter case (cf. Savinov, 1969b).

Acanthocephalans. Their euparatenic parasitism takes place in the stage of invasive larva (cystacanth, postlarva) in position Beta 2, between intermediate and definitive hosts, and *Centrorhynchus aluconis* is an example.

Trematodes. Euparatenic parasitism of trematodes may develop at three different levels of larval development, according to Savinov (1969b) (Fig. 18). The cercarial level is thought to apply to some representatives of the *Azygia* genus where euparatenic parasitism will occur in position Beta 2, between intermediate and definitive hosts. The range of euparatenic hosts in this context, however, is perhaps restricted to planarians, as reported by Stunkard (1950, 1956), with the cercarial bodies being harboured in the pharyngeal

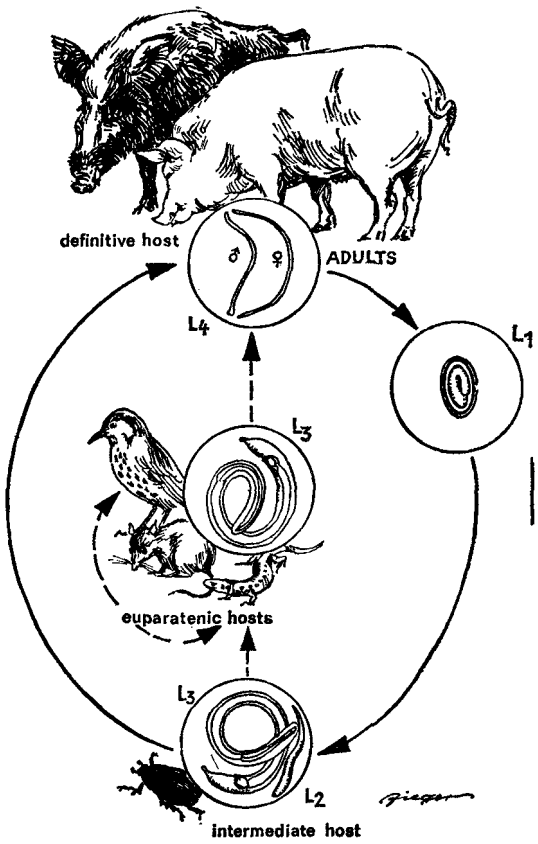

FIG. 21. Life-cycle of *Ascarops strongylina* (nematode). (Odening, 1968–69.)

pockets. (They might be transport hosts only, a question not elucidated as yet.) Certain authors have referred to fish as being paratenic hosts with small forms of *Azygia*, but this is more likely a group of paradefinitive hosts (p. 76).

Euparatenic parasitism of *Alaria* takes place on the mesocercarial level (Fig. 23; cf. Odening, 1961, 1963). The positions are Gamma 2, between second intermediate host and definitive host, or Gamma 3, between intermediate and metaparatenic hosts.

The metacercarial level applies to euparatenic parasitism of *Neodiplostomum*, including *Fibricola* (Fig. 22; cf. Pearson, 1961), *Pharyngostomum* (Gamma 2, between second intermediate and definitive hosts), and *Diplostomum flexicaudum* (Fig. 12; Beta 2, between intermediate and definitive hosts; cf. Becker and Brunson, 1966).

Cestodes. Savinov (1969b) found euparatenic parasitism of cestodes to occur at two larval levels, i.e. in the second period of stabilization (procercoid, cysticercoid) and in the third one (pleurocercoid, tetrathyridium) (Fig. 19).

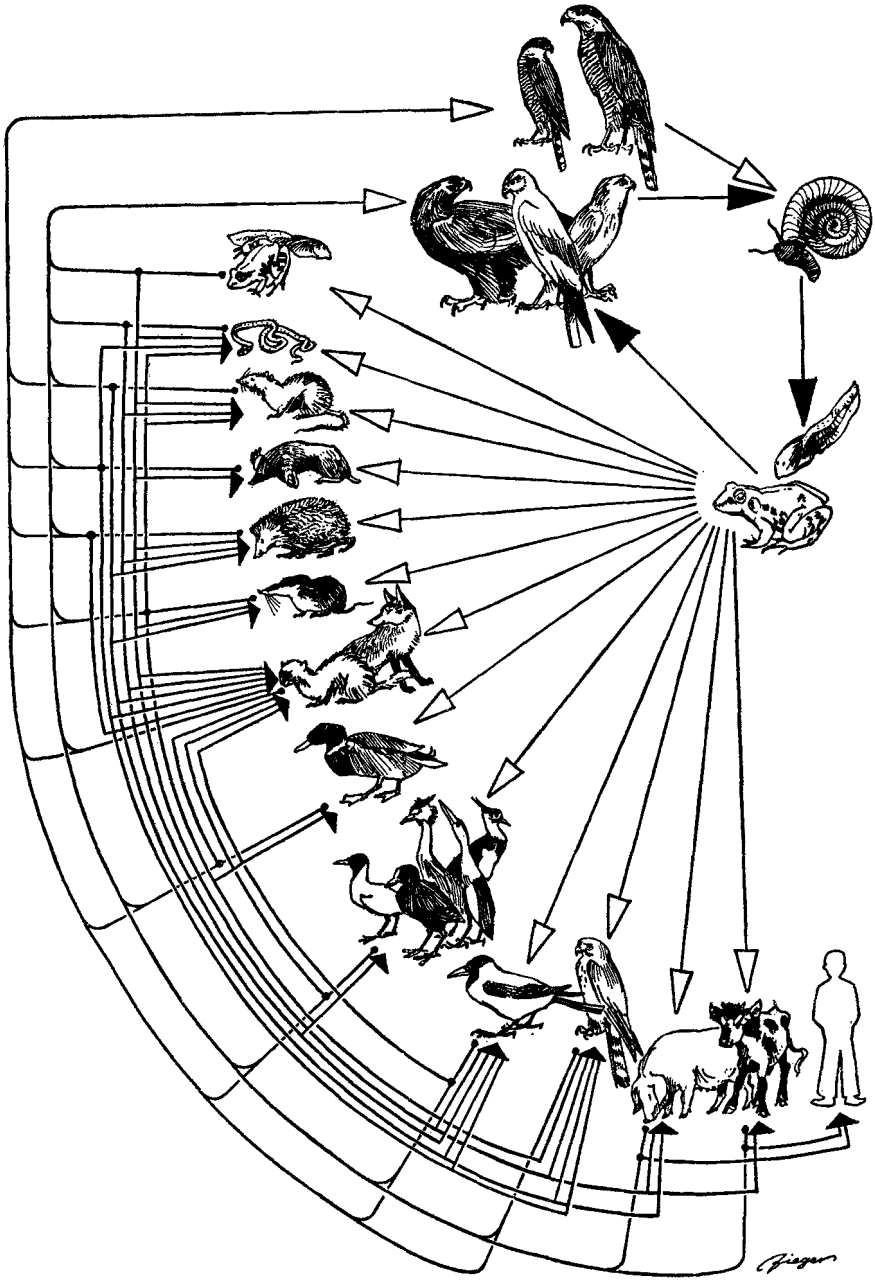

FIG. 22. Life-cycle of *Neodiplostomum spathoides* (trematode). Black arrow-heads: obligate three-host cycle with frog-eating Accipitriformes as phylogenetic primary definitive hosts; open arrow-heads: extended (phylogenetically secondary) parts of cycle with euparatenic hosts as well as primary and secondary non-frog-eating definitive hosts. (Odening, 1965a.)

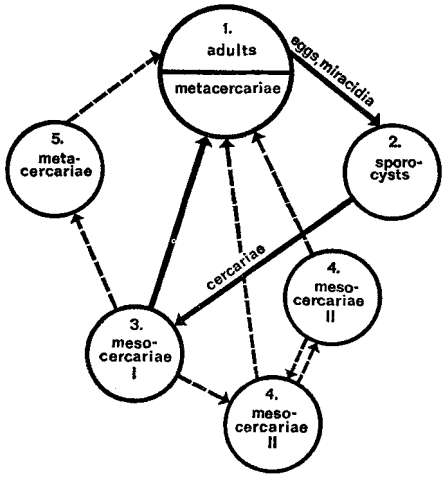

FIG. 23. Life-cycle of *Paralaria* (trematode). Solid lines: obligate host system; dotted lines: facultative host system. 1. Definitive host, 2. first intermediate host, 3. second intermediate host, 4. euparatenic host, 5. metaparatenic host. (Odening, 1974a.)

Position Beta 2 (between intermediate and definitive hosts) is the site for *Diplopylidium acanthotetra* as well as for many Hymenolepididae*. Position Gamma 2 is that of *Diphyllobothrium latum* (Fig. 29), *Spirometra*, *Nybelinia* and *Mesocestoides* (Fig. 24). A segment of the rear part of their body, which will degenerate in any case on invasion of the definitive host, will be cast off in the intestine of the euparatenic host in some of these cases. The cast rear part will then be regenerated in the euparatenic host (*Spirometra*). Position Gamma 2, between metaparatenic and definitive hosts, is the site of euparatenic parasitism of certain Proteocephalinae and Corallobothriinae (cf. Freze, 1965). The paratenic hosts of certain Nematomorpha should perhaps be mentioned in the same context (cf. Baer, 1951).

Saproreceptive euparatenic hosts. Larval stages are absorbed together with their dead hosts (saproinvasion). In many cases, only transport hosts seem to be invaded by saprosubstitution (cf. Ryšavý, 1964; on cysticercoids in the intestinal tract of water snails). *Neoechinorhynchus rutili* is likely to be a real euparatenic host (Fig. 25), although the molluscan hosts in this case were understood to be intermediate hosts by Petročenko (1956).

(b) *Stadiogenic additional hosts of helminth larvae.* These will harbour larvae or juvenile stages with a development period followed by a stabilization period.

(i) *Para-paratenic hosts* (Savinov, 1964b: pararezervuarnyj chozjain). Another stage of development will be reached in them, a development which had already started in the environment that is being substituted by the para-paratenic host (host or open environment). That development had been initiated, interrupted, and is now being resumed in the para-paratenic host. Development will here range from an interrupted development phase to the next higher stage. The final function of the substituted medium (carriership

* In certain cases, they should be transport hosts rather than euparatenic hosts.

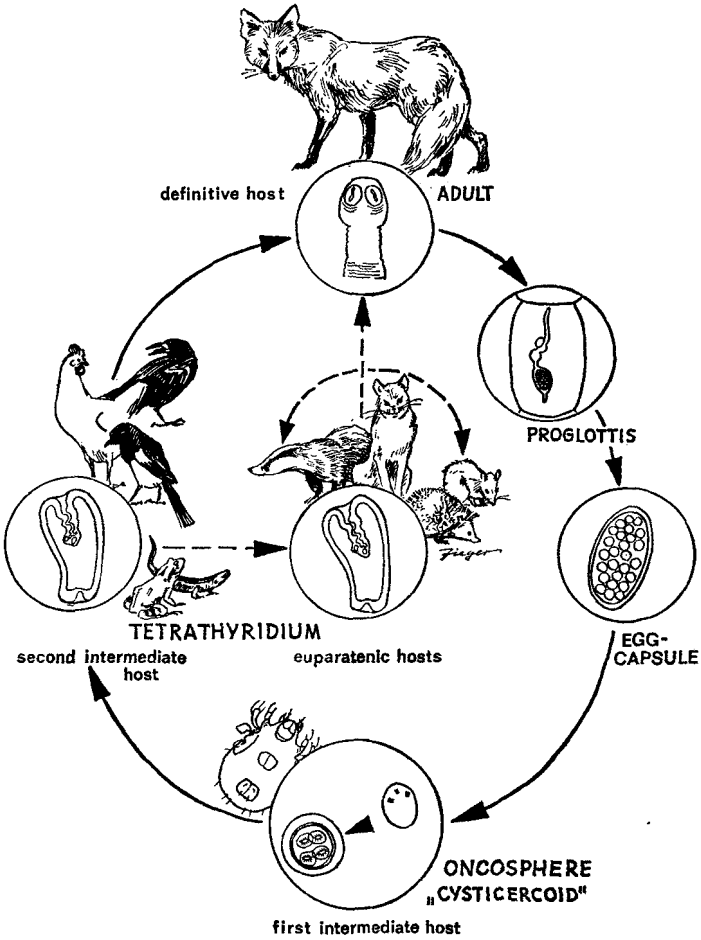

FIG. 24. Life-cycle of *Mesocestoides lineatus* (Cestoidea). (Odening, 1968–69.)

of the fully developed stage) will not be accomplished in substitution until the stage has been completely developed. The host, consequently, will be substituted along with the development of larval or juvenile stages (except pre-adults in the definitive host) (Fig. 26). Most of the para-paratenic hosts are bioreceptive, and few of them (for some nematodes, probably) are georeceptive.

These hosts are identical with some of the "bioreceptive reservoir hosts of the spirocercoidal type", according to Šumakovič and Ryžikov (1954) and Ryžikov (1954).

Acanthocephalans. Fish may be paratenic as well as definitive hosts of *Leptorhynchoides* (Fig. 27), and therefore it is not surprising that one and the same fish may be infested by invasive larvae or adults. Its actual role will

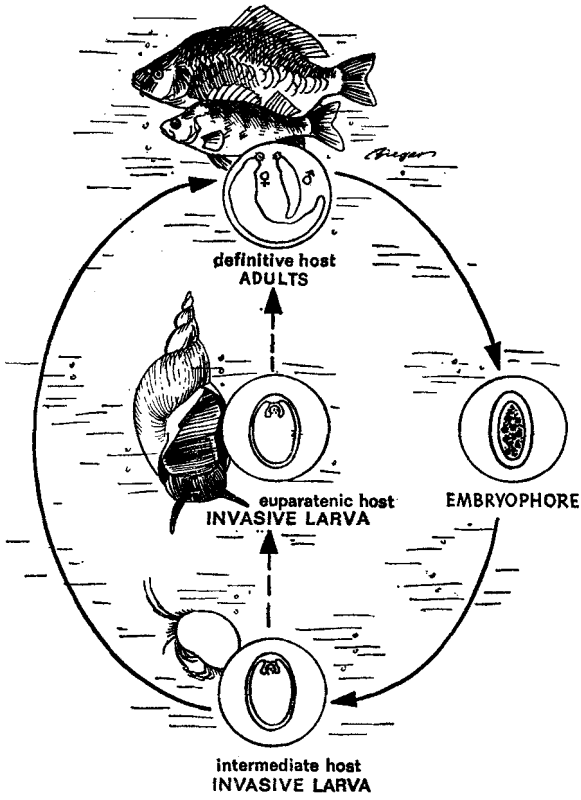

FIG. 25. Life-cycle of *Neoechinorhynchus rutili* (Acanthocephala). (Odening, 1968–69.)

depend on whether completely developed or developing (acanthellae) invasive larvae have been absorbed (De Giusti, 1949).

Trematodes. The *Strigea* genus has an obligate four-host cycle (Fig. 8). Euparatenic powers are not present in mesocercariae or in metacercariae. Both larval forms, however, may be substituted during their own development (Odening, 1967; Fig. 28). Developing mesocercariae of *Neodiplostomum* and *Alaria alata* were also successfully relocated from an interrupted second intermediate host (tadpole of *Rana*) to a paraparatenic host (*Rana*) in the framework of so far unpublished experiments.

Cestodes. *Diphyllobothrium latum* has both euparatenic and paraparatenic powers (Fig. 29; cf. Hobmaier, 1927). Against the background of fairly slow development of the plerocercoids, there will be some problems in finding out whether paraparatenic or euparatenic infestation will take place. Nor can the presence of paraparatenic powers in addition to euparatenic potentiality be ruled out for *Mesocestoides*.

Pentastomids. Infestation of paraparatenic hosts seems to be possible as well (cf. Brumpt, 1927).

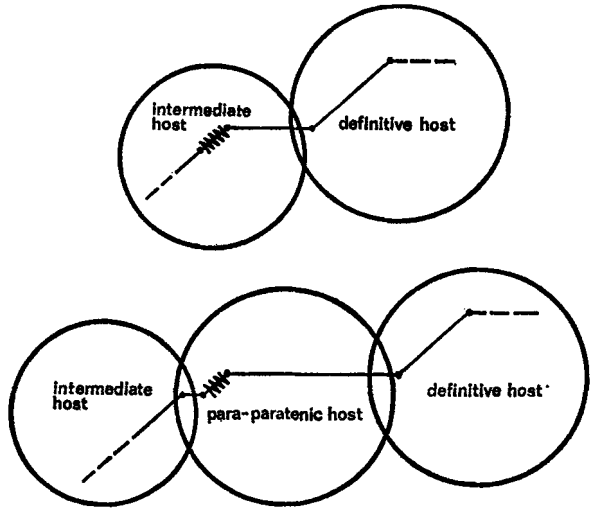

FIG. 26. Development with and without paraparatenic host; part of development phase marked by zigzag line being shifted to substituting host; development phases marked by diagonal lines; no-development periods marked by horizontal lines. (Savinov, 1964b.)

Nematodes. The stadiogenous paratenic hosts of *Spiroxys* depicted in Fig. 30 may also be interpreted as being paraparatenic hosts, but their interpretation as metaparatenic hosts would also be possible due to certain peculiarities of larval development.

(ii) *Metaparatenic hosts* (Savinov, 1964b: metarezervuarnyj chozjain). Part of the development will be brought forward from the obligate cycle in the definitive or solitary host to metaparatenic hosts (yet without any chance of having them converted to hosts of adults). The development taking place in metaparatenic hosts will start "at the very outset", i.e. from one stage to another at which it would not stop in an obligate host (definitive host, solitary host) (Fig. 31). A metaparatenic host, in other words, will not disrupt an initiated development and, consequently, is some sort of an alternative intermediate host.

Metaparatenic hosts are often "hosts of phylogenetic reminiscence": a host which originally used to act as an obligate intermediate host in phylogenesis has become dispensable (or alternative) to the cycle. A development which originally took place in the intermediate host has now become relocated alternatively to the definitive host (or now solitary host) (usually still detectable from body migration), but it can take place in a metaparatenic host, too. Metaparatenic hosts are the only additional hosts which satisfy all cyclic functions of an obligate host, in that they ensure complete development from one stage to another, the next higher, and serve as carriers of that fully developed stage.

There is some similarity between metaparatenic hosts and "unfit or abortive hosts". Development in unfit hosts will go on to a certain pre-adult stage, but no sexual maturity will be reached. Similar aspects apply to metaparatenic

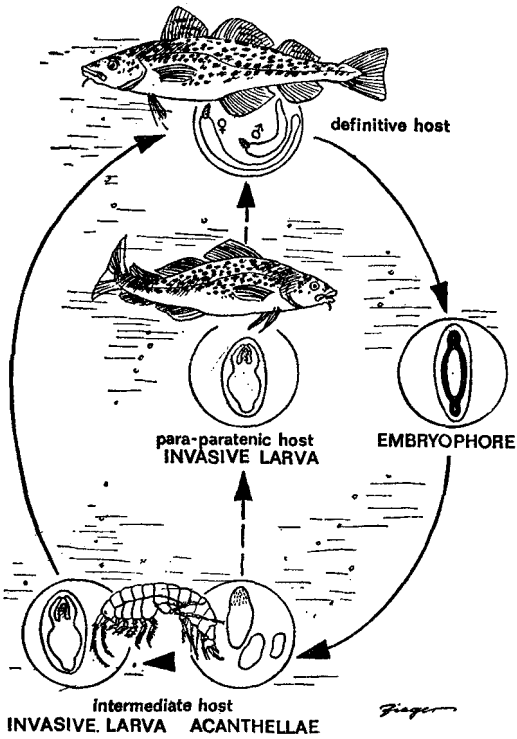

FIG. 27. Life-cycle of *Leptorhynchoides thecatus*; one and the same fish species becoming definitive or paraparatenic host, depending on its intake of completely developed or developing larvae. (Odening, 1968–69.)

hosts. The actual difference between the two will be understood only from the nature of the form with stopped development, and this can usually be elucidated from its further destination and localization. The localization of a pre-adult is equal or similar to that of an adult, and once a pre-adult is captured in an unfit host, it will end in death, sooner or later, whereas a long static period will go on in a metaparatenic host (with the typical hold-on stage being localized at a point different from that of the adult).

While both metaparatenic and unfit hosts can become sources for an infestation of hosts of adults, the metaparatenic host, as an alternative intermediate host, will be much more suitable for such a function. While the unfit host must be considered an unsuitable reproductive host which "must take its turn" in an obligate (minimum) consecutive occurrence of hosts (without being capable of fully accomplishing its task), the metaparatenic host is an additional host that is being intercalated in the cycle as a facultative host of larvae. An unfit host is no additional host at all.

Georeceptive metaparatenic hosts. Present homoxeny has been acquired secondarily, and former diheteroxeny has become facultative. Examples are various ascarids (Figs 17b,c, 31) and cestodes (e.g. *Hymenolepis nana* =

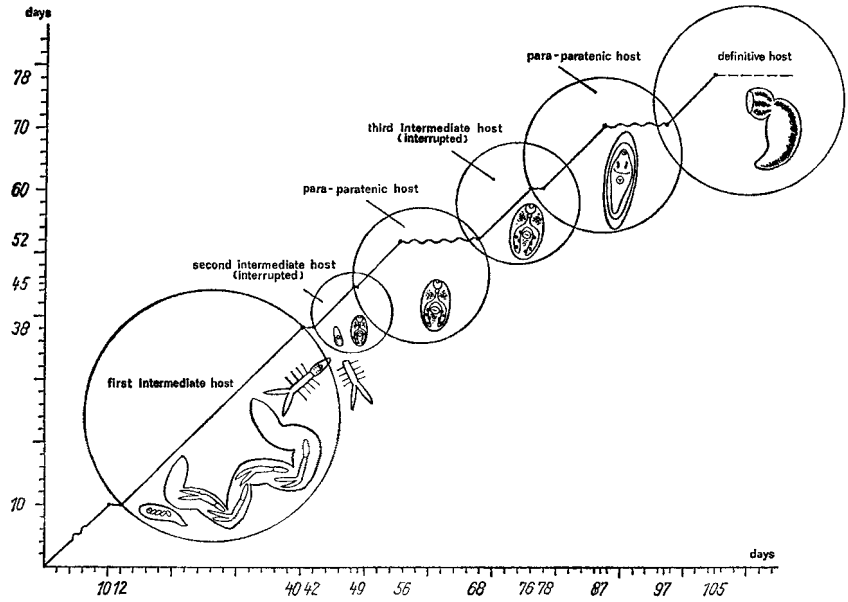

FIG. 28. Graph of phased-out development of *Strigea strigis* (trematode) in four-host cycle, with simple substitution of second and third intermediate hosts by paraparatenic hosts. Ordinate: actual time of development; abscissa: duration of whole cycle, including hold-on stages (horizontal meanders), assumed as an example, "hold-on phases" (temperature-borne delay of development, diagonal meander) and free-living stages (horizontal lines outside circles). (Odening, 1967.)

Rodentolepis straminea). These metaparatenic hosts are identical with the "georeceptive reservoir hosts of the toxocaroidal type", as described by Šumakovič and Ryžikov (1954) and Ryžikov (1966).

Bioreceptive metaparatenic hosts. The trematode genus *Alaria* used to be based on an obligate four-host cycle, before the third intermediate host dropped out, with its development being relocated to the definitive host. The third intermediate host of the subgenus *Paralaria* can be intercalated facultatively (alternatively) into the cycle as metaparatenic host (Fig. 23; cf. Johnson, 1968). Metaparatenic hosts may appear also in the cycle of *Opisthioglyphe* (cf. Grabda-Kazubska, 1969).

Examples to the same effect include some nematodes of suborders Camallanata (Fig. 32), Spirurata (Fig. 30) and Dioctophymata. Some of them, perhaps, are not reminiscence hosts. Yet no answer has so far been found to elucidate fully the question of how many larval stages actually exist in these cases and what they are. Some authors speak of no fewer than five larval stages. "Intermediate stage" L_{3-4}, provisionally assumed in this context, then should become L_4, and L_4 should appear as L_5 in Figs 30, 32 and 33. There are also interpretations which suggest that the depicted example would reflect the proper sequence, though with L_4 being followed by L_5. A peculiarity exhibited by Spirurata of this developmental type is that the "intermediate

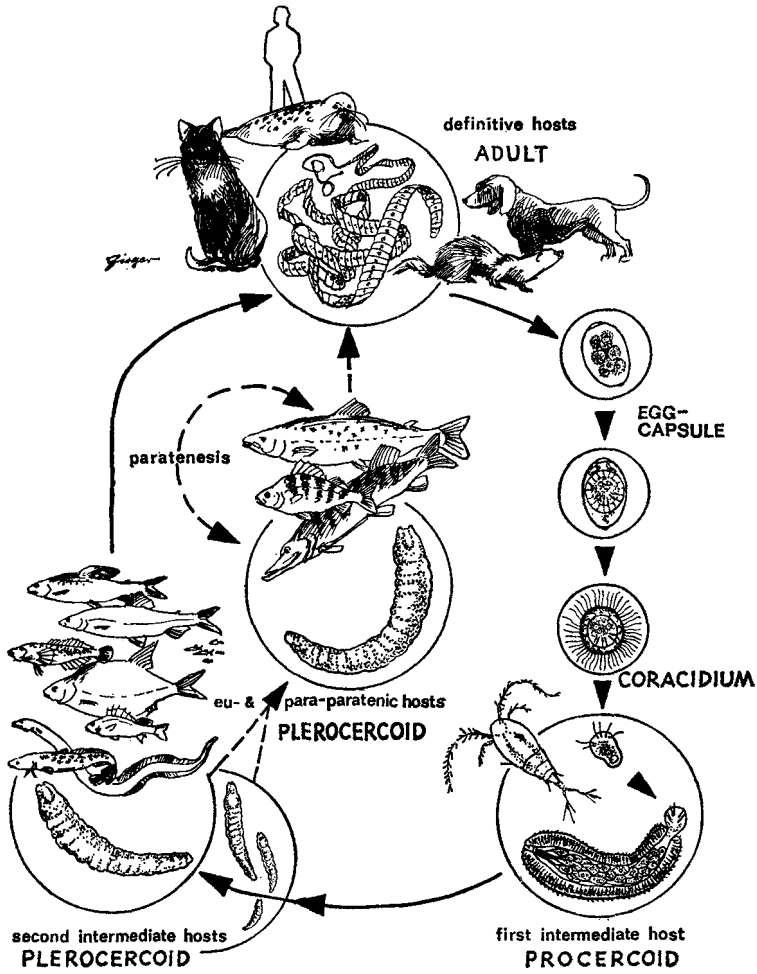

FIG. 29. Life-cycle of *Diphyllobothrium latum* (Cestoidea). (Odening, 1968–69.)

stage" (L_{3-4}) can be reached even in the intermediate host (Figs 30, 33). Hence, in addition to invasive larva (L_3), there will be another invasive form, different in quality from L_3, i.e. the "intermediate stage". It is precisely because of these peculiarities that the additional stadiogenous paratenic hosts of *Spiroxys* can as well be interpreted as paraparatenic hosts (cf. Savinov, 1964b; Ryšavý and Baruš, 1965). Even metaparatenic hosts, different in degree, may perhaps occur in one and the same cycle (*Gnathostoma spinigerum;* cf. Savinov, 1969b).

2. *Additional hosts of adults*

This phenomenon was known even to the earlier helminthologists. Looss (1894) mentioned the possibility of transmission between various hosts of

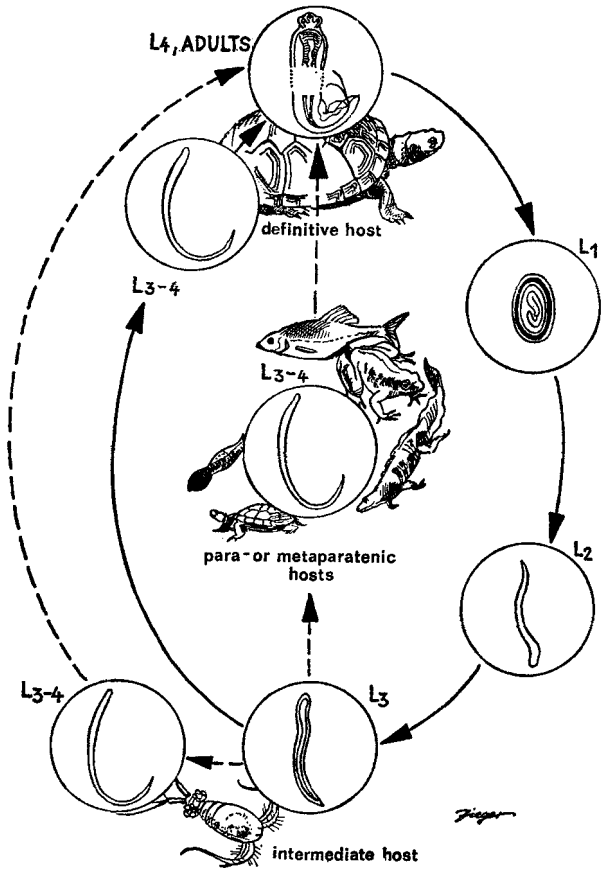

FIG. 30. Life-cycle of *Spiroxys contortus* (nematode). (Odening, 1968–69.)

adults, e.g. in the case of cannibalism. This possibility had been taken into account in Odening's ontogenetic classification of helminth hosts (1968–69), where comparison was undertaken between, basically, obligate (definitive) hosts and additional or facultative hosts of adults (substituting hosts of adults). Božkov (1969b) made a stringently delimited reference to relocation of sexually mature and ovulating helminths, and introduced the term postcyclic hosts. Odening (1974a,c), in his own reference to substituting hosts of adults, proposed subdivision by postcyclic and paradefinitive hosts (hosts that will substitute a definitive host, before the helminths harboured by the latter have acquired full sexual maturity; in other words, hosts into which pre-adults will be relocated). Careful differentiation is recommended between real parasitism in such substituting hosts of adults and incidental detection of helminths which would soon perish in their new host or be evacuted by it. The best possible assessment in such a case might be presence of pseudohosts.

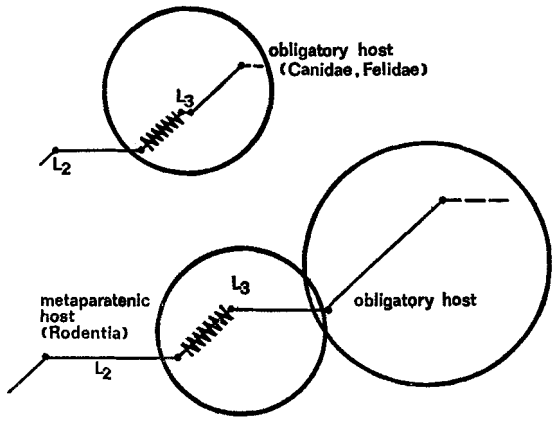

FIG. 31. Life-cycle of *Toxascaris leonina* (nematode) with and without metaparatenic host; development marked by zigzag line taking place either in host of adults or in metaparatenic host. (According to Savinov, 1964b, from Odening, 1968–69.)

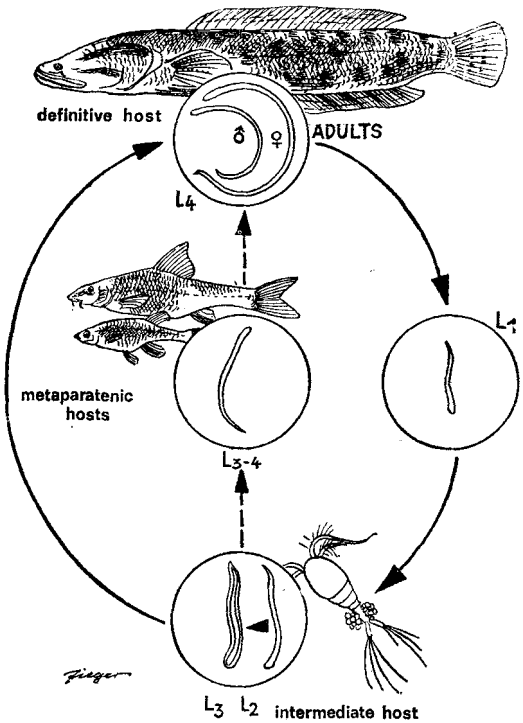

FIG. 32. Life-cycle of *Camallanus* (nematode). (Odening, 1968–69.)

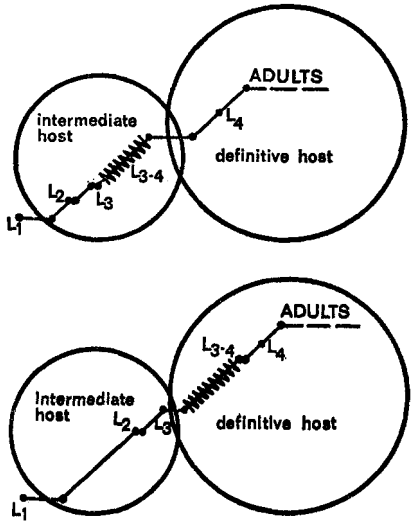

FIG. 33. Two possibilities for development of *Spirura rytipleurites* (nematode) in intermediate and definitive hosts: part of development marked by diagonal zigzag line taking place still in intermediate host or already in definitive host. (According to Savinov, 1964b, from Odening, 1968–69.)

(a) *Postcyclic hosts* (non-stadiogenous substituting hosts of adults). The host of adult helminths in which ovulation has already been started will be substituted. This may happen once or repeatedly but never in an unlimited manner, since a limitation is imposed by the life-span of the sexually mature helminths. Continuation or resumption of ovulation by the helminths in the postcyclic host is the essential factor here. Božkov (1969a) defined this phenomenon as postcyclic parasitism. The following routes of transmission must be reckoned with, according to Božkov (1975):

(i) Cannibalism (cf. Božkov, 1968); this kind of transmission of adult helminths has been recorded from Canidae, Ranidae and fish,

(ii) Predation; examples have become known of helminths of Canidae (Dubnickij, 1956), amphibians and reptiles (Božkov, 1975, 1974a) and fish (Odening, 1974d, c),

(iii) Eating of fresh carcass (Dubnickij, 1956),

(iv) Vomiting of feed by parents during nursing; a potential route for transmission of *Toxocara* and *Toxascaris* (cf. Šul'c and Gvozdev, 1972; for more examples cf. Dubinin, 1949),

(v) Intake from open environment of adult helminths that have left the body of their dead or sick host; a route established for *Azygia lucii* (cf. Odening, 1974d),

(vi) Swallowing of excrements which contain living adult helminths; a route reported by some authors for *Trichinella* (cf. Matov, 1961). Božkov (1975) pointed out that in nature, postcyclic parasitism was probably much more common than was generally known.

The term postcyclic host does not mean the same as the "accidental host" of Sprent (1963b): "It should be mentioned here that it sometimes happens that a mature parasite of an ingested animal may survive for a period in the alimentary tract of a predator. Thus, if a raptorial bird is examined shortly after swallowing a lizard, the latter's parasites may be found still living in the alimentary tract. This host is termed an *accidental host*." (cf. Božkov, 1975). Sprent's "accidental host" seems to correspond with the phenomenon of pseudoparasitism; it may be designated as pseudohost (in differentiation from the accidental host of p. 18 and from the postcyclic host).

(b) *Paradefinitive hosts.* These will substitute a definitive host (or another paradefinitive host), before the helminths parasitizing on the latter have acquired sexual maturity. In other words, it is relocation of pre-adults. There is no involvement of paratenic hosts, since the pre-imaginal phase is a phase of development rather than an independent larval or juvenile stage. In such substitution the substituted definitive host will never be a metaparatenic host either, since it would have acquired sexual maturity, which the metaparatenic host, defined as host of larvae, could not. Stadiogenous substituting hosts of adults differ from the non-stadiogenous variety, in that they are not postcyclic hosts. Examples and routes of transmission will be closely similar to those of postcyclic hosts. A great role is played by paradefinitive hosts in the cycle of *Azygia* (Fig. 34; cf. Odening, 1974d).

3. *Applicability of the additional host term to subjects outside helminthology*

The categories of additional hosts of helminths are obviously linked up

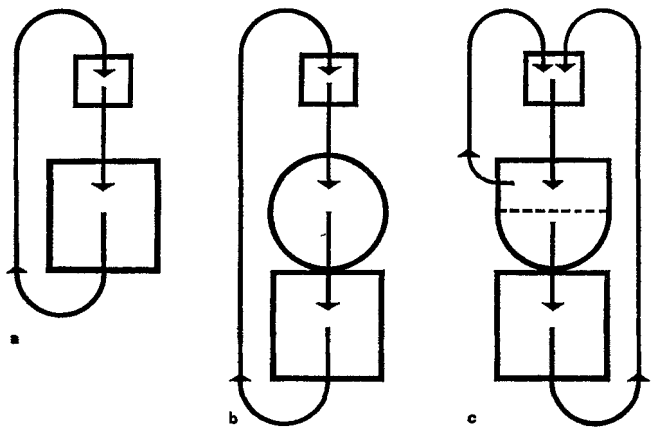

FIG. 34. Expansion of obligate two-host cycle of (a) *Azygia lucii* (trematode) through additional hosts of adults; (b) a paradefinitive host (lower square) substituting interrupted definitive host (circle) prior to sexual maturation of parasite; prevented definitive host thus will turn into transmissive host and cannot accomplish its definitive function; (c) a postcyclic host (lower square) substituting definitive host during reproduction period of parasite; definitive host, consequently, had been first disseminator (angular section) and later transmissive host (round section); upper square with a–c = intermediate host. (Odening, 1974b.)

to the peculiarities of helminthic ontogenesis which have already been described in great detail, namely, to individual development and non-development periods. Therefore, most of the notions involved will not be applicable to microparasites with "supra-individual" life-cycles. There are few instances, indeed, in which reference other than helminthological to additional hosts is possible at all. One example is protozoon *Schellackia bolivari* (Fig. 9), with the mite obviously not being an obligate host. There is neither development nor propagation of harboured sporozoites in the mite, which would thus be comparable to an euparatenic host (although the latter is defined as a host of larvae, and no larvae in this sense are present in protozoa). Trypanosomes with no propagation in the mammal, however, will present a somewhat different picture (*Trypanosoma conorhini*, *T. rangeli*). They will undergo intramammalian growth accompanied by some kind of metamorphosis, with certain triatomids acting as obligate hosts. The forms likely to grow in mammals are non-existent in triatomids, which should suggest some overlapping between additional host and alternative cycles. The mammals concerned may be considered as a kind of paratenic host, because there is no propagation of parasites in them (presence of individual development ruling out equalization with euparatenic host); at any rate they must be considered as an additional host.

D. HOST POLYVALENCE

When it comes to certain heteroxenous helminths, one and the same host species or even one and the same individual host may serve different ontogenetico-cyclic categories for one helminth species. This has given rise to the term polyvalent host (Božkov, 1969a, 1970a, 1971, 1973; Sudarikov, 1971). Host polyvalence (polyvalent receptivity or amphixeny, as termed by Sudarikov, 1971) can be facultative or obligate (Božkov). Two different levels, isochronous and heterochronous amphixeny, according to Sudarikov, must be distinguished in facultative host polyvalence. Heterochronous host polyvalence of helminths is always associated with secondary shortening of cycle.

1. *Facultative potentially isochronous host polyvalence*

One and the same host species or individual host may serve, even at one and the same time, as host for different categories of a helminth species. These different categories are not linked with each other, as a matter of principle, with regard to the host species or individual concerned. The different components of a polyvalent host need not be part of one and the same cycle, on the basis of the host species concerned, but they can be quite independent of each other. They will be independent at any rate, whenever one and the same individual host is involved. If several individuals of one and the same species are involved, participation of the polyvalent host species in one and the same cycle will be possible but not imperative.

Examples to this effect may be recorded from a wide range of trematodes. There are certain frog species that may serve as second or third intermediate hosts or even as paraparatenic hosts in the four-host cycle of *Strigea* (cf.

Odening, 1967). Certain bird species (*S. falconispalumbi*) can act as third intermediate or definitive host. Certain frog species are second intermediate hosts or euparatenic or paraparatenic hosts in the three-host cycle of *Neodiplostomum* and *Alaria* (Pearson, 1956; Savinov, 1954; Odening, 1965a,b).

Rainbow trout can be second intermediate or euparatenic host for *Diplostomum flexicaudum* (Fig. 12) (Becker and Brunson, 1966). In the context of *Telorchis bonnerensis* a snail can be first and second intermediate host, the latter being transitory in this rare case (Schell, 1962). Certain snail species were found to be first or second intermediate hosts for *Echinostoma revolutum*, *Echinoparyphium recurvatum*, *Neoacanthoparyphium echinatoides* and other Echinostomatidae, as well as for *Opisthioglyphe locellus*, *Leucochloridiomorpha*, representatives of the subgenus *Cotylurus*, and also some Cyathocotylata and Monorchiidae. There is one frog species that may be both second intermediate or definitive host for *Opisthioglyphe ranae*, *O. rastellus* and *Cephalogonimus americanus* (Grabda-Kazubska, 1969; Lang, 1968). Certain fish species (e.g. rainbow trout and pike) may be definitive, paradefinitive and postcyclic hosts for *Azygia lucii*. Certain fish species may be used as definitive or postcyclic hosts by cestodes of the genus *Proteocephalus*.

Many of the hosts which are involved in postcyclic parasitism are polyvalent and actually have the potential to serve as definitive, postcyclic, and perhaps even as paradefinitive hosts. More examples of potentially isochronous polyvalent hosts may be seen from Figs 8, 22, 27 and 29.

2. *Facultative (or alternative) heterochronous host polyvalence*

This form of heterochronous host polyvalence always relates to one and the same individual host which in a naturally established temporal sequence may be host to several categories. Polyvalent hosts of this kind can be found in certain alternative cycles of trematodes, e.g. that of *Gymnophallus choledochus*, with the snail being first and, later, second intermediate host (Fig. 13; Loos-Frank, 1969). Such alternative cycles, after all, may be observed in all those numerous cases in which cercariae get facultatively encysted in a host snail without parting from the latter (e.g. also in cases of *Fasciola hepatica* in *Lymnaea truncatula*, cf. Wigand and Mattes, 1958; or *L. stagnalis*, cf. Furmaga and Gundlach, 1967). Sudarikov (1971) mentioned *Taenia solium* in this context where, in some rare cases, man can be definitive and, later, intermediate host. However, such a situation is not necessarily linked to and dependent on one and the same individual, and therefore might serve also as an example of the above group of potentially isochronous polyvalent hosts. In addition, facultative heterochronous polyvalent hosts may be found to act in all cycles into which metaparatenic hosts can be, but are not, intercalated (e.g. in the alternatively homoxenous cycle of *Hymenolepis nana*, *H. diminuta* and ascarids and other nematodes, in the alternatively diheteroxenous cycle of *Opisthioglyphe* or in the alternatively triheteroxenous cycle of the subgenus *Paralaria*).

3. *Obligate heterochronous host polyvalence*

Obligate polyvalent hosts of helminths are always indicators of secondarily

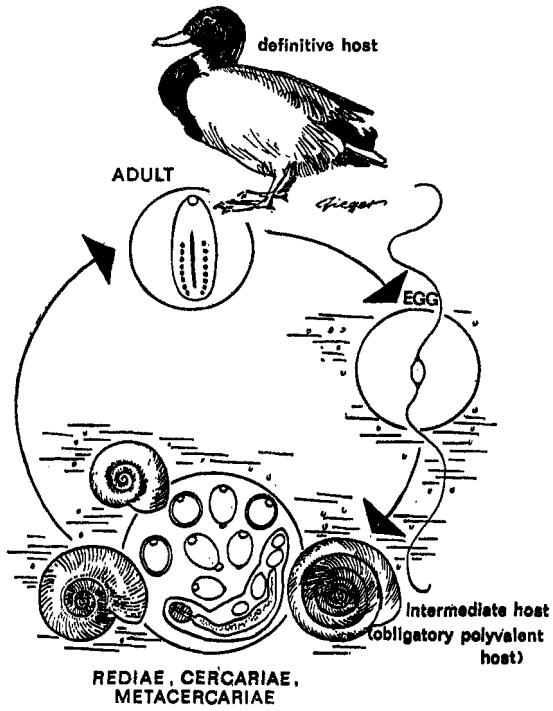

FIG. 35. Life-cycle of *Catatropis verrucosa* (trematode): two-host cycle with obligate heterochronic polyvalent intermediate host (cf. Fig. 4). (Odening, 1966.)

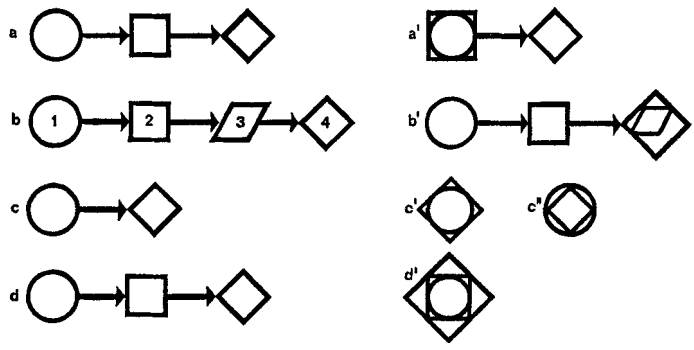

FIG. 36. Possible shortening of cycle due to appearance of polyvalent hosts for helminths. (a)–(d) Cycles without polyvalent host; (a′)–(d′) cycles with polyvalent host; (a a′) Microphallidae; (b′) subgenus *Alaria;* (c c′) *Hymenolepis nana;* (c″) *Trichinella;* (d′) *Paralepoderma progenetica.* 1, First (or only) intermediate host; 2, second intermediate host; 3, third intermediate host; 4, definitive host. (Modified from Božkov, 1973.)

shortened life cycles (Fig. 36; Božkov, 1971, 1973). *Trichinella spiralis* is a classical example to this effect, with every host, in principle, being definitive host first and intermediate host later on. Examples in the context of trematodes include representatives of the families of Microphallidae, Cyclocoelidae, Notocotylidae (Fig. 35), Leucochloridiidae and Brachylaemidae (coincidence of action as first and second intermediate hosts); *Haplometra cylindracea* (presumably obligate coincidence of action as second intermediate and definitive hosts) (cf. Combes, 1968; Grabda-Kazubska, 1970); as well as subgenus *Alaria* (coincidence of action as third intermediate and definitive hosts) (cf. Belopol'skaja, 1968; Ginecinskaja, 1968; Pearson, 1956).

Obligate heterochronous host polyvalence may be assumed to be present in rust fungi, if one and the same plant serves as host of uredospores and host of teleutospores, in this order. This kind of amphixeny, however, was found to differ from the conditions generally recordable from helminths, in that it has nothing to do with shortening of cycle. Host polyvalence of rust fungi plays quite a particular role, which may be seen, last but not least, from the fact that by distribution to several individual hosts the same case can be coordinated with the group of facultative isochronous host polyvalence.

4. *Relationship between heterochronous host polyvalence and secondary homoxeny of helminths*

The position held by homoxeny in the evolution of helminthic life-cycles may be seen from Table IV (with no consideration of changes in the framework of heteroxeny). Transmissive secondary homoxeny was called autoheteroxeny by Baer, as it constituted an extraordinary case of heteroxeny for its transmissive nature (one and the same individual host being, consecutively, definitive and intermediate hosts, e.g. *Trichinella*). Another form of secondary homoxeny is likely to display an opposite situation, with one and the same individual host being "intermediate host" (which here is quite unimportant and irrelevant to functionality and ecology, in contrast to autoheteroxeny,

TABLE IV

Position of helminth homoxeny in regard to the evolution of life-cycles (without consideration of changes within heteroxeny)

primary homoxeny (nematodes) (disseminative, georeceptive)

↓ host addition

heteroxeny
- 1 transmissive = bioreceptive (nematodes, cestodes, acanthocephalans; secondarily in trematodes)
- 2 disseminative (trematodes)
- 3 combined transmissive/disseminative (trematodes)

↓ host reduction

secondary homoxeny
- 1 autoheteroxeny (transmissive) (*Trichinella*)
- 2 disseminative (e.g. *Ascaris, Hymenolepis nana, Paralepoderma progenetica*)

where such relevance *does* exist) and, subsequently, "definitive host" (nematodes such as *Ascaris* and cestodes such as *Hymenolepis nana*).

Secondary homoxeny is a consequence of host reduction in the cycle. Such reduction may result from change to polyvalence of another host or, simply, from drop-out of a host (e.g. due to neoteny of helminth larvae). The intermediate which has become a definitive host in such cases cannot actually be considered as a polyvalent host in the strict sense, since the stage typical of the former definitive host has completely disappeared (cf. Buttner, 1950–51, 1955).

E. COMMON PRINCIPLES IN HOSTS OF THE LIFE-CYCLE

The majority of helminthological host categories do not have universal applications. The following aspects remain to be considered for general listing of hosts by ontogenesis, life cycle and parasitism:

1. Presence of parasite–host relation (true parasite hosts)	1′. Absence of parasite–host relation	[1]transport hosts [2]temporary or intermittent hosts
1a. Obligate	1a′. Facultative	[1]facultative parasitic [2]additional
1b. Stationary	1b′. Transitory	
1c. Non-stadiogenous	1c′. (Individually) stadiogenous	
1d. Non-reproductive	1d′. Reproductive	[1]propagative [2]multiplicative

The terms of stadiogenous and non-stadiogenous hosts are seen to be open to expansion and translation, but subdivision into these two categories alone is not sufficient for universal application to all groups of parasites, as these are categories which reflect typical aspects of helminthology. Addition of complementary terms, reproductive and non-reproductive, therefore proves necessary, and reproductivity has to be further subdivided into propagative and multiplicative reproduction. Stationary parasites may be coordinated with stationary or transitory hosts. The time of parasite residence in both of them will be extended. Temporary hosts would be hosts of those temporary parasites which are characterized by very short residence on the host. All three apply to the individual parasite rather than to a sequence of individuals or to the totality of descendants (propagative forms) which, by the way, will often part from the host. The terms are of particular relevance to parasitic metazoa.

Individual development can hardly be distinguished from reproduction in the cases of bacteria and viruses, since in the latter these processes will be much shorter than they are in metazoa and protozoa, and differentiation often becomes difficult due to poor range of forms. Environment-borne propagative forms, bacteriospores and viruses outside organisms, are the hold-on stages; and division, growth and mature divisible stage, and division again, is the typical rhythm of bacterial development. Viruses are based on alternation of infectious stages with phases of intracellular propagation which,

after destruction of or departure from the host cell, will give another sequence of infectious stages, and so on. Viruses and the majority of bacteria, including protozoa, typically are characterized by a rhythm where in one and the same host simple reproduction (e.g. simple or multiple division or reduplication) can be followed by numerous repetitive appearances of one and the same stage or one and the same generation: stage (generation)—reproduction/development—stage (generation). Helminths and many heteroxenous protozoa are characterized by development (individual or in generational cycles) from one stage (or one generational type) to another stage (or generational type), all in one and the same host.

This actually makes the differences that exist between development and reproduction modes of the large groups of parasites, and it is likely to offer an explanation for the differentiated nature of their parasitism, hosts, and host alternation, with some of these differences being reflected in the terminology. Helminthic cycles are found to exhibit a highly articulated set-up of individual development. One individual is usually required to pass through different phases of development in different hosts, organs or whatever environments. Development by individual cycles (stadiogenicity) is combined with development by generational cycles, more or less, only in trematodes. The developmental cycle of heteroxenous protozoa may be assumed to be accompanied by generational cyclicity (sporozoa) or by homogenous reproduction or by cyclic reproduction of heterogeneous forms (flagellates), and this is so in all cases; the same is believed to apply to most cases of host alternation. Viruses and bacteria (according to latest knowledge) are based exclusively on homogeneous-reproductive development (individual reproduction with no alternation of generations or forms and qualitatively unaffected by alternation of hosts). The bisexual type of a trematode generation (adults) will be represented by one single generation which has its habitat on one or on several different hosts (definitive hosts or second and, perhaps, third intermediate hosts). The parthenogenetic generational type usually has few consecutive generations in one and the same host (rarely beyond two to six). This type, also, can include different forms which often follow each other in an established sequence. Cyclic development of generations and forms is usually accompanied by homogeneously multiplicative reproduction in one host. Combination of generational alternation with homogeneously multiplicative reproduction, against the background of much stronger reproductivity, is typical of the schizogonial hosts of heteroxenous sporozoa.

The following types of "hosts of development" can be differentiated in a wider context:

1. Individually stadiogenous, non-reproductive hosts
2. Reproductive hosts
   - (a) Multiplicative
     - (aa) Homogeneous-multiplicative
     - (bb) Heterogeneous-multiplicative
       - A (generational cycles)
       - B (form cycles)
   - (b) Propagative

(aa) Homogeneous-propagative
(bb) Heterogeneous-propagative
A (generational cycles)
B (form cycles)

All obligate reproductive hosts are "individually stadiogenous hosts".

The postcyclic hosts of helminths, however, are propagative-reproductive hosts (homogeneous-propagative for the majority of nematodes as well as for all cestodes and acanthocephalans; cyclogenerational-heterogeneous-propagative for trematodes) rather than individually stadiogenous hosts.

The intermediate hosts of nematodes, acanthocephalans and the majority of cestodes, as well as the second (and third) intermediate hosts of trematodes, are individually stadiogenous but non-reproductive hosts. The intermediate hosts of some of the cestodes (*Echinococcus* etc.) are individually stadiogenous with homogeneous-multiplicative reproductivity. Larvae of Hypodermatidae, Mermithoidea, Nematomorpha, and the larvae of unionids, for example, have stadiogenous, non-reproductive, transitory hosts.

All definitive hosts, coupled hosts of adults, and solitary hosts of adults of helminths, as well as the first intermediate hosts (partheno-intermediate hosts) of trematodes, are reproductive hosts. Form-cyclic multiplicative, homogeneous-multiplicative, and cyclogenerational-propagative are the attributes of the partheno-intermediate hosts of trematodes. The above definitive hosts (in analogy to postcyclic hosts) are homogeneous-propagative or cyclogenerational-heterogeneous-propagative, as far as parasite reproduction is concerned, but they differ from postcyclic hosts in that they are individually stadiogenous.

Protozoa, fungi, bacteria and viruses usually have no individually stadiogenous and non-reproductive hosts, because of less articulated ontogenesis (the only exception being trypanosomes without intramammalian reproduction, cf. p. 77). All their obligate hosts are reproductive.

Homogeneous multiplicativity has been found to be the predominant form of reproduction among viruses and bacteria, but form-cyclic propagative reproduction is often found to occur as well among protozoa and certain bacteria (development of propagative forms, such as cysts and spores). Haemoflagellates will undergo reproduction in either mode, form-cyclic multiplicative reproduction and the homogenous-multiplicative variant. Haemosporidia have homogenous-multiplicative reproduction (schizogony), but this is sometimes supplemented by form-cyclic multiplicative reproduction (macroschizogony and microschizogony) or by cyclogenerational-propagative reproduction (gametogony and sporogony).

Reproduction among hosts of parasitic fungi usually is cyclogenerational-propagative or homogeneous-propagative. Reproduction of heteroxenous rust fungi is cyclogenerational-propagative (aecidial host and host of teleutospores), but homogeneous-propagative reproduction may be observed too (through uredospores in the host of uredospores and teleutospores). Form-cyclic multiplicative reproduction, through formation of pyknospores, is recordable from the aecidial host. The intra-host processes of individual growth are clearly distinguished from those of multiplicative reproduction

in monocellular species and metazoa, but they will unite in (polycellular) fungi.

The hosts of stationary-ectoparasitic arthropods (e.g. mites, lice, Mallophaga) are homogenous-multiplicative. Propagative and multiplicative reproduction are identical with each other in cases of exclusively homogeneous-multiplicative reproduction, that is in the absence of distinct propagative forms (viruses, a wide range of bacteria and protozoa, stationary-ectoparasitic arthropods).

Hosts of parasitoids are stadiogenous, with some of them (e.g. Chalcididae) being reproductive (homogeneous-multiplicative: polyembryonism). They are transitory as well.

VI. Conclusions

A distinction must be made between the various approaches to the host notion in parasitology.

1. The level or degree of association is one of the major criteria for general consideration in terms of ecology, and covers *polyhospitalism* (one visiting parasite having several hosts, satisfying the conditions of temporary or intermittent parasitism) and *monohospitalism* (one parasite having only one host, at least during a demarcated span of life, somatóxeny or body-contact relation, meeting the conditions of stationary parasitism, including transitory parasitism). Therefore, differentiation is necessary between *temporary* or *intermittent hosts*, on the one hand, and *stationary* as well as *transitory hosts*, on the other.

2. Another differentiation is made by quality of body-contact relation, i.e. the degree of intimacy of a somatoxenous parasite–host relation, with two types of hosts being involved, *transport hosts* (with ecologically determinate carrier function) and *parasite hosts* (with ecophysiologically determinate parasite–host relation).

3. Hosts are further differentiated by their inherent modes of transmission or propagation and, consequently, can be *disseminating* (dissemination of parasites by discharge into open environment) or *transmissive* (delivery of parasites by physical contact with next host). There are contaminative (tactile, excretory and lymphatic), phagous (peroral) and inoculative transmissive hosts. They can be either anadaptive (ectohaptic or entotransitive) or adaptive transmissive.

4. All hosts with transmission to an epidemiological (epizootiological, epiphytological) reference host may be called *vectors*. Epidemiological (and other) terms (e.g. for certain diseases) which are host-related may include the usage of words derived from the Greek xenos which, in turn, may imply two meanings, xenos = stranger or xenos = host. Names with reference to the host meaning include quiescent carrier, donator or donor, receptor or recipient, reservoir and reservoir or reserve host, and are used mainly in an epidemiological context.

5. Several host categories are differentiated by their suitability for certain parasites as well as by their occurrence and adaptation. Expressions used

in this context should be free of all possible ambiguity. Yet present usage still suffers from quite a bit of overlapping between common terms. Adequate unambiguity, for example, may be obtained by juxtaposing *well adapted, badly adapted* and *not adapted hosts*, or by speaking of *real hosts* versus *potential hosts*. Relatively good plausibility will be obtained as well by suggesting as an order *regular, preferred* or *principal hosts, incidental* or *by-hosts, occasional, accidental* or *sporadic hosts, unfit* or *abortive hosts*, as well as *blind-alley* or *captive hosts* (Table I).

6. Actual host specificity has led to subdivision into *stenoxenous* (monoxenous and stenoligoxenous) and *euryxenous* (euroligoxenous and polyxenous) parasites. However, *monoxeny* must not be confused with homoxeny or with monohospitalism, nor is such confusion permissible between *polyxeny* or *oligoxeny*, on the one hand, and heteroxeny or polyhospitalism, on the other.

7. *Homoxeny* and *heteroxeny* (*diheteroxeny, triheteroxeny, tetraheteroxeny*) will be juxtaposed according to whether just one or several different host categories are involved in the life-cycle of a parasite. Use of "monoxeny" instead of homoxeny or "polyxeny" instead of heteroxeny may lead to misunderstanding.

8. Every host will be a *facultative host* to a facultative parasite (i.e. in all cases of facultative parasitism). An obligate parasite will always need one host. Yet in the course of one complete cycle there may necessarily be obligate hosts (*necessary minimum*) and facultative hosts (*beyond indispensable minimum*). The latter group will include either *alternative* or *additional hosts* (with some overlapping between the two groups in certain areas). If alternative hosts are involved, a certain development or a host-borne alternative cycle, typically, will take place in these hosts in a manner different from what would have happened without their involvement. Additional hosts, however, will not change a mode of development in the cycle.

9. The following distinctions should be made according to the parasitic stages or forms harboured by the hosts concerned, and according to parasitic developments in them:

(a) For flagellates (and piroplasmids?) (cf. Table II): avertebrate hosts (obligate), vertebrate hosts (obligate or alternative or additional), and plant hosts (obligate or alternative).

(b) For sporozoa: schizosporogonial hosts (obligate or alternative), schizogonial hosts (obligate), sporogonial hosts (obligate), cystical hosts (alternative), and hosts of sporozoites (additional).

(c) For rust fungi: aecidial hosts (obligate), hosts of uredospores (alternative), and hosts of teleutospores (obligate/alternative).

(d) For helminths (applicable also to some pentastomids and isopods): hosts of adults (obligate, additional), hosts of parthenitae (obligate), and hosts of larvae (obligate, additional).

10. The common obligate host categories of heteroxenous helminths are usually *definitive hosts* and (*first, second, third*) *intermediate hosts*. Intermediate hosts include a category which is further subdivided into *partheno-intermediate* and *larvo-intermediate hosts*. These terms are perhaps also applicable to heteroxenous arthropods, say Epicaridae, but application

to parasite groups different from these will be problematic or even impossible. Heteroxenous flagellates require juxtaposition of *avertebrate host* to *vertebrate* or *plant host*, haemosporidia of *schizogonial* and *sporogonial host*, and Uredinales of *aecidial host* and *host of teleutospores* (cf. Table II).

11. Alternative hosts may include *vertebrate hosts* for flagellates (as well as for heteroxenous viruses and bacteria) or *cystical* and *schizosporogonial hosts* for *Toxoplasma* (cf. Table II).

12. Additional hosts will occur mainly in connection with helminths and include additional hosts of larvae or paratenic hosts *sensu lato* and additional hosts of adults. In *euparatenic hosts* helminth larvae will remain unchanged, with a non-development period being included. *Paraparatenic hosts* are the sites on which development of helminth larvae will be completed, after having been initiated but then interrupted by relocation in the paraparatenic hosts. Larval development which otherwise would have taken place in an obligate host of adults will occur in *metaparatenic hosts* which may be interpreted also as alternative intermediate hosts. There are many more terms often used for paratenic hosts and their three types, but these are ambiguous. Development of pre-adult helminths which had begun in an obligate host of adults will be completed to sexual maturity in *paradefinitive hosts* (cf. Table III). Continued existence of sexually mature helminths, after relocation, is ensured in *postcyclic hosts*. Protozoa may have additional vertebrate hosts (flagellates) and additional hosts of sporozoites (sporozoa) (cf. Table II).

13. Important parameters by which to characterize ontogenetico-cyclic parasite hosts are stadiogenous (with "ascending development"), non-stadiogenous (without individual development), and reproductive or non-reproductive, each of the two latter homogeneously or heterogeneously propagative or multiplicative.

14. Hosts (mainly of helminths) which are capable of acting in several ontogenetico-cyclic host categories, usually separated in a cycle, are called *polyvalent hosts*.

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Host–Parasite Relationships in the Alimentary Tract of Domestic Birds

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I. Introduction

One purpose of animal ecology is to discover the reasons for the distribution and numbers of animals in nature (Elton, 1927) and this suggestion has stimulated many studies of communities. A community is an assemblage of species from the same habitat (see Kontkanen, 1950) and a habitat is an area which seems to possess a certain uniformity with respect to some quality which the investigator decides is important in the study of a community (Andrewartha and Birch, 1954). The parasitologist may claim that the functioning of the digestive system justifies assigning the status of a habitat to the alimentary tract and, therefore, that of a community to the organisms living therein. Many investigators of the microflora of the vertebrate alimentary tract have developed an ecological approach to their studies (Haenel, 1961; Dubos and Schaedler, 1964; Luckey, 1970; Barnes, 1972; Fuller, 1973). We have attempted to follow their example by analysing and integrating knowledge of the biology of the parasites with information about the events and conditions occurring in the alimentary tracts of domestic birds.

In its widest sense, a domestic animal is one which is maintained by man for his use, and game birds, falcons, ostriches, little egrets and cormorants could have been included within the scope of this review. Several opinions, however, indicate that domestication primarily involves the management of animals for man's requirements by means of careful selection, breeding and husbandry (Wood-Gush, 1964; Campbell and Lasley, 1969; Morris, 1972). Thus, we have restricted ourselves to a discussion of relationships between certain parasites and the domestic duck (*Anas platyrhynchos* L.), goose (*Anser anser* (L.)), fowl (*Gallus gallus* (L.)), turkey (*Meleagris gallopavo* L.) and pigeon (*Columba livia* Gmelin). The characteristics of these domestic species have probably become considerably modified from those of their wild ancestors as a result of the programme of selective breeding imposed on them by man. There appears to be little information available to indicate how the nutritional requirements and digestive physiology of birds have changed during domestication. Similarly, little is known about how the biology and physiology of the inhabitants of their alimentary tracts have responded to domestication. Nevertheless, the possible consequence of domestication on host–parasite relationships should not be ignored by those who may be confronted with infections acquired by domestic birds after exposure to wild relatives or by those who infect domestic birds in the laboratory with parasites isolated from wild hosts.

An estimation of the importance of domestic birds to the world's human population, which was reckoned to be 3782 million in the mid-1972 census (United Nations, 1973), is given in Table I. These numbers are probably conservative estimates since many of them represent a census taken at one time and give little indication of the annual turnover of birds in a continent. Some concept of the extent of the habitat for parasites provided by the alimentary tract of domestic birds is given by the observation that the absorptive surface area of the tracts of the total number of birds shown in Table I is similar to the surface area of England and Wales or the state of Illinois.

TABLE I

Estimated populations of some domestic birds

	Ducks	Geese	Fowls	Turkeys	Total
Africa[a]	5,189,000	4,614,000	410,364,000	1,734,000	421,901,000
Asia[a]	64,209,000	2,429,000	757,289,000	3,038,000	826,965,000
China[a, b, c]	21,060,000	3,510,000	1,119,690,000	25,740,000	1,170,000,000
Europe[a]	28,700,000	14,055,000	1,206,659,000	13,594,000	1,263,008,000
North and Central America[a]	674,000	365,000	302,481,000	8,782,000	312,302,000
Oceania[a]	1,147,000	228,000	29,882,000	717,000	31,974,000
South America[a]	9,249,000	102,000	457,992,000	4,858,000	472,201,000
U.S.A.[c, d, e]	12,508,000	1,069,000	3,387,953,000	120,085,000	3,521,615,000
U.S.S.R.[a, b, c]	10,800,000	1,800,000	574,200,000	13,200,000	600,000,000
TOTALS	153,536,000 (1·8%)	28,172,000 (0·3%)	8,246,510,000 (95·7%)	191,748,000 (2·2%)	8,619,966,000 (100%)

[a] F.A.O. "Production Yearbook" 25 (1971).

[b] Estimates of species not given by F. A. O. Total numbers of domestic birds for these regions were calculated by us according to the proportions found in the world.

[c] Not included in continental location.

[d] U.S. Department of Commerce "U.S. Census of Agriculture" (1964) Vol. II, Ch. 1 for ducks and geese.

[e] U.S. Department of Agriculture "Agricultural Statistics" (1972) for fowls and turkeys.

The literature relevant to the subject of this review is enormous and we regret that we have ignored many more papers than we have examined. Several articles, dealing in detail with related topics, have already been published in earlier volumes of "Advances in Parasitology".

II. Aspects of the Nutrition of Domestic Birds

The nutrition of the domestic duck, fowl and turkey has been intensively studied for the past 50 years and comprehensive discussions of this topic, particularly with respect to the domestic fowl, have been published by Scott *et al.* (1969) and the National Academy of Sciences–National Research Council (1971).

A. Chemical Nutrients

The food of birds must supply at least 40 chemical compounds or inorganic elements that have been shown to be dietary essentials. The qualitative and approximate quantitative dietary requirements for these nutrients are shown in Tables II, III and IV. These requirements apply mainly to the fowl, but may be similar for other domestic birds. Occasionally, there are some quantitative differences in dietary requirements between individual species that are important. Turkeys and quail, for example, require more protein and amino

Table II

Protein, amino acid and energy requirements of fowls and turkeys

Nutrient[a]	Growing chickens 0–6 wks of age[b]	Turkey poults 0–4 wks of age[b]	Laying hens[c]
Protein	20	28	16
ME, kcal/day	78[d]	124[d]	310[d]
Lysine	1·1	1·5	0·64
Arginine	1·2	1·6	0·8
Histidine	0·4	0·55	0·3
Leucine	1·4	1·9	1·2
Isoleucine	0·75	1·1	0·8
Valine	0·85	1·2	0·8
Threonine	0·7	1·0	0·56
Tryptophan	0·2	0·26	0·16
Phenylalanine	0·7	1·00	0·7
Tyrosine	0·6	0·80	0·32
Methionine	0·4	0·52	0·32
Cystine	0·35	0·35	0·26

[a] Except for metabolizable energy (ME), requirements are expressed as approximate percentages of diet.

[b] Estimates from N.A.S.–N.R.C. "Nutrient Requirements of Domestic Animals: Nutrient Requirements of Poultry" (1971).

[c] Estimates from Scott *et al.* (1969).

[d] Estimates for daily energy required are for a 250 g chick about 3 weeks of age, a 400 g turkey about 3 weeks of age and a mature hen weighing about 1·8 kg.

TABLE III

Approximate minimum needs of vitamins of domestic fowls[a]

Vitamin	Growing chickens (0–6 wks)	Laying hens (for eggs and reproduction)
	amount per kg diet	
Vitamin A (retinal), IU	1500	4000
Vitamin D_3 (cholecalciferol), IU	200	500
Vitamin E (α-tocopherol), IU	10	10[b]
Vitamin K_1 (phytyl menaquinone), mg	0·53	0·5[b]
Thiamin, mg	1·8	0·8
Riboflavin, mg	3·6	3·8
Nicotinic acid, mg	27	10
Pantothenic acid, mg	10	10
Pyridoxine, mg	3·0	4·5
Vitamin B_{12}, mg	0·009	0·003
Folic acid, mg	1·2	0·35
Biotin, mg	0·09	0·15
Choline, mg	1300	—
Linoleic acid, g	12[b]	10[b]

[a] See footnote b, Table II.

[b] Represents estimates by authors of amounts which are considered adequate, but for which accurate data on minimum requirements are lacking.

TABLE IV

Approximate minimum needs of inorganic elements of growing and laying fowls[a]

Inorganic element	Growing chickens (0–6 wks)	Laying hens (for eggs and reproduction)
	%	%
Calcium	1·0	2·75
Phosphorus	0·7	0·6
Sodium	0·15	0·15
Potassium	0·30[b]	0·30[b]
Chlorine	0·15[b]	0·15[b]
	mg/kg	mg/kg
Magnesium	500	500[b]
Manganese	55	33
Zinc	50	30[b]
Iron	80	40[b]
Copper	4	5[b]
Molybdenum	0·2	0·2[b]
Selenium	0·1	0·1[b]
Iodine	0·35	0·3

[a] See footnote b, Table II.

[b] Estimates made by Scott *et al.* (1969).

acids per unit of diet than fowls to grow normally during early stages of life. Ducks and turkeys require considerably more nicotinic acid per unit of diet than fowls, and various domestic species of birds seem to have a striking variation in their requirements for vitamin D (N.A.S.–N.R.C., 1971).

Quantitative nutritional requirements change during the growth and development of young birds. The data in Table V show the growth pattern, energy and dietary protein requirements for the growth of a fowl from hatching to sexual maturity. During rapid early growth, diets must contain more protein relative to energy than in later stages when energy needs predominate. This pattern probably applies to the nutrient needs of most species of either domestic or wild birds which show a rapid early growth of young. Grimshaw (1911), for example, found that young red grouse, *Lagopus scoticus*, are mainly insectivorous during the first 3 weeks of life, after which the dietary importance of insects seems to decline. Insects are relatively high in protein (Table VI) and thus the food pattern of *L. scoticus* seems to follow the dietary requirements which have been demonstrated in laboratory experiments, and which probably apply to wild birds (Martin *et al.*, 1951). The high protein requirement of young birds, ingested in the form of invertebrates, may render them particularly vulnerable to infection with those parasites which develop in an invertebrate intermediate host.

Although the data show that dietary protein requirement per unit weight of diet declines with age, the total daily requirement is relatively constant throughout the growing period of the fowl. Thus the amount of protein and amino acid entering the tract is relatively constant over the period of growth.

B. ENERGY REQUIREMENTS

The energy needs of animals appear to be related to the mechanisms controlling food intake. Many investigators have found that food intake is regulated in domestic birds and, if birds are offered diets of different energy values per unit weight, food intake tends to become modified towards achieving more uniform energy intake as opposed to consumption of a given weight of food (Hill and Dansky, 1954). This tendency is never exact, however, since more energy than is required by a bird is usually consumed when highly concentrated diets are fed.

The daily energy requirement of a bird is a function of the amounts of energy required for basal metabolic rate, for activity and for growth and reproduction. The basal or resting metabolic rate is not constant per unit of body weight and small animals have a much higher metabolic rate per unit weight than do larger animals. Since the logarithm of metabolic rate is a linear function of the logarithm of body weight (Kleiber, 1932), it follows that metabolic rate is proportional to a given power of body weight. Kleiber (1947), in his review of resting metabolic rate of a large number of species of homeotherms, recommended that the 3/4 power of body weight be used to estimate the metabolic body size of an animal. He also concluded that the metabolic rate of adult homeotherms, from mice to cattle, averages 70 kcal per $kg^{3/4}$ per day.

TABLE V

Calculated feed consumption, energy and protein requirements of White Leghorn pullets (from Scott *et al.*, 1969)

Age	Body gains		Feed consumed per pullet per day[a]	Metabolizable energy (ME) required per pullet per day	Protein required per pullet per day[b]	Protein requirement in diet[a]
	Average weight	Gain per day				
wks	g	g	g	kcal	g	%
1	70	5·7[c]	8	24	1·7[d]	21·5
2	125	7·9	13	39	2·8	21·5
3	190	9·3	19	57	4·1	21·5
4	265	10·7	27	81	5·4	20·0
5	350	12·1	32	96	6·2	19·5
6	450	14·3	41	125	7·4	18·5
7	550	14·3	48	140	7·8	16·5
8	640	12·9	49	148	7·8	16·0
9	730	12·9	49	148	7·8	16·0
10	820	12·9	53	160	7·8	15·0
11	895	10·7	57	170	7·3	13·0
12	960	9·3	59	177	7·3	12·5
13	1025	9·3	60	180	7·3	12·5
14	1090	9·3	61	183	7·3	12·0
15	1155	9·3	63	189	7·3	11·5
16	1220	9·3	64	192	7·3	11·5
17	1270	7·1	65	195	7·0	10·5
18	1320	7·1	66	198	7·0	10·5
19	1365	6·4	67	201	7·0	10·5
20	1410	6·4	68	204	7·0	10·5
21	1455	6·4	69	207	7·0	10·5
22	1500	6·4	70	210	7·0	10·5

[a] Diet assumed to contain 3000 kcal ME/kg and to be adequate in all essential amino acids and other nutrients.

[b] Assuming White Leghorn pullets utilize dietary protein with an efficiency of 55%.

[c] Assuming 36 g chick at start, containing 5 g of spare yolk (yolk sac) which is used up during the week.

[d] Represents protein of 1·91 g minus 0·21 g from spare yolk. Spare yolk contains 30% protein, or a total of 1·5 g of protein, which supplies the newly hatched chick with an average amount of 0·21 g protein per day for first week.

A similar observation has been made on a variety of birds, including domestic birds, by King and Farner (1961) and Lasiewski and Dawson (1967). King and Farner found that the equation $\log M = \log 74{\cdot}3 + 0{\cdot}744 \log W \pm 0{\cdot}0074$ described the relationship between body weight and resting metabolic rate of the birds they examined, where M refers to the metabolic rate in kcal/24h and W is equal to body weight in kg.

The implications of the relationship between metabolic rate and body size to digestive physiology have not been investigated in detail. In addition to

needing less energy per kg of body weight, large birds also require less food per kg of body weight than small birds. A preliminary study of the food intake, size of the alimentary tract and body weight of four species of galliform birds, which were fed the same diet, has been made recently by Nesheim and Kuenzel (unpubl. obsvns). The logarithms of body weight and tract weight (Fig. 1) were observed to be linearly related ($r = 0{\cdot}996$) over a range of birds varying in size from Japanese quail, *Coturnix coturnix*, weighing about 125 g, to domestic turkeys weighing up to 15 kg. According to these measurements, the relationship between the weight of the body and the weight of the tract is exponential, with the exponent being 0·859. It is not unreasonable to assume that similar results would be obtained if birds of the same species but varying in age and size were fed the same diet. The parasitological significance of this relationship is that the tracts of small birds, irrespective of age, and of the young stages of a species, which is large when adult, may be expected to contain more space and more food per unit of the host's body weight. There may also be correspondingly more digestive activity in a small bird than in a large one.

The energy needed for the maintenance of resting metabolic rate is only one component of the total energy needs of birds. Evidence demonstrating the amount of energy needed in connection with environmental temperature on a bird's energy budget has been obtained for the fowl. Leeson *et al.* (1973)

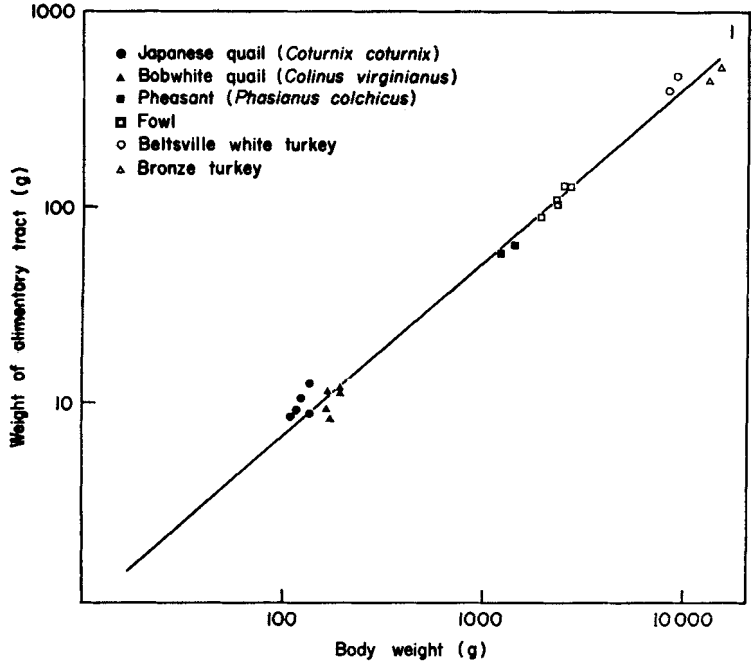


FIG. 1. Graphical representation of the results of a preliminary study of the relationship between the weight of the alimentary tract and the weight of the body of galliform birds (Nesheim and Kuenzel, unpubl. obsvns.)

have made detailed predictions about the energy requirements of fowls and have calculated that fowls maintained at different environmental temperatures need to ingest a further 20 kcal of metabolizable energy per day for each 10°C reduction of environmental temperature. This situation may also affect the alimentary tract as an environment for parasites; provided that food is available *ad libitum*, a host kept at a relatively low temperature will eat and digest more food per unit of body weight than an equivalent host offered the same diet at a higher temperature.

On theoretical grounds, it is unlikely that the energy requirements of a parasite of the alimentary tract will have a serious effect on the host's energy budget. The weight of a heavy parasitic burden forms a very small proportion of the total tissue involved in a host–parasite relationship (see von Brand, 1973). The indirect effects of certain parasites, however, may be severe in terms of the energy expenditure of the host to compensate for tissue damage, haemorrhage and other pathogenic effects.

C. NATURE OF DIET

The substrates for enzymes present in the alimentary tract of domestic birds are relatively few. From the information summarized in Table VII, the

TABLE VI

Energy values of some potential foods for birds

	Energy content (kcal/g)		%
	Gross	Metabolizable[a]	Protein
Maize	4·5	3·4	8·7
Oats	4·2	2·6	11
Barley	4·4	2·8	11·5
Dried alfalfa	4·2	1·4	17
Sunflower seeds	5·6	2·5	16
Soybeans	5·3	3·5	38
Soybean oil	9·4	8·9	—
Soybean meal (fat extracted soybeans)	4·7	2·2	45
Heather (Moss and Parkinson, 1972)	5·3	1·4[b]	6·4
Fly pupae (dried) (Calvert *et al.*, 1969)	5·5[c]	2·5[d]	63·1
Grasshopper (*Melanoplus* sp.) adult (dried) (Uvarov, 1966)	4·9[c]	3·3[e]	75·3

[a] Metabolizable energy represents energy ingested minus energy recovered in faeces and urine. Values are from Scott *et al.* (1969), except where indicated, and were determined with young chicks.

[b] Determined metabolizable energy using red grouse.

[c] Estimated by M.C.N. from proximate composition: protein 5·43 kcal/g, fat 9·4 kcal/g and carbohydrate 4·0 kcal/g.

[d] Determined value from Teotia and Miller (1970).

[e] Estimated by M.C.N. on the basis of 90% digestibility of fat and 85% of protein.

(All other values were obtained from the Department of Poultry Science, Cornell University, Ithaca, N.Y., U.S.A.)

TABLE VII

Observations on some digestive enzymes of domestic birds

Enzyme	Substrate	Notes	References
Carbohydrases			
Amylase	Starch	Produced by pancreas, at least two isoenzymes present in pancreas of fowl. Presence reported in saliva, bile and mucosa	Laws and Moore (1963a); Heller and Kulka (1968); Ziswiler and Farner (1972)
Isomaltase	Isomaltose	Most activity in anterior small intestine, with with more activity in mucosa than lumen	Siddons (1969, 1970)
Lactase	Lactose	Present in low quantity and of microbial origin; mainly found in caeca	Siddons (1969); Siddons and Coates (1972)
Maltase	Maltose	Most activity in anterior small intestine, with more activity in mucosa than lumen	Laws and Moore (1963a); Siddons (1969, 1970)
Sucrase	Sucrose	Most activity in anterior small intestine, with more activity in mucosa than lumen	Siddons (1969, 1970)
Lipid-Hydrolyzing Enzymes			
Cholesterol esterase	Cholesterol esters	Cholesterol esters hydrolyzed by extracts of pancreas, but not by small intestine	Laws and Moore (1963b)
Esterase	Water-soluble esters, triacetin, methylbutyrate	Probably originates in pancreas and small intestine. Activity throughout small intestine, but highest in duodenum	
Lipase	Triglycerides	Activity confined mainly to pancreas, but also found in small intestine, especially upper region	
Phospholipase	Lecithin	Lecithin hydrolyzed by extracts of pancreas, but not by small intestine	

Proteases			
Aminopeptidase Carboxypeptidase	Proteins	Not yet purified, activity measured by DeRycke	See Ziswiler and Farner (1972)
Chymotrypsin A Chymotrypsin B	Proteins	Purified from pancreas of fowl and turkey	Ryan (1965); Pfleiderer *et al.* (1970)
Elastase	Proteins	Originates in pancreas of fowls	Marrama *et al.* (1959)
Pepsin	Proteins	Evidence exists for at least five pepsinogens in proventriculus of fowl	Herriott *et al.* (1938); Green and Llewellin (1973)
Peptidases	Peptides	Many are probably present; few have been measured	Kuznetsov and Scherbakov (1971); Ugolev *et al.* (1971)
Trypsin	Proteins	Isolated from pancreas of fowl and turkey	Ryan (1965)

digestive enzymes present in the tract appear to be sufficient only to hydrolyze completely starch, sucrose, triglycerides and phospholipids for major sources of energy and proteins for amino acids. Simple sugars are useful energy sources although the value of pentose sugars is limited (Wagh and Waibel, 1966). Other enzymes needed to hydrolyze cellulose, various pentosans or other complex carbohydrates do not seem to be produced by domestic birds. Consequently, the types of foods that birds can assimilate efficiently are the seeds or grains, which have a relatively high starch and fat content, the oil-bearing seeds and animal foods such as insects, fish or other small animals. Many foods may be quite similar in combustible energy, but because digestibility can vary, they differ markedly in their metabolizable energy value (Table VI).

Commercial feeds for domestic birds are primarily made up of grains or grain by-products and oil seed residues from which vegetable oils have been extracted. Various supplements are usually included to offset nutritional deficiencies (Scott *et al.*, 1969).

Wild birds usually have broader food habits than their domestic relatives. Mallard, *Anas platyrhynchos*, for example, consume large quantities of grasses, seeds, insect larvae and small molluscs (Olney, 1964). The red grouse, *Lagopus scoticus*, subsists largely on a diet consisting of heather (*Calluna vulgaris* (Eastman and Jenkins, 1970). These diets contain little starch or sugars that are readily hydrolyzed by the enzymes present in the alimentary tract of the bird. Two major adaptations appear to have evolved to enable birds feeding on these types of diet to meet their energy requirements. First, in birds which feed mainly on herbage, for example geese, the low content of digestible matter appears to be compensated for by a very large capacity to consume food and to pass it quickly through the digestive system (Mattocks, 1971a). The food intake of grouse fed heather (25% digestible) was 70 g per day, while food intake of grouse fed a mixed diet (50% digestible) was only 30 g per day (Moss, 1972). The ability to vary food intake in response to the usable energy concentration of the diet is important for a bird consuming a varied type of food over a period of time. Secondly, digestion may be aided by enzymes provided by micro-organisms which can degrade cellulose and other complex carbohydrates to volatile fatty acids. The main sites of volatile fatty acid production are the caeca, in which concentrations up to 128 mmol/kg of luminal contents were found in 16-week-old fowls by Annison *et al.* (1968).

The evidence suggests, particularly from wild birds, that microbial digestion can be energetically important. The digestive system of the adult grouse represents an extreme adaptation to a vegetarian diet composed of over 79% lignin, holocellulose and α-cellulose. These components are not digested by enzymes normally present in the tract of birds, but the grouse has two caeca which may be up to 75 cm each in length (Fig. 13). The combined length of the two caeca is about 50% greater than the length of the small intestine (Moss, 1972), and since about 24% of the α-cellulose and 22% of the holocellulose in the heather were digested (Moss and Parkinson, 1972), it is possible that micro-organisms in the caeca were involved. Domestic birds

consuming commercial diets probably have little dependence on microbial fermentation for digestion (McNab, 1973) because the diets have a high energy content and a relatively low cellulose content. It is likely, however, that foraging domestic birds may ingest much plant material and may obtain significant amounts of energy through microbial activities. These considerations illustrate how the host's response to the nature of the diet may affect the alimentary tract as an environment for parasites.

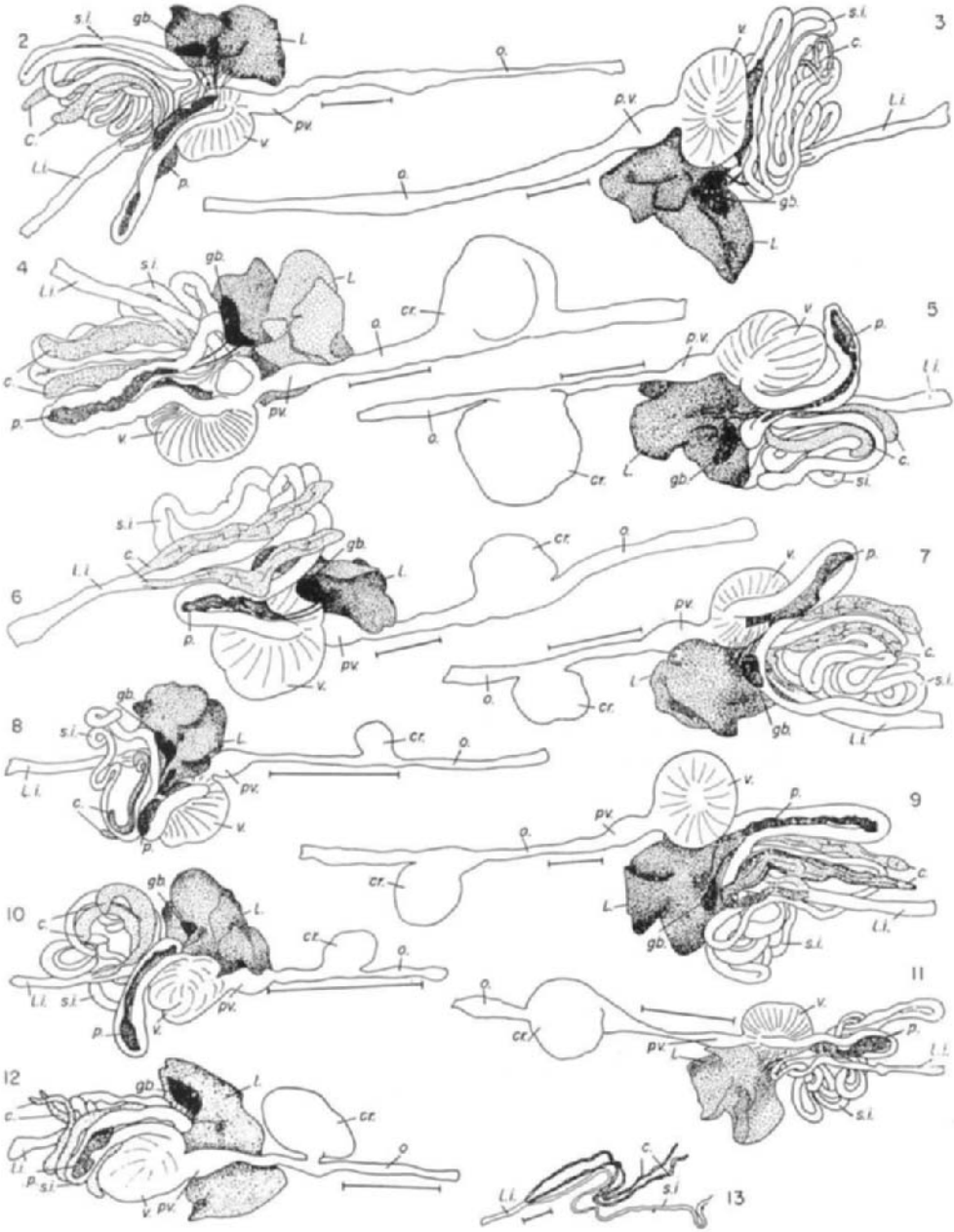
III. The Alimentary Tract of Domestic Birds as a Habitat for Parasites

Many references both to general information about the alimentary tract of vertebrates and to the conditions prevailing in the environments of endoparasites were cited by Crompton (1973) in a discussion of the distribution of helminths in the alimentary tract. Detailed descriptions of the alimentary tract of birds are given by Farner (1960), Sturkie (1965) and Ziswiler and Farner (1972), and of the domestic birds considered in this review by Calhoun (1933, 1954), Bradley (1938), Bolton (1969), Scott *et al.* (1969) and Hill (1971a,b) among others. Some aspects of the vertebrate alimentary tract have been discussed with a parasitological interest by Read (1950, 1971), Smyth (1969), Crompton (1969, 1970) and Mettrick and Podesta (1974).

A. MORPHOLOGY

The major part of the avian alimentary tract is derived embryologically from endoderm with small portions of the oral cavity and cloaca being formed from ectoderm (Romanoff, 1960). The general morphology of the alimentary tracts of adult domestic birds and of some galliform species of interest to man is shown in Figs 2–13, and estimations of the dimensions of the tracts of domestic ducks, geese, fowls, turkeys and pigeons are given in Table VIII. There is no crop in the duck and goose (Figs 2 and 3), both of which have an extensible oesophagus. The crop is a thin-walled pouch which extends ventrally from the surface of the oesophagus. The alimentary tract of the pigeon (Fig. 11) differs from that of the other birds under consideration in that there is no gall-bladder and the caeca are minute and are unlikely to have any digestive function. An impression of the arrangement of the main blood vessels of the tract is depicted in Fig. 14.

The caudal or lower oesophagus opens into the proventriculus which in turn leads into the ventriculus or gizzard. The ventriculus connects, by means of the pyloric opening, with the small intestine, which is coiled and folded in a manner characteristic of the phylogeny of the bird (Gadow, 1879). There is little evidence to justify identifying sections of the small intestine as the jejunum and ileum although the duodenum is generally obvious. In this review, the duodenum is defined as the anterior part of the small intestine extending from the pylorus to the point where the biliary and pancreatic secretions are discharged. The duodenum is generally of greater diameter than



FIGS 2–13. The general morphology of the alimentary tracts of some domestic and galliform adult birds. Some organs have been displaced to reveal certain features. Scale represents 5 cm; *c.*, caecum *cr.*, crop; *gb.*, gall-bladder; *l.*, liver; *l.i.*, large intestine; *o.*, oesophagus; *p.*, pancreas; *pv.*, proventriculus; *s.i.*, small intestine; *v.*, ventriculus.

TABLE VIII

Estimations of the size of the alimentary tracts of adult domestic birds

	Duck[1]	Goose[2]	Fowl[3]	Turkey[4]	Pigeon[5]
Weight of bird (kg)	1·5	4·9	2·5	11·9	0·5
Weight of tract (g)	127	663	111·5	446	48
Total length (cm)	185	279·5	161·25	252	117
Oesophagus (and crop) (cm)	25	35	17	26	12
Proventriculus and ventriculus (cm)	10	16·5	6	13	8
Small intestine[a] (cm)	141·5	211	127	201	92
Caecum[b] (cm)	15	20	17	31	V
Large intestine (cm)	8·5	17	11·25	12	5
Post-pyloric length (cm)	150	228	138·25	213	97
Weight of ventricular grit (g)	5·1	15·5	15·2	40	1·26

1. Crompton, Harrison and Lackie; Crompton and Nesheim (unpubl. obsvns).
2. Roberson and Francis (1965); Mattocks (1971b).
3. Scott and Heuser (1957); Crompton (1973); Nesheim and Kuenzel (unpubl. obsvns).
4. Scott and Heuser (1957); Crompton and Nesheim; Nesheim and Kuenzel (unpubl. obsvns).
5. Crompton and Nesheim (unpubl. obsvns).

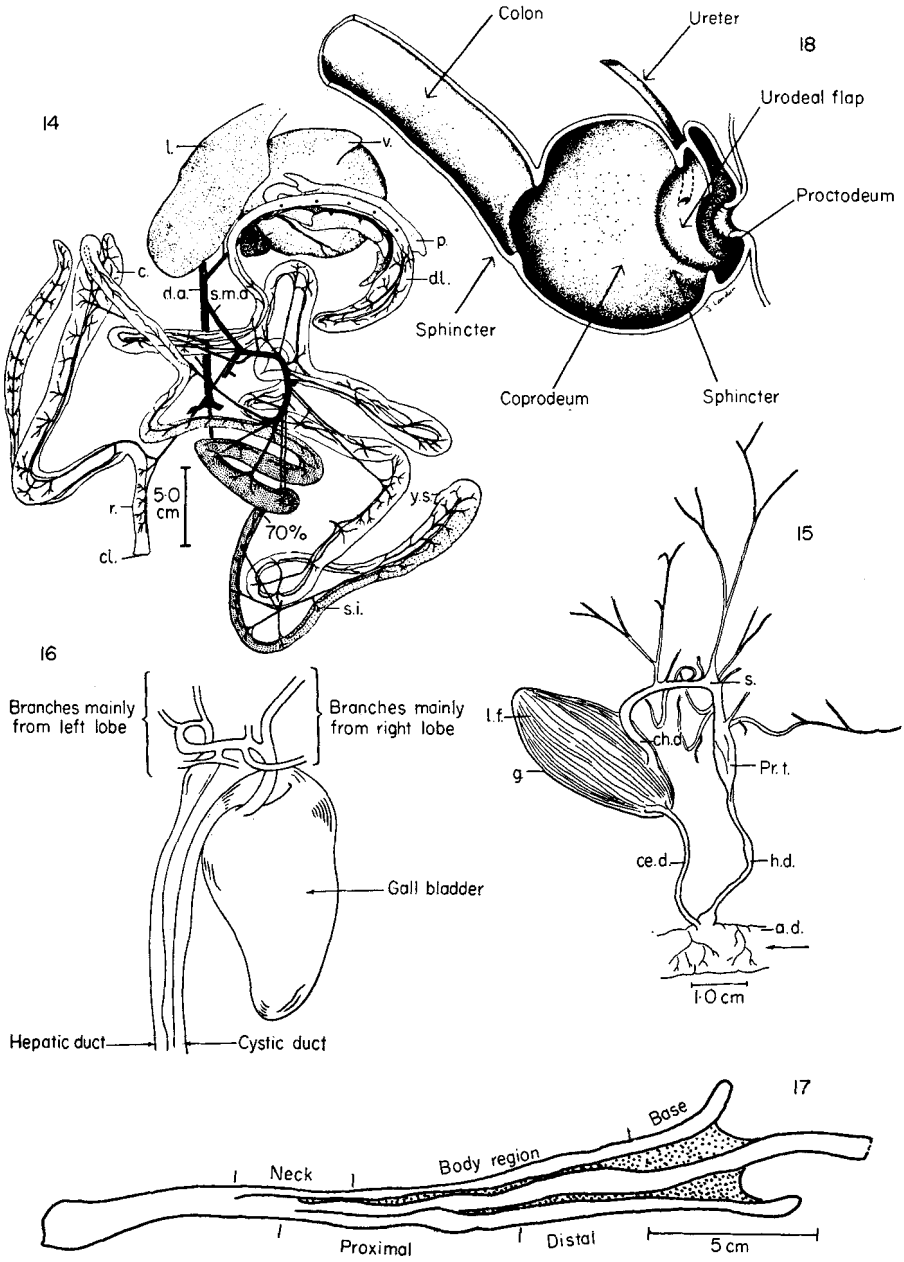
[a] Pylorus to ileo-caecocolic junction.

[b] Not included in post-pyloric length.

(V represents vestigial)

the rest of the small intestine and the pancreas is usually situated in the loop formed from the descending and ascending limbs (Fig. 14). The pancreas of the domestic fowl consists of three lobes which are usually connected with the duodenum by three ducts (Calhoun, 1933; Hill, 1971a). This situation probably occurs in ducks, geese, turkeys and pigeons, but the presence of three ducts cannot be relied on and parasitological experiments involving ligation of the pancreatic ducts of a fowl failed on one occasion when three had been expected, but four were present (Levine, 1942). The two bile ducts connect with the duodenum in the same region as the pancreatic ducts, and in the duck, goose, fowl and turkey the cysto-enteric duct drains the gall-bladder (Fig. 15). It can be seen from Figs 15 and 16 that in ducks (Crompton and Nesheim, 1972) and fowls (Hill, 1971a; Clarkson and Richards, 1971) the biliary systems of the left and right lobes of the liver are connected by a vessel or sinus normally embedded within the liver tissue. Thus, any parasite which enters the liver via the bile ducts may gain access to both lobes by means of either duct.

(2) Domestic duck. **(3)** Domestic goose. **(4)** Guineafowl (*Numida meleagris*). **(5)** Domestic fowl. **(6)** Peafowl (*Pavo cristatus*). **(7)** Pheasant (*Phasianus colchicus*). **(8)** Partridge (*Perdix perdix*). **(9)** Domestic turkey. **(10)** Japanese quail (*Coturnix coturnix*). **(11)** Domestic pigeon. **(12)** Captive red grouse (*Lagopus scoticus*). **(13)** The post-pyloric portion of the alimentary tract of a captive *L. scoticus* arranged to show the length of the caeca.



FIGS 14–18. Aspects of the morphology of the alimentary tracts of domestic birds. **(14)** Dissection of alimentary tract of a domestic duck after injection of Prussian blue into the superior mesenteric artery. Close stippling indicates the main attachment zone of *P. minutus*; *c.*, caecum; *cl.*, cloaca; *d.a.*, dorsal aorta; *d.l.*, duodenal loop; *l.*, liver; *p.*, pancreas; *r.*, rectum (large intestine); *s.m.a.*, superior mesenteric artery; *s.i.*, small intestine; *v.*,

The blind-ended tubular caeca (Fig. 17) arise at the junction of the small and large intestines of the duck (Crompton and Whitfield, 1968), goose (Mattocks, 1971a), fowl (Calhoun, 1933; Akester *et al.*, 1967; Hill, 1971a) and turkey (Durant, 1930). Each caecum of the fowl consists of a narrow neck, a wider body region and a round-ended base (Hill, 1971a), and in the goose the left caecum has been observed to be shorter than the right (Mattocks, 1971a). The region of the small intestine to which the caeca are connected is the ileo-caecocolic junction. The relatively short, large intestine or colon (Table VIII) extends from this junction to the cloaca (Fig. 18). There is a muscular sphincter at the entry of the large intestine into the coprodeum and this sphincter, the pylorus and the ileo-caecocolic junction (Yasukawa, 1959; Akester *et al.*, 1967) affect the movement of ingesta in the tract of the fowl.

The manipulation of the specimens for the preparation of Figs 2–13 necessitated displacement of the liver to reveal the gall-bladders and disturbance of the loops of the small intestine to show features like the junction of the caeca with the small intestine. The most careful removal of the tract from the bird destroys the elements which hold the tract in position, and it is difficult to envisage how the tract is arranged *in vivo*. In an attempt to overcome this conceptual problem, a male domestic fowl was processed and longitudinal serial sections were prepared. A section from the middle of the bird is shown in Fig. 19 and the relationships of the anterior part of the tract, the proventriculus, ventriculus, liver and small intestine to themselves and to the other organs of the bird can be seen. Until the tract is observed in this manner, it is difficult to appreciate how much or how little of the bird is composed of unoccupied body cavity. Those parasites that reach maturity in a particular organ of the body, after emigrating through the body cavity from the small intestine, may undergo a relatively short emigration if they have become adapted to penetrate the intestinal wall in a region located near their target organ. Other features of the alimentary tract of the domestic fowl are illustrated in Figs 20–26.

B. HISTOLOGY

Details of the histology of the alimentary tract of the domestic birds can be seen in the series of photomicrographs prepared by Calhoun (1933), and in Figs 22–26. The plates show not only the surface of the host that is in

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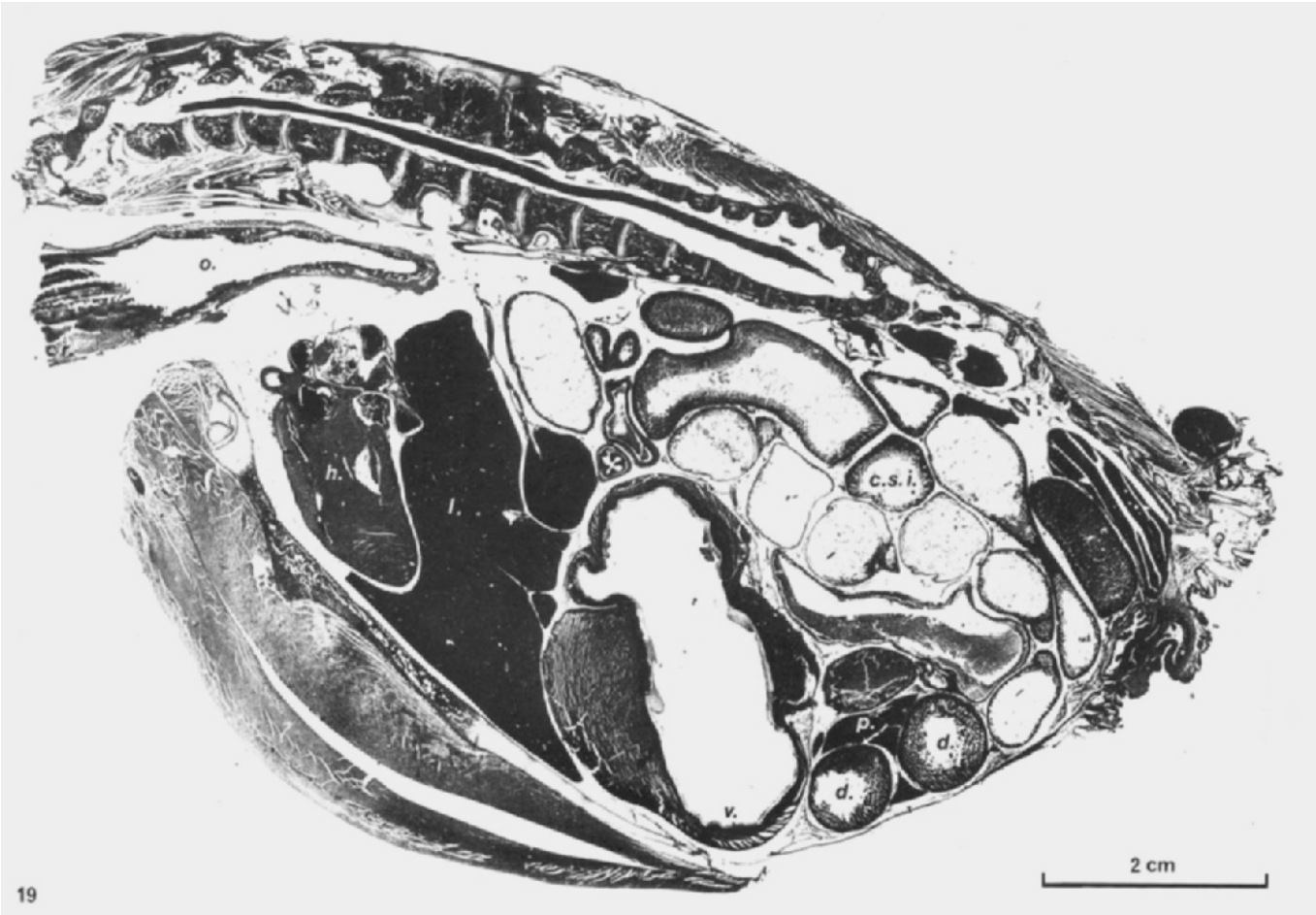
ventriculus; *y.s.*, yolk stalk (Meckel's diverticulum) (Fig. 1, Crompton and Whitfield, 1968). (**15**) The gall-bladder, bile ducts and principal biliary collecting vessels of a domestic duck. The arrow indicates the direction of intestinal flow. *a.d.*, ascending limb of duodenum; *ce.d.*, cysto-enteric duct; *ch.d.*, cysto-hepatic duct; *g.*, gall-bladder; *h.d.*, hepato-enteric duct; *l.f.*, longitudinal fold; *?r.t.*, possible reabsorptive tissue; *s.*, sinus (Fig. 1, Crompton and Nesheim, 1972). (**16**) Hycar latex cast of gall-bladder and biliary system of 8-week-old Light Sussex cockerel (Fig. 3, Clarkson and Richards, 1971). (**17**) The caeca of a domestic duck. (**18**) The relationship of the right ureter, cloaca and colon (domestic fowl). The ureters normally discharge on to the cranial side of the urodeal flap (Fig. 1, Akester *et al.*, 1967).



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contact with the organisms living in the lumen of the tract, but also the extent to which the folding of the surface and the components of the wall of the tract provide shelter for the smaller organisms. In birds, as in all vertebrates, five layers consisting of serosal connective tissue, longitudinal muscle, circular muscle, and submucosal and mucosal tissue, are present in most parts of the wall of the tract. The following account is based largely on the domestic fowl, the differences between this and the other species under consideration being minimal.

Descriptions of the mouth and salivary glands of the domestic fowl and goose have been written by Hill (1971a) and Mattocks (1971b). In ducks and geese, the lining of the oesophagus appears to be similar to that of the oesophagus and crop of galliform birds, both regions displaying a structure commensurate with their property of great entensibility (Fig. 22). The lining is made up of much-folded, stratified squamous epithelium through which mucus is discharged from sub-epithelial glands (Hill, 1971a). Examination with the electron microscope indicates that the crops of the turkey and pigeon are like that of the fowl (Crompton, Nesheim and Price, unpublished observations), but, during the breeding season, the lining of the crop of pigeons contains fat-laden cells which contribute a major portion of the "milk" secretion (Ziswiler and Farner, 1972).

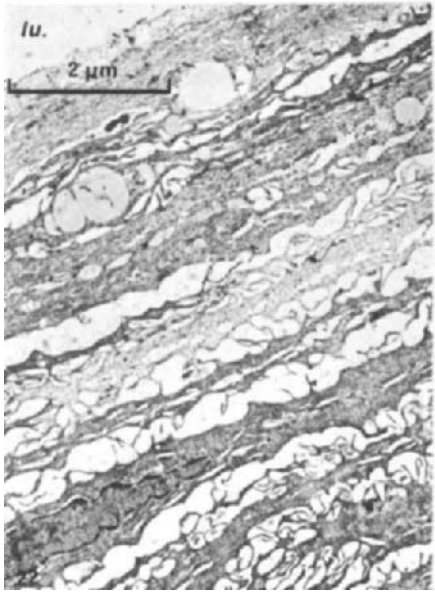
The proventriculus is the site of gastric secretions and the ventriculus is the site of trituration and peptic hydrolysis (Figs 20 and 23). The mucosa of the proventriculus consists of columnar epithelial cells surrounding the ducts draining the glands composed of alveolar, oxyntico-peptic cells which are now considered to secrete both hydrochloric acid and pepsinogen. The properties of these cells during and between phases of digestion have been considered by Hill (1971a). The most striking features of the ventriculus are the hard, extra-cellular keratin-like deposit or koilin lining on the surface of the mucosa and the muscle development of the wall. The lining, which in adult birds appears to be secreted by mucosal glands at about the same rate as it is abraded, protects the mucosa from chemical damage by peptic hydrolysis and from mechanical and abrasive damage as the food and grit in the lumen of the ventriculus are pulverized. Passage through the lumen of the ventriculus must be a major hazard for many parasites of the alimentary tract of domestic birds that are kept under range conditions and have access to all types of abrasive material (Fig. 21). Some information about the grit content of the ventriculus is given in Table VIII.

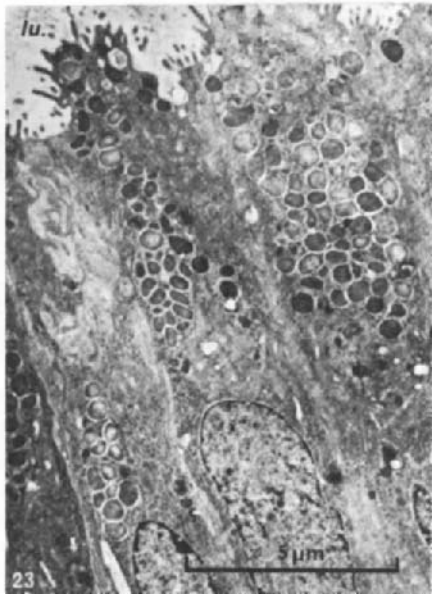
The mucosa of the small intestine of domestic birds is modified into a series of folds, villi and crypts, and the surfaces of the epithelial cells lining the villi have microvilli characteristic of cells involved in absorption (Fig.

FIG. 19. Photograph of a longitudinal section of a 32-day-old domestic fowl (Rhode Island Red × Light Sussex) prepared by Mr J. Cash, Department of Anatomy, University of Cambridge. The specimen was fixed in Bouin's fluid, the skin was removed, and then dehydration was achieved in a series of solutions of ethanol before embedding in paraffin wax (56°C). Sections were cut at 10–15 μm and stained with a trichrome stain. Note how the alimentary tract almost fills the body cavity. *c.s.i.*, coiled small intestine; *cr.*, crop; *d.*, duodenum; *h.*, heart; *l.*, liver; *o.*, oesophagus; *p.*, pancreas; *v.*, ventriculus.


pv.
v.
1 cm
20


1 cm
21


lu.
2 µm
22

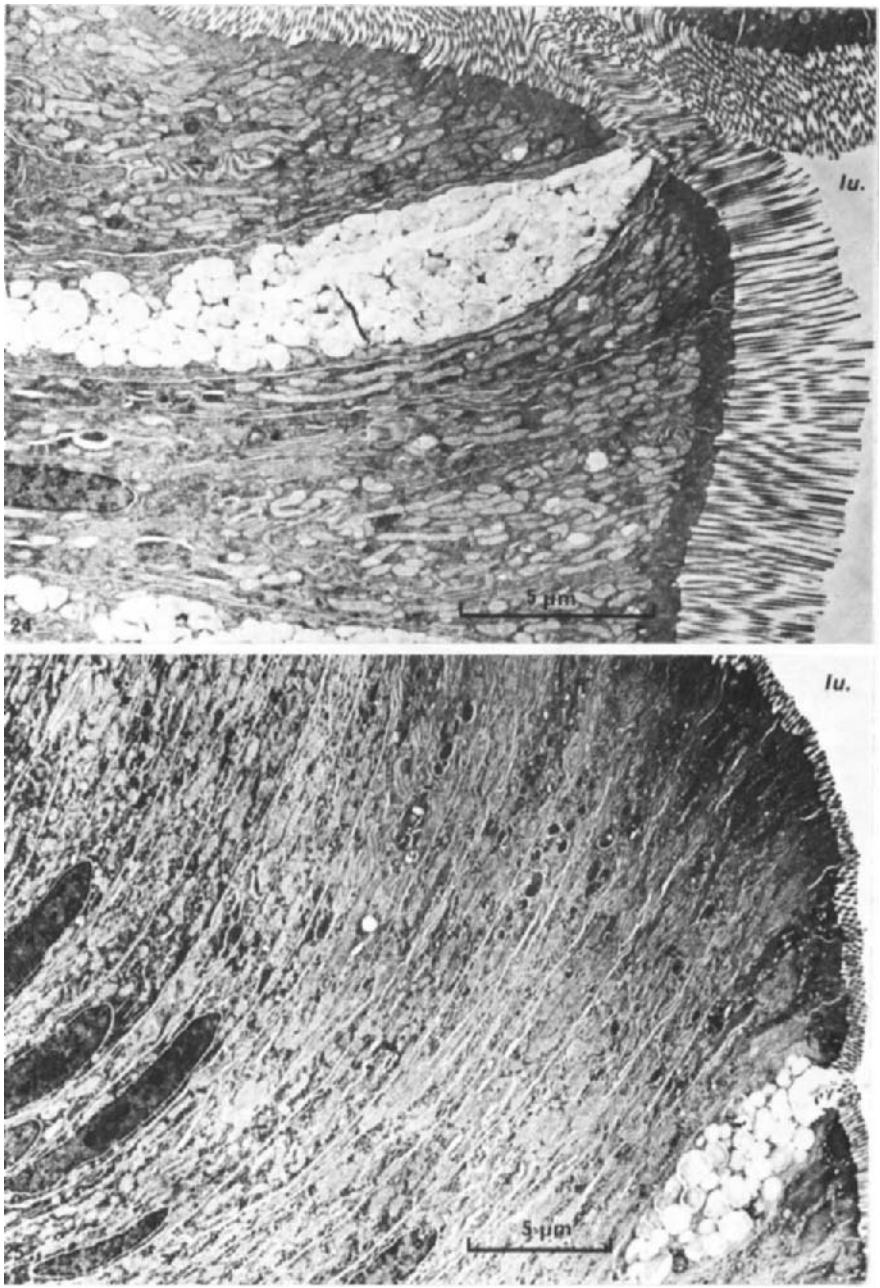

lu.
5 µm
23

24). Goblet cells and other secretory cells are present together with glands in the mucosal tissue (Calhoun, 1933). In most respects the histology of the small intestine of adult birds is like that of non-ruminant mammals. The mucosa of the large intestine is folded and possesses villi and cells with a microvillar brush border. There are also numerous goblet cells in this region (Fig. 26). The wall of a caecum resembles that of the small intestine, but the villi change from being like those of the small intestine in the narrow, proximal region, to shorter more sparsely distributed projections in the middle region (Fig. 25) and a virtual absence in the distal portion (Calhoun, 1933). The cloaca is lined with columnar epithelium, which forms short villus-like structures in the coprodeum, and the vent is lined with stratified squamous epithelium (Hill, 1971a). As in other homeotherms, the epithelial lining of the alimentary tract is continuously replaced, the cell turnover time in young fowls being approximately 48 h for epithelial cells from the middle of the region extending from about the pancreatic ducts to the yolk stalk (Imondi and Bird, 1966). The cell turnover time in the duodenum and posterior ileum was longer. The autolysis and digestion of these cells contributes to the endogenous nitrogen in the lumen of the tract and the significance of this factor in the environments of endoparasites living in non-ruminant mammals has been discussed by Read (1971) and Mettrick and Podesta (1974). The cell turnover rate is also of interest when considering the developmental stages of *Eimeria* spp. (Table XV) which are intracellular parasites of the epithelium of the small and large intestines and caeca. The problem arises of explaining how the intracellular parasites survive if the cells in which they are living are continuously being lost at a rapid rate. Perhaps the timing of the different generations of schizonts and merozoites has become synchronized with that of cell turnover during the course of evolution in a manner that avoids much loss to the parasite, or perhaps the presence of an intracellular parasite alters the rate of the epithelial turnover.

C. PHYSIOLOGY

Comparisons between any recent review of the digestive physiology of birds (Hill, 1971b; Ziswiler and Farner, 1972) and accounts of the physiology of their intestinal parasites indicates that current knowledge about the host is far in advance of that of the parasite. Consequently, in this discussion of the physiology of avian digestion, we have selected those topics which we

FIGS 20–23. Morphological and histological features of the alimentary tracts of birds. (**20**) Photograph of a longitudinal section through the proventriculus (*pv.*) and ventriculus (*v.*) of the fowl used in the preparation of Fig. 19. (**21**) Grit from the ventriculus of a mallard (*Anas platyrhynchos*). (**22**) Electron micrograph of the tissue lining the crop of a pheasant (*Phasianus colchicus*). Specimens for this and for Figs 23, 24, 25 and 26 were obtained by biopsy and were immediately fixed for 1½ h in ice-cold 2·5% glutaraldehyde made up in 0·2M sodium cacodylate buffer adjusted to pH 7·2. Post-fixation was carried out in ice-cold 2% OsO_4 for 1h followed by dehydration in acetone and embedding in Spurr resin. *lu.*, lumen of the alimentary tract. (**23**) Electron micrograph of the epithelial surface of the proventriculus of a domestic fowl. Figs 22–26 are from Crompton, Nesheim and Price (unpubl. obsvns).



FIGS 24 and 25. Electron micrographs of the epithelial surface of the middle region of the small intestine (**24**) and the body region (see Fig. 17) of a caecum (**25**) of a domestic fowl. *lu.*, lumen of the alimentary tract.

consider to be most helpful in understanding host–parasite relationships. We have ignored control mechanisms, the effects of parasympathetic drugs, innervations and so forth, not because we feel they are unlikely to contribute eventually to knowledge of the relationship, but because the essence of the ecological approach is to integrate information about the host's physiology with that of its parasites.

It appears that in domestic birds, the general process of digestion, the secretions involved and the sites of absorption in the tract are similar to those of non-ruminant mammals except that evidence of a cephalic phase of digestion in birds is not so well established (Hill, 1971b). Digestion has been described from a parasitologist's standpoint by Crompton (1973), and evidence is presented by Crompton (1969; 1970), Crompton and Edmonds (1969) and Crompton and Nesheim (1970) to show that different conditions occur in different regions of the lumen of the alimentary tract of domestic ducks allowed food and water *ad libitum*. This conclusion probably applies to the alimentary tracts of many birds.

1. *Events occurring during and between phases of digestion*

(a) *Motility of the alimentary tract.* In the domestic fowl and, presumably, in other birds with a crop, the boluses of food, which have been lubricated with saliva in the mouth and oesophagus, pass into the crop. If food has been withheld for 24 h, the first three or four boluses pass directly into the proventriculus (Henry *et al.*, 1933). Once food is present in the ventriculus, the boluses are diverted into the crop from which food is released intermittently as the ventriculus empties. When food is delayed in the crop, carbohydrate digestion may occur as a result of the activities of salivary secretions and micro-organisms (Bolton, 1965; Pritchard, 1972). It has been implied that the control over whether a bolus passes to the crop or the proventriculus may be located in the caudal oesophagus. Radiographic studies have revealed a large bolus in the caudal oesophagus being divided by peristaltic contractions and the anterior portion was then moved back to the crop while the posterior portion moved down to the proventriculus (see Hill, 1971b). If this activity occurs frequently, the passage into the small intestine of infective stages located in the portion of a bolus, which is returned to the crop, may be affected and delayed.

Some of the observations recorded in the literature on the rate of passage of food down the alimentary tracts of domestic birds are summarized in Table IX and sets of observations for the fowl are shown in Figs 28 and 29. Many factors appear to influence the rate of food passage, including the length of time since the birds last received food, the feeding routine, the amount of food consumed at a meal, the texture, composition and moisture content of the food and the inhabitants of the tract. The references cited in Table IX are the source of this conclusion. The effects of these factors probably influence the length of time for which the food is processed in the ventriculus. The longer the time in the ventriculus, the longer the time before a meal is exposed to the duodenal and intestinal secretions and the longer the time before the crop is emptied. For example, Groebbels (1930) found that

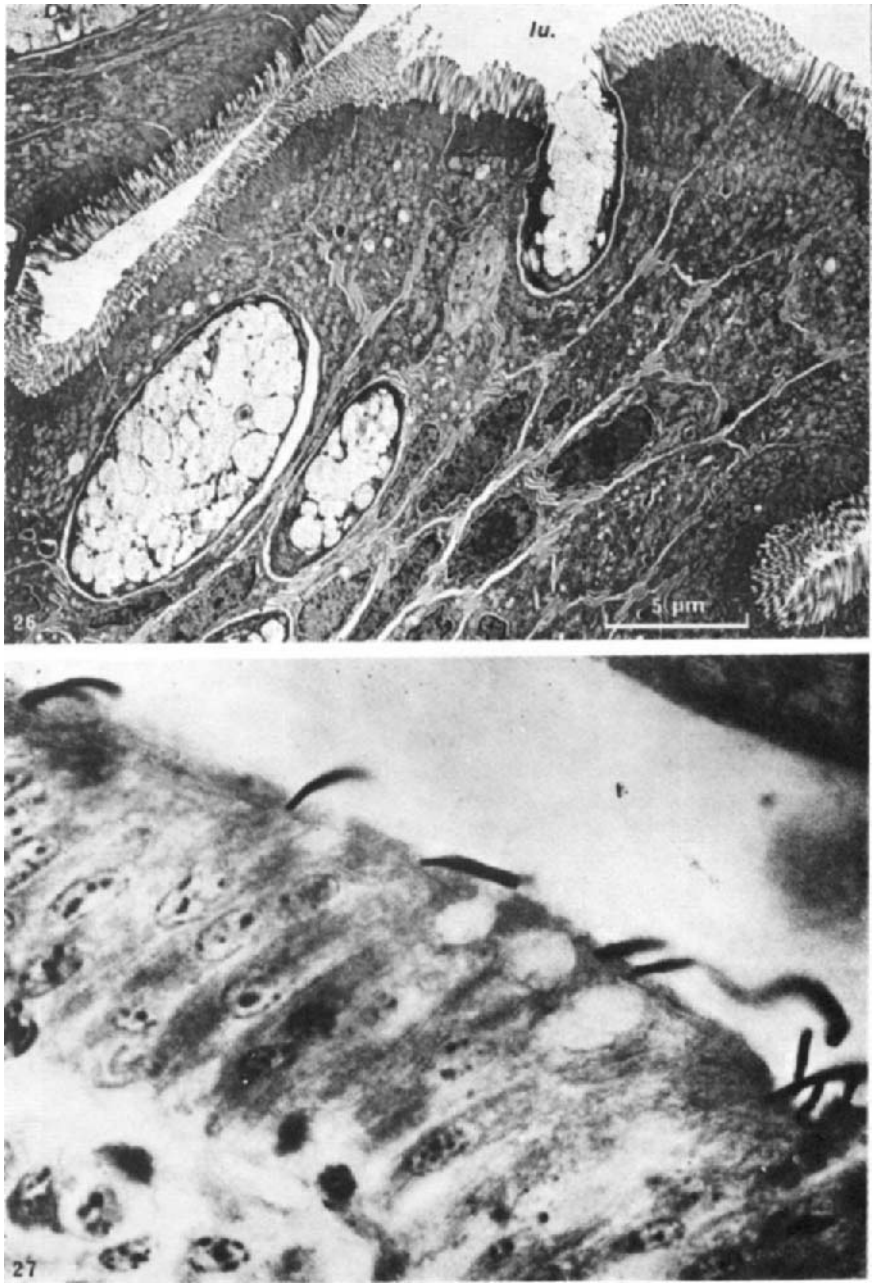


FIG. 26. Electron micrograph of the epithelial surface of the large intestine of a peafowl (*Pavo cristatus*). *lu.*, lumen of the alimentary tract.

FIG. 27. Photomicrograph of a Gram stained section through the ileum (posterior half of

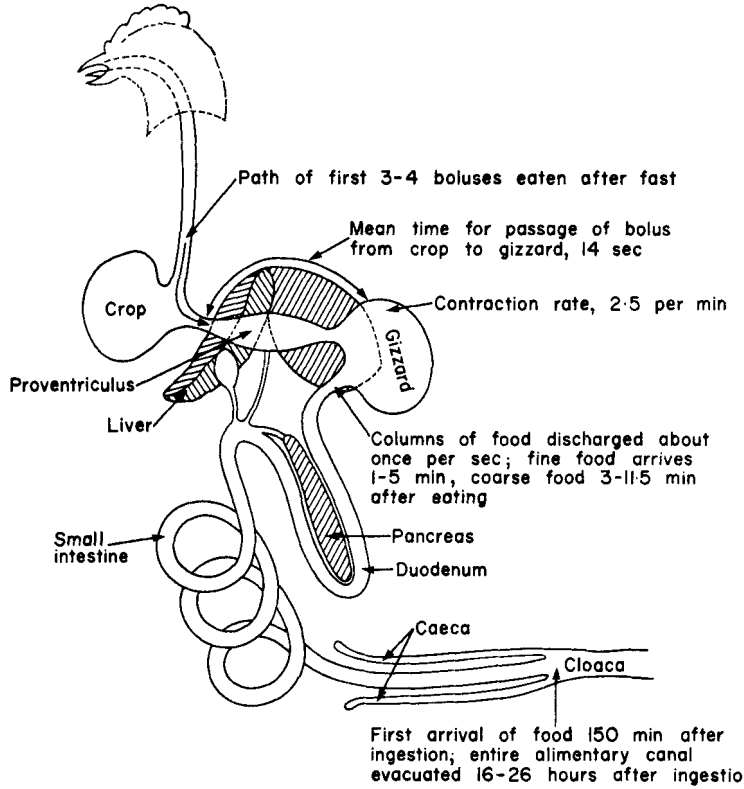


FIG. 28. Diagrammatic representation of the passage of food down the alimentary tract of the domestic fowl (Fig. 12, Henry *et al.*, 1933).

dry foods remained longer in the tracts of domestic geese than wet foods; Browne (1922) and Heuser (1945) made similar observations on fowls. Water appears to pass straight through the proventriculus and ventriculus of geese (Groebbels, 1930) and of domestic ducks (Crompton, 1966) on drinking.

Coarse and hard foods are retained in the ventriculus, which generates a mean pressure of 178 mmHg and 257 mmHg during contraction in ducks and geese respectively (Kato, 1914). There are usually about four contractions per minute in ducks and geese (Kato, 1914) and about two and a half in fowls (Henry *et al.*, 1933), but the frequency of contractions is related to the hardness of the food (Groebbels, 1930). In the ventriculus of turkeys, mechanical activity capable of cracking pecan nuts within 1 h and hickory nuts within 30–32 h has been described by Schorger (1960). The fact that the ventriculus is firmly held in position in the bird's body results in a rotary

small intestine) of a young domestic fowl to show bacteria attached terminally to columnar epithelial cells (after Plate 2, Fuller and Turvey, 1971).

TABLE IX

Observations on the passage of ingesta down the alimentary tract of domestic birds

Age	Diet	Marker	Observations	References
Duck				
Adult	Commercial feed	Coloured string	c. $3\frac{3}{4}$ h from mouth to faeces	Crompton (1964)
36 days	Commercial feed	Carbon particles	Total emptying time, 3 h	Macy *et al.* (1968)
7–12 days	Commercial feed	Cystacanths of *Polymorphus minutus*	c. 20–30 min from mouth to yolk stalk	Lingard and Crompton (1972)
Goose				
31 days	Commercial feed	Carbon particles	Total emptying time, 2·25 h	Macy *et al.* (1968)
Adults	Grass	—	Through-put time, 1 h 11 min	Mattocks (1971a, b)
	Meal	—	Through-put time, 44 min	
	Grass to meal	—	Through-put time from change of diet, 1 h 59 min	
	Meal to grass	—	Through-put time from change of diet, 2 h 37 min	
	Grass or meal	—	Caecal discharge early each morning; defecation (intestinal contents) every 24 min on average	
Fowl				
Adults	Oats	Methylene blue	Entire meal of oats (crop full) passes through in 27–28 h	Browne (1922)
Growing	Wheat middlings and corn meal	Lamp black	3 h 52 min from mouth to faeces	Kaupp and Ivey (1923)
Laying			3 h 46 min from mouth to faeces	
Not laying	after 24 h fasting		8 h from mouth to faeces	
Broody			11 h 44 min from mouth to faeces	
Adults	Commercial feed after 24 h fasting	Barium sulphate	First material arrives in cloaca 150 min after ingestion; entire alimentary tract evacuated 16–26 h after ingestion	Henry *et al.* (1933)
	Commercial diet after 18–20 h fasting	Dyes	Caeca cleared of dyes 120 h after cessation of feeding marked diet	Olson and Mann (1935)

	Commercial feed	*Lycopodium* spores	95% of spores evacuated from caeca in the 2 days after cessation of feeding marked diet	Harwood (1937)
	Variety of cereals	—	30–40% of feed still in crop after 12 h; crop practically empty after 24 h	Heuser (1945)
Pullets	Variety of diets	—	Caecal pouches usually evacuated after sunrise and shortly before sunset	Johansson *et al.* (1948)
Layers	Commercial feed	Ferric oxide or charcoal	3 h 42 min	Hillerman *et al.* (1953)
Non-layers			3 h 50 min	
Pullets			3 h 12 min	
Cocks			3 h 20 min	
			average food-passage time	
Young birds	Commercial diet	Charcoal and *Lycopodium* spores	2 to 3 days for disappearance of marker from caecal contents: observations on rhythm of caecal evacuation	Ikeda (1957)
Pullets	Commercial feed	Ferric oxide	Average passage time, 1 h 34 min in presence of other birds and 2 h 18 min when isolated	Tuckey *et al.* (1958)
21 days	Commercial feed	Carbon particles	Total emptying time, 1·15 h	Macy *et al.* (1968)
Broilers	Commercial feed	Dyes	Average passage times of 2 h 2 min to 4 h 21 min	Aylott *et al.* (1968)
Turkeys				
♀, 4–5 mon.	Commercial feed	Ferric oxide or charcoal	2 h 27 min	Hillerman *et al.* (1953)
♀, 1½–2½ yr			3 h 52 min	
Layers			3 h 13 min	
Non-layers			4 h 16 min	
			average passage time	
6 weeks	Commercial feed	$^{51}CrCl_3$	Minimum and maximum passage times, 1·2 h and 13·5 h; maximum time of caecal clearance, 36 h	Duke *et al.* (1969)
8–9 weeks			Minimum and maximum passage times, 1·5 h and 13·1 h; maximum time of caecal clearance, 60 h	

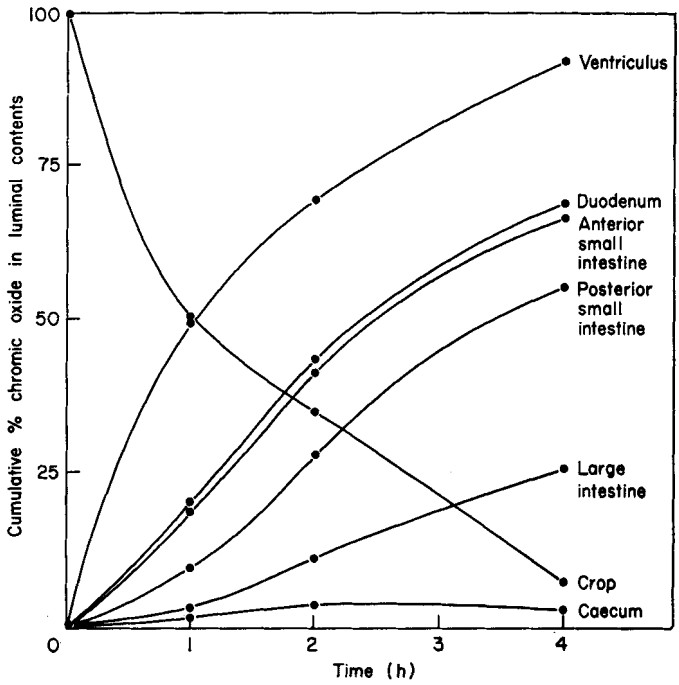


FIG. 29. Graph showing the rate of passage of chromic oxide down the alimentary tract of 6½- to 7-month-old fowls which were fasted for 15 h before being force-fed 13·5 g of experimental ration. Each point is the mean result from three birds. The curve for the crop is an output curve; the remainder are input curves (Bensadoun and Ichhponani, pers. comm.).

movement of the luminal contents when the contractions occur (Browne, 1922); the rotary activity will increase the efficiency of the contact between food and the abrasive material in the ventriculus (Fig. 21; Table VIII). Browne (1922) discussed the interesting point that domestic fowl appear to be capable of retaining grit and sand in the ventriculus not only when triturated food is passed into the duodenum, but also when they are deprived of a source of replacement. There are records of sand remaining in the ventriculus seven months after it had been withheld from the diet and of as much as 6 g of sand being passed daily in the faeces of fowls with an unlimited supply.

It is also apparent that the rate of passage may vary with the physiological condition and age of the bird (Table IX) and with its psychosomatic state (Browne, 1922; Groebbels, 1930; Tuckey *et al.*, 1958). The average rate of passage of food in chicks in brooders to which they were acclimatized was found to be 1 h 34 min, and in similar chicks, which had been disturbed and isolated, the average time was found to be 2 h 18 min (Tuckey *et al.*, 1958). Nearly all the experimental determinations of passage time have involved either radiography or the inclusion of marker substances like ferric oxide,

dyes, carbon particles or *Lycopodium* spores (Harwood, 1937; Ikeda, 1957) and it is recognized that some markers can interfere with the rate of passage. For example, Kaupp and Ivey (1923) found that methylene blue induced constipation in domestic fowls. The handling and immobilizing of birds during radiography may retard the rate of passage and these technical difficulties should not be ignored when attempts are made to apply data of the type shown in Table IX either to parasitic infections or to established infections in birds in the laboratory. Irrespective, however, of whether the published rates have natural physiological significance or not, sufficient information has been presented to illustrate the complexity of this factor in the environments of organisms in the alimentary tract of domestic birds.

(b) *Secretions and physico-chemical conditions occurring in the alimentary tract.* During its passage from the mouth to the ileo-caecocolic junction in domestic birds, the food is subjected, at a temperature of about 41 to 42°C (Sturkie, 1965), to the effect of a variety of secretions including mucus, salivary enzymes, gastric juice, bile, pancreatic juice, which consists of several enzymes and bicarbonate, and enzymes originating in the mucosa of the small intestine. The stimulation of the secretions, their properties and the optimum condition for their activity in the domestic fowl are described by Hill (1971b).

The time for which the food and the organisms living in the lumen of the tract are exposed to the activity of the secretions is directly related to the motility of the tract and the many factors that affect motility including the nature of the food. Thus, the infective stages of parasites that become activated on exposure to a particular set of conditions resulting from digestion may be over- or underexposed depending upon the motility (see later, p 158). The combination of the composition of the food, the digestive secretions and the motility contribute to the physico-chemical conditions in the tract and the time for which they are present in a given region. Measurements of the

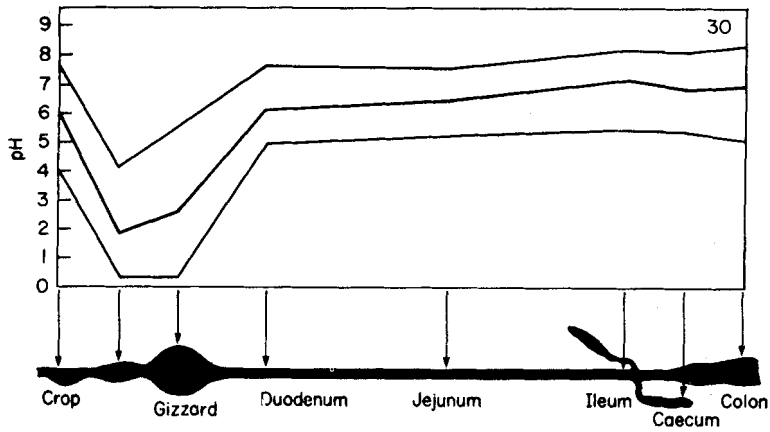


FIG. 30. Mean pH, with maximum and minimum deviation, of the contents of the different regions of the digestive tract of the fowl (Fig. 8, Hill, 1971b, after Herpol and Van Grembergen, 1967).

TABLE X

Measurements of pH in lumen of alimentary tract of domestic birds allowed to feed ad libitum *on standard diets*

Age	pH							References
	Oesophagus	Crop	Provent.	Ventric.	Small int.[d]	Caecum	Large int.	
Duck								
37 days[a]	—	—	2·50	5·52	6·84–7·44	8·19	8·41	Macy *et al.* (1968)
Adult[b]	4·92	—	3·41	2·33	6·01–6·87	5·88	6·73	Farner (1942)
Adult[b]	5·0	—	—	4·5	6·1–8·0	—	—	Smith (1965a)
Goose								
31 days[a]	—	—	1·75	2·30	6·37–7·21	7·83	8·11	Macy *et al.* (1968)
Fowl								
24 h[a, c]	—	—	2·48	3·50	6·69–7·27	6·97	7·68	Macy *et al.* (1968)
21 days[a]	—	—	2·86	2·40	6·25–6·50	6·73	6·80	Macy *et al.* (1968)
Adult[b]	—	4·51	4·40	2·60	5·76–6·42	5·71	6·26	Farner (1942)
Adult[b]	—	5·37	4·69	4·06	6·31–7·59	7·10	7·31	Olson and Mann (1935)
Adult[b]	—	4·9	—	4·2	5·8–7·8	7·0	—	Smith (1965a)
Various[b]	—	4·1–7·8	0·3–3·0	0·4–3·8	5·6–8·5	5·6–8·1	5·3–8·4	Herpol and Van Grembergen (1961)
Turkey								
Adult[b]	—	6·07	4·72	2·19	5·82–6·95	5·86	6·46	Farner (1942)
Pigeon								
Squabs[b] and adults	—	4·28	4·80	2·00	5·23–5·89	—	5·43	Farner (1942)

[a] Measured under anaesthesia *in vivo*.
[b] Measured in samples from freshly-killed birds.
[c] Allowed no food after hatching.
[d] From pylorus to ileo-caecocolic junction; pH value lower in anterior part of small intestine than in posterior part.

hydrogen ion concentration in the tract of domestic birds are depicted in Fig. 30, summarized in Table X and described in detail by Herpol (1966), and information about the enzymes and bile salts are given in Table VII and XI respectively.

The organisms which live in the tissues of the wall of the alimentary tract will not be exposed to the direct action of motility, and their environmental conditions will be relatively stable and mainly dependent on the host's vascular and lymphatic systems instead of on the reactions occurring in the lumen of the tract. Conditions in certain parts of the lumen are bound to fluctuate and the temporary changes which may be expected to occur in the osmotic pressure of the intestinal contents of domestic ducks when the birds drink illustrate this point (Crompton, 1966; Crompton and Edmonds, 1969).

Some small organisms, however, inhabit the region of the lumen of the small intestine extending from between the villi and folds of the mucosa to a distance of about 1 mm from the mucosal surface. This region has been called the paramucosal lumen (Read, 1950) and the physico-chemical conditions there originate from substances which have diffused from the capillary bed of the mucosa and the components of the lumen. The pulsations of the villi (Bell *et al.*, 1968) may be expected to cause mixing; intestinal motility probably has little direct effect on organism living in the paramucosal lumen because the ingesta in this region will move with a low velocity (Crompton, 1973). Evidence that conditions in the paramucosal lumen differ from those in the remainder of the lumen was obtained by Crompton *et al.* (1965), who used an oxygen electrode to record oxygen tensions varying from 25 mmHg close to the villi to less than 0·5 mmHg in the centre of the lumen of the small intestine of the domestic duck. There has been much debate about whether metazoan parasites of the alimentary tract utilize oxygen during energy metabolism (see von Brand, 1973). If the measurements of oxygen tension made in the duck are reliable, it is apparent that adequate oxygen is available for many types of parasites. There are, however, some regions of the tract of domestic birds for which a complete absence of oxygen must be predicted, since obligate anaerobic micro-organisms can only be isolated provided that rigid precautions are taken to exclude oxygen at every stage of the procedure (Barnes and Impey, 1970). The presence or absence of oxygen also affects the oxidation–reduction potential of the luminal contents. Some recent measurements of oxidation–reduction potential in the tracts of domestic ducks, geese and fowls have been obtained by Macy *et al.* (1968).

2. *Intestinal caeca*

Many birds, including four of the species under consideration in this review, possess a pair of intestinal caeca (Figs 2, 3, 5 and 9) which are important in the ecology of many organisms (Tables XIII–XVI). The problem of attempting to study the relationship between the organisms living in the caeca and the physiology of their hosts is particularly difficult because the precise functions of the caeca are still incompletely understood (McNab, 1973). Under experimental conditions, caecectomy has been carried out on fowls (Mayhew, 1934; Couch *et al.*, 1950; Thornburn and Willcox, 1965; Nesheim

TABLE XI

Observations on the bile and bile acids of domestic birds

	Bile acids isolated	Notes	References
Duck	Cholic, chenodeoxycholic, lithocholic and unidentified acids	Gall-bladder bile contained 58·9% chenodeoxycholic, 16·6% lithocholic, 16·3% cholic and 8·2% of other bile acids. Bile acids in the chyme varied from 1·7–5·18 mg/g.	Crompton and Nesheim (1970)
Goose	Chenodeoxycholic acid		see Anderson *et al* (1957)
Fowl	(a) Cholic, chenodeoxycholic, isolithocholic, 3α-hydroxy-7-oxocholanic, 3-oxochola-4 : 6-dienic and tetrahydroxynorsterocholanic acids		see Anderson *et al.* (1957)
	(b) Cholic, chenodeoxycholic and lithocholic acids	In gall-bladder bile of male chicks chenodeoxycholic found in amounts 2–3 times that of cholic acid. Lithocholic represents 5–10% of bile acids found	Serafin and Nesheim (1970)
	(c)	Gall-bladder bile contained 126 mmol/l of bile acids. Adult males secreted 193 ml bile/24 h containing 7800 mg bile acids	Hurwitz *et al.* (1973)
Turkey	Cholic, chenodeoxycholic acid		Haslewood (1967)

and Carpenter, 1967) and turkeys (Durant, 1930) with apparently no deleterious effects over a long period.

In addition to the probable involvement of the caeca in cellulose degradation (Section II), the caeca have also been implicated in vitamin synthesis, the digestion of nitrogenous substances, the development of immune responses and water absorption (Mattocks, 1971a,b; McNab, 1973). The role of the caeca in water absorption is supported indirectly by the observation that the faeces of caecectomized fowls are usually wetter than those of control birds (Thornburn and Willcox, 1965), and by the results of the radiographic study of urine flow in the fowl undertaken by Akester *et al.* (1967). When radio-opaque substances were injected into the vascular system, they were observed to collect in the ureters and eventually to reach, by means of retroperistalsis, the colon and the lumen of each caecum, but never the ileum. These observations provide a physical basis for the post-renal absorption of urinary water by the caecal and colonic tissues of the fowl.

The observations of Akester *et al.* (1967) are also of interest because they suggest an explanation of how the caeca become filled apart from the sucking mechanism (Browne, 1922) assumed to operate like the action of the teat on a pipette. If localized contraction occurs in the intestinal wall just anterior to the ileo-caecocolic junction, subsequent retroperistaltic contractions would carry urine and the contents of the coprodeum and large intestine into the caeca provided that the caeca were empty or incompletely filled and that their entrances were open. This type of process, which must require complex innervation, may be important in the entry of organisms into the caeca and in the replenishment of their environment. Investigators of avian digestion will be aware that the caecal contents may differ in texture, colour and odour from those of the rest of the tract (Browne, 1922; Mattocks, 1971a). Similarly, the caecal contents appear to be discharged with little contamination from the remainder of the tract since faecal masses composed of caecal contents alone are found (Table IX).

The fact that the texture of caecal contents is different from that of the intestine may be connected in part with its relatively long exposure to myriads of resident micro-organisms (Table XIV). The length of exposure may be assumed because the caeca do not become emptied as frequently as the rest of the small and large intestines (Table IX). The discharge of the caecal contents, which is probably accomplished by muscular contractions in the caecal wall (Ikeda, 1957), must present a serious hazard to the organisms living in the caecal lumen. The difference in texture may also result from the observation that some type of filtration occurs during the entry of material into a caecum. Browne (1922) described experiments in which sand grains of various sizes and coloured particles included in the diet of fowls failed to enter the caeca.

D. THE PLASTICITY OF THE AVIAN ALIMENTARY TRACT

Various observations indicate that the avian alimentary tract changes not only during growth and development, but also in response to dietary and other

stimuli. This plasticity may explain how the mechanical damage expected to result from the presence of large numbers of parasites is reduced and tolerated in certain parts of the tract. For example, an estimated 20 000 individuals of the cestode *Hymenolepis carioca* were retrieved from a domestic fowl by Adams and Geiser (1933) and 3261 individuals of the nematode *Heterakis gallinarum* from the caeca of another fowl by Owen (1951). Many digenetic trematodes appear to have become modified for mucosal feeding (see Crompton, 1973) and the rapid turnover time of the epithelial cells will compensate for their activity and repair the damage which must result from the presence of large populations of parasites.

The morphology of the tract appears to respond to the composition and quantity of the diet. When red grouse, *Lagopus scoticus*, which feed chiefly on heather, are bred and kept in captivity on a diet of pellets of much higher nutritional value than heather, the length of the small intestine changes from 99 ± 1 cm, in male wild birds, to 72 ± 3 cm, in male captives, and the combined length of the caeca (Fig. 13) changes from 144 ± 5 cm to 78 ± 3 cm (Moss, 1972). Related observations have been made with young pheasants, *Phasianus colchicus*, in which the ventriculus increases in size when fibre is included in the diet (Scott *et al.*, 1954). Lepkovsky and Furuta (1971) forcibly fed excessive amounts of food to domestic fowls and noted that the size of the crop and the length of the small intestine increased. When the routine of forced feeding was stopped the abnormalities were quickly rectified. Mattocks (1971a,b) discussed the force feeding of domestic geese which may swallow up to 1·5 kg of food per day. Presumably the tract increases in size to cope with this quantity of food.

The enzymic secretions also may respond to the composition of the diet. For example, significantly more chymotrypsin activity was detected in the duodenal contents of domestic geese fed a diet containing 28% protein than in geese fed a diet containing 16% protein (Nitsan *et al.*, 1973).

The alimentary tract of domestic birds is very tolerant of surgical cannulation (Crompton *et al.*, 1965; Crompton and Edmonds, 1969; Crompton and Nesheim, 1972), caecectomy (Durant, 1930; Nesheim and Carpenter, 1967) and many other operations. Even the ventriculus may be removed with survival of the bird (Ikeda, 1956a).

IV. The Alimentary Tract of Germ-free Domestic Birds

The influence of the microflora of the alimentary tract on the morphology, histology and physiology of the host is substantial and an important factor in the interplay of events that determine the nature of the habitat for other parasites. The contribution of micro-organisms to the conditions found in the tract can be studied by comparisons made between domestic birds with a conventional microflora and those raised under conditions which exclude microbial contamination. These germ-free birds represent a highly artificial state, but they are very useful for the study of the interaction between micro-organisms and host morphology and physiology. Several papers have reviewed this subject in detail, and should be consulted for more extensive information

than can be presented here (Coates and Jayne-Williams, 1966; Jayne-Williams and Fuller, 1971; Coates; 1973; Eyssen, 1973).

A. MORPHOLOGY, HISTOLOGY AND PHYSIOLOGY

Some differences between the tract of conventional and germ-free fowls are summarized in Table XII. The small intestine of germ-free birds generally weighs less, is slightly shorter and contains less tissue per unit length than the small intestine of conventional birds (Coates and Jayne-Williams, 1966). The intestine of conventional birds inhabited by micro-organisms had a greater cross-sectional area occupied by lamina propria than that of germ-free birds (Gordon and Bruckner-Kardoss, 1961). The amount of lamina propria which made up the core of the villus was particularly enhanced in the presence of the microflora. Intestinal tissue from conventional birds also contained greater numbers of lymphocytes, plasma cells and Schollen leukocytes (globule leucocytes) compared to tissue from germ-free birds. Similar observations have been made in the caeca by Visco and Burns (1972), who reported that caecal mucosa and submucosa were less densely cellular and contained fewer centres of lymphocyte accumulation in germ-free than in conventional fowls. The caecal epithelium was more columnar in appearance in germ-free fowls than in those with an established microflora. These observations on the small intestine and caeca from germ-free fowls are also representative of findings for germ-free turkeys (Doll and Franker, 1963).

Digestive enzymes of fowls seem to be affected relatively little by the presence of the microflora in the alimentary tract. After measuring the activity of pancreatic enzymes in contents collected from different regions of the post-pyloric tract of germ-free and conventional chicks, Lepkovsky *et al.* (1964) concluded that the distribution of enzyme activity was largely unaffected by the microflora. Similar results have also been obtained from fowls by Coates *et al.* (1970) and Siddons and Coates (1972). In the latter studies, intestinal disaccharidase activity was the same in conventional and germ-free birds except for lactase, which is apparently only of microbial origin. Lactase activity was detected in the caeca and the large intestine of conventional fowls, but not in the small intestine.

Micro-organisms are also responsible for considerable transformation of the chemical conditions in the alimentary tract. Fowls are capable of secreting primarily chenodeoxycholic acid, cholic acid and allocholic acid in bile conjugated mainly with taurine (Table XII) when no bacteria are present (Haslewood, 1967). In conventional fowls, other bile acids, especially lithocholic acid, are produced from the primary bile acids by the action of the microflora (Table XI). Microflora also possess enzymes that are able to deconjugate bile acids to release free bile acids in the intestinal contents (Eyssen, 1973). Reabsorption of bile acids is reduced and considerably more bile acids are excreted in faeces in conventional than in germ-free birds. Volatile fatty acids are found throughout the alimentary tract of fowls (Annison *et al.*, 1968), but in germ-free tracts, these compounds are present at very low levels.

TABLE XII

Features of the alimentary tracts of germ-free (GF) and conventional (C) fowls

		GF	C	References
Dimensions	body weight (g)	265	255	Coates and Jayne-Williams (1966)
	small intestine: cm	84·8	89·2	
	small intestine: g	8·74	10·96	
	small intestine: g/cm	0·103	0·123	
Distribution of tissue in small intestine as % of total cross-sectional area	villi	26·1	23·4	Gordon and Bruckner-Kardoss (1961)
	crypts	4·2	5·0	
	lamina propria	13·6	18·5	
	muscularis mucosa	5·4	5·6	
	muscle	48·2	45·5	
	serosa	2·4	1·9	
Total lymphocytes, plasma cells and Schollen leukocytes, per mm^2 tissue in 4 μm sections of small intestine		930	2860	Gordon and Bruckner-Kardoss (1961)
Relative rate of cellular migration from base to tip of villus in small intestine		1	1·7	Cook and Bird (1973)
pH	duodenal contents	6·11	6·39	Springer *et al* (1970)
	caecal contents	6·90	6·19	
Redox potential (mV)	duodenal contents	+122	+ 95	
	caecal contents	+112	−218	
Bile acids		Primary bile acids only (cholic, chenodeoxycholic and allocholic acids)	Primary bile acids and secondary bile acids particularly lithocholic acid	Haslewood (1964); Serafin and Nesheim (1970)

The caeca of conventional fowls at 5 weeks of age were found to contain 70 mmol of volatile fatty acids per kg of caecal contents, whereas only 3·5 mmol/kg of caecal contents were found in germ-free fowls (Coates and Jayne-Williams, 1966). The presence of these volatile fatty acids may be responsible for the lower pH of caecal contents reported for conventional, compared to germ-free fowls (Springer *et al.*, 1970).

In conventional fowls the caeca have been observed to be larger and their contents to include a higher percentage of dry matter compared to caeca from germ-free birds (Reyniers *et al.*, 1960). Some of this dry matter is probably protein since Lepkovsky *et al.* (1964) found large amounts of protein in caecal contents from conventional fowls while almost none was present in the contents of caeca from germ-free birds. The oxidation-reduction potential in caecal contents from conventional fowls was strongly negative according to measurements made by Springer *et al.* (1970), while the potential from caecal contents obtained from germ-free chicks was positive. Visco and Burns (1972) noted that the caecal contents of germ-free fowls are greener than those from conventional birds, probably because of an accumulation of biliverdin which is not further transformed by the microflora (Huhtanen and Pensack, 1965b).

Nitrogenous compounds arising from the diet and from secretions of the alimentary tract are altered by the microflora. Amounts of ammonia in the portal blood of germ-free guinea pigs are considerably lower than those from conventional guinea pigs (Warren and Newton, 1959). Much of this ammonia probably comes from the hydrolysis of urea in the tract by the action of microbial urease (Levenson *et al.*, 1959). Visek (1972) has concluded that micro-organisms acting on nitrogenous substrates are the major source of ammonia outside of tissues themselves. Ammonia levels in intestinal contents of conventional fowls have been shown to be very variable (Harbers *et al.*, 1963; Alvares *et al.*, 1964), but have been reported to be above 1 mm in all segments of the tract and up to 6–10 mm in the caeca (Prior *et al.*, 1974). Comparable measurements were not made in germ-free chicks. One source of ammonia in conventional birds might be uric acid which has entered the caeca (Akester *et al.*, 1967) and may then be degraded by micro-organisms (Barnes, 1972).

Salter and Coates (1971) found that 8·2% of the nitrogen in the excreta of conventional chicks was ammonia compared to 3·6% in the excreta of germ-free fowls. The presence of elevated ammonia levels and deconjugated bile acids in the alimentary tract of conventional animals has been considered by Visek (1972) as being a major factor in the increased turnover rate of their mucosal cells compared with those of germ-free animals. If the concentrations of ammonia in the lumen of the tract have the marked effects suggested by Visek (1972), it should be realized that parasites living in the intestinal lumen might also be affected by similar factors. Similarly, it is possible that pharmacologically active amines, capable of affecting intestinal motility, might be produced in the intestine through the action of micro-organisms, although food transit time in germ-free fowls was observed to be the same as that in conventional birds (Ford, 1971).

B. NUTRITION

The nutrient requirements of the host are extensive, with many compounds being essential elements of the diet (Tables II, III and IV). Many intestinal micro-organisms, however, are probably autotrophs with the ability to synthesize vitamins and amino acids that are not made by the tissues of the host. Potentially, the microflora could provide many nutrients for the host because of its greater synthetic ability. Such a mutually beneficial relationship is well established in ruminants and other animals that have significant pre-gastric fermentation capability (Hungate, 1966).

In domestic fowls, micro-organisms are not essential for the growth and reproduction of the host, provided that the diet fed to the germ-free fowl contains the known nutritional essentials (Reyniers *et al.*, 1960). A general finding by many investigators has been that germ-free fowls grow more rapidly in early life than their conventional counterparts (Coates *et al.*, 1963), and there is an extensive literature describing the stimulation of growth rate of fowls and turkeys by feeding them diets containing antibacterial agents (Jukes, 1955).

The contributions of the microflora to nutrition of the host may be important, however, if diets containing marginally adequate amounts of some vitamins are fed. Coates *et al.* (1968) fed conventional and germ-free fowls diets devoid of individual vitamins and assayed the caecal contents of the birds for the omitted vitamin. Significantly more riboflavin, vitamin B_6, pantothenic acid, vitamin B_{12}, folic acid, nicotinic acid, thiamin and biotin were found in the caeca of conventional birds compared to germ-free birds where, in most instances, vitamins in the caeca were barely detectable. There was little evidence that appreciable quantities of the vitamins synthesized in the birds with a conventional flora were absorbed because the body weights of the young fowls fed the deficient diets differed little from those of the germ-free birds. Measurements of vitamin storage in the liver also revealed that the amounts stored were not appreciably different between conventional and germ-free birds. Vitamin synthesis in the lower tract by micro-organisms may be of considerable significance to foraging birds which may also practice coprophagy. Presumably, parasites located in the caeca and lower intestine could also be assured of a vitamin supply if the host's diet were deficient.

While we have not found any direct evidence that the activity of micro-organisms is of significance in the nutrition of eukaryotic and metazoan parasites in the tract, it has been established that entire microbial cells are regularly ingested by parasites (Section V).

C. MICRO-ORGANISMS AND OTHER INHABITANTS OF THE TRACT

Experimental evidence suggests that in certain cases the presence of the microflora is associated in some unexplained manner with the development of relationships between domestic birds and habitual parasites of the alimentary tract (Wescott, 1970). When germ-free and conventional fowls were

fed sporulated oocysts of *Eimeria tenella* (Table XV), the parasite became established and completed its life cycle in both types of host (Clark *et al.*, 1962), but the disease appeared to be less severe in the germ-free than in the conventional hosts (Visco and Burns, 1972). Of 229 conventional birds, 139 died of coccidiosis whereas none out of 89 germ-free birds was lost. Increasing the number of oocysts given to the germ-free hosts to 125 000 resulted in haemorrhage, although none of the 12 experimental birds died compared with 23 out of 30 conventional birds which had ingested the same number of oocysts. This observation suggests that the caecal microflora may have an enhancing rather than an essential effect on the development of the disease. Clark *et al.* (1962) and Visco and Burns (1972) observed that the early development of *Eimeria tenella* was delayed in germ-free fowls and, since oocyst production was similar in both types of host, they concluded that caecal micro-organisms favoured the development of the parasite. The weight of infected germ-free hosts was not depressed as much as that of conventional fowls (Visco and Burns, 1972). In a similar investigation involving *E. brunetti* (Table XV) Hegde *et al.* (1969) found that the course of infection and the pathogenicity of the parasite were the same in germ-free and conventional fowls; the mortality rate of both types of host was not significantly different. At present, insufficient information is available to enable these observations on *Eimeria tenella* (Visco and Burns, 1972) and *E. brunetti* (Hegde *et al.*, 1969) to be explained or reconciled, but the finding that changes occur in the populations of caecal micro-organisms in young fowls infected with *E. tenella* (Johansson and Sarles, 1948) and *E. brunetti* (Hein and Timms, 1972) may be relevant.

Equally complex relationships appear to exist between micro-organisms in the tract, the nematode *Heterakis gallinarum* and the protozoan *Histomonas meleagridis* during the development of enterohepatitis in turkeys (Reid *et al.*, 1967; Reid, 1972). When 12 germ-free and 12 conventional turkeys were each fed 1000 eggs of *H. gallinarum* infected with *H. meleagridis*, one of the germ-free hosts showed signs of enterohepatitis whereas 11 of the conventional hosts died of the disease (Doll and Franker, 1963). Since no stages of *H. gallinarum* were retreived from the germ-free birds at autopsy, the possible failure of the hatching of the eggs of the nematode in the sterile small intestine may have accounted for the absence of the disease. Further work demonstrated that some degree of enterohepatitis developed in a few individuals from groups of gnotobiotic turkeys harbouring *Escherichia intermedia* or *Streptococcus faecalis* alone, but not in gnotobiotic turkeys harbouring *Bacillus cereus* or *Lactobacillus fermenti* (Franker and Doll, 1964). A more severe form of the disease developed in gnotobiotic turkeys infected with *E. intermedia* together with *B. cereus* or *L. fermenti;* correspondingly more nematode larvae were recovered in these cases than in the hosts harbouring one bacterial species. Generally similar observations were made by Bradley and Reid (1966) using gnotobiotic turkeys infected with eggs of *Heterakis gallinarum* and *E. coli* and *Clostridium perfringens* or *B. subtilis*. Evidence for the disease was not obtained from germ-free turkeys which ingested surface-sterilized eggs of *H. gallinarum* alone, or eggs and either heat-killed *E. coli* or filtered

nutrient medium in which the bacterium had been grown. Observations by Springer *et al.* (1970) indicated that different species of micro-organisms are involved in the development of enterohepatitis in fowls compared with those in turkeys. They also found that *Eimeria adenoeides* (Table XV) survived in the caeca of germ-free turkeys which ingested oocysts of the coccidium and eggs of *H. gallinarum* concurrently; no nematodes were recovered from these hosts. Some survival of *Histomonas meleagridis* occurred, however, but there were no signs of enterohepatitis.

The attempts to infect germ-free birds with *H. meleagridis* by feeding eggs of *Heterakis* have established that the course of infection with the nematode *H. gallinarum* is also dependent on the microflora of the alimentary tract. Johnson and Reid (1973) fed varying numbers of surface-sterilized eggs of *Ascaridia galli* (Table XVI) to germ-free, gnotobiotic and conventional fowls. The results showed that the eggs of the nematode hatched in the small intestine irrespective of the type of host, but significantly fewer worms were recovered from germ-free as compared with conventional hosts. In one experiment, 81 worms were retreived from 29 germ-free fowls and 218 were retreived from 20 conventional birds. The number of worms recovered from gnotobiotic hosts harbouring *Bacillus cereus*, *B. subtilis* or *Penicillium* sp. were usually more than from germ-free, but less than from conventional hosts. The mean lengths of *Ascaridia galli* from the different hosts were not significantly different. Experiments involving the cestode *Raillietina cesticillus* indicated that the presence or abscence of the microflora of the tract of the fowl had no detectable effect on the establishment of infection and growth of the parasite (Reid and Botero, 1967). *Raillietina cesticillus*, however, normally occupies a site in which microbial activity may be minimal in conventional fowls (Tables XIV and XVI).

V. The Observed Distribution of Parasites in the Alimentary Tract of Domestic Birds

Observations on the distribution of a selection of parasites in the alimentary tract of domestic birds are presented in Tables XIII, XIV, XV and XVI. The information has been organized in a similar manner to that of Crompton (1973) whose teminology for site, emigration and migration has also been adopted in this review. The protozoan parasites have been classified according to Levine (1973) and the helminths according to Yamaguti (1958, 1959, 1961 and 1963). It is considered that while describing the sites occupied by parasites in the tract provides a record of their distribution within the host, distribution depends on the effects of all facets of the biology of each parasite and its interaction with its environment. That idea has been developed in detail for free living animals by Elton (1927) and Andrewartha and Birch (1954).

In a discussion of aspects of the ecology of adult helminths in the alimentary tract of vertebrates, Crompton (1973) proposed that ideally, in addition to details of the host's age and sex, the description of a site should include information about (1) the parasite's linear distribution, (2) the parasite's radial

distribution, (3) the length of time which passed between the death of the host and finding the parasites, (4) the time of day when the search was made, (5) the stage of digestion in progress on the death of the host, (6) the season of the year when the parasites were observed, (7) the parasite's reproductive state and (8) details of the parasite burden and other parasites present in the alimentary tract of the host. It was implied that this range of information was necessary before a description of an adult helminth's site in its host could be accepted, and supporting evidence was presented (Crompton, 1973).

The same elements are equally desirable when studying the distribution of all parasites, including micro-organisms and protozoa in the tracts of domestic birds for which details of the diet and feeding routine should also be available. For example, the numbers of different micro-organisms present in the tract of 2-week-old chicks varies according to the level of fish meal in the diet (Barnes *et al.*, 1972; Table XIV). The site of *Entamoeba gallinarum* in the caeca of fowls and turkeys changes soon after the death of the host (Tyzzer, 1920) and the location and identity of the protozoan *Cochlosoma* sp. from turkeys cannot be determined unless the hosts are still warm during *post mortem* examination (Campbell, 1945). The presence of *Eimeria necatrix* results in a significant decrease in the rate of passage of food along the tract of fowls (Aylott *et al.*, 1968; Fig. 38); any interference in intestinal motility may be expected to affect the activities of other parasites. The site of the cestode *Raillietina georgiensis* varies according to its age (Reid and Nugara, 1961). Young ducks are more susceptible to infection with the cestode *Schistocephalus solidus* than older hosts (McCaig and Hopkins, 1963); one consequence of an increase in population density for parasites in the tract is an extension of site (Crompton, 1973). Many parasites show marked interspecific reactions (Crompton, 1973) and various effects have been demonstrated between the nematodes *Ascaridia galli* and *Heterakis gallinarum* in the fowl (Madsen, 1962a). This brief discussion, and many of the other references cited in Tables XIII, XIV, XV and XVI, illustrate the need for the inclusion of much more than the spatial position of a parasite in the description of its site.

A. THE DISTRIBUTION OF MICRO-ORGANISMS

1. *General observations*

Some observations of the distribution of certain micro-organisms in domestic ducks, geese and fowls are listed in Tables XIII and XIV. The numbers of micro-organisms in a particular location have been expressed to the nearest order of magnitude per g wet weight of intestinal contents; the reader must consult the original references for accurate information and for details of the techniques and culture media used, since these affect both the numbers and types of organism detected (Ochi and Mitsuoka, 1958; Huhtanen and Pensack, 1965a; Jayne-Williams and Coates, 1969; Barnes and Impey, 1971). Most workers appear to agree that, irrespective of the methods used, there are more micro-organisms in the posterior part of the tract and particularly in the caeca than elsewhere (Tables XIII and XIV). There may also

TABLE XIII

Observations on the distribution of certain micro-organisms in the alimentary tracts of the domestic duck[a] and the domestic goose[b]

	Estimations of the numbers of micro-organisms/g wet weight of contents											
	Oesophagus		Ventriculus		Ant. sm. intest.		Post. sm. intest.		Caecum		Large intest.	
	D	G	D	G	D	G	D	G	D	G	D	G
Coliforms	n.t.	10^5	n.t.	10^4	n.t.	10^3	n.t.	—	n.t.	n.t.	n.t.	—
Escherichia coli	10^2	n.t.	N	n.t.	10^2	n.t.	10^5	n.t.	10^7	n.t.	10^6	n.t.
Clostridium welchii	N	n.t.	N	n.t.	10	n.t.	10	n.t.	10^4	n.t.	10^4	n.t.
Clostridia	n.t.	10^5	n.t.	10^6	n.t.	10^5	n.t.	10^4	n.t.	10^8	n.t.	10^5
Streptococci and faecal streptococci	10^5	—	10^6	—	10^6	—	10^7	—	10^9	10^5–10^8	10^8	—
Pepto-streptococci	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	10^8–10^9	n.t.	n.t.
Lactobacilli	10^5	—	10^5	—	10^6	—	10^7	—	10^9	0–10^4	10^8	—
Bacteroides	N	n.t.	N	n.t.	N	n.t.	N	n.t.	10^9	10^5–10^7	10^8	n.t.
Yeasts	N	n.t.	N	n.t.	10^2	n.t.	10^3	n.t.	10^5	n.t.	10^4	n.t.

[a] Adult duck (Smith, 1965a).
[b] Adult goose (Mattocks, 1971a,b).
(n.t. = not tested; N = None found in 0·02 g of chyme).

develop large populations of micro-organisms, especially lactobacilli (Bolton, 1965; Fuller, 1973), in the crop of galliform birds (Table XIV; Figs 2–13). Two of the factors which seem to influence the distribution of micro-organisms are pH and intestinal motility (Smith, 1965a; Jayne-Williams and Coates, 1969; Jayne-Williams and Fuller, 1971). The slower the movement of material down the tract, the longer the time available for microbial populations to develop. Thus, the infrequent emptying of the caeca relative to the remainder of the tract (Table IX) will facilitate bacterial development. Similarly, under certain conditions material may remain for a comparatively long time in the crop (Table IX; Heuser, 1945) with the result that micro-organisms flourish and a form of digestion analogous to that in the ruminant stomach begins to occur (Bolton, 1965).

Although many studies have been made of the micro-organisms from the lumen of different parts of the tract of domestic birds, surprisingly little information is available about their radial distribution. Recently, Fuller and Turvey (1971) and Fuller (1973) have begun to describe the flora associated with the surface of the epithelium of the crop, the posterior half of the small intestine and the caeca of the fowl. Samples of tissue were fixed in Schaudinn's fluid before examination by light microscopy. Bacterial cells were observed to form an almost complete layer, 2–3 cells thick, attached to the surface of stratified squamous epithelium of the crop, and to the brush border of the epithelial cells covering the top third of the villi of the posterior half of the small intestine (Fig. 27), and to form a layer of about 200 cells thick over the surface of the caecal mucosa (Fuller and Turvey, 1971). The possibility that these attachments were fixation artifacts was dispelled not only by observation of the living adherent micro-organisms with the phase microscope, but also by a test of bacterial adhesion based on the culture of micro-organisms from macerated tissue which was vigorously washed several times before maceration. Incidentally, the micro-organisms adhering to the epithelial cells of the small intestine are living in a region of reduced motility since they are inhabiting the paramucosal lumen where the plug flow of ingesta would have little effect (Crompton, 1973).

A more detailed study of the adhesion of lactobacilli to the epithelium of the crop of the fowl was made by Fuller (1973). Few adherent micro-organisms were detected after the host had fasted for 16 h, but when food was available the bacteria flourished and adhesion occurred irrespective of the composition of the eight diets used or the age of the hosts. When epithelial cells from the crop were maintained *in vitro*, adhesion was demonstrated provided that the lactobacilli tested had been isolated from an avian host. Lactobacilli from ten species of mammal did not adhere to chicken cells *in vitro*, a result indicating some degree of specificity in this particular host–parasite relationship.

2. *Observations on the establishment of the microflora in the alimentary tract*

Micro-organisms may be introduced in the alimentary tract of domestic birds by way of the diet, water, faecal particles, airborne agents (Jayne-Williams and Coates, 1969) and numerous other environmental contacts.

TABLE XIV

Observations on the distribution of certain micro-organisms in the alimentary tract of the domestic fowl

Micro-organism and host details	Estimations of the numbers of micro-organisms/g wet weight of contents								References
				Small intestine				Large	
	Crop	Ventric.	Duod.	ant.	mid.	post.	Caec.	int.	
Lactobacilli									
2 weeks[a]	—	—	10^5–10^8	—	10^7–10^8	—	10^8–10^9	—	Barnes *et al.* (1972)
2 weeks[b]	—	—	10^5–10^8	—	10^6–10^8	—	10^8–10^9	—	Barnes *et al.* (1972)
12-week broilers[c]	—	—	10^6	—	—	—	10^6–10^8	—	Barnes and Shrimpton (1957)
pullets[d]	—	—	10^5	—	10^7	—	10^{10}	10^9	Johansson *et al.* (1948)
adult[e]	10^9	10^7	10^8	10^8	10^8	10^9	10^9	10^9	Smith (1965a)
6 h[e]	N	N	—	N	—	N	N	—	Smith (1965b)
24 h[e]	N	N	—	N	—	N	N	—	Smith (1965b)
6 days[e]	10^5	10^4	—	10^4	—	10^4	10^8	—	Smith (1965b)
24 days[e]	10^8	10^5	—	10^7	—	10^6	10^8	—	Smith (1965b)
Streptococci and faecal streptococci									
2 weeks[a]	—	—	$< 10^3$	—	$< 10^3$–10^5	—	10^6–10^8	—	Barnes *et al.* (1972)
2 weeks[b]	—	—	$< 10^3$–10^5	—	10^5	—	$< 10^3$–10^6	—	Barnes *et al.* (1972)
12-week broilers[c]	—	—	10^3–10^5	—	—	—	10^5–10^6	—	Barnes and Shrimpton (1957)
adult[e]	10^4	10^4	10^4	10^4	10^4	10^4	10^7	10^7	Smith (1965a)
6 h[e]	10^4	10^3	—	N	—	N	N	—	Smith (1965b)
24 h[e]	10^8	10^4	—	10^7	—	10^9	10^9	—	Smith (1965b)
6 days[e]	10^6	10^3	—	10^4	—	10^4	10^6	—	Smith (1965b)
24 days[e]	10^4	N	—	10^3	—	10^4	10^8	—	Smith (1965b)

Clostridium welchii									
2 weeks[a]	—	—	$<10^2$	—	$<10^2$–10^4	—	10^4–10^9	—	Barnes *et al.* (1972)
2 weeks[b]	—	—	$<10^2$	—	$<10^2$	—	$<10^2$–10^4	—	
adult[c]	N	N	N	N	N	N	10^2	10^2	Smith (1965a)
6 h[c]	N	N	—	N	—	N	N	—	Smith (1965b)
24 h[c]	N	10^4	—	10^5	—	10^6	N	—	
6 days[c]	10^2	10^2	—	10^2	—	10^2	N	—	
24 days[c]	N	N	—	10^2	—	N	10^2	—	
Total Clostridia									
2 weeks[a]	—	—	10^2–10^3	—	$<10^2$–10^5	—	10^5–10^9	—	Barnes *et al.* (1972)
2 weeks[b]	—	—	10^2–10^4	—	10^3–10^4	—	10^6–10^7	—	
12-week broilers[c]	—	—	10–10^3	—	—	—	10^3–10^7	—	Barnes and Shrimpton (1957)
Coli-aerogenes									
2 weeks[a]	—	—	$<10^3$	—	$<10^3$–10^8	—	10^8–10^{10}	—	Barnes *et al.* (1972)
2 weeks[b]	—	—	$<10^3$	—	$<10^3$–10^5	—	10^7–10^8	—	
12-week broilers[c]	—	—	10^4–10^5	—	—	—	10^6–10^8	—	Barnes and Shrimpton (1957)
Escherichia coli									
adult[c]	10^2	N	10^2	10^2	10^2	10^3	10^6	10^6	Smith (1965a)
6 h[c]	10^7	10^5	—	10^6	—	10^7	10^8	—	Smith (1965b)
24 h[c]	10^8	10^4	—	10^8	—	10^9	10^{10}	—	
6 days[c]	10^6	10^3	—	10^6	—	10^6	10^9	—	
24 days[c]	10^5	N	—	10^3	—	10^5	10^8	—	
Yeasts									
pullets[d]	—	—	10^2	—	10^3	—	10^4	10^4	Johansson *et al.* (1948)
adults[e]	10^3	N	10^2	N	10^2	N	10^2	10^2	Smith (1965a)

[a] Commercial feed + 9% fish meal.
[b] Commercial feed + 25% fish meal.
[c] Commercial feed.
[d] Laying ration.
[e] Cereal + 10–15% fish meal.
(N = None found in 0·02 g chyme).

In small non-ruminant mammals, maintained under controlled conditions in the laboratory, the microflora of the large intestine appears to become established in a definite sequence (Schaedler, 1973). Lactobacilli and anaerobic streptococci arrive first, followed by coliforms and enterococci and eventually by a very heterogeneous assemblage of obligate anaerobes whose growth is associated with weaning and the ingestion of solid food by the host. Once this microfloral community has become established, it is very difficult for pathogenic forms, for example *Vibrio cholera* and *Salmonella* sp., to invade the host.

There is probably a similar sequence in the colonization of the tract of domestic birds by micro-organisms. Smith (1965b) studied the development of the flora in the tract of the fowl by analyzing samples from different regions, 6 h, 12 h, 1, 2, 3, 4, 6, 8, 11, 14, 17 and 24 days after hatching. The trend of some of his results is given in Table XIV and it would appear that much of the flora is established by about 8 days after hatching. Fuller (1973) pointed out that the lactobacilli, which become established early in the crop of the fowl, are well situated with respect to their activities having a possible regulatory effect on the other members of the flora. If the flora of the tract of the bird also become established in a definite sequence to form a community which then contributes to the host's well-being by impeding the development of pathogenic micro-organisms, it is possible that the activities of protozoan and metazoan parasites, many of whom have a close relationship with micro-organisms in the tract (p. 132), may disturb the indigenous flora. Perhaps such disturbance may be an indirect element in the pathogenicity of parasites like *Histomonas meleagridis* in turkeys or *Trichomonas gallinae* in pigeons.

B. THE DISTRIBUTION OF PROTOZOA

1. *General observations*

Some observations on the sites occupied by protozoan parasites in the tract of domestic birds are given in Table XV and the references cited should be consulted if information about the radial distribution is required. The radial distribution is of great importance in understanding the ecology of protozoan parasites; nearly all the examples given live in association with the mucosa and the pathogenicity of parasites of the genus *Eimeria* may be partly related to the extent of their radial distribution within the mucosa. Clarkson (1958) compared *E. adenoeides* and *E. meleagridis*, both of which live in mucosal cells of the caeca and large intestine of turkeys (Table XV). The very pathogenic *E. adenoeides* was found to inhabit the cells of the villi and the glands deep in the mucosa, whereas the slightly pathogenic *E. meleagridis* was confined to cells at the tips of the villi. Some assessment of the pathogenicity of some protozoan parasites is given in parentheses in Table XV.

Two main conclusions may be drawn from Table XV. First, although it is not claimed that all the types of protozoa found in the tract are represented in the Table, it would appear that relatively few genera of protozoa and, apart from the genus *Eimeria*, relatively few species inhabit the tract of domestic birds. An individual species, however, may be present in enormous numbers, as Hinshaw *et al.* (1938) observed with *Hexamita* sp. in turkey

poults. Second, the greatest variety of extracellular protozoa, which live in the lumen or paramucosal lumen of the tract, have been found in the caeca. This suggests that these protozoa may have an environmental requirement for reduced intestinal motility either directly or indirectly depending on whether they also require an association with micro-organisms which seem to thrive in regions where motility is minimal (Tables XIV and XV). The amoeba *Endolimax gregariniformis*, living in the caecal lumen of fowls, ingests bacteria (Tyzzer, 1920; McDowell, 1953), whereas the amoeba *Entamoeba gallinarum* from the same site shows a much greater dietary range with some trophozoites containing as many as 23 specimens of *Trichomonas gallinarum* (McDowell, 1953). The protozoan *Chilomastix gallinarum* from the caeca of turkeys devours bacteria which are directed towards the cytosome by the action of the undulating membrane (Boeck and Tanabe, 1926), and the stages of *Histomonas meleagridis* and *Parahistomonas wenrichi* from the caecal lumen feed mainly on bacteria (Tyzzer, 1934; Wenrich, 1943; Lund, 1963). The other region of the tract where motility is often reduced, apart from the caeca, is the crop of galliform and columbid birds where extracellular protozoa can develop (Table XV) in association with micro-organisms (Table XIV). These inter-relationships between micro-organisms and protozoa suggest that ecological communities exist in the tract; the varied feeding habits could explain how closely related species might co-exist within the same habitat (see Lack, 1971).

2. *The distribution and site specificity of* Eimeria *spp. in the tract*

Parasitologists have long been intrigued by the distribution of species of *Eimeria* in the tracts of fowls and turkeys (Table XV). Tyzzer (1929), Tyzzer *et al.* (1932), Davies *et al.* (1963), Long (1967a), Reid (1968, 1972) and Clarkson (1958, 1959a,b) have provided diagrammatic representations of the regions of the tract parasitized by the different species, and Marquardt (1973) has discussed this topic in detail. It is important to recognize, however, that a diagram may suggest that the limits of the parasitized region are more sharply defined than is actually the case; this impression is not given by the texts of the references cited in Table XV. It is also a general observation that heavy infections result in an extension of the parasite's site in the tract (Marquardt, 1973).

(a) *Longitudinal distribution.* Several proposals have been made to explain the observed longitudinal distribution of *Eimeria* spp. in the tract of fowls. Infection is achieved when the sporozoites become established in the appropriate region of the mucosa. This cannot occur until the sporocysts have been released from the ingested oocysts and the sporozoites from the sporocysts. These processes take time and require the participation of the host's digestive physiology (Section IV). Hammond (1971), in a summary of observations made *in vivo* and *in vitro* on *Eimeria* spp. from mammalian and avian hosts, pointed out that the rate of excystation of oocysts may be correlated with the distance which must be travelled in the digestive tract to reach the usual site of development. Consequently, a species developing in the anterior or middle portions of the small intestine may be expected to excyst faster than species

TABLE XV

The distribution of the sites occupied by some protozoa in the alimentary tract of domestic birds

Parasite	Infective stage	Host[a]	Site	References
ZOOMASTIGOPHORASIDA				
RETORTAMONADORIDA				
RETORTAMONADIDAE				
Chilomastix gallinarum	? cyst	F, T	Caecal mucosa; division stages in crypts	Martin and Robertson (1911); Boeck and Tanabe (1926); McDowell (1953)
COCHLOSOMATIDAE				
Cochlosoma anatis	—	D	Caeca and large intestine	Travis (1938); see Levine (1973)
Cochlosoma sp.	—	T	Mucosa of anterior half of small intestine (catarrhal enteritis)	Campbell (1945)
DIPLOMONADORIDA				
HEXAMITIDAE				
Hexamita columbae	unresolved (see Levine, 1973)	P	Mucosa of posterior two-thirds of small intestine and large intestine (catarrhal enteritis and ulceration)	McNeil and Hinshaw (1941a)
Histomonas meleagridis	unresolved (see Levine, 1973)	T, F	Close application to mucosal surface of anterior small intestine (catarrhal enteritis, and elsewhere in post-pyloric tract	Hinshaw *et al.* (1938); McNeil and Hinshaw (1941b)
TRICHOMONADORIDA				
MONOCERCOMONADIDAE				
Histomonas meleagridis	associated with *Heterakis gallinarum*	F, T	Lumen, mucosa and tissues of caeca (necrosis; invade deep-body tissues in turkeys)	Smith (1915); Tyzzer (1934); Wenrich (1943); Lee (1971); Lund (1963); Levine (1973)
Parahistomonas wenrichi	associated with *Heterakis gallinarum*	F, T	Caecal mucosa	

TRICHOMONADIDAE				
Tetratrichomonas (= *Trichomonas*) *anatis*	trichomonad	D	Posterior part of small intestine	Kotlán (1923)
T. gallinarum	trichomonad	F, T	Caecal mucosa	Martin and Robertson (1911); Richardson (1934); McDowell (1953)
Trichomonas diversa	trichomonad	T	Mucosa from the oesophagus to proventriculus (catarrhal exudate occludes oesophageal lumen)	Hawn (1937)
T. gallinae	trichomonad	F, T, P	Mucosa of anterior part of tract from mouth to proventriculus (caseation and occlusion)	Stabler (1954); Mesa *et al.* (1961); Levine and Brandly (1940)
Tritichomonas (= *Trichomonas*) *eberthi*	trichomonad	F, T	Caecal mucosa	Martin and Robertson (1911); McDowell (1953)
RHIZOPODASIDA				
AMOEBORIDA				
ENDAMOEBIDAE				
Entamoeba gallinarum	cyst	D, G, F, T	Caecal mucosa	Tyzzer (1920); Richardson (1934); McDowell (1953)
Endolimax gregariformis	cyst	D, G, F, T	Caeca	Tyzzer (1920); McDowell (1953); Levine (1973)
SPOROZOASIDA				
EUCOCCIDIORIDA				
EIMERIIDAE				
Eimeria acervulina		F	Usually mucosal cells of anterior small intestine (+ +)[b]	1, 2, 3, 4, 5, 6; Tyzzer (1929); Doran (1966b)
E. adenoeides		T	Mucosal cells of caeca, posterior small and large intestines (+ + + +)	2, 3, 4, 6; Clarkson (1958)
E. anseris		G	Usually mucosa of posterior small intestine, but may extend throughout post-pyloric tract	2; see Levine (1973)

TABLE XV—*continued*

E. brunetti	sporulated oocyst	F	Usually mucosal cells of posterior small intestine; also caeca and large intestine (+ + +)	1, 2, 3, 4, 5, 6; Ryley *et al.* (1972)
E. danailova		D, G	Mucosal cells of small intestine	3
E. dispersa		T	Usually mucosal cells of anterior small intestine, but also remainder of small intestine (?+)	2, 3, 4, 6; Hawkins (1952)
E. gallopavonis		T	Mucosal cells of posterior small intestine and large intestine; sometimes caeca (+ +)	2, 3, 4, 6; Hawkins (1952); Farr and Doran (1962)
E. hagani		F	Mucosal cells of anterior half of small intestine	2, 3, 5, 6
E. innocua		T	Mucosal cells of small intestine (?+)	2, 3, 6; Moore and Brown (1952)
E. kotlani		G	Mucosal cells of large intestine and neck of caeca (+ + +)	see Levine (1973)
E. labbeana		P	Mucosal cells of small and large intestine (+ + + +)	2; see Levine (1973)
E. maxima		F	Mucosal cells of entire small intestine, but usually in middle portion (+ + +)	1, 2, 3, 4, 5, 6; Tyzzer (1929)
E. meleagridis		T	Mucosal cells of caeca (+)	2, 3, 6, 7; Tyzzer (1927); Hawkins (1952); Clarkson (1959b)
E. meleagrimitis		T	Mucosal cells of anterior small intestine (+ + + +)	2, 3, 4, 6, 7; Hawkins (1952); Clarkson (1959a); Farr and Doran (1962)
E. mitis		F	Mucosal cells of anterior half of small intestine; occasionally remainder of small intestine, large intestine and proximal caeca (+)	1, 2, 3, 4, 5, 6; Tyzzer (1929)
E. mivati (= *E. acervulina* var. *mivati;* Long, 1973)		F	Usually mucosal cells of anterior small intestine, but moves into remainder of small intestine, large intestine and caeca (+ +)	3, 4, 5, 6, 7
E. necatrix		F	Asexual stages in mucosal cells of small intestine; sexual stages in caeca (+ + + +)	1, 2, 3, 4, 5, 6, 8

E. nocens		G	Mucosal cells of pasterior port of small intestine; may extend throughout post-pyloric tract (+ +)	2; see Levine (1973)
E. parvula		G	Mucosal cells of posterior part of small intestine	2; see Levine (1973)
E. praecox		F	Mucosal cells of anterior third of small intestine (?+)	1, 2, 3, 4, 5, 6, 8
E. saitamae		D	Middle and posterior part of small intestine	Inoue (1967)
E. stigmosa		G	Mucosal cells of anterior part of small intestine: may extend into posterior part	see Levine (1973)
E. subrotunda		T	Mucosal cells of anterior half of small intestine (?+)	2, 3, 6; Moore *et al.* (1954)
E. tenella		F	Usually mucosal cells of distal caeca, but sometimes in posterior small intestine and large intestine (+ + + +)	1, 2, 3, 4, 5, 6, 7
Wenyonella gallinae	sporulated oocyst	F	Mucosa of posterior part of small intestine (+ +)	3; Ray (1945)
W. philiplevinei	sporulated oocyst	D	Mucosal cells of posterior half of small intestine and large intestine (+)	Leibovitz (1968)
Tyzzeria anseris (? = *T. parvula*)	sporulated oocyst	G	Small intestine	Farr and Wehr (1952); see Levine (1973)
T. perniciosa	sporulated oocyst	D	Mucosa and sub-mucosa of small intestine, especially in anterior half (+ + + +)	3; Allen (1936)
CRYPTOSPORIDIIDAE				
Cryptosporidium meleagridis	unresolved	T	Mucosa of posterior third of small intestine	3; Slavin (1955)
C. tyzzeri (= *C. parvum* in part)	unresolved	F	Mucosal cells of proximal part of caeca	6; see Levine (1973)

[a] D = duck; G = goose; F = fowl; T = turkey; P = pigeon.
[b] Pathogenicity; (+ + + +) = high; (+) = low.

1. Boles and Becker (1954)
2. Davies *et al.* (1963)
3. Levine (1973)
4. Long (1967a)
5. Reid (1968)
6. Reid (1972)
7. Tyzzer (1929)
8. Tyzzer *et al.* (1932)

developing in the posterior small intestine or the large intestine. Under the experimental conditions employed, sporozoites of *E. acervulina* (Doran and Farr, 1962) were detected in the small intestine of fowls after a shorter time and more anteriorly than sporozoites of *E. tenella* (Goodrich, 1944); *E. acervulina* is usually found in the anterior small intestine and *E. tenella* in the caeca (Table XV). The studies of Farr and Doran (1962) with *E. acervulina* and *E. tenella* from fowls and with *E. meleagrimitis* and *E. gallopavonis* from turkeys may be interpreted in this way. Further indirect evidence in support of the hypothesis favouring the importance of excystation time and intestinal motility, are the observations made after the artificial introduction of sporozoites of several species in carefully controlled experiments into abnormal regions of the tract of fowls in which deep anaesthesia had been induced. When sporozoites of *E. brunetti* and *E. necatrix* were injected into the distal portion of a caecum ligated half-way along its length, the parasites completed their development in the caecal mucosa and oocysts were produced (Horton-Smith and Long, 1965), despite the fact that ligation must have impaired the normal functioning of the caecum. Sporozoites of *E. mivati* were also found to complete their life cycle when injected into the caecum (Horton-Smith and Long, 1966). These observations may be taken to indicate that some species of *Eimeria* have no preference for a particular region of the intestine and thus the time taken for excystation may be important in influencing the penetration of a particular region of the tract. On the other hand, infections of *E. acervulina* and *E. maxima* failed to develop in the caeca of fowls when sporozoites were injected directly into this site, whereas intestinal infections were successful in control birds which received an injection into the duodenum (Horton-Smith and Long, 1966).

Although time and motility must be considered in a discussion of the distribution of *Eimeria* spp. in the tract, the hypothesis is too simple to account for all the observations made on all the species infecting galliform birds. For example, it is known that on infection of the fowl, sporozoites of *E. tenella* are liberated in the small intestine, sometimes as anteriorly as the duodenum (Rose, 1967), but establishment in the intestinal mucosa rarely occurs if the caecal mucosa is available (Table XV). This finding suggests that some form of site selection is inherent in *E. tenella* and that the role of intestinal motility is to convey sporozoites as far as the junction of the caeca and small intestine. Conversely, it could be argued that any sporozoites of *E. tenella* liberated in the small intestine will soon perish and only those sporozoites which happen to have been released in the vicinity of the caecal entrances will enter the caeca and their mucosal tissues. That point is open to experimental test and it appears to be supported by the work of Leathem (1969), who fed about 1×10^6 sporulated oocysts of *E. tenella* to 3-week-old chicks from which the caeca had been removed one week before infection. *Eimeria tenella* in the experimental birds completed normal development in the mucosal cells of the large intestine near the caecal openings, but the parasites were either less pathogenic in this site or fewer became established because control birds had to be given half the number of oocysts in an attempt to reduce mortality resulting from caecal lesions. Leathem's work was an

expanded and confirmatory study of earlier investigations by Mayhew (1934) and Ikeda (1957). Thus, in the case of *E. tenella* removal of caeca appears to remove from the host the stimuli for the parasite's site-finding activity, and in fowls without caeca the effects of time and motility may then become more important.

An interesting observation was made by Ikeda (1957) on the site specificity of *E. tenella*. The proximity of the distal ends of the caeca to the duodenum and other regions of the tract of the fowl (Figs 5 and 19) facilitated the surgical technique needed to divert the intestinal contents through the distal end of one caecum while the other remained undisturbed. Following the ingestion of sporulated oocysts, infections became established in the complete caecum, and in those caeca which were united to the posterior part of the small intestine and contained ingesta from that region. *Eimeria tenella* did not infect the mucosal tissue of caecal pouches receiving material from the duodenum; it is possible that the ingesta were at a low pH because they had been diverted anterior to the entry of the pancreatic ducts.

Some observations, however, obtained during experimental studies on the distribution of *Eimeria* spp. in the tract of fowls, stress the potential importance of the parasites' site-finding characteristics irrespective of intestinal motility. Sporozoites of *E. praecox*, a parasite of the anterior third of the small intestine (Table XV), were injected by surgical methods into the caeca of fowls aged 6 days and 3 weeks and inoculations of equivalent numbers of sporozoites were placed directly into the duodenum of each of the control birds (Long, 1967b). Infections developed in the small intestines of both groups of birds, but not in the caeca. The parasites had left the caeca of the experimental birds and had reached their normal site, presumably by moving against the direction of intestinal flow, unless they had emigrated through the caecal wall and body cavity to another part of the tract (Fig. 19). Sporozoites of *E. praecox* were introduced into the cloacae of 4-week-old fowls and the mouths of others. In both cases, infections developed in the anterior half of the small intestine and not in the caeca. Provided that oral contamination or emigration through the body cavity had not occurred, the sporozoites which survived the journey from the cloaca must have moved at least 50 cm against the direction of intestinal flow. These results may be interpreted as indicating that under certain circumstances, which may be artificial and unnatural, the sporozoites of at least one species of *Eimeria* may reach the region of their site without total dependence on excystation time or intestinal motility.

Observations have also been made on the establishment of infections of *Eimeria* spp. in their usual sites after experimental manipulations which must have separated the parasites from intestinal factors for a considerable time. Joyner and Norton (1972) managed to establish light infections of *E. acervulina* in the caeca of young fowls by injecting large numbers of sporozoites by a procedure similar to that used (unsuccessfully in this case) by Horton-Smith and Long (1966). Joyner and Norton found that great care had to be taken to avoid contamination of the peritoneal cavity with sporozoites which could emigrate to establish infections in the intestine and

especially in the normal site in the anterior part of the small intestine (Joyner, pers. comm.). This finding emphasizes again the need to consider the arrangement of the intestinal loops and the volume of unoccupied body cavity *in vivo* (Fig. 19). Normal infections of *E. acervulina*, *E. maxima*, *E. necatrix* and *E. tenella*, resulting in schizogony in normal sites (Table XV) and in the production of oocysts, were obtained in fowls which received inoculations of oocysts by a variety of parenteral routes (Davies and Joyner, 1962). Similar results were obtained by Sharma (1964) with seven species of *Eimeria* in fowls. One conclusion which may now be drawn from the discussion of the longitudinal distribution of *Eimeria* spp. in fowls is that while excystation time and intestinal motility are important factors in the establishment of the parasites in their sites under natural conditions, the parasites themselves appear to possess considerable powers of emigration and site detection.

(b) *Radial distribution.* The radial distribution of species of *Eimeria* in the mucosal tissue is as diverse as the longitudinal distribution. The different species may not only occupy epithelial cells from different regions along the length of a villus (Clarkson, 1958, 1959a,b, p. 140) and different positions with respect to the nucleus within the cells (Tyzzer, 1929; see Levine, 1973), but also the different stages in the life cycle of the same species may have a different radial distribution. Gametogony in *E. maxima* is preceded by three generations of schizonts. The first generation occupies the deep glands of the mucosa, the second the cells near the openings of the crypts of Lieberkühn and the third the cells along the sides and sometimes tips of the villi (Millard *et al.*, 1972). There are at least three cycles of asexual schizogony before gametogony in *E. brunetti*, in which the first generation inhabit epithelial cells at the base of the villi while schizonts of the second and third generations inhabit the cells at the tips of the villi (Ryley *et al.*, 1972).

The radial distribution of the developing parasites does not necessarily reflect the region of the mucosa which was originally penetrated by the sporozoites on the infection of the host. On the basis of fixed intestinal tissue examined with the light microscope, Van Doorninck and Becker (1957) concluded that sporozoites of *E. necatrix* first invaded the lamina propria of the mucosa, and that some were engulfed by macrophages and were then transported by these cells to the glandular region of the epithelium. A fairly similar sequence of observations was made by Pattillo (1959) in a study of the establishment of *E. tenella* in the caecal mucosa, the lamina propria again being the initial location of the sporozoites after they had bored through or between the epithelial cells. Gill and Ray (1957) noted that sporozoites of *E. tenella* pierced the lamina propria and were engulfed by macrophages, but apparently they attached little significance to their observation.

3. *The distribution and site specificity of* Histomonas meleagridis *in the tract*

Histomonas meleagridis is a common parasite of the caeca of fowls and turkeys, in which it is involved in a very severe disease. In natural infections in turkeys from which the caeca have been removed, the signs and symptoms of the disease are not observed. Durant (1930) separated the caeca from the

small intestine of turkeys and closed the free ends of the caeca and the remains of their junction with the intestine. The caecal vascular system was left intact and the caeca continued to grow apart from the small intestine. Several of the operated birds died from peritonitis, but the survivors proved very resistant to infection with *H. meleagridis* when introduced into a flock of control turkeys which were suffering from the disease. Schlotthauer *et al.* (1933) and Delaplane and Stuart (1933) also found that the majority of turkeys whose caeca had been occluded or abligated by surgical techniques will survive in an environment in which unoperated turkeys will succumb to the effects of *H. meleagridis*. These results suggest that either the protozoan has an obligatory requirement for certain caecal conditions including an association with the caecal flora, or that the disease stems from interactions between *Histomonas* and other caecal inhabitants. *Trichomonas* sp. and *Chilomastix* sp. (Table XV) also fail to become established after natural and experimental attempts to infect chicks from which the caeca have been removed (Richardson, 1939).

Histomonas meleagridis is pleomorphic and its different forms appear to occupy different regions of the caecum. There is some disagreement about the number of forms of *H. meleagridis* (see Levine, 1973). Lee *et al.* (1969) described the location and feeding habits by the amoeboid invasive stage and the more quiescent vegetative stage in the caecal tissues, and Wenrich (1943) described a stage from the luminal contents.

C. THE DISTRIBUTION OF HELMINTHS

Observations on the distribution of sexually mature helminths in the alimentary tract of domestic birds are given in Table XVI. The references cited in the Table should be consulted for more detailed descriptions of the sites and particularly for accounts of the radial distribution of the parasites. The articles by Cram (1927), Lapage (1961), Madsen (1952), Reid (1962), McDonald (1969a,b), Owen (1951), Byrd (1972), Todd (1946, 1948) and Wehr 1972a,b) give much information about the parasites of domestic birds and their wild relatives which may carry infections into flocks of poultry. Apart from *Polymorphus* spp. (Table XVI), which is an important parasite of ducks (Petroschenko, 1956; Hynes and Nicholas, 1963; Romanovski, 1964), acanthocephalans are rarely encountered in domestic birds. Some acanthocephalans, however, become established in domestic birds in the laboratory and thus have the potential to infect foraging poultry (Van Cleave, 1947; Schmidt and Olsen, 1964). The following comments and the general conclusions drawn from Table XVI are extensions of those expressed by Crompton (1973).

1. *Longitudinal distribution*

The distribution of the adult stages of the digenetic trematodes mentioned in Table XVI shows that they may be found in the paramucosal lumen and in association with the mucosal surface of most regions of the alimentary tract. The adult stages of the Nematoda have an equally extensive longitudinal distribution and the most diverse radial distribution of all the metazoan

TABLE XVI

The distribution of the sites occupied by some adult helminths in the alimentary tract of domestic birds

Parasite	Infective stage and (intermediate host)	Host[a]	Site	References
DIGENETIC TREMATODA				
PROSOSTOMATA				
STRIGEIDAE				
Apatemon gracilis minor	"tetracotyle" (leeches)	D	Anterior third of small intestine	Blake (pers. comm.); Öhman (1966)
Strigea falconis meleagris	"tetracotyle" (?)	T	Upper third of small intestine	Harwood (1932)
CYATHOCOTYLIDAE				
Cyathocotyle bushiensis	metacercaria (snails)	D	Mucosa of caeca	Erasmus and Öhman (1965)
Holostephanus lühei	metacercaria	D	Mucosa of large intestine; not in cloaca	Erasmus (1962)
ECHINOSTOMATIDAE				
Acanthoparyphium spinulosum	metacercaria (snails)	F	Anterior part of small intestine	Martin and Adams (1961)
Echinostoma revolutum	metacercaria (snails; tadpoles)	F	Posterior half of small intestine, caeca and cloaca	Beaver (1937); Fried and Weaver (1969);
		P	Small and large intestines	Beaver (1937)
Echinoparyphium recurvatum	metacercaria (?)	T	Upper portion of small intestine	Annereaux (1940)
Hypodaerium conoideum	metacercaria (snails)	D	Lower part of small intestine (localized inflammation)	Vevers (1923)
PSILOSTOMIDAE				
Psilostomum ondatrae	metacercaria (fish)	D, F, P	Proventriculus; each worm forming a deep pit in the mucosa	Newsom and Stout (1933); Beaver (1939); Riggin (1956)
Sphaeridiotrema globulus (= *S. spinoacetabulum*)	metacercaria (snails)	D, G, F	Caeca; mucosal region (haemorrhage and ulceration)	Burns (1961); Macy *et al.* (1968)
BRACHYLAEMIDAE				
Harmostomum (= ?*Brachylaema*) *horizawi*	?	F	Caeca	Ishii (1933)
Postharmostomum gallinum	metacercaria (snails)	F	Caeca (inflammation and haemorrhage)	Alicata (1940)

OPISTHORCHIIDAE				
Metorchis orientalis	metacercaria	D	Biliary system	Ishii and Matsuoka (1935)
HETEROPHYIDAE				
Euhaplorchis californiensis	metacercaria (fish)	F	Posterior part of small intestine	Wong (1954)
MICROPHALLIDAE				
Levinseniella amnicolae	?	D	Caeca	Etges (1953)
Maritrema obstipum	?	D	Middle third of small intestine; also rest of small and large intestines	Etges (1953)
NOTOCOTYLIDAE				
Catatropis johnstoni	metacercaria	F	Caeca; mucosal surface	Martin (1956)
Notocotylus seineti	?	D	Caeca and large intestine	Harper (1929)
Zygocotyle lunatum	metacercaria	D	Usually near distal end of caecum; also posterior small intestine	Gower (1938); Willey (1938)
CESTODA				
PSEUDOPHYLLIDEA				
DIPHYLLOBOTHRIIDAE				
Schistocephalus solidus	plerocercoid (fish)	D	Posterior half of small intestine (sometimes in caecum)	McCaig and Hopkins (1963)
		F	Middle of small intestine	
		P	Anterior half of small intestine	
CYCLOPHYLLIDEA				
DAVAINEIDAE				
Davainea dubius	cysticercoid (?)	F	Duodenum	Meggitt (1916)
D. meleagridis	cysticercoid (?)	T	Duodenum	Jones (1936)
D. proglottina	cysticercoid (slugs)	F	Duodenum; posterior extension of site in heavy infestations	Owen (1951)
Raillietina cesticillus	cysticercoid (beetles; flies)	F	Anterior part of small intestine	Foster and Daugherty (1959); Gray (1972a, b)
R. echinobothrida	cysticercoid (ants)	F	Posterior part of small intestine and caeca	Todd (1946, 1948)
R. georgiensis	cysticercoid (ants)	T	Posterior third of small intestine	Reid and Nugara (1961)
R. tetragona	cysticercoid (ants)	F	Posterior part of small intestine	Todd (1946)

TABLE XVI—*continued*

DILEPIDIDAE				
Amoebotaenia sphenoides (= *A. cuneata*)	cysticercoid (earthworms)	F	Duodenum; strobila "shelters" among villi	Meggitt (1916); Ferry (1934)
Choanotaenia infundibulum	cysticercoid (beetles, flies)	F	Posterior half of small intestine; "strobila generally anterior to attachment point"	Todd (1946)
HYMENOLEPIDIDAE				
Fimbriaria fasciolaris	cysticercoid (crustaceans)	D	Posterior part of small intestine	Whitfield (pers. comm.)
Hymenolepis cantaniana	cysticercoid (beetles)	F	Duodenum	Jones and Alicata (1935)
H. carioca	cysticercoid (beetles, flies?)	F	Duodenum	Ferry (1934); Todd and McSpadden (1947)
H.(? = *Dicranotaenia*) *coronula*	cysticercoid (crustaceans)	D	Upper portion of small intestine	Schofield (1931)
H. exigua	cysticercoid (amphipods?)	F	Duodenum	Alicata and Chang (1939)
NEMATODA				
RHABDIASIDEA				
STRONGYLOIDIDAE				
Strongyloides avium		F	Caecal mucosa (serious effect on caecal wall)	Cram (1929)
Strongyloides sp.		F	Anterior two-thirds of small intestine	Cram (1936b)
TRICHURIDEA				
TRICHURIDAE (see Madsen, 1951)				
Capillaria annulata	egg (?earthworms)	F, T	Mucosa and inner lining of oesophagus and crop (pathological lesions)	Cram (1928, 1936a); Todd (1948)
C. caudinflata	egg (?earthworms)	F, T	Small intestine, particularly anterior half and duodenum	Morehouse (1944); Owen (1951)
C. collaris (= *C. retusa*)	egg	F	Caeca	Owen (1951)
C. columbae (= *C. obsignata*)	egg	F	Mucosa of lower two thirds of small intestine; also duodenum and caeca	Levine (1938a); Wakelin (1965)
		P	Mucosa of anterior half of small intestine (destruction of mucosa)	Wehr (1939)

C. contorta	egg (?earthworms)	D, T	Mucosa and submucosa of mouth, oesophagus and crop (inflammation, thickening and starvation)	Cram (1936a); Emmel (1939)
C. dujardini		F	Duodenum and anterior half of small intestine	Owen (1951)
C. perforans		T	Under mucosa of oesophagus and crop	Cram (1936a)
C. retusa (? = *C. collaris*)	egg	F	Caeca; embedded in mucosa of proximal region and free in the lumen of distal portion	Todd (1948)
STRONGYLIDEA				
TRICHOSTRONGYLIDAE				
Amidostomum anseris	larva	G	Embedded in tissue of ventriculus (destroys koilin lining)	Jerstad (1936)
A. raillieti	larva	F	Under epithelium of ventriculus	Leiby and Olsen (1965)
A. skrjabini	larva	D, F	Epithelial lining of ventriculus	Leiby and Olsen (1965)
Epomidiostomum uncinatum	larva	D, F	Lining of ventriculus	Leiby and Olsen (1965)
Ornithostrongylus quadriradiatus	larva	P	Small intestine, particularly anterior portion (haemorrhage and anaemia)	Komarov and Beaudette (1931)
Trichostrongylus tenuis	larva	D, Dm, F	Mucosal surface of caecum (haemorrhage and desquamation of epithelium)	Cram and Cuvillier (1934); Owen (1951); Soliman (1955)
OXYURIDEA				
HETERAKIDAE				
Heterakis gallinarum (= *H. gallinae*)	egg	D, G, F, T	Caeca	Jerstad (1936); Farr and Wehr (1952); Roberts (1937); Todd (1948); Owen (1951)
SUBULURIDAE				
Subulura brumpti	larva (beetles)	F, T	Caeca	Cram (1926); Cuckler and Alicata (1944)
S. minetti		F	Caeca	Nath and Pande (1963)
ASCARIDIDEA				
ASCARIDIDAE				
Ascaridia galli (= *A. lineata*)	egg	F	Anterior half of small intestine, but also in posterior half	Ackert (1931); Owen (1951); Todd and Crowdus (1952)

TABLE XVI—*continued*

SPIRURIDEA				
SPIRURIDAE				
Gongylonema ingluvicola		F, T	Burrows in mucosa of crop	Wehr (1972a)
Seurocyrnea (= *Cyrnea*) *colini*	? (cockroaches)	F, T	Wall of proventriculus at junction with ventriculus	Cram (1931)
THELAZIIDAE				
Oxyspirura (= *Cheilospirura*) *hamulosa*	larva (grasshoppers)	F, T	Tissue of ventriculus, under corneous (koilin) lining	Cram (1931)
PHYSALOPTERIDAE				
Streptocara sp.	larva (amphipods)	Dm	Wall of the tract anterior to pylorus	Lancaster (1973)
ACUARIIDAE				
Dispharynx nasuta	?	P	Embedded in mucosa of proventriculus (inflammation and desquamation)	Hwang *et al.* (1961)
D. spiralis	larva (isopods)	F, T, P	Deeply buried in wall of proventriculus	Cram (1931)
Echinuria (= *Acuaria*) *uncinata*	larva (cladocerans)	D	Proventriculus (laceration, thickening and obstruction)	Buxton *et al.* (1952)
TROPISURIDAE				
Tetrameres (= *Tropisurus*) *americana*	larva (grasshoppers)	F, P	Glandular tissue and mucosa of proventriculus (necrosis)	Cram (1931); Raggi and Baker (1957)
T. crami	larva (amphipods)	D	Proventriculus	Swales (1936)
T. fissispinus	larva (crustaceans)	D	Glandular tissue of proventriculus	Soliman (1955)
ACANTHOCEPHALA				
PALAEACANTHOCEPHALA				
FILICOLLIDAE				
Filicollis anatis	cystacanth (amphipods)	D, Dm	Posterior two-thirds of small intestine	Soliman (1955)
POLYMORPHIDAE				
Polymorphus minutus	cystacanth (amphipods)	D	Posterior part of small intestine (Fig. 14)	Crompton and Whitfield (1968)

[a] D = duck; Dm = muscovy duck; G = goose; F = fowl; T = turkey; P = pigeon.

parasites of the tract. The adult stages of the Cestoda and Acanthocephala, however, are restricted to the lumen and paramucosal lumen of the small intestines of their hosts. The distribution of most helminths in the tract of domestic birds can be explained in terms of the nutritional requirements and feeding habits of the parasites (Crompton, 1973). Many of the digenetic trematodes appear to have become specialized for browsing on the mucosal tissues in their sites by ingesting cells, blood, mucus, products of the host's digestion and their own histolytic secretions in a manner similar to that described for *Apatemon gracilis minor* on the intestinal villi of the domestic duck (Öhman, 1966). The small size of many trematodes may reflect an evolutionary adaptation towards dependence on the mucosa. The smallness and shape of many larval and adult nematodes and their retention of an alimentary tract, which may enable them to feed on a variety of materials including chyme, blood, lymph, histolytic products and bacterial suspensions (Ackert and Whitlock, 1940), appear to have contributed to their colonization of nearly all the tissues of the tract. Adult cestodes and acanthocephalans have no alimentary tract and feed by absorbing through their surfaces products of their hosts' digestive processes on which they are dependent.

Interesting and unexplained observations have been made about some of the species listed in Table XVI. Martin (1956) noticed that the trematode *Catatropis johnstoni* became established in roughly similar numbers in both caeca of fowls instead of one caecum becoming very heavily infected. Todd (1948), in a survey of helminths from fowls, found the nematode *Capillaria retusa* as often in the distal portion of a caecum as in the proximal portion, but the worms tended to be free in the distal lumen and embedded in the proximal mucosa. Todd (1948) also observed mature specimens of the cestode *Raillietina echinobothrida* not infrequently with their scoleces attached to the caecal mucosa while their strobilae extended out of the caeca and anteriorly along the small intestine.

The observation made by McCaig and Hopkins (1963) on the cestode *Schistocephalus solidus* from ducks, fowls and pigeons and from sites in these hosts in which physico-chemical conditions are believed to be different (Table XVI), indicates that some helminths must not have precise environmental requirements. Cram (1936b) discovered that the nematode *Strongyloides* sp. occurred primarily in the anterior two-thirds of the small intestines of naturally infected fowls, but exclusively in the caeca of experimentally infected hosts. No explanation for this change in longitudinal distribution has been offered, although the effects of different infection procedures, the age of the hosts, the age of the infective stages and intra- and interspecific reactions were explored.

2. *Emigrations and changes in longitudinal distribution during the course of the infection*

Examination of the sources mentioned in Table XVI indicates that adult helminths rarely emigrate or change their sites compared with immature stages (Crompton, 1973). Most changes in longitudinal distribution appear to be associated with emigrations in the direction of gastrointestinal flow. For

example, 272 specimens of the trematode *Echinostoma revolutum* were recovered from the posterior small intestine, 163 from the caeca and 241 from the large intestine of 40 fowls infected for 14 days, and 105 from the posterior small intestine, 102 from the caeca and 193 from the large intestine of 29 fowls infected for 22–35 days (Fried and Weaver, 1969). The cestodes *Raillietina cesticillus* in fowls (Foster and Daugherty, 1959; Gray, 1972b) and *R. georgiensis* in turkeys (Reid and Nugara, 1961) move down the small intestine during the course of infection, the mature worms being situated more posteriorly than the immature. Small specimens of *Choanotaenia infundibulum* occurred more anteriorly in the small intestine of fowls than specimens believed to be older (Todd, 1946). In these cases, the posteriorly directed emigrations could be correlated with the possible requirement of mature individuals for the more stable conditions considered to exist in the posterior small intestine (Crompton, 1970, 1973).

Many emigrations in the direction of gastrointestinal flow appear to occur during the arrival of certain parasites in their sites. After activation, during the process of infection (Section VI), in some regions of the small intestine, the immature stages of helminths from the caeca and posterior part of the tract (Table XVI) must either emigrate or be propelled with the ingesta down the tract. After the ingestion of infective eggs by fowls, second-stage larvae of the nematode *Heterakis gallinarum* may be recovered within 2 h from the region of the small intestine extending from the bile ducts to the yolk stalk. Within 4½ h the larvae have reached the posterior part of the small intestine and 2 h later some have entered the caeca where development continues (Roberts, 1937). Presumably, immature stages of the trematodes from the caeca undergo a similar emigration unless they penetrate the intestinal wall and cross the body cavity of the host. From the present state of knowledge, it is almost impossible to make any generalizations from observations which are relevant to topics like emigrations, changes in longitudinal distribution and the arrival of helminths in their site. In some cases, however, the arrival of a helminth in its site seems to be related to the rate of activation of the infective stage and the distance which that stage will have been propelled by the intestinal motility of the host during the time required for activation (Lingard and Crompton, 1972; Asanji and Williams, 1974).

Few observations have been found of helminths undergoing emigrations against the direction of gastrointestinal flow, although those which enter the caeca from the small intestine make an anteriorly directed emigration and those parasites of the oesophagus, crop, proventriculus and ventriculus may also undergo a considerable anterior emigration if their infective stages are not activated until they pass the pylorus (Table XVI). Wehr (1939) noted that mature *Capillaria columbae* were generally to be found more anteriorly than immature forms in the small intestine of pigeons, an observation suggesting the occurrence of an emigration against the direction of flow.

The site of a helminth may change if the longitudinal distribution becomes extended in response to competition for a limiting environmental resource resulting from an increase in population density. The site of the trematode *Echinostoma revolutum* may become extended both anteriorly and posteriorly

in the tract of pigeons (Beaver, 1937), that of the cestode *Davainea proglottina* may extend posteriorly in fowls (Owen, 1951), and that of the acanthocephalan *Polymorphus minutus* may be expected to extend posteriorly in domestic ducks as it does in mallard, *Anas platyrhynchos* (Crompton and Harrison, 1965; Fig. 14).

3. *Radial distribution*

An impression of the significance of radial distribution in the biology of helminths and of their effects of domestic birds may be obtained from observations on adult nematodes. *Ascaridia galli* (= *A. lineata*) occupies the lumen of the small intestine of fowls (Ackert, 1931), *Ornithostrongylus quadriradiatus* lives in association with the mucosal surface and in the paramucosal lumen of pigeons (Komarov and Beaudette, 1931), and species from the anterior tract burrow, with severe consequences (Section VII), into the walls of the ventriculus, proventriculus, crop, oesophagus and mouth (Table XVI). In addition to unfavourable physico-chemical conditions in many parts of the anterior lumen, the motility is probably too strong and erratic for helminths to occupy the lumen. It is of interest that the larval stages of those nematodes from beneath the tough koilin lining of the ventriculus, for example *Amidostomum raillieti*, *A. skrjabini* and *Epomidiostomum uncinatum* in ducks, fowls and pigeons, gain entry by penetrating the lining where it is thin and relatively soft at the junction of the proventriculus and ventriculus (Leiby and Olsen, 1965; Fig. 20).

The larval stages of several namatodes have been found to have a radial distribution associated with the mucosal tissues, and this phase may have evolved in some species as an adaptation to offset the effects of intestinal motility. According to Roberts (1937), the larvae of *Heterakis gallinarum* immediately invade the mucosa on entering the caeca, an event which would clearly reduce heavy losses when the caeca evacuate. The association of the larval stages of *A. galli* with the mucosal tissues is variable. Ackert (1931) and Tugwell and Ackert (1952) observed that immature stages of *A. galli* lived in the lumen for their first 9 days in the fowl and then emigrated into the mucosa for the next 9 days before returning to the luman. In contrast, Todd and Crowdus (1952) examined the tracts of 54 month-old fowls each of which had been given 50 eggs of *A. galli* a fortnight previously. Of the 817 worms recovered, 723 (ca. 88·5%) were in the lumen and 94 (ca. 11·5%) in the tissues of the wall. Madsen (1962b) discussed accounts of the tissue phase of nematodes, and in an attempt to reconcile these conflicting observations on *A. galli* suggested that emigrations into the mucosa depended on the interplay between host resistance and larval viability.

Differences between the radial distribution of adult male and female nematodes of the same species may occur in domestic birds as well as between stages of the life cycle. Adult male *Tetrameres crami* are found on the mucosal surface of the proventriculus of ducks and adult females are found in the crypts (Swales, 1936). Similarly, adult females of *T. americana* emigrate deep into the glandular tissue of the proventriculus of fowls while the males remain at the surface (Todd, 1948).

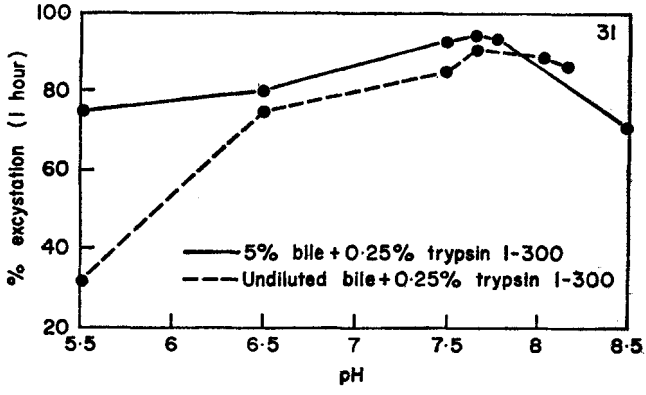
VI. Relationships between the Infective Stages of Parasites and Digestive Physiology

This section of the review is concerned with the role of the digestive physiology of the host in the establishment of parasites whose infective stages enter the host orally. The chances of successful transmission from one host to the next appear to have been increased by the evolution of resting infective stages within toughened egg shells, cysts, cast cuticles or the tissues of the intermediate host. Infection, however, requires the activation of the resting parasite and its release from encasing tissues into a region of the alimentary tract where a favourable environment exists. These requirements appear to be achieved by the relationship between the digestive physiology of the host and the responses of the parasite. The range of infective stages of some protozoan and helminth parasites of domestic birds is summarized in Tables XV and XVI; few observations appear to have been made on the process of infection of domestic birds with micro-organisms.

A. PROTOZOAN PARASITES

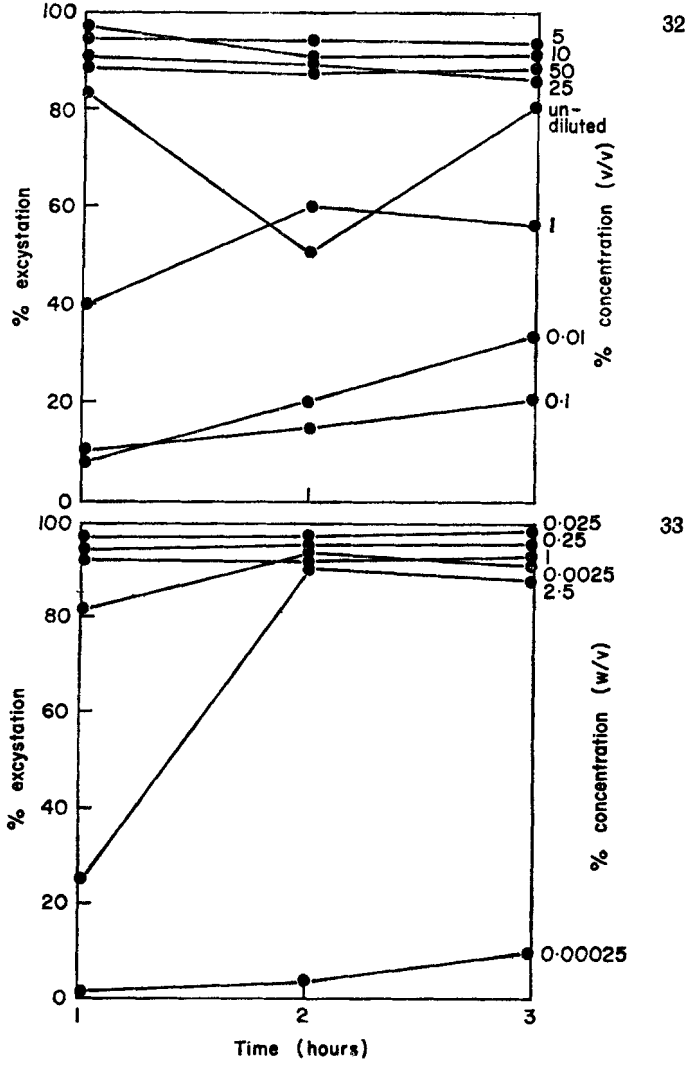
1. Eimeria *spp.*

The wall of the oocyst of *Eimeria tenella* consists of at least two layers. The outer layer of the wall, which forms about 20% of the dry weight of the complete unsporulated oocyst, is composed of carbohydrate and a protein characterized by its high proline content. The main components of the inner layer are protein and lipid (Ryley, 1972). Excystation is the term for the



Figs 31–33. Observations on the excystation of oocysts of *Eimeria acervulina in vitro*. (31) Effect of hydrogen ion concentration on excystation. (32) (upper, p. 159) Effect of bile concentration on excystation. Constant: 0·25% trypsin 1–300; pH 7·5–7·7. Mean values of 2–4 determinations. (33) (lower, p. 159) Effect of trypsin 1–300 concentration on excystation. Constant: 5% bile; pH 7·5–7·6 (Figs 1, 2 and 3 respectively, Doran and Farr, 1962).

biphasic release of the four sporocysts from the oocyst and the two sporozoites from each sporocyst. When sporulated oocysts of *Eimeria hagani*, *E. maxima*, *E. mitis*, *E. necatrix*, *E. praecox* and *E. tenella* were fed to fowls with ligated pancreatic ducts, infections were not established and the oocysts were believed to have passed through the tract unchanged since they could be recovered from faeces and used to infect other fowls (Levine, 1942). Infections of these species of *Eimeria* became established when oocysts were fed to control birds in which the pancreatic ducts were open. By establishing infections from merozoites given to control birds, Levine demonstrated that,



for the duration of the experiments, pancreatic interference had not adversely altered the environments of the parasites within the mucosal cells. He concluded, therefore, that the host's pancreatic juice was necessary for excystation of the oocysts, and Pratt (1937) had already found "spores" (sporozoites) of *E. tenella* in the posterior part of the small intestine of a fowl 20 min after sporulated oocysts had been injected into the duodenum, which had first been ligated at its junction with the pylorus. This experimental approach was extended by Ikeda (1955a,b), who studied the relationship between *E. tenella* and fowls. Infection occurred in hosts in which the bile ducts had been ligated before the oocysts were given. Infections were essentially unsuccessful in fowls with closed pancreatic ducts and open bile ducts, but not in similar fowls which received oocysts suspended in a pancreatic extract, unless the extract or individual enzymes under test had been heated before use (Ikeda, 1956b).

Excystation, however, is a more complex process than is suggested by these experiments. After sporulated oocysts of *E. tenella* were fed to three young fowls, which were killed at intervals of 1 h, 1¾ h and 3 h, the walls of nearly all the oocysts retrieved from the small intestine were observed to be fractured (Goodrich, 1944) and it appeared that ventricular grinding was responsible. Goodrich concluded that, as far as she could make out, after studying thousands of oocysts of different species of *Eimeria*, unless the spores were freed in some mechanical way from the impervious "ectocysts" (oocyst wall) there was little chance of their being able to germinate. Evidence supporting both this opinion and the view of Pratt (1937), Levine (1942) and Ikeda (1955a, b) that pancreatic secretions of the host participate in the release of sporozoites, was obtained from *E. acervulina* and *E. tenella* in fowls and *E. meleagrimitis* and *E. gallopavonis* in turkeys by Doran and Farr (1962) and Farr and Doran (1962). They found sporocysts, but not sporozoites, anterior to the pyloric sphincters of the birds. The efficiency of ventriculus is shown by the presence of sporozoites of *E. acervulina* in the duodenum 15 min after ingestion of the oocysts.

Studies have been undertaken *in vitro* with oocysts which have first been treated by processes designed to simulate ventricular grinding, in attempts to elucidate how the sporozoites escape from the sporocysts. Excystation rates as high as 95–98% occurred with oocysts of *E. acervulina* after incubation for one h at pH 7·3–7·6 in 5% fowl bile and 0·25% trypsin at physiological temperatures (Doran and Farr, 1961, 1962), and some excystation took place after 5–10 min in these conditions. Insignificant rates were observed when pancreatic enzymes, bile or bile salts were tested alone. Hydrogen ion concentration is important and although pH values of 7·3 and above may be uncommon *in vivo* (Table X; Fig. 30) it is clear from Fig. 31 that a considerable number of sporozoites would be expected to be released under duodenal conditions. The optimum concentration of bile in the incubation medium was found to be 5% (Fig. 32); at higher concentrations the sporozoites appeared to clump together and at lower concentrations the excystation rate was reduced. The optimum concentration of trypsin (Fig. 33) was between 0·025 and 1% (s/v), higher concentrations appearing to have a deleterious

effect on the sporozoites. Excystation was obtained *in vitro* not only with trypsin but also with enzyme preparations and mixtures of trypsin and lipase in the presence of a trypsin inhibitor. This finding indicated that in addition to trypsin, other factors which are likely to occur in the digestive secretions of fowls could be involved in the excystation of oocysts of *E. acervulina*, unless the sporozoites themselves produce secretions to aid in escape from the sporocyst. Further experiments *in vitro* confirmed that trypsin and chymotrypsin in the presence of bile, but not carboxypeptidase or lipase, induced the release of sporozoites from liberated sporocysts (Doran, 1966a). Ikeda (1960), however, observed the excystation *in vitro* of oocysts of *E. tenella* in intestinal fluid containing trypsin from fowls, without any pretreatment to affect the wall of the oocyst.

The results from studies *in vitro* of *E. acervulina* correspond with many of the observations made on the process of infection *in vivo* and with knowledge of the digestive physiology of domestic birds (Section III). Many of these findings have been confirmed in principle for *E. acervulina* (Farr and Doran, 1962; Hibbert and Hammond, 1968; Hibbert *et al.*, 1969; Bunch and Nyberg, 1970) for *E. necatrix* (Hibbert and Hammond, 1968; Hibbert *et al.*, 1969) and for *E. tenella* (Farr and Doran, 1962; Nyberg *et al.*, 1968; Hibbert *et al.*, 1969) from fowls and for *E. gallopavonis* and *E. meleagrimitis* from turkeys (Farr and Doran, 1962). There now seems to be reasonable agreement that when healthy domestic birds, which are receiving an adequate diet under normal feeding conditions, ingest sporulated oocysts of *Eimeria* spp., ventricular grinding cracks the wall of some of the oocysts and thus exposes their sporocysts and sporozoites to the activity of bile and pancreatic secretions (Ryley, 1972, 1973; Marquardt, 1973). Under certain circumstances *in vivo*, sporozoites may be observed to have become active within apparently unbroken oocysts (Lotze and Leek, 1968), although the same workers stated that they had inoculated more than 200 fowls with sporulated oocysts of *E. tenella* and observed that large numbers were broken in the ventriculus. Ryley (1972) suggested that bile might activate the sporozoites and alter the properties of the sporocyst wall. It is clear, however, from the work of Doran and Farr (1962) that the rise in environmental temperature experienced by ingested oocysts is important in excystation and this factor may be of primary importance in the activation of the sporozoites.

Various observations and experiments have already been cited in this account to indicate that there are exceptions to the generalization about the excystation of sporulated oocysts in domestic birds. The oocysts of several species of *Eimeria* which infect mammalian hosts have been found to be rendered susceptible to the activity of bile and enzymes after exposure to carbon dioxide (Hibbert and Hammond, 1968; Nyberg *et al.*, 1968; Bunch and Nyberg, 1970; Hammond, 1971). Their work has also established that excystation of oocysts of *E. acervulina*, *E. necatrix* and *E. tenella* will occur *in vitro*, without any mechanical stresses on the oocysts, after prolonged exposure to carbon dioxide. It is possible that carbon dioxide may have this function in domestic birds, particularly in those species with a crop in which the diet may be retained for several hours (Table IX; Heuser, 1945; Bunch

and Nyberg, 1970). Perhaps carbon dioxide or some unrecognized manipulative factor was involved when *E. tenella* became established in fowls in which the oocysts had not passed through the ventriculus (Pratt, 1937; Ikeda, 1956a).

2. *Genera other than* Eimeria

In a study of the oral infection of fowls with caecal protozoa, one cyst of *Chilomastix gallinarum*, about 240 cysts of *Endamoeba* (= *Entamoeba*) *gallinarum* and about 200 000 trichomonads of *Trichomonas* (= *Tetratrichomonas*) *gallinarum* (Table XV) were found to be the minimum infective doses required to establish the parasites in the caeca of fowls (Richardson, 1934). These observations demonstrate the protective properties of a cyst and the vulnerability of naked protozoa to detrimental conditions in the anterior part of the tract. It is possible that pepsinogen secretion in the proventriculus or low pH (Table X) destroys many of the trichomonads. Normally *Histomonas meleagridis* passes through the anterior part of the tract of its host within the resistant egg of the nematode *Heterakis gallinarum* (Lee, 1971), but infections can become established if the flagellate stage from the caecal lumen is swallowed by fasting fowls or by feeding fowls which are given alkaline material immediately after the parasites (Horton-Smith and Long, 1956).

Trichomonas gallinae is highly pathogenic in young pigeons and to a lesser extent in other domestic birds (Table XV; Stabler, 1947, 1954). Initially, the parasite inhabits the mouth, oesophagus and crop, and the trichomonads are transferred directly to the squabs in secretions of the crop of the parent bird (Stabler, 1954), without exposure to any adverse environmental conditions.

B. HELMINTH PARASITES

1. *Infective eggs*

Relatively few investigations have been made of the process of infection of domestic birds apart from studies on the nematodes *Ascaridia galli* and *Heterakis gallinarum* from fowls (Table XVI). The eggs of *A. galli* hatch in the duodenum (Ackert, 1931), the egg-shell having protected the larval nematodes from adverse conditions in the region of the tract anterior to the pylorus, although the elevated temperature in the avian host may have been having some undetected effect. Eggs of *A. galli* sometimes hatch in damp conditions, but infections are rarely established when fowls are fed larval stages which must pass through the proventriculus and ventriculus (Ackert, 1931; Hansen *et al.*, 1956). Normal infections are established when either the eggs or the larvae, which have been obtained from eggs *in vitro*, are injected by surgical techniques directly into the duodenum of a fowl (Hansen *et al.*, 1956). Some of the physico-chemical conditions of the duodenum (Section III) can be simulated *in vitro* and used to initiate the hatching of the eggs (Rogers, 1960; Fairbairn, 1961; Hass and Todd, 1962). A high precentage of eggs of *A. galli* will hatch *in vitro* after exposure at 37°C to bicarbonate–

carbon dioxide buffers containing reducing agents under a mixture of nitrogen and carbon dioxide, provided that the concentration of undissociated carbonic acid is about $0{\cdot}25 \times 10^{-3}$ M at pH 7·3. Rogers exposed eggs to these conditions for 3 h, a time which may be much longer than many eggs will spend in the duodenum *in vivo* (Table IX; Figs 28 and 29). These complex conditions probably function as a stimulus since the larvae of related nematodes have been observed to escape from their egg-shells on being transferred, after a short period in a similar experimental solution, to physiological saline (Rogers, 1960). The stimulus initiated the activity of an enzymatic secretion, which attacks the inner side of the egg-shell (Fairbairn, 1961; Rogers, 1963) after having passed through the impermeable vitelline membrane (Fairbairn, 1961). The larvae are motile by this stage and the figures of Ackert (1931) depict how they force their way through the altered shell. The larval stages of *H. gallinarum* escape from the egg-shells in the duodenum, or anterior small intestine (Uribe, 1922; Clapham, 1933; Roberts, 1937). The exposure of the eggs *in vitro* to a medium at 40°C containing sulphur dioxide, sodium chloride and sodium bicarbonate under an atmosphere of 5% carbon dioxide and 95% nitrogen, resulted in the hatching of 70% to 95% of the sample (Hass and Todd, 1962).

2. *Larval stages*

The metacercaria of the trematode *Sphaeridiotrema globulus* (Table XVI) consists of the larval parasite surrounded by four layers of varying properties and thicknesses, embedded in the tissues of a snail (Macy *et al.*, 1968). When natural infections of ducks and geese occur, the metacercariae will be exposed to a temperature of about 42°C, and it may be assumed that ventricular grinding will smash the gastropod's shell and expose the tissues to the activity of pepsin at a relatively low pH. The outer layer of the metacercarial cyst was found to be attacked by 1% solution of acid pepsin, and the other layers by 1% solution of trypsin at pH 7 or 8 and temperatures of 37–42°C. The evidence from these studies *in vitro* also indicated that the activation of the parasite, a process which led to its emergence through the partially digested, enveloping layers, was pH dependent. Macy *et al.* (1968) noted that, after exposing metacercariae for 30 min in 1% acid pepsin, the parasites became active on subsequent treatment with 1% trypsin at pH 8·0, but not if the pH of the trypsin solution was lowered to 7 and the treatment continued for 8 h. The paper of Macy *et al.* also contains an attempt to integrate the process of infection in ducks, geese and fowls with the results of the *in vitro* studies. The escape of the parasite from the cyst in the host appears to depend on changes occurring in the cyst wall after exposure to the activity of pepsin and trypsin for periods related to intestinal motility, which is influenced in turn by many factors (Table IX). It is clear that alterations to the feeding routine and diet of domestic birds affect the excystation or establishment of the trematodes *Euhaplorchis californiensis* (Wong, 1954) and *Acanthoparyphium spinulosum* (Little, 1970) in the tract of fowls (Table XVI). Recently, Asanji and Williams (1974) have studied the excystation of five species of digenetic trematode in the tract of young fowls and have described some degree of correlation between

the region of the tract in which excystation was considered to occur and the digestion of the layers of the metacercarial cysts.

Some metacercarial stages have been observed to respond to temperature, and the strigeid *Holostephanus lühei* (Table XVI) became active and revolved within its cyst in warm saline (Erasmus, 1962). The excystation of *H. lühei* was promoted within 30 min to 1 h *in vitro* after placing metacercariae, which had been obtained by incubating the tissues of infective fish in a solution of acid pepsin, in saline containing 1% sodium bicarbonate, 1% trypsin and 0·5% sodium "tauroglycocholate" at 37°C. Similar conditions were found to contribute to the excystation *in vitro* of *Cyathocotyle bushiensis* (Table XVI), and the presence of bile salt increased the rate of excystment (Erasmus and Bennett, 1965). Some infective stages of digenetic trematodes are not encysted in a complex manner in the tissues of their intermediate hosts, although ventricular grinding and the digestive enzymes of the definitive hosts probably contribute to the release of the parasites into the alimentary tract. In the case of *Diplostomum phoxini*, which matures in ducks, incubation of the infected tissues of the intermediate host in saline at 40°C resulted in the emergence of the parasites (Bell and Hopkins, 1956).

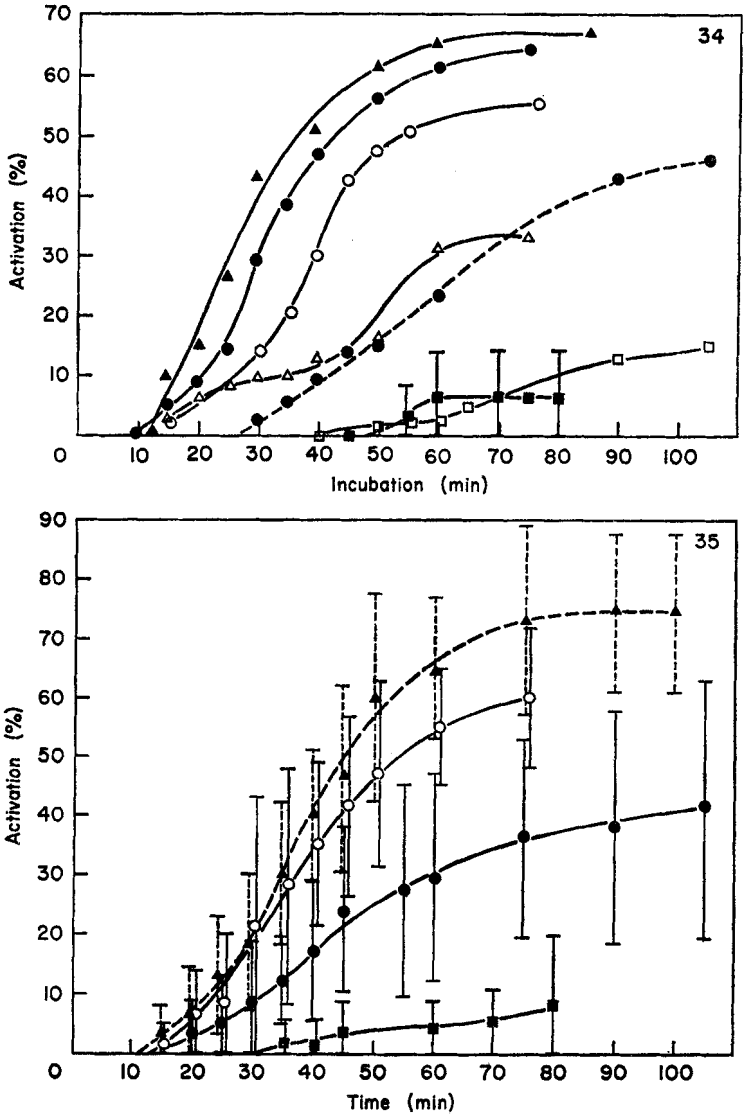
The process of infection of domestic birds with cestodes and acanthocephalans often involves the evagination of an inverted attachment organ, but if evagination were to occur in the contracting ventriculus it is possible that the parasite would be damaged. The scoleces of cysticercoids of the cestode *Hymenolepis exigua* were observed not to be everted in the tract of week-old fowls until the parasites had passed out of the ventriculus and reached the duodenum (Alicata and Chang, 1939). *In vitro* evagination of cysticercoids of the cestode *Raillietina cesticillus* occurred more readily in saline at physiological temperature in the presence of bile salts than in their absence (Edgar, 1941). The most successful evagination of the scoleces from cysticercoids of *R. kashiwarensis* was obtained by incubation at pH 7 and a temperature of 40–42°C in saline containing 0·1% pancreatin (Sawada, 1959), which is a crude preparation containing amylase, trypsin, lipase, ribonuclease and other enzymes. Trypsin appeared to be the most important of the enzymes and evagination did not occur at room temperature or as readily at lower pH. Extrapolation of Sawada's results suggests that evagination would not occur if the pH were to be adjusted to that of the ventriculus of fowls (Table X). All these observations, and the fact that *R. cesticillus* becomes attached initially to the mucosa of the anterior part of the small intestine of fowls (Foster and Daugherty, 1959; Gray, 1972b), lend support to the hypothesis that postpyloric conditions in the tract are important in the establishment of cestodes and acanthocephalans in birds.

The proboscides of cystacanths of the acanthocephalan *Polymorphus minutus* were never observed to become everted until they had passed out of the duck's ventriculus, which sometimes crushed them (Lingard and Crompton, 1972). Passage through the region of tract anterior to the pylorus was found to be unnecessary for eversion and the establishment of infection, apart from liberating the cystacanths from the tissues of the intermediate host, *Gammarus* spp. Proboscides became everted when cystacanths were

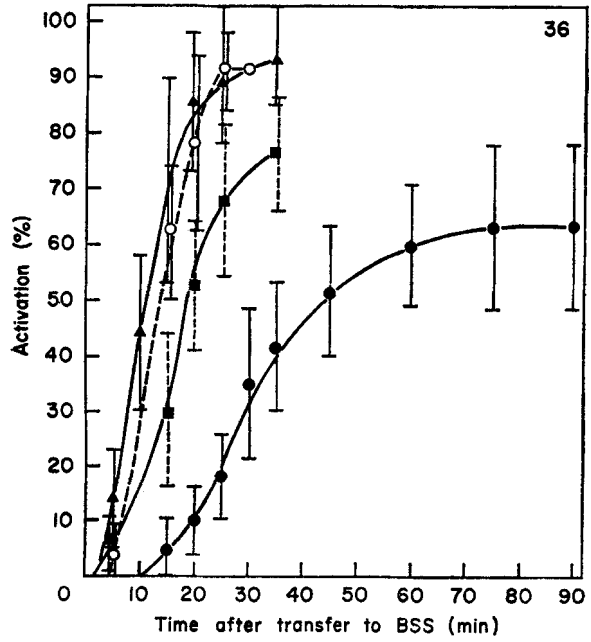
introduced directly into the small intestine through a perspex cannula. These findings led to a detailed investigation *in vitro* of the factors involved in the activation of cystacanths of *P. minutus* (Lackie, 1974a,b). Under the conditions used, the optimum temperature for activation was found to be 42–44°C (Fig. 34), the optimum pH was 7·0 (Fig. 35) and a significant enhancement in the rate of activation was observed when cystacanths were exposed for a few minutes to bile, bile salts (Fig. 36) or chromatographically pure sodium taurocholate. Lackie also studied the effect of the osmotic pressure of the medium on activation (Fig. 37), a factor which is likely to be important for invertebrate animals whose movements depend on a hydrostatic skeleton. These observations on activation *in vitro* correspond fairly closely with the observations made *in vivo* (Lingard and Crompton, 1972) and with the physico-chemical conditions prevailing in the duck's small intestine (Crompton, 1969, 1970). Although activation will occur in the absence of bile salts, by the time the cystacanths have been propelled to the site of the adult worms (Table XVI; Fig. 14) the proboscides of relatively few cystacanths would have become everted. The increase in rate of activation in the presence of bile salts (Fig. 36) may explain how a greater number of cystacanths are ready to become attached to the mucosa by the time they have been carried to the site of the adult parasite.

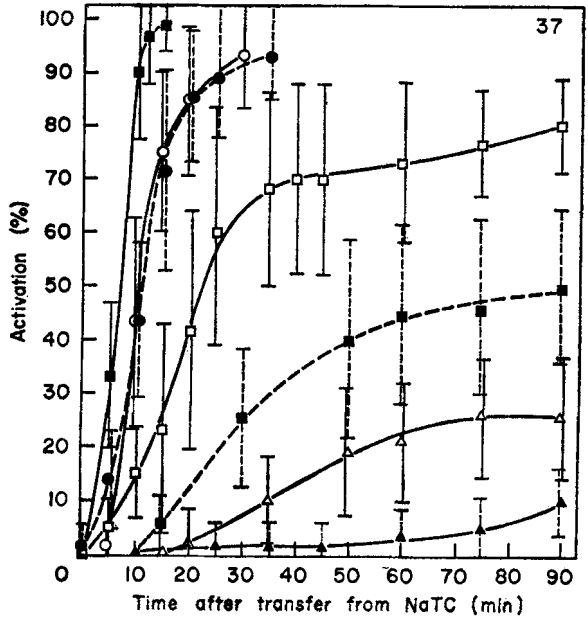
VII. Further Relationships between Parasites and the Digestive Physiology and Nutrition of Domestic Birds

In an attempt to simplify a complicated subject in which much of the research effort appears to have been concerned with the accumulation of primary observations and not with attempts to discover the basic mechanisms involved, we have classified information into two categories depending on whether the parasite or the host was the object of the investigation. This abritrary division tends to ignore the fact that, in the context of this review, the term "parasite" is a relative one which is of no significance without the host and vice versa. That conclusion is well illustrated by the immune responses of hosts against parasites. Although immune responses are evoked by some parasites of the alimentary tract of domestic birds, they have not been discussed in this review since their effects on digestive physiology and nutrition are indirect. For example, in a study of a possible relationship between dietary vitamin A and *Eimeria acervulina* and *E. tenella*, Erasmus *et al.* (1960) presented measurements showing that experimental chicks weighed 252 g on average at the time of infection and control chicks weighed 259 g. After a week, the infected birds had gained 10 g while the uninfected birds had gained 32 g, but during the next fortnight the infected birds gained a further 325 g as compared with 323 g by the uninfected birds. This recovery in the growth rate of the infected fowls is probably correlated with the effectiveness of the host's immune response (Farr, 1943; Reid, 1972), which may have been followed by a return to normal digestion and absorption of nutrients. The explanation does not lie in the ingestion of more food by the infected birds (Erasmus *et al.*, 1960).



FIGS 34–37. Observations on the activation of cystacanths of *Polymorphus minutus in vitro*. (**34**) The effect of temperature on cystacanth activation. □———□, 49°C; ●- - - -●, 48°C; ○———○, 47°C; ▲———▲, 44°C; ●———●, 42°C; △———△, 40°C; ■———■, 39°C. (**35**) Effect of pH on cystacanth activation. ○, pH 7·4; ▲, pH 7·0; ●, pH 6·7; ■, pH 6·0. (**36**) Effect of different concentrations of NaTC (sodium taurocholate) on cystacanth activation. ▲, 0·05% NaTC; ○, 0·01% NaTC; ■, 0·002% NaTC; ●, control. (**37**) Effect of osmotic pressure on activation. ■———■, 100 mM; ○———○, 150 mM; ●- - - -●, BSS; □———□, 180 mM; ■- - - -■, 190 mM; △———△, 220 mM; ▲———▲, 250 mM. Osmolarity of solutions expressed as equivalent to mmoles NaCl/l. Bars show standard deviations in all figures. (Figs 1, 2, 3 and 6 respectively, Lackie, 1974a.)


36
Activation (%)
Time after transfer to BSS (min)


37
Activation (%)
Time after transfer from NaTC (min)

A. HOST EFFECTS ON PARASITES

1. *Feeding routine of the host*

Some observations indicate that interference with the feeding routine of domestic birds may affect intestinal parasites in a variety of ways. For example, domestic fowls, from which food was withheld for about 12 out of every 24 h, appeared to be more favourable hosts for *E. tenella* than were fowls offered food *ad libitum* (Edgar and Herrick, 1944). On the other hand, the fasting of fowls, infected with the cestode *Davainea proglottina*, for 24 h, was accompanied by a decrease in the rate of explusion of proglottides from the host (Levine, 1938b). Nearly the entire strobila of *Raillietina cesticillus* was lost when food was withheld from fowls for up to 48 h (Reid, 1942a,b). The scoleces of some of the cestodes were still present in the host after 20 days' fasting, but their growth and that of newly established *R. cesticillus* were arrested until food was given to the host. The explanation for these observations is probably related to the parasite's requirement of carbohydrate (von Brand, 1973; see later).

The acanthocephalan *Polymorphus minutus* is also adversely affected within a short time when food is withheld from domestic ducks (Hynes and Nicholas, 1963). Four adult *P. minutus* out of a possible 90 worms were recovered from three ducklings which had fasted for 48 h before being killed, and 34 adults out of a possible 87 were recovered from three hosts which had not fasted. In another experiment in which two ducks each ingested 200 *Gammarus pulex* infected with *P. minutus*, two 3-week-old worms were recovered from the host which had fasted for at least 24 h before infection, and 181 3-week-old worms were recovered from the host which had been offered food. This observation is difficult to interpret; 200 amphipods probably constitute a large enough meal to stimulate the release of bile and any other secretions involved in the process of infection (Lackie, 1974a).

The naturally acquired feeding routine of the host appears to affect the release of proglottides by adult cestodes. Reid *et al.* (1938) observed that nearly all proglottides of *R. cesticillus* were to be found in faeces discharged by fowls in the afternoon and evening. Levine (1938b) found that about 80% of proglottides of *D. proglottina* were contained in the faeces discharged during the last 5 h of daylight. After artificially reversing the time for which the hosts were exposed to light, he concluded that the discharge of proglottides depended upon the feeding time and activity of the host and not on any innate characteristics of the cestode. The observations of Reid *et al.* (1938) and Levine (1938b) confirmed earlier reports by Wetzel, whose work they discuss.

2. *Nutrition*

There is a paucity of information on nutritional requirements of parasites inhabiting the alimentary tract of animals. One factor contributing to this lack of information is the difficulty of culturing these organisms in chemically defined media which are necessary for careful nutritional studies. Information on the parasites' nutritional requirements can only be indirectly obtained by

observing the establishment, growth and reproduction of parasites in hosts fed diets lacking in nutrients. The results of such studies are often difficult to interpret because the nutrient deficiencies also have marked effects on the physiology of the host. It seems probable, however, in view of the well established requirement for vitamins, amino acids and inorganic elements by many organisms (see Beerstecher, 1964), that many nutrients required by parasites will be found to be similar to those needed in the diet of the host.

In cestodes, particularly *Hymenolepis diminuta*, from the rat's small intestine, host dietary carbohydrate has been shown to be very important for growth and development of the parasite (Mettrick and Podesta, 1974). Such detailed information is not available for parasites of domestic birds, although it is likely that a similar requirement for carbohydrate may be shown for avian cestodes.

Twenty hours of starvation of the fowl were sufficient to reduce the glycogen stores of *Raillietina cesticillus* to one eleventh of the level in worms from fully fed hosts (Reid, 1942a,b). The glycogen level in the worms varied with time of day, being highest at 6 p.m. and lowest at 6 a.m., presumably because of the low feed consumption by the host during the night. Levine (1938b) also observed that segment discharge from fowls infected with *Davainea proglottina* was reduced by feeding the hosts diets made up of sawdust, or high proportions of wheat bran and cellophane. Although these diets are low in available starches and sugars, other factors than carbohydrate may have influenced Levine's results.

There are similar problems in the interpretation of studies by Little (1970) on the influence of various dietary alterations of fowls infected with the trematode *Acanthoparyphium spinulosum*. More flukes were recovered from hosts fed a diet with starch rather than sucrose as the principle carbohydrate. However, when a high sucrose diet lacking in protein or vitamin A was fed, worm recoveries were as good as when starch was the source of carbohydrate. The diets used by Little may have been lacking in some nutrient, because chicks died after a few days of feeding a diet in which sucrose was the carbohydrate or when no carbohydrate was fed. This seems unusual; in our experience young fowls normally grow well when feeding on purified diets containing sucrose. These details are mentioned to emphasize that adequate control of the host's nutrition is essential before effects on parasites can be interpreted.

Some early studies on the influence of diet on parasitism in the alimentary tract were made by Ackert *et al.* (1931) and Ackert and Nolf (1931). Groups of young fowls were fed diets with or without added cod-liver oil or yeast and were infected with a known number of eggs of *Ascaridia galli*. The number and sizes of worms recovered were greater in chicks fed the diets without cod-liver oil; the chicks without the cod-liver oil showed signs of vitamin A deficiency. In birds fed the diets without yeast, greater numbers of worms were recovered, but they were of similar size to those recovered from birds receiving yeast. The diet of chicks receiving no yeast was probably deficient in several of the B-complex vitamins. Folic acid deficient fowls were also found to be better hosts for *A. galli* by Sadun *et al.* (1950). In chicks fed the folic acid deficient

diet, an average of 17·3 worms was recovered, while 4·9 worms were found in birds receiving 200 μg of supplemental folic acid per 100 g of diet. Similarly, young fowls fed a pyridoxine deficient diet were more susceptible to infection with *A. galli* than chicks fed adequate amounts of vitamin B_6 (Brody, 1954).

No mechanism has been found to explain how vitamin deficient hosts might provide a better environment for parasites, although the authors postulated that the deficient diets may have lowered host resistance to the parasite. In the studies cited above, the hosts fed the deficient diets were reported to grow more slowly than controls. No feed consumption measurements were reported, but it is very likely that food intake of the deficient chicks was lower than that of the controls in each case. A very useful control in such studies would be a pair-fed group that received the same amount of food as was consumed by the deficient birds. Although the nutrient deficiencies may have been the primary factor affecting the biology of the parasite, reduction in food intake and consequent effects on the rate of food passage (see Section III) cannot be excluded as a cause for the effects observed.

When a vitamin B_{12} deficient diet was fed to chicks, relatively little growth reduction was observed compared to control birds, and the deficient birds were not appreciably better hosts for *Ascaridia galli* than the controls (Hansen *et al.*, 1954). In these experiments, worms from birds fed a diet supplemented with vitamin B_{12} were significantly larger than those from the deficient hosts. This may indicate that vitamin B_{12} is required by *A. galli*. A similar requirement for calcium and phosphorus by *A. galli* may exist. Gaafer and Ackert (1953) recovered 3·3 worms of 13·3 mm average length from chicks fed a diet low in phosphorus but high in calcium, compared to 9·7 worms of 21 mm in lengths from birds fed a diet with normal levels of calcium and phosphorus. When a calcium deficient diet was fed to young fowls, an average of 5·5 worms, 15·8 mm long, was recovered compared with 11·8 worms, 25·4 mm long, from hosts receiving adequate amounts of calcium.

A number of dietary changes aimed at investigating the influence of various protein sources or amino acid supplements on the establishment and growth of *A. galli* have been reported (Riedel, 1950, 1954a,b, 1955; Riedel and Ackert, 1950, 1951; Todd, 1951). In general, no consistent effects have been observed, although evidence of amino acid deficiency in the basal rations studied is often lacking. Some alterations of dietary protein sources have been found to affect the number and growth of *A. galli* recovered, but usually multiple changes were made in the diet and no assessment can be made about the nutritional factors involved.

Dietary factors affecting coccidiosis in domestic birds have been reviewed by Joyner (1963), and the relationship of vitamin A and coccidiosis is discussed elsewhere (p. 173). A relationship between dietaryprote in levels and infection with *Eimeria tenella* was found by Britton *et al.* (1964). Young fowls, fed diets either low or free of protein, were very poor hosts in experimental infections. Trypsin activity was found to be low in the intestine contents of chicks fed the low protein or protein-free diets. The authors related the low rate of infection in chicks fed the low protein diets to the lack of sufficient trypsin activity needed to contribute to the excystation of the oocysts. When trypsin

was added to the inoculum of oocysts, no influence of dietary protein on the infection was observed. In a similar study, low levels of dietary calcium resulted in lower mortality of young fowls from coccidiosis (Zucker, 1964), an effect attributed to the role of calcium in trypsin activation.

B. PARASITIC EFFECTS ON HOSTS

1. *General effects on digestive physiology*

The literature on the alimentary tract of domestic birds abounds with qualitative descriptions of the damage and destruction which result from the combined effects of host responses to parasitic activity. In addition to the pathological conditions associated with *Histomonas meleagridis* (Lund, 1972) and *Eimeria* spp. (Reid, 1972), protozoa of the genera *Cochlosoma*, *Hexamita* and *Trichomonas* have been found to have deleterious effects on the tracts of domestic birds. Infections of *Cochlosoma* sp. (Table XV) in turkey poults are associated with oedema, dilation and loss of tone in the wall of the small intestine, catarrhal enteritis on the mucosal surface, diarrhoea and anorexia (Campbell, 1945); and infections of *Hexamita meleagridis* (Table XV) in the same host have been described in the same terms (Hinshaw *et al.*, 1938). Virulent strains of *Trichomonas gallinae* (Table XV) penetrate the wall of the upper alimentary tract of pigeons and probably gain access to other parts of the host through the vascular system (Mesa *et al.*, 1961). Great destruction and necrosis of the wall of the oesophagus and crop occurs in turkeys infected with *T. diversa* (Hawn, 1937).

Irritation, haemorrhage, ulceration, laceration, congestion and desquamation are some of the words used in descriptions of the effects of helminths on the alimentary tracts of domestic birds, and leukocytic infiltration, chronic catarrhal enteritis and hypersecretion of mucus are some of the terms used to describe the responses of the host. It appears, however, that those helminths which infect the region of the tract anterior to the pylorus (Table XVI) are the most pathogenic, probably because they inhabit the tissues of the tract wall rather than the lumen. Sometimes, the combined effect of the numbers of worms present and the inflammatory response of the host is to reduce the diameter of the lumen so that food becomes impacted (Buxton *et al.*, 1952). The koilin lining (Fig. 20) of the ventriculus of domestic geese is destroyed by the burrowing of *Amidostomum anseris* beneath it (Jerstad, 1936).

More subtle effects probably exist. For example, Hurwitz *et al.* (1972a) found that *A. galli* seemed to cause a greater secretion of nitrogen into the duodenum than was detected in uninfected fowls, but there was no obvious effect on the digestion of protein and absorption of amino acids by the host. Activities of trypsin, chymotrypsin and amylase were reduced in the intestinal contents of infected birds compared to controls, but these reduced enzyme levels apparently had no influence on digestion (Hurwitz *et al.*, 1972b). Some effects of parasites on alimentary physiology in the fowl have been reviewed in detail by Crompton (1976).

2. *Growth and nutrition*

(a) *General effects.* Uninfected chicks of various breeds grew from 2·3% to

32·8% faster than those infected with the cestode *Raillietina cesticillus* (Harwood and Luttermoser, 1938), while fowls infected with *Davainea proglottina* were always somewhat lighter than uninfected controls (Levine, 1938c). Another cestode *Hymenolepis carioca*, did not appear to affect growth of fowls at the level of infection studied (Luttermoser, 1940). Young fowls infected with the nematode, *Ascaridia galli*, grew about 20% more slowly than uninfected controls (Ackert and Herrick, 1928), while chicks infected with a controlled infection of *A. galli* grew about 8% more slowly than controls (Hurwitz *et al.*, 1972a). A similar general effect on the growth of the host is also caused by *Eimeria* spp. (see below) and by the presence of a microflora in the alimentary tract of the host (Section IV). The reasons for the slower growth rate of fowls harbouring parasites in their alimentary tracts are not understood. The reduced growth rate would be expected to be accompanied by reduced food consumption, but food intake has not always been measured in studies of this type.

Single or mixed infections with several species of *Eimeria* (Table XV) cause severe damage to the alimentary tracts of domestic birds (see Reid, 1972), and the absorption and utilization of several nutrients are often impaired. The reduction of food and water intake associated with coccidial infections (Reid and Pitois, 1965) results in a reduction in the intake of all nutrients required by the host. These changes in food intake must be considered in investigations of the influence of coccidial infections on nutritional requirements, but studies have often been undertaken in which no measurement of food intake are given. In a typical course of a controlled coccidial infection in the laboratory, a fall in food intake is observed to occur 5–7 days after the host has ingested the oocysts, and weight gains are reduced markedly compared with non-infected controls (Reid and Pitois, 1965; Sykes, 1970). After 2–3 days of reduced food intake, the infected birds that recover, rapidly increase food intake so that by the 9th–10th day of the infection the hosts consume as much or more than the controls. Turk (1972) pointed out that young fowls recovering from coccidial infections frequently gain weight more rapidly than uninfected birds, and that they may weigh as much as control chicks by 4–6 weeks after the start of the infection. During the acute stages of the infection, fowls seem to make use of dietary energy rather inefficiently because pair-fed controls gain more weight than infected birds, even though they receive the same amount of feed (Erasmus *et al.*, 1960; Sykes, 1970). Measurements of the metabolizable energy value of diets being consumed by birds in the acute stages of a coccidial infection have not been reported, so that it is not possible to determine if the reduced efficiency is due to impaired digestion or to an increased energy need of the infected birds.

Turk (1974) has recently summarized his extensive studies on the absorption of nutrients by young fowls infected experimentally with *Eimeria* spp. The observed effects of the parasite depended upon the nutrient studied, the site of the parasites (Table XV), the developmental stage of the parasite, and the intensity of the infection. When young fowls which harboured *E. acervulina* in the duodenal tissues, were given a dose of radioactive ^{65}Zn, only about a third as much radioactivity compared with controls appeared in the blood

during the acute phase of the infection. Reductions in blood levels of radioactivity of a similar magnitude were observed when radioactive oleic acid or calcium were administered, while uptake of radioactivity from protein and amino acids was relatively unaffected by the parasite. Infection with *E. necatrix*, which inhabits the mid-portion of the small intestine, resulted in a 40–70% reduction in blood radioactivity during the acute phase when radioactive zinc and protein were given, but the reduction in uptake of radioactive calcium and oleic acid was only about 25–35% compared with results obtained from uninfected controls. When infections with *E. brunetti* and *E. tenella* inhabiting the posterior part of the intestine and the caeca were studied, only slight effects were observed on the transfer of radioactive zinc, oleic acid, protein and amino acids to the blood. Uptake of radioactive calcium was slightly reduced by infection with *E. brunetti*.

A common finding in many of Turk's (1974) studies was an increased rate of uptake of several nutrients by birds recovering from coccidial infections, particularly by about 10 days after inoculation with oocysts when tissue repair was probably occurring. In some cases, a striking feature of infection with coccidia appears to be a marked increase in the time required for food to pass through the intestine (Aylott *et al.*, 1968; Fig. 38). When young fowls were infected with *E. necatrix*, the passage time increased as the dose of oocysts given was increased, and was double that of controls during the acute phase of the disease. Similar, but less striking effects, were observed with infections of *E. maxima* and *E. tenella*. These changes in rate of passage are consistent with the reduced motility of the crop, small intestine and caeca of fowls infected with *E. tenella* (Schildt and Herrick, 1955).

(b) *Vitamin A and parasitic infections.* The severity of infections of *Eimeria* spp. and the vitamin A status of the host have been the subject of several investigations. Much of this work seems to have been prompted by the association of vitamin A deficiency with damage to the epithelial tissues of the body (Scott *et al.*, 1969). Since coccidiosis damages the intestinal mucosa, the vitamin A status of the fowl might be expected to influence both the degree of the damage during the acute phase of the disease and the repair of the tissues once it has passed. This notion has been tested on several occasions (Erasmus *et al.*, 1960; Waldroup *et al.*, 1963; Pande *et al.*, 1964; Singh and Donovan, 1973), but the results are difficult to interpret. The observations seem to demonstrate the following relationships. When chicks receiving diets deficient in vitamin A are inoculated with oocysts of cultures containing *E. acervulina* or mixtures of *E. acervulina* and *E. necatrix* or *E. tenella*, mortality is usually greater among infected birds than uninfected birds fed the same diet. The storage of vitamin A in the liver is usually less in infected birds compared to uninfected birds receiving diets with comparable vitamin A levels even when food intake is the same. Coles *et al.* (1970) and Singh and Donovan (1973) considered that oocyst production was greater from infected chicks receiving low levels of vitamin A in their diet than from infected chicks receiving higher levels of the vitamin. Some results suggest that the reduction in growth rate and feed consumption associated with coccidiosis is alleviated to some extent by high dietary levels of vitamin A in the diet of the infected host. The

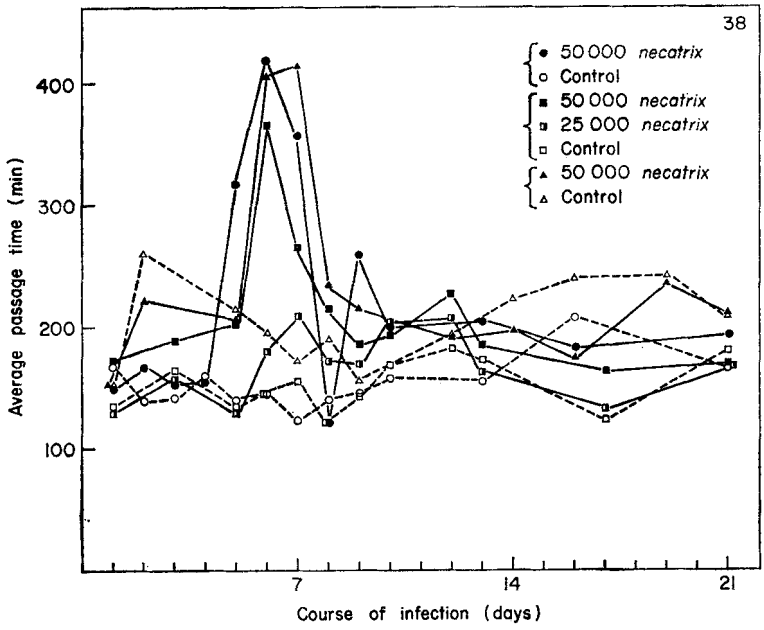


FIG. 38. Graphical representation of the effect of *Eimeria necatrix* on the rate of passage of material down the alimentary tract of the domestic fowl. The figure was prepared from tabulated observations of Aylott *et al.* (1968); consult paper for details of variation within groups of birds.

evidence for this conclusion is not very convincing and the effects reported are relatively small. There seems to be some evidence, however, to indicate that a relationship exists between the vitamin A status of the host and the effects of infection with some species of *Eimeria.*

The absorption of vitamin A and carotene into the intestinal wall of the anterior part of the small intestine was shown to be lowered on the 5th day following infection of young fowls with *E. acervulina* (see Kouwenhoven and Van der Horst, 1969, 1972). The low pH of the intestinal contents during the acute phase of the infection was believed to be involved in the poor absorption of carotene and vitamin A. The reduced absorption of vitamin A by infected fowls may explain the apparent increase in the requirement of infected fowls for this vitamin.

Infection with the nematode *Capillaria obsignata* (Table XVI) has been observed to be associated with reduced liver stores of vitamin A and with reduced levels of carotenoids and vitamin A in blood plasma (Chubb *et al.*, 1964). The damage to the intestinal wall by the parasite and the changes in vitamin A and carotenoid concentration were correlated.

VIII. CONCLUSION

The results of studies on the distribution, process of infection and nutrition of organisms living in the alimentary tract of domestic birds have demon-

strated, in a relatively superficial manner, something of the complexity of the various host–parasite relationships. The evidence shows that many parasites are dependent on the digestive physiology of their hosts and that some of the inhabitants of the tract also appear to be dependent on the activities of others, although it is not clear whether this dependence is direct or mediated through the host or some other environmental factor. The nature of the alimentary tract as an environment needs further investigation, and more studies should be undertaken to discover how the digestive physiology of the host and the intraluminal conditions of the tract are affected by dietary changes and by parasites. Information of this type will facilitate both the design of experimental studies of host–parasite relationships and the *in vitro* cultivation of protozoan and metazoan parasites under chemically defined conditions. Satisfactory methods for *in vitro* cultivation are a pre-requisite of direct nutritional studies of parasites.

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Nematode Sense Organs

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I. Introduction

Nematodes have been found to respond to a wide range of physical and chemical stimuli and must, therefore, possess a variety of sensory receptors coupled with a high degree of nervous co-ordination. Owing to the limitations of the light microscope (LM), early studies on nematode sense organs were principally confined to the larger parasitic species such as *Ascaris* and *Parascaris*, which could be readily sectioned for microscopical examination. These pioneer observations were most creditable, however, and have provided a sound basis for a subject which has only recently come to the forefront of research into nematode physiology. The introduction of the

electron microscope (EM) as a laboratory tool has extended these early observations to a wide range of smaller nematodes, so that structures whose existence was merely assumed on the basis of comparison with *Ascaris* have now been identified. Electron microscopy has also revealed the existence of several hitherto unrecognized sensory receptors.

In this review I have included detailed accounts of the various types of nematode sense organs recognized to date, and this has, in some instances, necessitated the introduction of unpublished preliminary information. I have also attempted to correlate the ultrastructural observations with cytochemical and behavioural studies, in order to assess the possible functional significance of individual receptors.

II. Cephalic Sense Organs

A. Labial and cephalic papillae

1. *Morphological studies*

In the primitive state the nematode mouth is surrounded by six lips; two of these are lateral, two sub-dorsal and two sub-ventral in position. A number of papillae are associated with these lips but there are two schools of thought as to the generalized distribution of these sense organs: (i) de Coninck, 1942, 1950, 1965; (ii) Chitwood and Wehr, 1934; Chitwood and Chitwood, 1950. These differing views arise from conflicting theories on the evolution of the Nematoda, and the arguments for and against these theories should be consulted in the original. The plan suggested by de Coninck (1942) has generally gained the widest acceptance and this proposes a total of 16 papillae or setae, arranged in three concentric circles around the mouth. Each of the six lips thus bears an inner and an outer labial papilla, and the four additional papillae are situated behind the lips (Figs 1 and 2). In marine nematodes these papillae often take the form of long setae or bristles, but in terrestrial and parasitic forms fusion and/or reduction in size of the papillae often occurs, and this results in modification of the basic hexaradiate symmetry.

The vast majority of early publications on labial and cephalic papillae were concerned merely with the observed arrangements in selected species. Goldschmidt (1903) examined the papillae of *Ascaris* in more detail, however, and recorded structural differences between the outer labial and the cephalic papillae of this worm; he also noted that the cuticle covering these sense organs is appreciably thinner than elsewhere on the head. According to Goldschmidt, in the region of each outer labial papilla a nerve fibre approaching the head of the worm becomes thinner, and then terminates in a dilated tip beneath the surface layer of cuticle. The cephalic papillae differ, however, in that the nerve fibre forms a lenticular swelling beneath the cuticle, before giving rise to a sensory hair which enters a narrow canal in the outer cuticle. In both cases the nerve fibre was reported to be accompanied by two non-nervous cells; an inner "supporting cell" which surrounds the fibre, and an outer "escort cell".

With the advent of electron microscopy the labial and cephalic papillae of

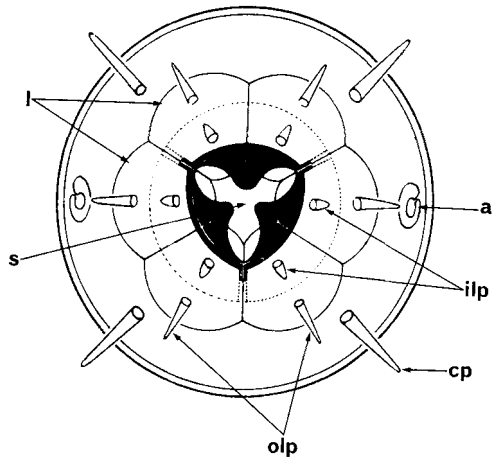
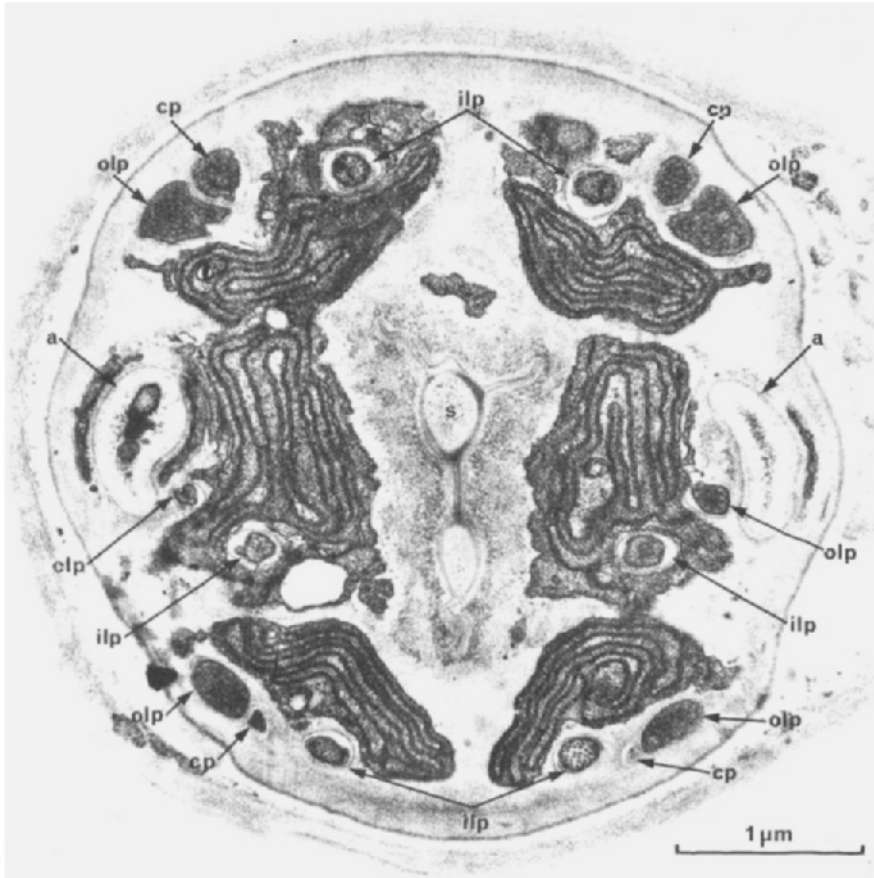


FIG. 1. *En face* diagram of a nematode head showing the symmetrical distribution of the amphids (a), inner labial papillae (ilp), outer labial papillae (olp) and cephalic papillae (cp). Lips, l; stoma, s. (From de Coninck, 1965.)

several free-living and parasitic nematodes have been re-examined at the ultrastructural level. In every case the papillae have been found to be innervated by one or more nerve axons which usually terminate as modified cilia. The discovery that nematodes possess ciliated sense organs (Hope, 1965) inevitably led to a series of short publications describing the existence, structure and number of modified cilia in the papillae of a variety of nematode species: *Xiphinema index* (Roggen *et al.*, 1966, 1967); *Haemonchus contortus* (Ross, 1967); *Neoaplectana carpocapsae* (Poinar and Leutonegger, 1968); *Trichodorus christiei* (Hirumi and Chen, 1968; Hirumi *et al.*, 1970); *Trichodorus allius* (Raski *et al.*, 1969); *Longidorus elongatus* (Hirumi *et al.*, 1970). It was soon apparent that these cilia differ from typical kinetocilia in three respects: (i) they do not show the usual 9+2 arrangement of microtubules, (ii) they do not have typical basal bodies, (iii) basal rootlets may or may not be present. Other structural features of the papillae in these nematodes were not recorded in any detail.

Perhaps the first nematode papillae to be examined critically were those of the filarial worm *Dipetalonema viteae* (McLaren, 1970, 1972a). In filariae the lips fuse completely to form a united ring around the mouth and this is surrounded by eight large papillae arranged in two concentric circles. McLaren has examined the ultrastructure of these papillae in microfilariae (1969, 1972b), larval stages (1971) and adult worms (1970, 1972a) of *Dipetalonema viteae* The structure of the adult papilla is shown diagrammatically in Fig. 4. The papillae do not protrude from the surface of the worm, but the cuticle overlying each papilla is much thinner than elsewhere on the head. Each papilla is innervated by a single nerve axon originating from the region of the nerve-ring. The axon gives rise to a single modified cilium, which contains large, randomly distributed microtubules. At the base of the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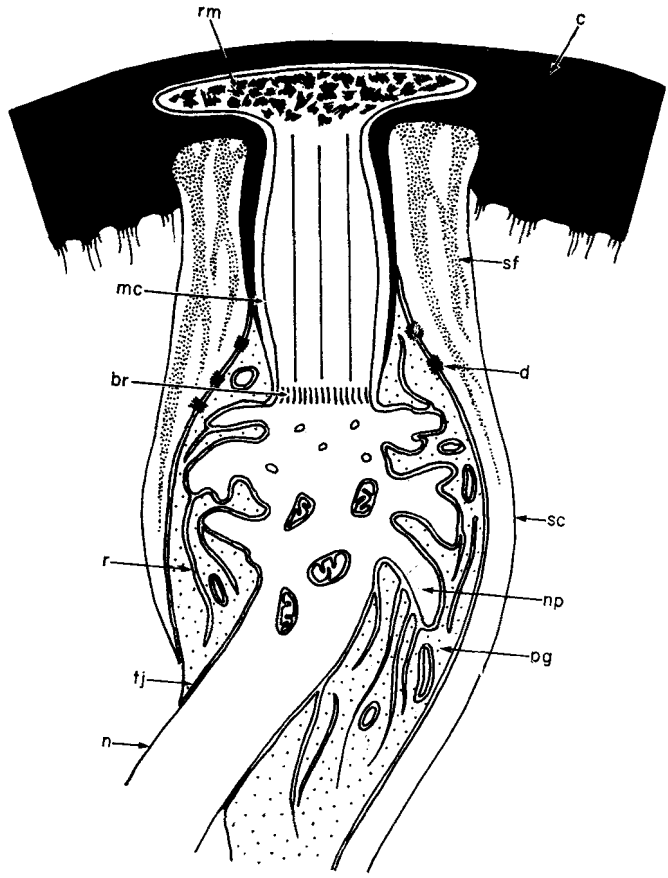

FIG. 4. Diagram showing a longitudinal section through one of the four papillae located on the head of adult *Dipetalonema viteae*. Cuticle, c; basal region of cilium, br; desmosome, d; modified cilium, mc; nerve axon, n; nerve process, np; papillary gland, pg; reticulum, r; reticulate material, rm; supporting cell, sc; supporting fibres, sf; tight junction, tj.

cilium these microtubules are organized along a lamellar structure and this presumably represents a basal body. Rootlets have not been observed, but just posterior to the basal region the axon develops bulbous peripheral processes, and contains vesicles resembling synaptic vesicles (Fig. 3). The

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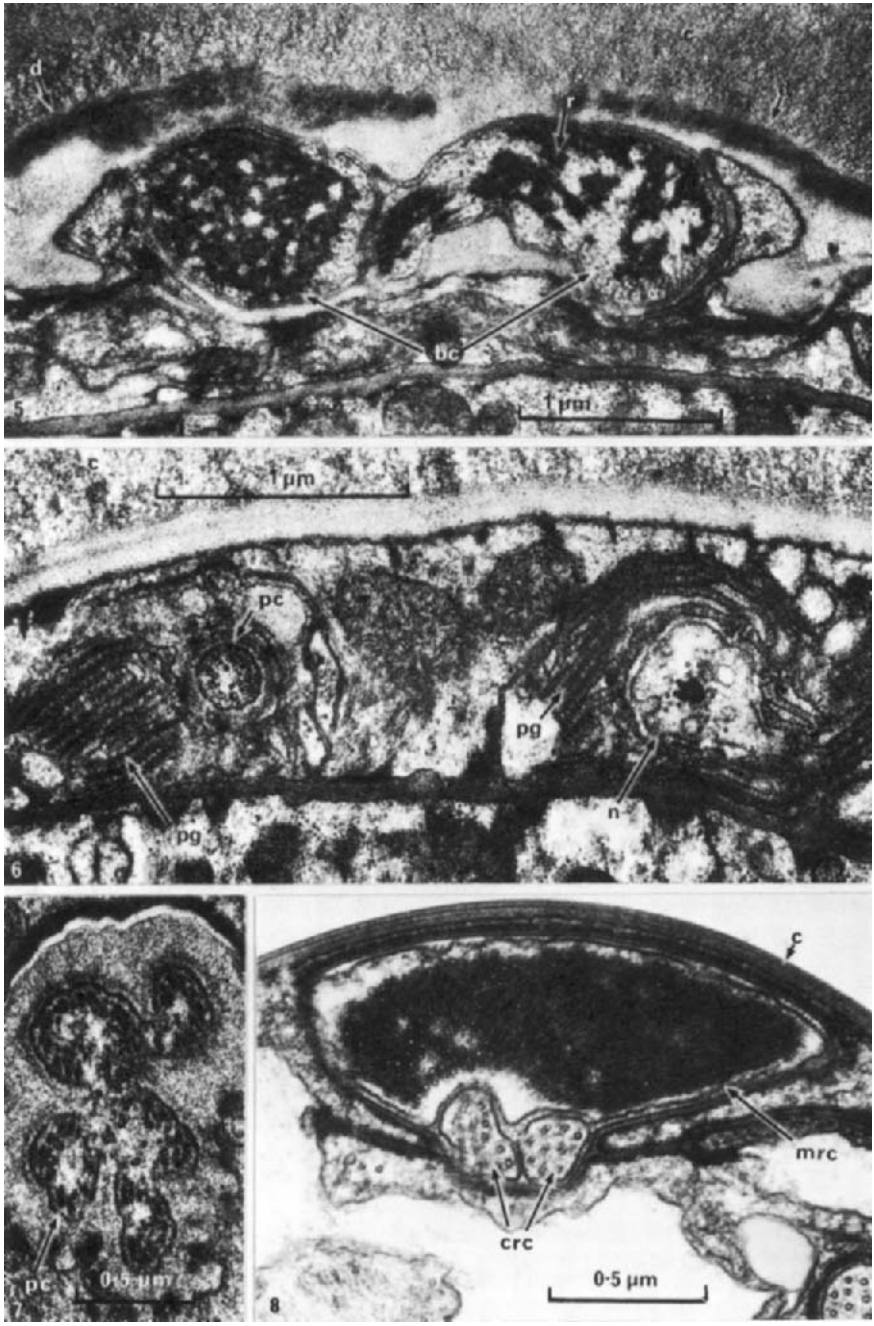
FIG. 2. Transverse section through the anterior end of the infective larva of *Necator americanus* showing the distribution of the sense organs. Amphid, a; cephalic papilla, cp; inner labial papilla, ilp; outer labial papilla, olp; stoma, s.

FIG. 3. Transverse section through the nerve axon which innervates the papillary cilium of *Dipetalonema viteae*. The nerve axon (n) is surrounded consecutively by the papillary gland (pg) and the supporting cell (sc). Nerve processes, np; reticulum, r; supporting fibres, sf; vesicles, v. (From McLaren, 1972a.)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cilium lies within a "mushroom-shaped" channel which does not open to the exterior via a pore (Fig. 4). The base, or "stalk", of the channel is lined with cuticle continuous with the outer cuticle of the worm, while the distal part of the channel takes the form of a flattened, circular cavity, or "mushroom cap", within the thin surface cuticle. This cavity appears lenticular in longitudinal sections. The tip of the cilium spreads out to fill this cavity and thus takes the form of a flattened disc. The ciliary microtubules spiral and then fan out so as to be arranged around the periphery of the disc; they enclose a central mass of dense reticulate material which has been believed to assist in transmission of the stimulus. The "stalk" part of the channel is surrounded by a single cell containing bundles of fibres, which presumably have a supporting function. It is therefore considered more appropriate that this cell be herewith designated the supporting cell. A small cell containing a limited complex of agranular reticulum encloses the base of the channel, and part of the nerve axon (Fig. 3); the membranes of the reticulum are continuous with the inner limiting membrane of the cell. Structurally this cell is remarkably similar to, although much smaller than, the amphidial gland of this worm (see Fig. 15), and will therefore be tentatively termed the papillary gland. The base of the supporting cell expands to surround this gland (Fig. 3). The papillae of the microfilarial and larval stages of *D. viteae* are, with minor differences, structurally similar to those of the adult. A papillary gland has not been observed in the microfilarial papilla although it is definitely present in the infective larva, where it contains a more highly developed reticulum than in the adult worm. Poinar and Leutonegger (1968) also noted that the papillae of the third-stage larva of *Neoaplectana carpocapsae* were considerably larger than in other larval stages. This feature is possibly associated with the task of locating and penetrating a new host. In *D. viteae*, the cuticular lining of the ciliary channel and the dense reticulate material filling the tip of the cilium are both shed during the moult to the subsequent larval stage.

In the trichostrongyles *Nippostrongylus brasiliensis*, *Nematospiroides dubius* and *Trichostrongyuls colubriformis*, certain of the head papillae are similar to those described in the filariae (McLaren, pers. obsvn). The distal tips of the papillary cilia are bulbous, as opposed to discoidal, and they have dense material dispersed amongst the microtubules (Fig. 5). In *N. brasiliensis*, the cilia within two papillae of each quadrant pass anteriorly in close apposition, and once inside the surface cuticle the bulbous tips of these two cilia bend

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FIG. 5. Transverse section showing the bulbous distal tips of the papillary cilia (bc) of adult *Nippostrongylus brasiliensis* within the outer cuticle (c) of the worm. The ciliary microtubules are connected by dense, reticulate material (rm). An elongated dense structure (d) can be seen in the cuticle overlying each cilium.

FIG. 6. Transverse section showing a papillary nerve axon (n) and a papillary cilium (pc) in adult *Nippostrongylus brasiliensis*. The papillary glands (pg) contain parallel arrays of paired membranes. Cuticle, c.

FIG. 7. Section showing the multilobed papillary cilium (pc) of adult *Necator americanus*.

FIG. 8. Section through a type II sense organ of *Capillaria hepatica* showing the two chemosensory cilia (crc) and the one greatly enlarged mechanoreceptive cilium (mrc). Outer cuticle, c. (From Wright, 1974.)

towards each other, and their membranes appear to fuse (Fig. 5). The gland cells associated with these papillae contain an extensive complex of reticulum (Fig. 6).

Each of the four cephalic papillae of the oxyurid pinworm *Syphacea obvelata* contain a single cilium (Dick and Wright, 1973) which is structurally similar to that of the trichostrongyles. The tip of each cilium is bent so as to be orientated parallel to the body surface, and a crescent-shaped dense structure is present in the surface cuticle medial to the bend in the cilium. Dick and Wright suggest that these two features may be related to the magnitude and direction of pressures received by the sense organ. A similar dense structure has also been identified in the cuticle overlying the trichostrongyle papillae (Fig. 5; McLaren, pers. obsvn). Neither supporting cells nor gland cells have been described in association with the cephalic papillae of *S. obvelata*.

In the adult strongyle *Necator americanus*, lips are also absent, the oral opening being modified to include teeth and cutting plates. Nevertheless, the full complement of papillae are present. The papillae of the adult worm include the same components as those described for filariae, but the individual components show minor structural differences (McLaren, pers. obsvn). The gland cell contains a sparse complex of reticulum, and has a central, irregularly shaped duct. A nerve axon containing numerous mitochondria penetrates into this duct, develops processes which interdigitate with the cell membrane lining the duct, and then takes the form of a modified cilium. In some papillae the cilium is a typically straight dendrite, but in other papillae it appears as a multilobed structure, in which the microtubular arrangement is difficult to recognize (Fig. 7). The cilium enters a short cuticle-lined channel, surrounded by a supporting cell, and becomes flattened at its tip. The dense reticulate material seen in the papillary cilia of filariae has not as yet been observed in adult *N. americanus*. In the infective third-stage larva, which has not yet developed a globular buccal capsule, the papillary nerve axons develop numerous, very long processes, so that in transverse sections the head of the worm seems to be filled almost entirely with nervous tissue (McLaren, pers. obsvn). The cilia are unlobed, and have a distal cap of dense reticulate material. The gland cell contains a more developed complex of reticulum than that seen in the adult worm. The papillae of the fourth-stage larvae more closely resemble those of the adult, and the cilia seem to contain a greater number of microtubules.

Studies on the strongyle *Syngamus trachea* (Jones, 1974) have revealed that each of the cephalic and lateral papillae contain three modified cilia. Jones suggested that each cilium represents a reduced papilla; thus, hypothetically one cilium each from the labials and cephalics have become fused into each cephalic papilla, and the lateral papillae have received one cilium from one of the labials and two cilia from the other labial. In each cephalic papilla (Fig. 9) the outermost cilium extends straight up the centre of the sense organ, and acquires a cap of dense material which lies at right angles to the spatulate tip of the cilium. The median cilium separates from the outermost cilium half way along its length, acquires a dense cap and terminates below the cap of the outermost cilium. The innermost cilium separates completely from the

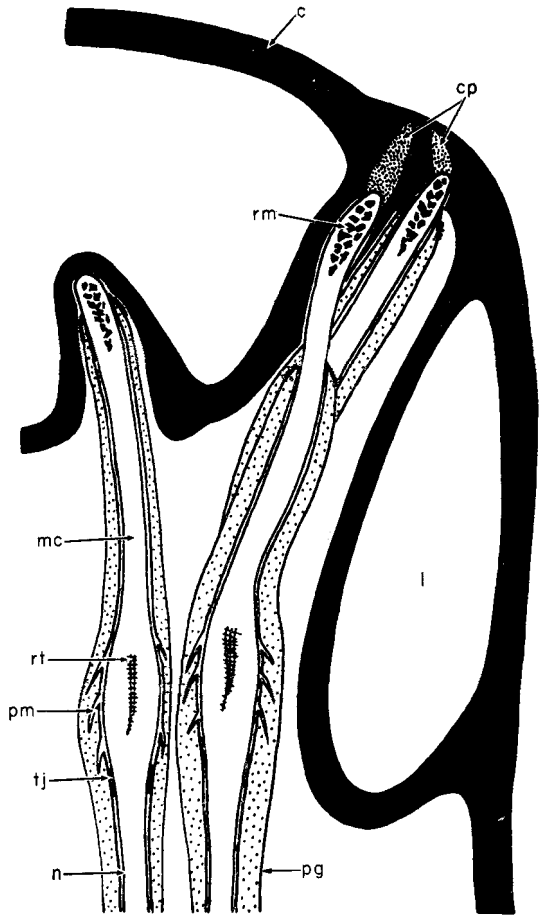


FIG. 9. Diagram showing a longitudinal section through a cephalic papilla of *Syngamus trachea*. Cuticle, c; ciliary cap of dense material, cp; junctional complex, jc; lumen of cephalic inflation, l; modified cilium, mc; nerve axon, n; papillary gland, pg; paired membranes, pm; reticulate material, rm; rootlet, rt. (Courtesy G. M. Jones.)

other two and follows the line of the buccal capsule to emerge on the inner surface of the papilla; it does not seem to have a dense cap. The three cilia all have basal rootlets and they are each surrounded by supporting cells. The nerve axons which innervate the cilia are surrounded by cells which contain elements of reticulum. The papillae themselves have not been seen to open to the exterior.

Studies on the adult worms (Baldwin and Hirschmann, 1973) and second-stage larvae (Wergin and Endo, 1974) of the plant parasitic nematode *Meloidogyne incognita* have shown that there are six inner labial papillae each containing two modified cilia, and four cephalic papillae each containing

a single modified cilium. The outer labial papillae are absent, and their cilia have presumably fused with those of the inner labials. The papillary cilia of the adult worm all have dense terminal caps, and are surrounded, proximally, by cells containing membranes and vesicles. These probably correspond to the gland cells of other nematode papillae. Rootlets and modified basal bodies have been identified in the second-stage larva. In the adult worm, the cilia of the cephalic papillae terminate within a thin layer of surface cuticle, as in the other nematodes described. The cilia of the labial papillae, however, are exposed to the external environment via a small pore in the outer cuticle. This observation is of particular interest, because nematode labial and cephalic papillae have traditionally been thought to function in a mechanoreceptive rather than a chemoreceptive capacity.

An even more unusual situation has been observed in the trichuroid nematode *Capillaria hepatica* (Wright, 1974). This nematode has a total of 18 cephalic sense organs, two of which, by their morphology, can clearly be identified as the amphids (Fig. 10). The remaining 16 sense organs, which

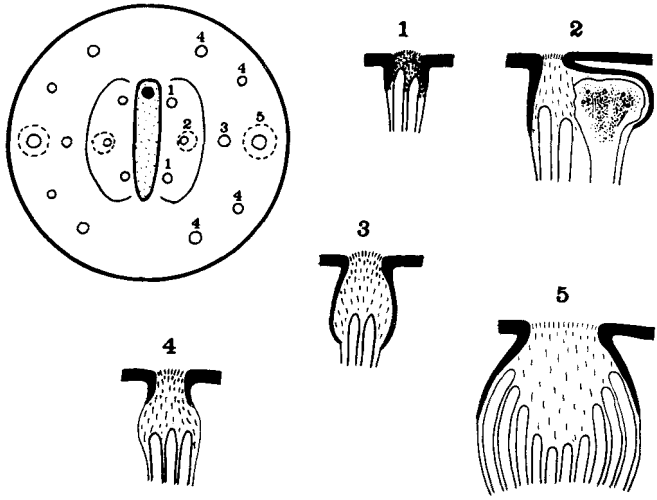


FIG. 10. Diagrams showing the distribution and structure of the five types of sense organs located on the head of *Capillaria hepatica*. (From Wright, 1974.)

presumably represent the papillae, all open to the exterior via a pore in the cuticle; Wright has classified these into four types (Fig. 10). There are four type 1 sense organs situated on the lateral oral elevations close to the mouth; they each contain two modified cilia of differing length. The two type 2 sense organs are lateral in position, also on the oral elevations, and they each contain three cilia. Two of these cilia terminate immediately beneath the pore, while the third cilium more closely resembles a typical papillary cilium, in that it forms a terminal bulb which lies beneath the invaginated surface cuticle (Fig. 8). The distal microtubules of this cilium are surrounded by dense material. There are two type 3 sense organs which lie in line with, but out-

side those of type 2; they closely resemble the type 1 sense organs, but the cilia terminate well below the pore and the cuticular lining extends further into the channel. The eight type 4 sense organs are sub-dorsal and sub-ventral in position, and each contains three cilia; they largely resemble the type 3 sense organs but the cuticular lining does not extend as far into the channel. Filamentous material surrounds the cilia in each type of sense organ. The cilia all have a basal region containing an outer ring of 9 doublet microtubules surrounding 0–3 singlets. Hypodermal cells enclose the cilia from their tips to a level below their basal regions, but posteriorly the axons lie freely in the pseudocoelom and form two sub-dorsal and two sub-lateral nerves. There are no specialized cells or glands associated with these sense organs. The papillae of *C. hepatica* all have a morphology which is suggestive fo a chemosensory rather than a mechanosensory capacity. Clearly one of the cilia in the type 2 sense organs shares many structural features with the cilia found in typical mechanoreceptive papillae, however, and it seems possible that the type 2 sense organs may therefore have a dual sensitivity.

Another trichuroid nematode, *Trichinella spiralis*, also has 16 possible chemoreceptive papillae in addition to the paired amphids (McLaren, pers. obsvn). In this nematode, however, each papillary sense organ contains three modified cilia. The sense organs located in the positions occupied by the type 2 sense organs of *C. hepatica* also contain one cilium with a possible mechanoreceptive capacity. The sense organs occupying the *C. hepatica* type 3 positions appear to have one very short cilium, so that only two cilia become enclosed within the channel. No gland or supporting cells have been observed.

The cephalic papillae of the mermithid *Mermis nigrescens* have been reported to differ significantly from those of other nematodes (Lee, 1974). Of particular interest is the fact that the dendrite innervating each sense organ ends in two dendritic processes, club-shaped structures which lie in a space in the hypodermis. Each process has a single basal plate from which well developed striated rootlets extend posteriorly into the dendrite. Other rootlets are associated with the walls of the dendritic process. Lee has concluded that these processes contain only numerous microtubules and do not appear to have the ciliary structure typical of other nematode sense organs. It is clear that the sensory cilia of those nematodes described to date are highly modified structures, however, and the fact that the dendritic processes of *M. nigrescens* include both a basal plate and striated rootlets suggests that it would not necessarily be inappropriate to describe these processes as modified cilia.

Contrary to expectations, scanning electron microscopy (SEM) has revealed very little information regarding the surface topography of head papillae, although the pores of the *Capillaria hepatica* sense organs have been clearly identified (Wright, 1974). This technique is likely to prove more useful for assessing the number and distribution of papillae for taxonomic purposes.

2. *Functional considerations*

The fact that most typical labial and cephalic papillae contain a modified cilium whose distal tip is overlaid by cuticle and is not, therefore, exposed to

the external environment, suggests that these particular sense organs are sensitive to mechanical pressures. The distal end of the cilium is often either bulbous or discoidal, a structural modification which probably serves to increase the area of sensitivity at the stimulus site. The microtubules within the distal tip of the cilium usually enclose, or are surrounded by, a mass of dense material. Similar dense, spongy material is characteristically associated with the modified cilia contained within arthropodan mechanoreceptors, where it is thought to act as a compressional component, responsible for transmitting the stimulus to the cilium (Thurm, 1968). An unusual dense structure has also been identified in the body cuticle overlying the ciliary tip of certain nematode papillae, and this has been suggested to confer directionality to the stimulus (Dick and Wright, 1973). Typical basal bodies are not found in nematode papillary cilia, although a basal region in which the microtubules show a more ordered arrangement, can usually be identified. The basal bodies of motile cilia are believed to possess mechanoreceptive properties (Thurm, 1968). The cilia within arthropodan mechanoreceptors also lack basal bodies, and Thurm (1968) has suggested that this may be because the site of mechanosensitivity is associated with the distal tip of a much reduced cilium.

In some nematodes the nerve axon innervating the papillary cilium is surrounded by a small cell which is structurally similar to the amphidial gland of filarial worms (McLaren 1972a). This cell contains a complex of reticulum, the paired membranes of which open into the space surrounding the nerve axon. In the case of filarial nematodes the enzyme acetylcholinesterase has been localized within the cisternae of these paired membranes (McLaren, 1972a). It is unlikely, however, that the enzyme constitutes an exosecretion, as is thought to be the case in the amphids, because the ciliary channel of the filarial papilla does not open to the exterior. Further use of histochemical procedures will undoubtedly increase our understanding of the functional significance of this cell.

Experiments have been performed to test the behavioural responses of nematodes to mechanical stimuli. Dickinson (1959) showed that when freshly hatched larvae of *Heterodera schachtii* are exposed to artificially prepared hydrophobic and hydrophilic nitro cellulose membranes, physical contact stimulates the larvae to penetrate the hydrophobic but not the hydrophilic surface. It follows, therefore, that other biological activities such as feeding, hatching, moulting, aggregation and swarming may also result from stimulation of the mechanoreceptive sense organs. LM studies have shown that the long setae and bristles found on the heads of many marine nematodes probably represent highly developed tactile receptors, but the experimental evidence supporting this idea is very limited.

Recent studies have revealed that in certain nematodes the sense organs in conventional papillary locations open to the exterior by a pore in the outer cuticle. Such sense organs seem more likely to have a chemoreceptive capacity. The reason for this variation is difficult to see, but it may depend on certain aspects of the biology of the nematode such as mode of feeding, habitat and life cycle. We shall only be able to assess the extent and significance of chemo-

receptive versus mechanoreceptive papillae, as and when other nematode species have been examined.

One particularly interesting observation to have arisen from these studies, is that the nerves which innervate the cephalic papillae of *Panagrellus silusiae* (Yuen, 1968), *Necator americanus* (McLaren, 1974) and *Capillaria hepatica* (Wright, 1974) have been found to join with those innervating the amphids before reaching the nerve-ring. This observation contradicts the traditionally accepted hypothesis that the amphids and papillae are innervated separately.

B. AMPHIDIAL SENSE ORGANS AND GLANDS

1. *Morphological studies*

In addition to the labial and cephalic papillae a pair of laterally positioned amphidial sense organs are located on the head of the nematode (Figs 1 and 2). Early light microscopists evidently encountered some difficulty in distinguishing the amphids from the papillae, and even when they were noted the amphids were usually referred to by the rather indefinite terms lateral organs (Bastian, 1865), dorso-lateral organs (Goldschmidt, 1903) and spiral-lateral organs (zur Strassen, 1904). It was Cobb (1913) who formally proposed that they be renamed the "amphids". He later reported (1923) that these structures are so widely distributed in the phylum Nematoda as to make them a distinctive feature of the anatomy, and that they show much variation in size and form in free-living species and are usually small and obscure in parasitic nematodes; he further commented that they are "doubtless, in part at least, sensory".

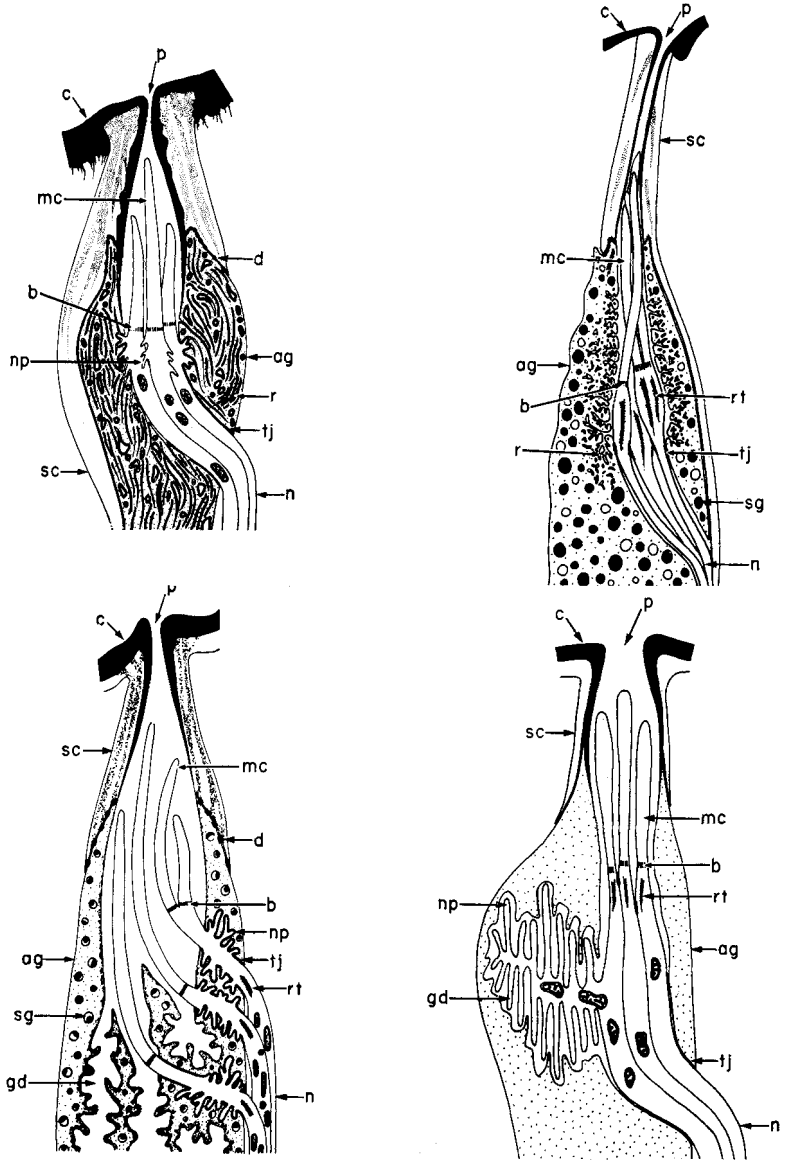
One of the earliest LM accounts of these sense organs was that of Goldschmidt (1903), who reported that the amphids of *Ascaris lumbricoides* contain not less than 12 nerve fibres enclosed within a "support cell". Anteriorly these nerve fibres were considered to fuse into a unified cone which projects through a pore in the surface cuticle. Hoeppli (1925) later recorded the existence of a second non-nervous cell in the amphid; he considered this second cell to be the "support cell" and the cell originally described by Goldschmidt to be the "escort cell". According to Chitwood and Chitwood (1950) the amphidial nerve approaches the head of the worm and enters an amphidial gland; the nerve processes then break up into an elongated sac termed the amphidial pouch. The specialized ends of individual neurones are referred to as "terminals" and a group of such terminals as a "sensilla". These authors also noted the presence of a short amphidial duct which runs anteriorly from the pouch and opens to the outside at the amphidial pore. Recent EM studies have shown this account to be remarkably accurate. The amphids of parasitic nematodes such as *Ascaris* are generally considered to be much reduced in form, however, and in certain free-living species the amphidial pouch is reported to open into a pocket, circle or elaborate spiral cuticular modification (Chitwood and Chitwood, 1950). As yet these more complex structures have not been investigated at the ultrastructural level. Accounts of the external morphology and evolutionary development of the amphids in marine and fresh water nematodes have been published by Schuurmans Stekhoven and de Coninck (1933) and de Coninck (1965).

One of the first EM studies of nematode amphidial sense organs was that of Hope (1965), who reported that the amphids of the free-living, marine nematode *Deontostoma californicum* contain structures resembling modified cilia. Subsequent observations on *Xiphinema index* (Roggen *et al.*, 1966), *X. americanum* (López-Abella *et al.*, 1967), *Haemonchus contortus* (Ross, 1967), *Neoaplectana carpocapsae* (Poinar and Leutonegger, 1968) and *Panagrellus silusiae* (Yuen, 1968) confirmed that the sensory terminals within nematode amphids take the form of highly modified cilia whose microtubular numbers and arrangements show extreme diversity. The amphids generally differ from the papillae in that each usually contains several modified cilia. The plant parasitic nematodes *Trichodorus christiei* (Hirumi and Chen, 1968; Hirumi *et al.*, 1970), *T. alluis* (Raski *et al.*, 1969), and *Longidorus elongatus* (Hirumi *et al.*, 1970; Taylor *et al.*, 1970) have unusually large numbers of cilia within each amphid, 22 and 23 cilia being found in *Longidorus* and *Trichodorus* respectively.

Details of structural features other than modified cilia are very limited in these early reports. The amphidial cilia of *Xiphinema index* (Roggen *et al.*, 1966) and *Haemonchus cortortus* (Ross, 1967) are said to be enclosed in vacuolar spaces, and in *X. index* this space is seen to have a highly folded wall. The amphidial pouch of the free-living nematode *Panagrellus silusiae* is reported to be composed of regularly arranged sheets of lamellae and bundles of longitudinal "tubes" (Yuen, 1968). The lamellae completely surround the thirteen amphidial cilia in the widest part of the pouch, but they decrease in numbers anterior and posterior to this region. The longitudinal "tubes" are most numerous near the basal regions of the cilia, where they become dispersed between the lamellae. It is perhaps worthy of note that Epstein *et al.* (1971) observed similar laminated structures and membranes in the anterior lateral hypodermal cords of another free-living nematode, *Caenorhabditis briggsae;* these authors suggested that the laminations form the lower sector of the amphidial pouch. A similarity between such laminated structures and the amphidial gland of adult filarial worms will become apparent later in this section.

Recent ultrastructural studies on the microfilaria of *Dirofilaria immitis* (Kozek, 1968, 1971) have revealed that the paired eosinophilic structures, or "röten Mundgebilde" (Fülleborn, 1913), reported to be present in the cephalic region of the larva by light microscopists, are in fact the amphidial sense organs. Kozek also found that the amphidial cilia of *D. immitis* lie within channels which are lined with cuticle different from the outer cuticle of the worm. The cilia themselves are of unequal length, there being a maximum of nine cilia at the base of each channel, whereas only three to five of these reach the channel orifice. At its base each cilium is connected to neighbouring cilia by tight junctions, and the ciliary membranes are covered with fine, filamentous material, which appears to accumulate as a dense plug in the channel orifice.

Subsequent studies on filarial nematodes (McLaren, 1969, 1970, 1971, 1972a,b) were aimed at elucidating the relationship between the amphidial sense organ and its associated cells. Figure 11 shows a diagrammatic recon-



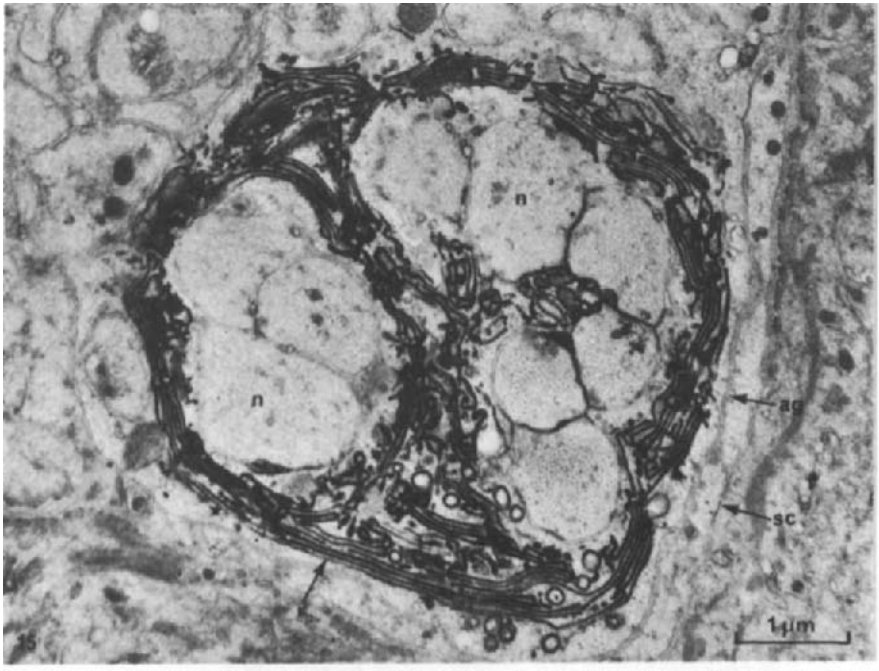
FIGS 11–14 Amphidial gland, ag; basal region of cilium, b; cuticle, c; desmosomes d; gland duct, gd; modified cilium, mc; nerve axon, n; nerve processes, np; pore, p; reticulum, r; rootlets, rt; supporting cell, sc; secretion granules, sg; tight junction, tj.

FIG. 11 (top left). Diagram showing a longitudinal section through the amphidial sense organ and gland of adult *Dipetalonema viteae*. (Redrawn from McLaren, 1972a.)

FIG. 12 (top right). Diagram showing a longitudinal section through the amphidial sense organ and gland of adult *Necator americanus*. (From McLaren, 1974.)

FIG. 13 (lower left). Diagram showing a longitudinal section through the amphidial sense organ and gland of adult *Syngamus trachea*. (Courtesy G. M. Jones.)

FIG. 14 (lower right). Diagram showing a longitudinal section through the amphidial sense organ and gland of the second-stage larva of *Meloidogyne incognita*. (Courtsey W. P. Wergin and B. Y. Endo.)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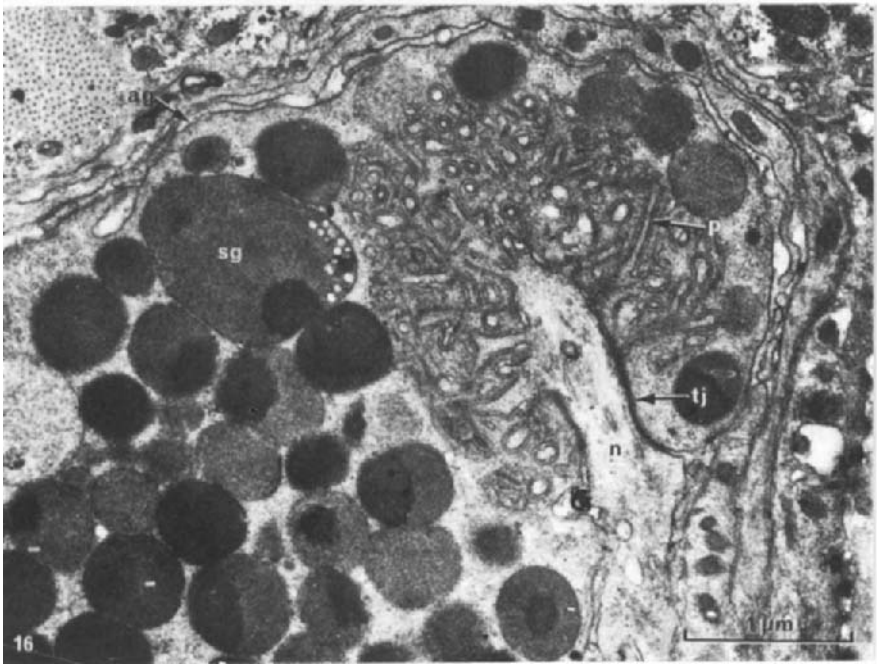
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struction of the amphids of the adult filarial worm *Dipetalonema viteae*. The paired amphidial channels are each lined with cuticle continuous with but structurally different from the outer cuticle of the worm. Each channel is surrounded by a cell which contains longitudinally orientated fibre bundles, and possibly, therefore, has a supporting function. The base of the channel is surrounded by a spindle-shaped cell containing vesicles and parallel arrays of paired membranes which resemble agranular endoplasmic reticulum (Fig. 15). This cell was originally designated the "multivesicular cell" (McLaren, 1970), but more recent studies have revealed that it is in fact the amphidial gland; the ultrastructural morphology of this cell indicates a possible secretory function. The cisternae of the paired membranes open into the central lumen of the gland and this facilitates the release of any secretions which they may synthesize. The supporting cell expands proximally to encompass the amphidial gland, and desmosomes mark points of close attachment between the apposed plasma membranes of the two cells. Transverse sections taken near the base of the amphidial gland show that at this level the supporting cell extends only half way around the circumference of the gland; it is closely applied to that half of the gland facing the centre of the worm. A bundle of nine axons, originating from the region of the nerve-ring, penetrates through the base of the gland, and almost immediately divides into two groups; one group comprises six axons and the other group three axons (Fig. 15). The limiting plasma membranes of gland and axon are closely apposed as the axons pass anteriorly through the gland cytoplasm. The axons then enter the base of the amphidial channel and take the form of modified cilia. These cilia do not have rootlets or typical basal bodies. At the base of each cilium, however, the microtubules become organized along both sides of a branching lamellar structure, and this may represent a specialized basal region. The microtubules arranged along the outer edge of the lamellar structure are usually in the form of doublets and these are invariably attached to the ciliary membrane by means of a Y-shaped structure. Similar "membrane–microtubule" complexes have been observed in the region immediately beneath the basal plates of both somatic cilia, and the modified cilia within certain insect mechanoreceptors (Gilula and Satir, 1972). Freeze-etching studies on the cilia of the lamellibranch gill have shown that in this region the fracture face of the ciliary membrane contains rows or strands of membrane particles that encircle the ciliary shafts; this arrangement has been referred to as the ciliary necklace, which may be involved in the control of localized membrane permeability (Gilula and Satir, 1972).

Just posterior to the basal regions of the cilia, the nerve axons of *D. viteae* develop long peripheral processes which lie within deep invaginations of the

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FIG. 15. Transverse section showing the amphidial gland (ag) and nerve axons (n) of adult *Dipetalonema viteae*. Cholinesterase is localized in the reticulum (r) of the amphidial gland. Supporting cell, sc. (From McLaren, 1972a.)

FIG. 16. Transverse section showing a nerve axon (n) penetrating into the amphidial gland (ag) of adult *Necator americanus* and sending out peripheral processes (np). Secretion granules, eg; tight junction, tj.

gland plasmalemma. In this region the axons contain mitochondria and vesicles resembling synaptic vesicles. The amphidial sense organ plus gland of adult *D. viteae* is in effect a surprisingly small structure; it occupies only 0·002% of the total length of the worm. Apart from size and degree of development of the amphidial gland reticulum, there are few structural differences between the amphids of microfilariae, larvae or adult filarial worms (McLaren, 1971). In the microfilaria one of the amphidial channels turns obliquely before reaching the anterior tip of the larva, so that the aperture of the sense organ lies immediately beneath the cephalic hook. This may possibly reflect a direct relationship between sensory perception and the use of the hook for penetrating through the tissues of the intermediate arthropodal host. The quiescent second-stage larva has a limited complex of reticulum within the amphidial gland but the infective third-stage larva has a well developed reticulum. The cuticular lining of the amphidial channel is shed at each moult.

Preliminary observations indicate that the trichostrongyles *Nippostrongylus brasiliensis*, *Nematospiroides dubius* and *Trichostrongylus colubriformis* have amphidial sense organs and glands which resemble those of filariae (McLaren, pers. obsvn). The glands contain much less reticulum, however, and each amphid includes 13 or 14 modified cilia. As has already been mentioned, the amphids of *Panagrellus silusiae* (Yuen, 1968) and *Caenorhabditis briggsae* (Epstein *et al.*, 1971) probably also have filarial-type amphidial glands.

Studies on the strongyle *Necator americanus* (McLaren, 1974) have revealed that while the sensory component of the amphid is structurally similar to that of filariae, the gland is not only considerably longer, but is also structurally different (Fig. 12). Indeed the sense organ and gland of this nematode occupy about 25% of the total body length. Each gland is intimately attached to the dorsal surface of a lateral hypodermal cord, and this unfortunately makes microdissection and analysis of the glands difficult. The glands are unicellular with a large nucleus situated at about the level of the nerve-ring. The posterior ends of the glands show signs of high metabolic activity, and it is here that the Golgi bodies synthesize electron-lucent secretion granules, which increase in density as they ascend the gland. At the anterior tip of the worm the gland envelops the base of the amphidial channel. Numerous, irregularly-shaped collecting ducts situated within the anterior cytoplasm of the gland, eventually unite to form a single wide duct which is continuous with the cuticle-lined amphidial channel. The secretion granules synthesized within the gland are thought to eliminate their contents into these ducts for release to the exterior via the amphidial pore. A supporting cell has also been identified in the *N. americanus* amphid; it has much the same structural features and the same relationship with the channel and gland as was described for filarial nematodes.

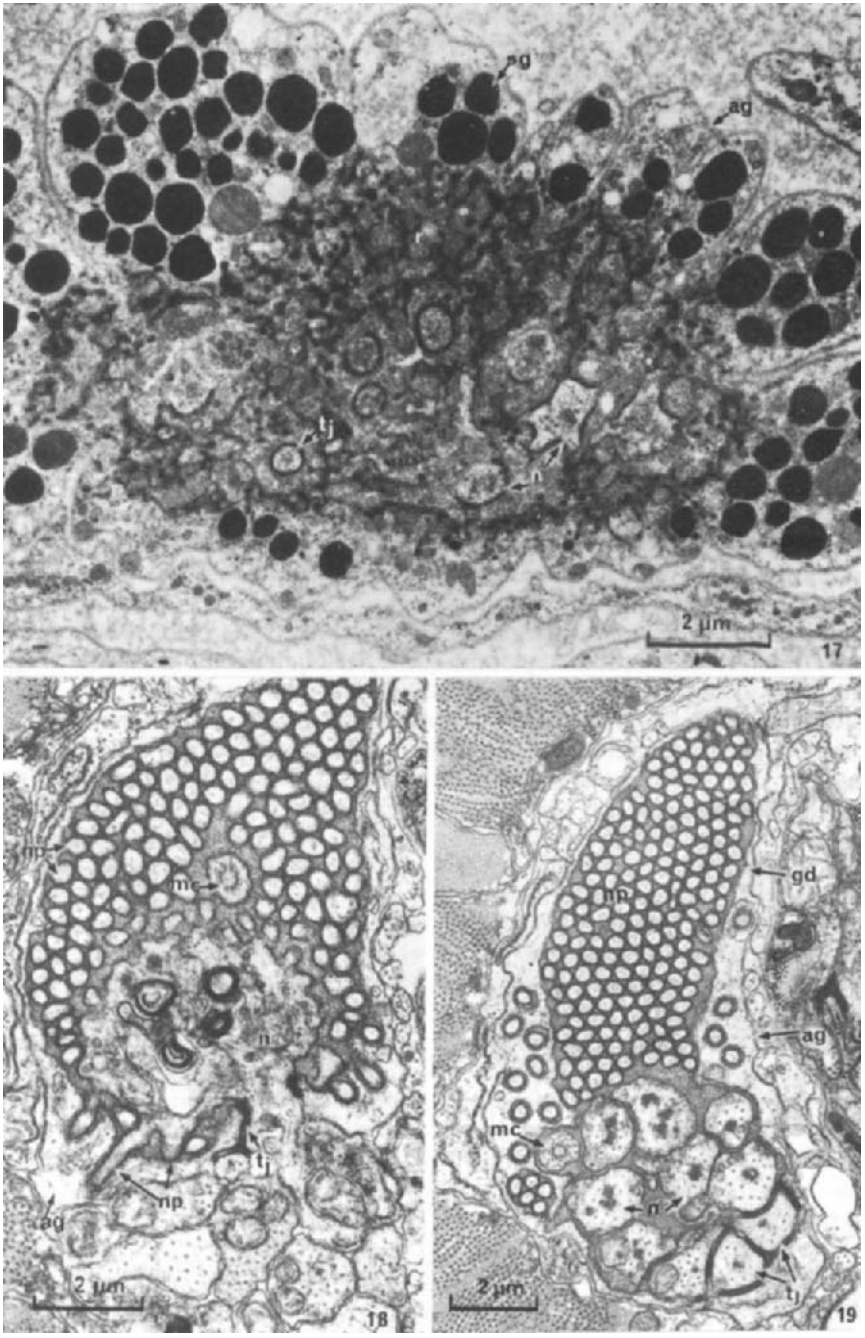
A bundle of nerve axons originating from the region of the nerve ring of *N. americanus* passes anteriorly in close apposition to the surface of the gland. Near the base of the buccal capsule some axons penetrate into the gland cytoplasm (Fig. 16), while the remainder continue forward to innervate the cephalic papillae. Tight junctions are clearly visible between axonal and gland

membranes at the points of penetration (Fig. 16). The axons then develop elongate processes which ramify around and between the collecting ducts of the gland (Fig. 16). In this region the axons contain vesicles resembling synaptic vesicles. All but one of the axons eventually penetrate either into the gland ducts or directly into the amphidial channel, and tight junctions again mark such points of penetration. Within the ducts the axons each give rise to one or more modified cilia, so that a total of 13 cilia enter the amphidial channel. These cilia have modified basal regions, and well developed striated rootlets. One nerve axon gives rise to a modified cilium whilst still within the gland cytoplasm; this cilium never enters the ducts or channel but continues anteriorly for some distance before terminating within the gland.

Studies on the larval stages of *N. americanus* have revealed that third-stage larvae, both before and after penetration of the vertebrate host, have small amphidial glands containing, not secretion granules, but a limited reticulum similar to that present in filariae (McLaren, pers. obsvn.). A transitional period, during which the gland contains both reticulum and secretion granules, occurs at about day 6 or 7 post infection, so that fourth-stage larvae have elongated glands containing only secretion granules. The significance of these structural differences would seem to be associated either with the change of environment which accompanies the migration from lungs to intestine or with the change in feeding habits which results from the development of an armed buccal capsule.

The amphids of another strongyle, *Syngamus trachea*, a parasite of rooks, have recently been studied by Jones (1973, 1974). The amphidial glands of the adult male worm occupy about 0.2% of the total body length, and those of the longer female about 0·1% of the body length. The structural arrangement of the sensory components of the amphid is similar to that described (Fig. 13). The amphidial cilia do not have typical basal bodies, although rootlets are present. Just behind the origin of each cilium the axon swells to form a nerve bulb which is variable in shape when seen in transverse section. Near the nerve bulb the axon gives rise to short, irregular processes more like those of filariae than those in *N. americanus*. Tight junctions mark points of close attachment between the plasma membrane of the gland and that of each nerve bulb (Fig. 17). In the region anterior to the nerve bulbs the gland contains an extensive reticulum, the cisternae of which open into the lumen of the amphidial channel (Figs 13 and 17). Transverse sections through this region show that the periphery of the gland is lobulated, the lobes containing dense secretion granules (Fig. 17) which are present along the entire length of the gland. In having both secretion granules and reticulum the gland of adult *S. trachea* resembles that of the 6 to 7-day-old third-stage larva of *N. americanus*. In fully mature *S. trachea* the lumen of the amphidial channel is swollen throughout most of its length and the cilia are located around the periphery, rather than in the central lumen, which is filled with dense homogeneous gland secretion.

The amphidial glands of young adult *S. trachea* recovered immediately after the fourth moult are fairly small; they show little evidence of secretory activity, having only a few paired membranes and very few, small secretion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granules. After the worms have paired, but before they have migrated to the trachea, at about day 6–7, there seems to be a sudden burst of activity within the glands, for the paired membranes expand to form a reticulum, and the secretion granules become more numerous. At this time the dense secretion material is first seen within the channel, the base of which is not swollen. The apparent increase in the secretory activity of the gland at this stage of the life cycle may possibly be associated once again with attachment to the host tissues and the onset of feeding.

An interesting modification of the basic structure of the amphidial sense organ has been observed in the plant parasitic nematode *Meloidogyne incognita* (Baldwin and Hirschmann, 1973; Wergin and Endo, 1973, 1974). The amphidial gland of the second-stage larva occupies less than 5% of the total body length (Wergin, pers. comm.), while that of the adult worm may be estimated to occupy about 1.0% of the total length of the nematode. In the adult worm (Baldwin and Hirschmann, 1973), the 13–15 nerve processes which constitute the lateral nerve bundle, penetrate into the amphidial gland. Seven or eight of these processes become associated and enter the amphidial channel in the form of modified cilia; the remainder are scattered within the gland cytoplasm, and either terminate therein as cilia, or leave the gland and continue anteriorly to innervate the cephalic papillae. The cilia do not have distinct basal bodies although rootlets have been identified. According to Baldwin and Hirschmann the posterior region of the adult amphidial gland contains numerous secretory organelles, while the central region contains "membraneous chambers" which are filled with longitudinally orientated "microvilli" and granular, extracellular material. The exact origin of the chambers and microvilli is not clearly demonstrated, although the authors state that "the 'microvilli' are physically distinct from the amphidial nerve bundle". Studies on the second-stage larva of *M. incognita* (Wergin and Endo, pers. comm.) have revealed, however, that the "microvilli" are elongated processes which arise from one or more well developed nerve axons, and that these processes lie within the ducts of the amphidial gland (Fig. 14). These observations contradict the results of Baldwin and Hirschmann, but are clearly substantiated by the relevant electron micrographs (Figs 18 and 19); they also agree with a brief report by Bird (1971) that the microvillar structures found in *M. javanica* amphids are nerve processes originating from the amphidial nerve axons. It would thus appear that the short nerve processes

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FIG. 17. Transverse section showing both secretion granules (sg) and reticulum (r) within the lobed amphidial gland (ag) of adult *Syngamus trachea*. Nerve axons, n; tight junctions, tj. (Courtesy G. M. Jones.)

FIG. 18. Transverse section showing a nerve axon (n) penetrating into the amphidial gland duct (gd) of a second-stage larva of *Meloidogyne incognita* and developing numerous elongated processes (np). Amphidial gland, ag; modified cilium, mc; tight junction, tj. (Courtesy W. P. Wergin and B. Y. Endo.)

FIG. 19. Transverse section showing nerve axons (n), nerve processes (np) and a modified cilium (mc) within the amphidial gland duct (gd) of a second stage-larva of *Meloidogyne incognita*. Amphidial gland, ag; tight junctions, tj. (Courtesy W. P. Wergin and B. Y. Endo.)

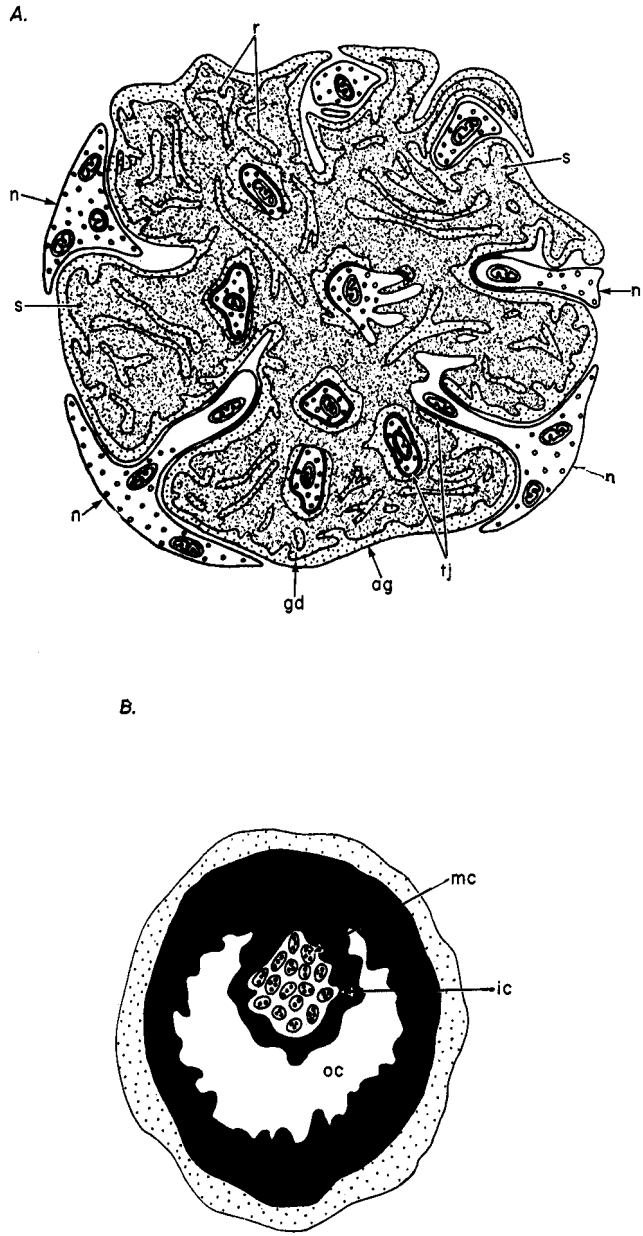


FIG. 20. Diagrammatic transverse sections through the amphidial gland (A) and double amphidial channel (B) of *Gastromermis boophthorae*. Amphidial gland, ag; gland duct, gd; inner channel, ic; modified cilium, mc; nerve axon, n; outer channel, oc; processes of reticulum, r; secretion, s; tight junction, tj. (Courtesy B. Batson.)

observed in filariae and strongyles have reached an as yet unparalleled development in *Meloidogyne* sp.; however, the reason underlying this exaggerated development has yet to be elucidated.

Preliminary observations on the amphids of the mermithid *Gastromermis boophthorae* (B. Batson, pers. comm.) have revealed hitherto unrecorded features. The amphidial glands contain an extensive reticulum similar to that seen in the filariae. Anteriorly, however, the gland duct becomes exceptionally large, and there is a corresponding reduction in the volume of gland cytoplasm; the duct is filled with a granular secretion (Fig. 20A). The amphidial nerve axons do not approach the surface of the gland in a discrete bundle, as in other nematodes, but are arranged around its circumference. Individual axons travel forward enclosed within peripheral pockets of the gland reticulum (Fig. 20A). The axons eventually become flattened against the surface of the gland and then penetrate into the gland cytoplasm (Fig. 20A). Tight junctions are visible between the apposed membranes of the gland and the penetrating axons. The axons eventually give rise to 16–19 modified cilia which enter the cuticle-lined amphidial channel. Within this channel the bundle of cilia becomes enclosed within a secondary channel which arises from the cuticular lining of the primary channel (Fig. 20B). The bundle of cilia remains confined within this secondary channel for some distance, but eventually the lumen of the secondary channel opens into that of the primary channel.

The secondary channel seems to be associated with retaining the cilia in a tight bundle, but because the two channels merge, this arrangement cannot serve, as it first seems, to separate the cilia from the gland secretions. Thick-walled contact chemoreceptors of arthropods are known to have similar double channels, and here also the modified cilia occupy the innermost lumen (Hansen and Heumann, 1971). Rees (1967) suggested that both channels of these arthropod receptors are involved in the transmission of receptor potentials from the receptor site. It must be emphasized that the observations recorded for *Gastromermis boophthorae* are of a preliminary nature, and it is to be hoped that future studies will help to elucidate the functional significance of this unusual structural arrangement in the Nematoda.

Studies on the marine nematode *Oncholaimus vesicarius* (Burr and Webster, 1971; Burr and Burr, 1975) have indicated that the amphids of this worm may possibly have a photoreceptive capacity. Dark red "eyespot granules" are concentrated anterolaterally within the most anterior of the radial oesophageal muscle cells of this nematode. Morphological studies (Burr and Webster, 1971) have shown, however, that the "eyespot" of this species is not itself a photoreceptive organ, but is more likely to serve as a shading structure for a neighbouring photoreceptor. Burr and Webster noted a marked swelling of the amphid immediately anterior to the region containing the highest concentration of "eyespot granules", and they therefore suggested that the amphidial sense organ should be examined for a possible photosensitive terminal. Such studies have shown that the amphids of *O. vesicarius* are each innervated by four nerve axons. These axons are unusual, however, in that each gives rise to several modified cilia (Burr and Burr, 1975). The 28–36

cilia which arise from three of these axons enter the cuticle-lined amphidial channel, and have been referred to as the amphidial cilia. Burr and Burr consider that these particular cilia resemble olfactory cilia. The fourth axon also gives rise to several modified cilia, but these project medially and end just anterior to the pigment spot (Fig. 28); it is these cilia which are presumed to have a photoreceptive capacity.

A "secretory cell" containing membrane-bounded granules, surrounds the base of the ciliary channel and ensheathes the nerve axons. This is presumably the amphidial gland. A "sheath cell" has also been recognized around the distal region of the ciliary channel and this is presumably the supporting cell. These studies indicate that the amphids of *O. vesicarius* may have a dual sensitivity, and this in turn has led Burr and Burr to suggest the possibility that nematode ciliary photoreceptors may have evolved from chemoreceptors.

That amphidial glands are not universally associated with amphidial sense organs has been demonstrated by studies on the free-living, aquatic nematode *Tobrillus aberrans* (Storch and Riemann, 1973; Storch, 1973) and the zooparasitic trichuroid nematodes *Capillaria hepatica* (Wright, 1974) and *Trichinella spiralis* (McLaren, pers. obsvn.). In *T. aberrans* each amphid includes sixteen modified cilia which have elongated basal plates, but no rootlets. The details published by Storch and Riemann (1973) and Storch (1973) are concerned primarily with the structure of the sensory components of the amphids, but the authors do state that they have not observed associated glial cells. In *C. hepatica* the nerve axons innervating the sense organ are enclosed by the processes of hypodermal cells underlying the surface cuticle; these cell processes contain groups of filaments. No other specialized cells or glands are found in the region of the amphids. Each amphidial channel contains ten modified cilia which are surrounded by filamentous material; the cilia have modified basal regions posterior to which are located a few coated vesicles. The amphidial nerve axons continue posteriorly for some distance enclosed within the lateral hypodermal cords, but they eventually enter the pseudocoelom where they join the sublateral nerves. The amphidial sense organs of *T. spiralis* are, not unnaturally, very similar to those of *C. hepatica.*

SEM has proved particularly useful for locating the exact position of the amphidial pores in those nematodes possessing complex buccal capsules, e.g. hookworms; it has also revealed how small these pores may be, i.e. 1·0 μm in diameter in *N. americanus.* In many species, however, these small pores are often rendered invisible by adherent host material, amphidial gland secretions, or by the materials utilized in the preparative techniques.

2. *Functional considerations*

Because the amphidial sense organs open to the exterior by a pore in the outer cuticle of the worm, they have long been regarded as chemoreceptors responsible for detecting changes in the external environment. Electrophysiological techniques for stimulating or inactivating these receptors have yet to be perfected, however, and we must therefore rely on the correlation of

morphological, cytochemical and behavioural studies for information on the mechanism of action of these sense organs.

It is clear that nematode amphids exist in a variety of forms and sizes; some appear to be purely chemosensory (e.g. *Capillaria hepatica*), some are chemosensory and have an associated secretory gland (e.g. *Necator americanus*), and others may be chemosensory, photoreceptive and have an associated gland (e.g. *Oncholaimus vesicarius*). Undoubtedly further types will be recognized as and when other nematode species are examined.

The relationship between what is evidently a highly active secretory gland and a sense organ thought to monitor changes in the environment, is of particular interest since it is evident that the sensory cilia will be exposed not only to external stimuli, but also to the gland secretions. McLaren (1974) suggested that the amphidial cilia of *N. americanus* may function as multipurpose receptors, and has proposed the following tentative hypothesis to explain their possible mode of action. The release of gland secretions to the exterior will render the amphidial cilia temporarily insusceptible to external stimuli, and it may be that during this period, some or all of the cilia are responsible for monitoring and regulating the output of gland secretion. There are no specific muscles associated with the amphidial pore, and indeed to use physical closure of the pore as a means of regulating the output of secretion would be impracticable, because this would also restrict the activity of the sense organ. The morphological features of the axon–gland relationship suggest that release of gland secretions may possibly be under direct nervous control. Once the nerve axons have penetrated into the amphidial gland they each send out numerous processes and thus produce a concentration of nervous tissue around the gland ducts. Possibly, therefore, these processes either release neurosecretions which stimulate the gland to release its secretions, or the stimulus might pass from axonal to gland membrane by electrical transmission. Thus, the amphidial cilia may function initially as receptors of external stimuli, and under certain conditions stimulate the nerve axons from which they are derived to respond in a motor capacity and switch on the secretory activity of the gland. The same, or perhaps other cilia may then monitor the output of secretion, and the motor apparatus would regulate the secretory activity of the gland. Receptor–effector pairs of cells such as these possibly represent were originally postulated as an intermediate stage in the evolution of the nervous system (Parker, 1919; Ramón y Cajal, 1952), but until recently their existence had not been conclusively demonstrated. Suggested examples have now been recorded in the marine mollusc *Aplysia californica* (Coggeshall, 1971), in *Hydra littoralis* (Westfall, 1973), in four species of ctenophores (Hernandez-Nicaise, 1974), and in a trichuroid nematode (Wright and Chan, 1974). The amphidial cilia and axons of *N. americanus* share certain features with all these examples, although typical dense-cored neurosecretory granules have not been identified in the peripheral nervous processes. Vesicles resembling synaptic vesicles have been observed in this region, however, and these may possibly serve a similar function. Another notable feature is the occurrence of numerous mitochondria in the nerve axon just posterior to these processes. Similar accum-

ulations of mitochondria are localized immediately posterior to the basal regions of the cilia which occur in arthropod chemoreceptors, insect mechanoreceptors and vertebrate retinal photoreceptors; the region containing these mitochondria has been considered as a possible site for the generation of the receptor potential (Thurm, 1968). We should also consider that stimuli detected by nematode amphidial cilia may be transmitted directly to the nerve-ring; this arrangement would correspond to the advanced stage in Parker's hypothesis, of an interneurone interposed between the receptor and effector cells. A neurone with similar features has been described in *Hydra littoralis* (Westfall, 1973), and in order to indicate the multifunctional nature of this unique cell type Westfall proposed the term sensory-motor-interneurone.

Necator americanus stores the secretions of the amphidial glands in the form of membrane-bounded granules. The amphidial gland secretions of filariae and certain trichostrongyles are apparently synthesized and released directly from a system of reticulum, while the strongyle *Syngamus trachea* utilizes both secretion granules and reticulum. However, it is clear that the basic structural features observed in *N. americanus* amphids are present in all these nematodes, and therefore the same mode of action could well apply. The exaggerated development of nerve processes recorded in *Meloidogyne incognita* by Wergin and Endo (pers. comm.) is more difficult to explain, particularly since the majority of these processes are located within the gland duct rather than in the gland cytoplasm. Baldwin and Hirschmann (1973) called these processes "microvilli" and suggested that they were comparable to microvillar projections which have been described in certain arthropod sense organs (Carthy and Newell, 1968; Harris and Mill, 1973) and which are thought to be responsible for secreting the fluids that lubricate the dendrites (Harris and Mill, 1973). The arthropod microvilli arise from adjacent cells rather than from the axons innervating the cilia, however, and it seems unlikely that an analogy may be drawn between these microvilli and the nerve processes of *M. incognita*.

Cytochemical studies have shown that the amphidial glands of adult *Dipetalonema vitae* (McLaren, 1972a) and adult *Necator americanus* (McLaren *et al.*, 1974) contain the enzyme acetylcholinesterase. In the filarial worm the enzyme is localized within the cisternae of the paired membranes, whereas in the hookworm it is distributed throughout the gland cytoplasm. That the nematode actively secretes this enzyme has been demonstrated by maintaining *N. americanus* in *in vitro* culture, and comparing the level of enzyme present in the worm at the onset of the experiment, with that detected in the culture medium on varying days thereafter (McLaren *et al.*, 1974). The amount of cholinesterase released after 24 h in culture is more than three times that initially present in the worms, and the enzyme is continually released for up to ten days at a rate equivalent to about twice that initially present. It should be mentioned here that the oesophageal glands of *N. americanus* also contain acetylcholinesterase (McLaren *et al.*, 1974) and it is therefore probable that some of the secreted enzyme originates from this source.

Attempts to detect the release of cholinesterase by cultured *Dipetalonema*

viteae have proved unsuccessful (J. Burt, pers. comm.); this may well be due to the fact that the amphidial glands of this nematode are exceptionally small, and possibly have a limited capacity for enzyme synthesis. Furthermore, the oesophageal glands of *D. viteae* do not synthesize cholinesterase. McLaren (1972a) suggested originally that the cholinesterase present in the amphidial glands of this worm might be involved in nervous transmission. However, the results obtained with *N. americanus* indicate that the filarial enzyme is more likely to represent a specific secretion, even though it is apparently synthesized at an exceptionally low level.

Cholinesterase has also been demonstrated cytochemically in the amphids of several plant parasitic nematodes, including *Trichodorus christiei*, *Pratylenchus penetrans*, *Xiphinema index*, *Helicotylenchus nanus* and *Dorylaimus* sp. (Rohde, 1960). Detailed ultrastructural studies have yet to be made on these worms, and we therefore have no information regarding the presence or absence of amphidial glands; however, cytochemical evidence suggests that they may be present. We have conclusive proof that amphidial glands are present in *Meloidogyne incognita* (Baldwin and Hirschmann, 1973; Wergin and Endo, 1974), and although cytochemical studies have not been made on this particular species, a positive reaction for esterase has been recorded in the amphidial glands of both larvae and adult worms of *M. javanica* and *M. hapla* (Bird, 1966).

Jones (1974) has been unable to demonstrate the enzyme cholinesterase in the amphidial glands of *Syngamus trachea*, although she has shown that the dissected glands contain an anticoagulant. Similarly, Thorson (1956) and Eiff (1966) showed that extracts of the amphidial glands of the dog hookworm *Ancylostoma caninum* also contain an anticoagulant, and preliminary results indicate that cultured *N. americanus* may also secrete low levels of this substance (Ogilvie, pers. comm.). It would seem, therefore, that nematode amphidial glands may synthesize more than one type of secretion. The role of the anticoagulant is clearly associated with the blood-sucking nutritional habits of these strongyles. The function of the cholinesterase is more difficult to assess, but the enzyme has been said either to serve as a biochemical holdfast (Lee, 1970; Ogilvie and Jones, 1971) or to alter the permeability of the host plasma membranes and thus facilitate the leakage of nutrients (Lee, 1969).

Not all nematode amphids have associated glands, and in species lacking a gland it seems likely that the amphids function primarily in a chemoreceptive capacity. We have also seen that the amphidial sense organs of another nematode, *Oncholaimus vesicarius*, may possibly include both olfactory and photoreceptive terminals. It seems possible, therefore, that nematode amphidial sense organs may well contain receptors which are sensitive to a number of different stimuli.

Additional clues as to the function of nematode amphids may be obtained by studying the behavioural responses of the worms to different chemical and physical stimuli. This subject has been reviewed elsewhere and should be consulted in the original (Croll, 1970). Briefly, chemical attraction is known to play an important role in orientation, host finding and pairing (Green,

1971). Plant parasitic nematodes have been shown to move towards a carbon dioxide gradient (Croll, 1970), but oxidizing agents, reducing agents, ions and amino acids have also been reported to serve as attractants to other nematode species (Croll, 1970). More recently Ward (1973) has shown that the free-living nematode *Caenorhabditis elegans* responds to specific anions and cations, to basic pH and to cyclic AMP. Furthermore, he has proposed that the orientation response of the nematode is mediated by comparisons of stimuli by the anterior sensory receptors at successive lateral sweeps of the head; morphological studies indicate that these receptors are likely to be the amphids.

El-Sherif and Mai (1968, 1969) found that the plant parasitic nematodes *Pratylenchus penetrans*, *Ditylenchus dipsaci* and *Tylenchorhynchus claytoni* are able to detect and migrate along a temperature gradient, produced by an infrared beam of radiation, with an increase in temperature as small as 0·033°C per 4·0 c.m. The authors suggest, therefore, that the sense organs of these nematodes should be examined to determine whether they have an infrared detector configuration. The pit organs of certain snakes are known to be infrared sensitive structures (Bullock and Cowles, 1952; Bullock and Fox, 1957; Terashima *et al.*, 1970). These pit organs consist of a membrane, about 10 μm thick, suspended in the pit cavity, which is innervated by a conglomeration of non-myelinated nerve branchlets (Terashima *et al.*, 1970). A structural similarity between these pit organs and nematode amphids is not immediately apparent, but the amphidial cilia appear to be receptive to diverse stimuli, and may well have the capacity to detect an infrared temperature gradient.

Behavioural responses to sex attractants have been recorded in male worms of the genera *Panagrolaimus* (Greet, 1964), *Pelodera* (Jones, 1966) and *Heterodera* (Green, 1966). Greet *et al.* (1968) have shown that a water soluble substance can be extracted from female *Heterodera* by continuously washing the worm with distilled water for 2 days. This substance cannot be detected by chemical or physical techniques, but will successfully attract the male worms; it is therefore one of the first nematode attractants which may be classed as a pheromone. Gereart (1965) noted a distinct difference in the degree of development of the amphids in male and female worms of *Meloidogyne* and *Tylenchus* spp., and suggested that this may reflect the role of these sense organs in sex attraction and contact between the sexes.

Nematode amphids are clearly very interesting multifunctional sense organs which are only now becoming the subject of detailed studies. Present concepts of the function and mode of action of these structures are based on circumstantial evidence, and the development of electrophysiological techniques is essential if these speculations are to be substantiated.

C. CEPHALIDS

1. *Morphological studies*

LM studies on the plant parasitic nematodes *Heterodera glycines* and *Hoplolaimus tylenchiformis* have revealed the presence of two highly refractile

structures in the cephalic region of the worm (Hirschmann, 1959). These structures have been called the anterior and posterior cephalids; they extend around the body of the nematode and appear bioconcave in longitudinal section (Fig. 21). In *H. glycines* the anterior cephalid is located under the second cuticular annule posterior to the lip region; it occupies from one half to one full width of the second annule and because of its size it is often difficult to detect. The posterior cephalid is more conspicuous and occurs at the sixth to eighth annule posterior to the lip constriction. In the region of each cephalid some layers of the outer cuticle are either missing or reduced in thickness. Close inspection has shown that the dorsal and ventral hypodermal cords appear to originate at the level of the posterior cephalid, while the two lateral hypodermal cords appear to originate at the level of the anterior cephalid.

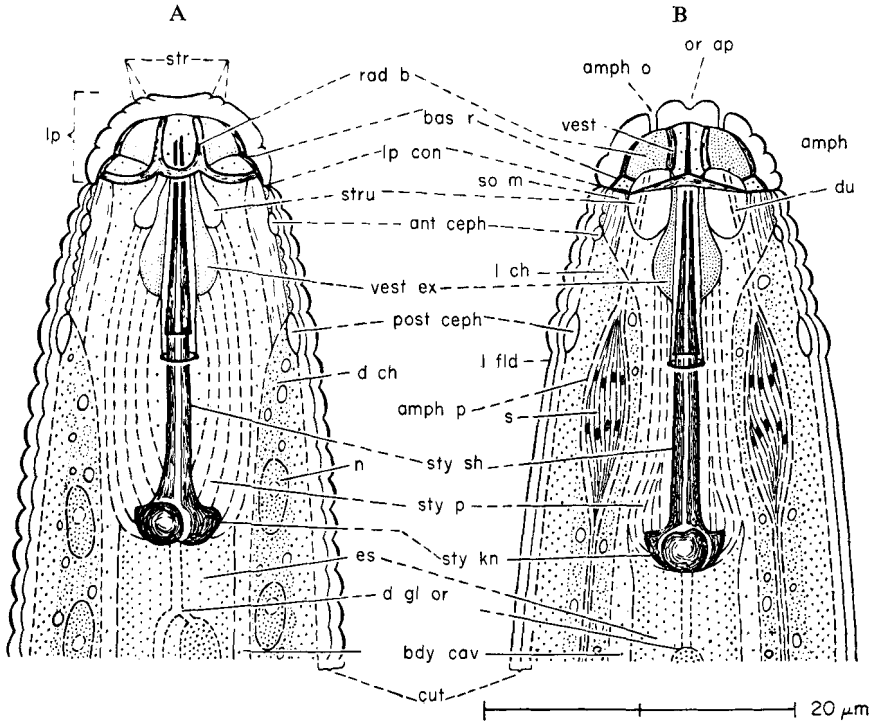


FIG. 21. Diagrams showing the position of the anterior and posterior cephalids in adult male *Heterodera glycines*. A, lateral view; B, dorsal view. Amphidial duct, amph du; amphidial pore, amph o; amphidial pouch, amph p; anterior cephalid, ant. ceph; basal ring, bas r; body cavity, bdy cav; cuticle, cut; dorsal chord, d ch; dorsal oesophageal gland orifice, d gl or; oesophagus, es; lip region, l; constriction of lip region, lp con; lateral field, l fld; lateral chord, l ch; nucleus, n; oral aperature, or ap; posterior cephalid, post ceph; radial blade, rad b; sensilla, s; somatic musculature, so m; striation, str; 8-shaped structure, stru; stylet knob, sty kn; stylet protractor, sty p; stylet shaft, sty sh; vestibule, vest; vestibule extension, vest ex. (From Hirschmann, 1959.)

2. *Functional considerations*

In the absence of the more detailed structural information to be gained from EM studies it is difficult to assess the biological significance of the cephalids. Hirschmann (1959) has suggested two possibilities. First, they may arise merely as a result of the attachment of the hypodermal cords to the body wall, and second, they may be associated with sensory perception, the stimuli being conducted alongside the hypodermal cords. Hirschmann commented upon a marked similarity between the cephalids and another highly refractile structure, the hemizonid, a ventro-lateral commissure located near to the excretory pore in many nematode species. The hemizonid of hookworms has recently been examined at the ultrastructural level (Smith, 1974) and has been shown to consist of nerve axons. Possibly, therefore, the anterior and posterior cephalids may also represent nervous commissures, and since the outer cuticle of the worm appears to be modified where it overlays these structures, the cephalids may conceivably function as pressure receptors.

III. Cervical Sense Organs

A. DEIRIDS

1. *Morphological studies*

Prior to 1923 these structures were called simply cervical papillae, but Cobb (1923) proposed that they be renamed "deirids" and defined them as "paired lateral organs, located in the vicinity of the central nervous system, which may vary in form from a papilla to a seta-like appendage". Goldschmidt (1903) considered them to be structurally similar to the external labial papillae of *Ascaris lumbricoides;* he reported that each sense organ is innervated by a branch of the nerve trunk which connects the medial externolateral ganglia with a nerve-ring, and that the sensory cell of each deirid lies within this ganglion.

Observations at the ultrastructural level are presently limited to those of Jones (1974) on the strongyle *Syngamus trachea.* The deirids of adult *S. trachea* are located just behind the nerve-ring, and each is innervated by a nerve axon which gives rise to a modified cilium (Fig. 22). The cilium itself is very short and its flattened distal end contains a dense, spherical inclusion body. The cilium lies within a cuticle-lined channel, but Jones has yet to determine whether this channel opens to the exterior. The small discontinuity seen in the cuticle overlying the ciliary tip suggests that a fine pore canal may in fact be present. Well developed rootlets are seen at the base of the cilium, and in this region the nerve axon gives off a large bulbous process. The nerve axon and the base of the channel are surrounded by a cell containing paired membranes which resembles the papillary gland cell. A supporting cell has also been observed. Prior to the eighth day of development the young adult worms of *S. trachea* have very narrow lateral alae and the sense organs which are destined to become the deirids of the mature adult worm are located within these alae. At this time the modified cilium has a cap of dense material, and in many ways resembles the cephalic papillary cilium of *S. trachea.* It is

only from the eighth day that the distal tip of the cilium becomes flattened and the characteristic dense inclusion body makes its first appearance.

It is convenient to mention here that a pair of similar, laterally positioned sense organs are located in the mid-region of the body of certain nematode species, and these are referred to as post-deirids. The post-deirids were first observed by Hesse (1892) in *Ascaris megalocephala*, but we have little infor-

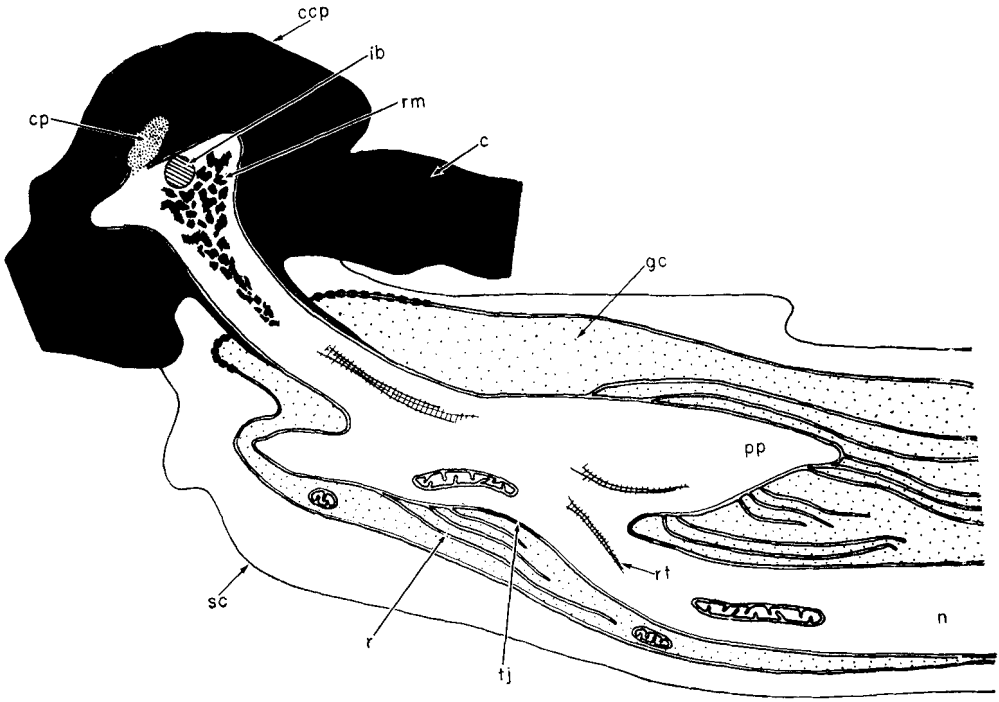


FIG. 22. Diagrammatic longitudinal section through a deirid of adult *Syngamus trachea*. Cuticle, c; conical projection of cuticle, ccp; ciliary cap, cp; gland cell, gc; inclusion body, ib; junctional complex, tj; nerve axon, n; posterior projection of nerve axon, pp; reticulum, r; reticulate material, rm; ciliary rootlet, rt. (Courtesy G. M. Jones.)

mation regarding their structure other than that in *A. lumbricoides* each post-deirid is innervated by a nerve fibre connected with a sensory neurone in the lateral cord (Goldschmidt, 1903).

2. *Functional considerations*

The paucity of information regarding the deirids and post-deirids makes it unrewarding to speculate on their possible function. However, their apparent structural similarity to the mechanoreceptive type of cephalic papilla suggests that they too may have similar capacity. The deirids of parasitic nematodes are often more conspicuous than are the labial and cephalic papillae, and it may be that they are responsible for determining whether or not the nematode can successfully pass through a restricted space.

B. HEMIZONID AND HEMIZONION

1. *Morphological studies*

The hemizonid (from the Greek, meaning "belt" or "girdle") was first observed by Goodey (1951) in plant parasitic nematodes belonging to the superfamily Tylenchoidea; it has since been recorded in many other nematode groups, however, and Timm (1960) has suggested that it might be universally present throughout the phylum. In whole mounts the hemizonid appears as a highly refractile, biconcave band apparently located between the cuticle and the hypodermis; it extends around the ventral side of the nematode and ends at the lateral fields. Where present, the deirids are located more or less opposite the ends of this structure (Goodey, 1951). The hemizonid is, in fact, a ventro-lateral commissure of the nervous system (Goodey, 1959), which joins the subventral nerve trunk with the lateral ganglia (Anderson and Das, 1967); it may be situated either anterior or posterior to the excretory pore (Caveness, 1961).

Observations at the ultrastructural level are limited. Bird (1968) identified a bundle of nerve fibres about 1·0 μm in width, in close association with a hypodermal duct of larval *Meloidogyne javanica;* he suggested that this structure might represent the hemizonid. Bird (1971) further suggested that a ventro-lateral commissure observed by Rogers (1968) posterior to the excretory pore in the infective larva of *Haemonchus contortus* might also be a hemizonid. Rogers reported that this commissure is composed of nerve axons containing neurosecretory granules. More recently, Smith (1974) has made a specific attempt to identify and describe the hemizonid in the second- and third-stage larvae of the cat hookworm *Ancylostoma tubaeforme* and in the third-stage larvae of the human hookworm *Necator americanus.* In both species the hemizonid is located just posterior to the excretory pore; it measures about 2·4 μm in width in *A. tubaeforme* and 2·9 μm in width in *N. americanus.* In longitudinal sections the hemizonid is seen as a double row of about 20 nerve axons, which are clearly separated from the cuticle by the hypodermis. Rogers (1968) reported that the outer cuticle of *H. contortus* is modified where it overlies the ventro-lateral commissure, but Smith (1974) could find no comparable cuticular modification adjacent to the hookworm hemizonid. Larval hookworms do have prominent lateral alae, however, and Smith finds that the striated basal layer of the cuticle is absent beneath these alae. Studies on the trichuroid nematode *Trichinella spiralis* have confirmed the nerve structure of the hemizonid (McLaren, pers. obsvn.). In adult *T. spiralis* the hemizonid consists of a group of about 12 nerve axons which contain microtubules, mitochondria and dense-cored vesicles resembling neurosecretory granules (Fig. 23). These axons are quite definitely situated between the hypodermis and the underlying somatic muscle cells, although the hypodermis is considerably narrower above the axons. The nerve axons comprising the ventro-lateral commissure of *H. contortus* were reported to merge into the cuticle without limiting membranes (Rogers, 1968). It is established that some free nerve endings do occur in the nematode cuticle (Bird, 1971), but the main bundle of nerve axons constituting the ventro-

lateral commissure of *H. contortus* appears, on close inspection of the published micrographs, to be overlaid by a very narrow layer of hypodermal tissue. The structural similarity between this commissure and the established hemizonids of hookworms and *T. spiralis* strongly supports Bird's contention that the ventro-lateral commissure described in *H. contortus* is in fact the hemizonid.

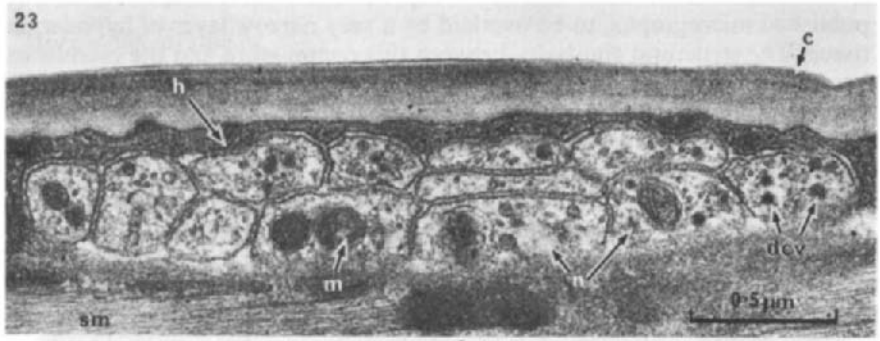
Hemizonion (the diminutive of hemizonid) was a term proposed by Caveness (1961) for a small ventro-lateral commissure located posterior to the hemizonid in various members of the Rhabditoidea, Tylenchoidea, Aphelenchoidea and Dorylaimoidea; it joins the sub-ventral nerve trunks with the posterior externo-lateral ganglia (Anderson and Das, 1967). Apart from its smaller dimensions the hemizonion, as seen with the LM, is apparently identical to the hemizonid; it has yet to be examined with the EM.

2. *Functional considerations*

Bird (1968) proposed that the nervous structure which he interpreted as the hemizonid of *M. javanica* might be a sensory receptor which triggers off the synthesis of protein granules in the sub-ventral oesophageal glands of this nematode. These protein granules are thought to contain enzymes utilized by the larva both in penetration of the egg-shell and the host root.

The exsheathing fluid produced by nematode infective larvae is known to be synthesized from a site near the excretory pore (Sommerville, 1957; Fisher, 1966; Rogers and Sommerville, 1960; Davey and Kan, 1967); it contains the enzyme leucine aminopeptidase (Rogers, 1965). The histochemical appearance of leucine aminopeptidase in the excretory glands of *Phocanema decipiens* may be correlated with the histochemical demonstration of neurosecretory activity in the dorsal and ventral ganglia of this worm (Davey, 1966). The hemizonid is located near the excretory pore, and has been shown to consist of nerve axons containing neuro-secretory granules (Rogers, 1968; McLaren, pers. obsvn.). Bird (1968, 1971) has suggested, therefore, that the hemizonid of zoo-parasitic infective larvae may be a receptor associated with neurosecretory activity and that the neurosecretions lead to the production of enzymes which initiate moulting.

Smith (1974) found that in larval hookworms, the characteristic striated layer of outer cuticle is absent in the region of the lateral alae. The hemizonid extends around the ventral side of the body and terminates beneath these alae, and Smith has suggested that the alae might represent an area of possible cuticular stimulation with the hemizonid providing the nervous connection to the circum-oesophageal ganglia. Whether or not there is any comparable modification of the lateral body cuticle in adult hookworms which lack alae has yet to be established. The general consensus of opinion seems to favour the idea that the hemizonid functions as a sensory receptor; possibly this receptor initiates different responses at differing stages of the life cycle. We have even less information regarding the structure of the hemizonion, but it seems at least possible that it may perform a similar role to the hemizonid.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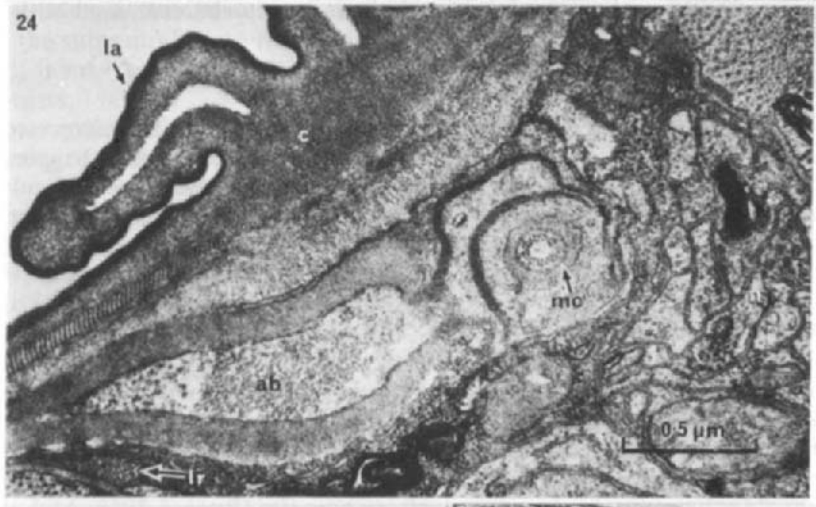
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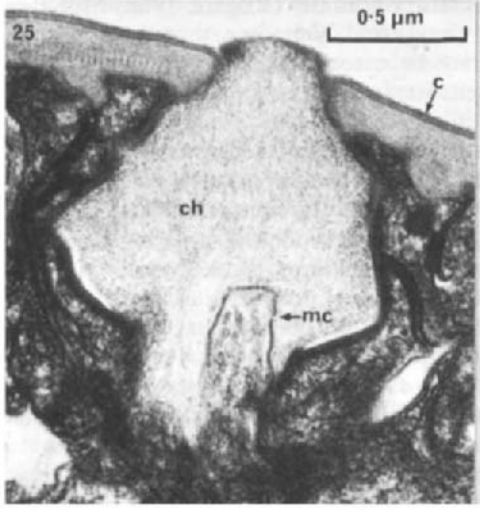
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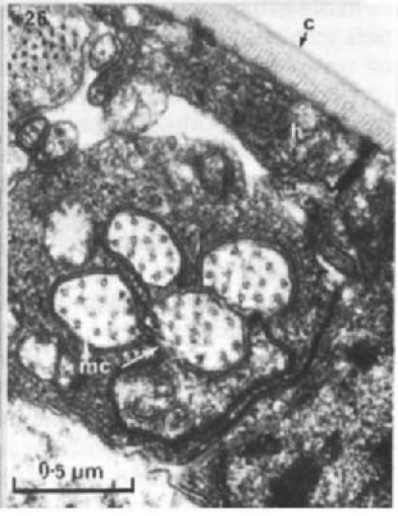
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C. LATERAL CERVICAL SENSE ORGANS IN LARVAL HOOKWORMS

1. *Morphological studies*

A single pair of lateral sense organs has been identified in the region adjacent to the excretory duct, in the third-stage larvae of both *Necator americanus* and *Ancylostoma tubaeforme* (Smith and Croll, 1975). Each sense organ contains a single modified cilium 3–4 μm in length which is orientated longitudinally beneath the lateral ala (Fig. 24); the cilium has a 9+0 arrangement of microtubular doublets. Each cilium lies adjacent to a discrete structure referred to as "the associated body", which is also orientated longitudinally beneath the lateral ala, and is seen in transverse sections as an elliptical cuticular structure with a cytoplasmic central core (Fig. 24). In some sections the cuticular outer layer of the "associated body" is in part continuous with the outer cuticle of the larva (Fig. 24). In other sections the outer cuticular layer of the "body" is missing in the vicinity of the cilium and in these circumstances the central cytoplasmic core of the "associated body" is continuous with the outermost of two cells surrounding the cilium (Fig. 24). The lateral nerves which run posteriorly down the length of the nematode appear to emerge from the nerve-ring in the region of the sense organs. Smith and Croll consider that the nerve axon which innervates the sensory cilium passes ventrally to join with the other nerve axons constituting the hemizonid. Furthermore, they have identified a nervous connection between the hemizonid and the ventral ganglion of the nerve ring.

Yet another nervous structure has been identified running parallel with the associated body and ala on each side of the nematode (Fig. 24); it has been traced from the anterior end of the larva and continues posteriorly beyond the associated body. The microtubules within this structure are arranged more regularly than in a typical nerve axon and Smith and Croll suggest that it might also function as a sensory receptor; they tentatively propose that it be called a "lateral longitudinal receptor". This proposed receptor has also been observed in other nematodes and Smith and Croll feel that it might be as widespread in the phylum as is the hemizonid.

2. *Functional considerations*

The occurrence of an internal ciliated sense organ beneath each lateral ala in this particular region of the larva has prompted Smith and Croll (1975) to speculate that a continuous morphological and sensory neural circuit

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FIG. 23. Longitudinal section showing the hemizonid of adult *Trichinella spiralis*. Cuticle, c; dense-cored vesicles, dcv; hypodermis, h; mitochondria, m; nerve axons, n; somatic muscle, sm.

FIG. 24. Transverse section showing the lateral cervical sense organ and lateral longitudinal receptor of an infective larva of *Necator americanus*. Associated body, ab; cuticle, c; lateral ala, la; lateral longitudinal receptor, lr; modified cilium, mc; nerves, n. (Courtesy J. M. Smith and N. A. Croll.)

FIG. 25 and 26. Transverse sections through the sense organ in the ventral anterior body wall of *Capillaria hepatica*. Cuticle, c; chamber of sense organ, ch; hypodermis, h; modified cilium, mc. (From Wright and Chan, 1974.)

may exist, which connects the alae to the nerve-ring by way of the associated body, the cilium and hemizonid. They further suggest that while the ciliary receptor may be sensitive to more than one modality, its morphology and relationship with adjacent structures is suggestive of a proprioceptor.

The "lateral longitudinal receptor" occurs along a greater length of the worm than does the ciliated receptor, and Smith and Croll suggest that this may be either a proprioceptor or a stretch receptor. A bilateral receptor system monitoring pressure, posture or mechanical deformity is suggested to exist in larval hookworms.

It is perhaps worthy of note that the deirids of the adult hookworm are located in much the same positions as those occupied by the larval ciliated sensory receptors. Furthermore, Jones (1974) has proposed that the cilia present in the lateral alae of young adult *Syngamus trachea* become the deirids of the mature adult worm. Thus, while the bilateral receptor system proposed by Smith and Croll (1975) is an attractive theory, there could be a much simpler explanation for the occurrence of sensory receptors in this localized region of the larval worm.

D. SENSE ORGANS IN THE BACILLARY BAND OF TRICHUROID NEMATODES

1. *Morphological studies*

In nematodes belonging to the superfamily Trichuroidea, regions of the lateral and median hypodermal cords are modified to form areas known as bacillary bands. The bacillary bands have been examined at the ultrastructural level in *Trichuris muris* and *T. vulpis* (Sheffield, 1963); *T. myocastoris* (Wright, 1968); *T. suis* (Jenkins, 1969); *Capillaria hepatica* (Wright, 1963) and *Trichinella spiralis* (Bruce, 1970); they have been found to consist of two cell types, (i) a non-glandular cell closely associated with the outer cuticle and (ii) a lateral hypodermal gland cell associated with a pore through the cuticle. The non-glandular cells are thought to function in cuticle formation and glycogen storage (Wright, 1968). The plasma membrane of the hypodermal gland cell is invaginated beneath the pore so that numerous long tubules extend deep into the cell cytoplasm. The morphology of these hypodermal glands is similar to that of known water- and ion-transporting cells, and Wright (1963, 1968) has suggested, therefore, that they might function in regulating the osmotic or ionic balance of the pseudocoelomic fluid of the nematode. More recently Wright and Chan (1973) have recognized an association between certain of the hypodermal gland cells and underlying sensory receptors in *C. hepatica* and *T. vulpis*. These receptors are restricted to the region between the nerve-ring and the most anterior stichocyte of the oesophagus. A receptor cell is closely applied to the base of each gland cell (Fig. 27); it contains the usual cytoplasmic organelles plus dense-cored vesicles resembling neurosecretory granules. Each receptor cell produces four to six modified cilia, which push into the cytoplasm of the gland cell and terminate beneath the infolded plasmalemma (Fig. 27). The shaft of each cilium contains between 5 and 12 microtubules while the basal region contains nine doublets surrounding one or two central singlets. The receptor cells do not appear to

be innervated by nerve axons and no synapses have been identified between these cells and other nervous tissues.

2. *Functional considerations*

Apart from the morphological characteristics, evidence supporting the idea that the hypodermal gland cells are involved in ionic or osmotic regulation has come from cytochemical experiments (Wright, 1963, 1968). Acid phosphate enzymes have been localized in the lamellar apparatus of the gland cell in *Trichuris* species, and the fact that colloidal gold particles are deposited on the boundary layer across the cuticular pore of the cell after gold has been injected into the host, suggests a possible filtering action for the gland cell.

The intimate association between the receptor cell and the hypodermal gland is, in some respects, reminiscent of the situation already described in nematode amphids, in particular those of filariae; possibly this may represent

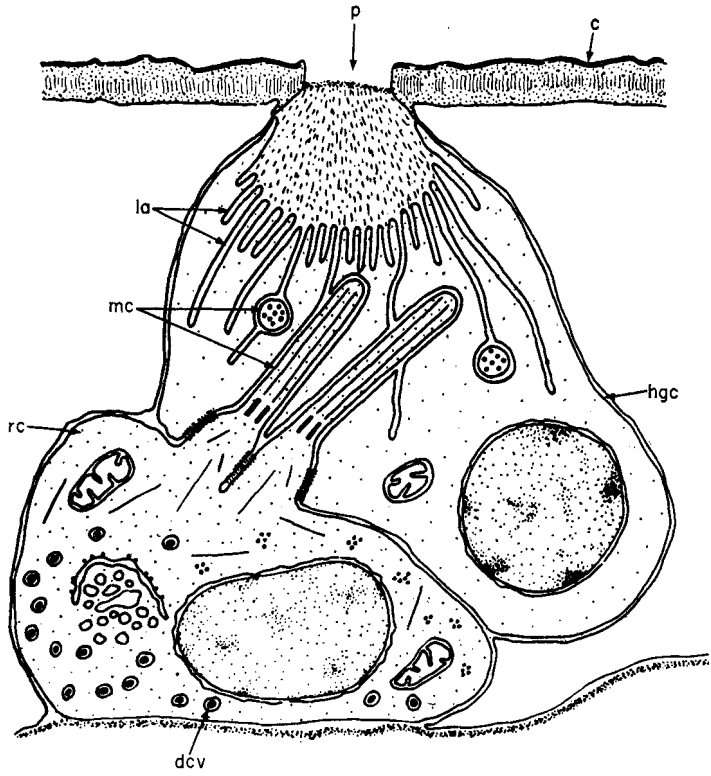


FIG. 27. Diagrammatic representation of the lateral hypodermal gland cell (hgc) and receptor cell (rc) of *Capillaria hepatica*. The receptor cell gives rise to modified cilia (mc) which push into the lamellar apparatus of the gland cell (la). Cuticle, c; dense-cored vesicles, dcv; pore, p. (From Wright and Chan, 1973.)

yet another example of a receptor–effector pair of cells. Wright and Chan (1973) concluded that the receptor cell is probably a sensory cell, capable of monitoring the environment within the extracellular channels of the lamellar apparatus in the gland cell. Thus, stimulation of the modified cilia might result in the release of neurosecretions from the supposed neurosecretory granules which occur in the receptor cell. The neurosecretions might then influence either the hypodermal gland cells themselves or even other body tissues. If this is indeed the case, then the receptor cells in the trichuroid bacillary band may be regarded as sensory-motor neurons. Wright and Chan have commented that the existence of a sensory motor-neuron in a tissue apparently involved in ion or water regulation is of particular interest, because an association such as this is as yet unparalleled in other animals.

E. A SENSE ORGAN ON THE VENTRAL ANTERIOR BODY WALL OF TRICHUROID NEMATODES

1. *Morphological studies*

In *Capillaria hepatica* a hitherto undescribed sense organ has been identified in the ventral body wall, behind the nerve-ring and about 55–60 μm from the head of the worm (Wright and Chan, 1974). This structure consists of a cuticle-lined chamber which opens to the exterior via a pore in the outer cuticle; the chamber contains extensive extracellular filamentous material (Fig. 25). Four modified sensory cilia (Fig. 26) enter the base of the chamber and terminate beneath the pore; their distal tips are bluntly truncated (Fig. 25). The axons innervating these cilia appear to travel anteriorly towards the nerve-ring enclosed within processes of the hypodermis. Wright and Chan have as yet identified but a single example of this sense organ in *C. hepatica* and suggest that it is either the only one present, or is one of very few.

An apparently identical sense organ has also been identified in the anterior ventral body wall of *Trichinella spiralis* (McLaren, pers. obsvn.). In this case the four nerve processes innervating the sense organ give rise to modified cilia which have conspicuous basal regions. Dense-cored vesicles resembling neurosecretory granules are seen within the nerve-axons posterior to these basal regions, and the filamentous material filling the chamber of the sense organ is much coarser and denser than that seen in *C. hepatica.* Random sections taken through several worms have included this sense organ on four occasions, which suggests that more than one example may be present in *T. spiralis.*

2. *Functional considerations*

The fact that this recently identified sense organ opens to the exterior via a pore in the outer cuticle indicates that it possibly functions as a chemoreceptor. However, it must be remembered that the sense organs occupying the positions of the labial and cephalic papillae of trichuroid nematodes also have a morphology indicative of a chemoreceptive capacity (Wright, 1974; McLaren, pers. obsvn.). This sense organ is clearly not one of the sense organs recently found to be associated with the bacillary band of *C. hepatica*

(Wright and Chan, 1973) because a characteristic hypodermal gland cell is not associated with the pore. Furthermore, the fact that it is ventral in position eliminates the possibility that this sense organ has any relationship with the laterally striated deirids. Speculations as to its function must await further observations on its relationship with other structures.

F. POSSIBLE GUSTATORY ORGANS ASSOCIATED WITH THE FEEDING APPARATUS OF *Longidorus leptocephalus*

1. *Morphological studies*

Recent ultrastructural studies on the plant parasitic nematode *Longidorus leptocephalus* (Robertson, 1974) have revealed the presence of a nervous connection between the central part of the feeding apparatus and the hypodermal cord. This connection comprises five nerve fibres, which lie freely within the pseudocoelom for part of their length, and are thus able to cope with protractions and retractions of the odontostyle. Robertson has suggested that these nerve fibres may provide a link between the nerve-ring and two sets of nerve processes, or modified cilia, which he has identified in the feeding apparatus; the two sets of nerve processes come together in the region of the odontophore. One set, comprising four small processes, extends anteriorly along the length of the stomodaeum in close association with the support membrane of the odontostyle protractor muscles; these nerve processes innervate the cheilostome. The nerve processes constituting the second set are located within the three sinuses of the odontophore, and are closely associated with pore-like regions which penetrate almost to the food canal.

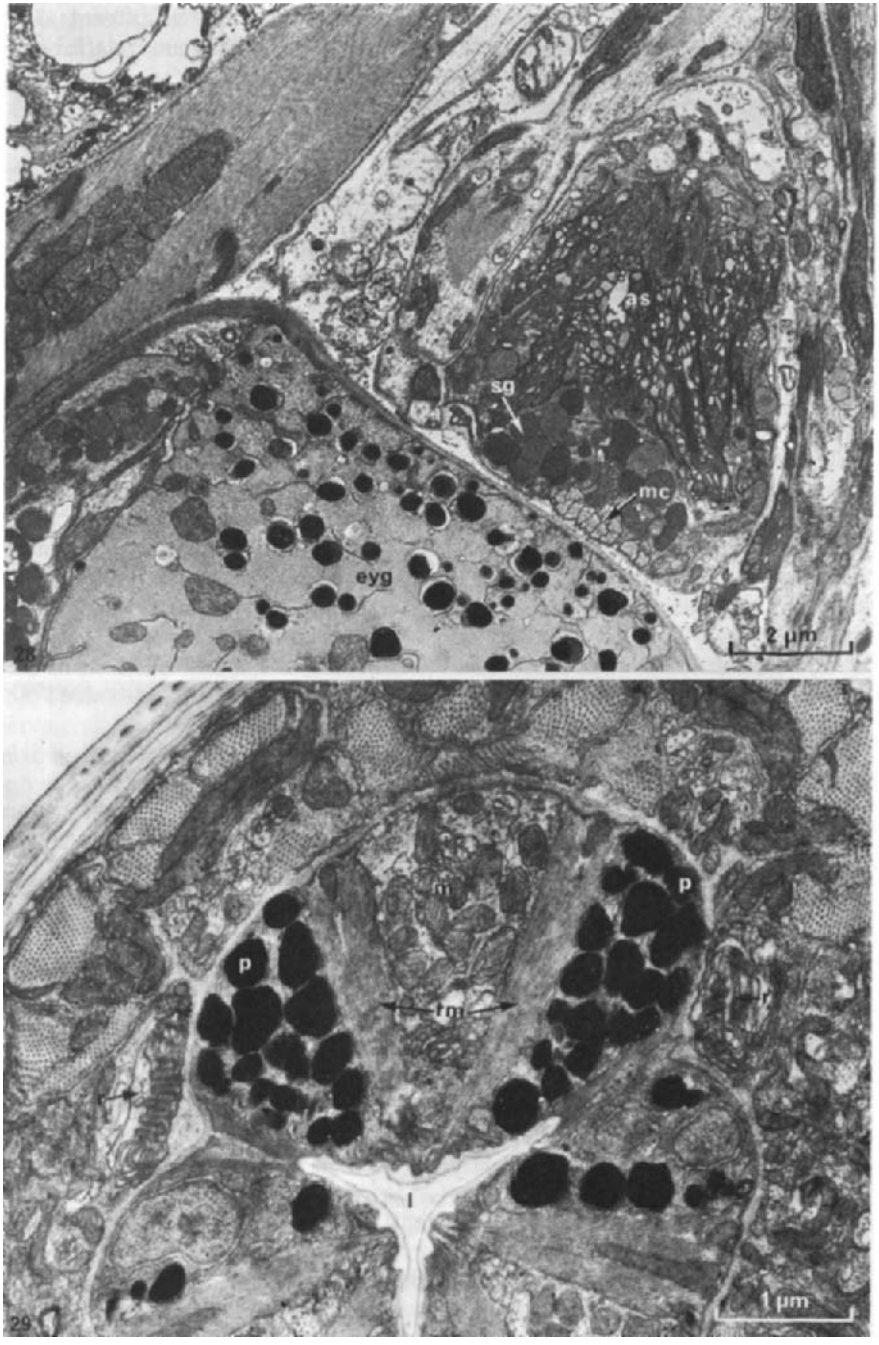
2. *Functional considerations*

On reaching the roots of a host plant the phytoparasitic nematode probes the root cells until it has located a suitable point for penetration, and then repeatedly thrusts its stylet against the cell wall so as to puncture the membrane. Secretions from the oesophageal glands are discharged through the stylet into the cell where they bring about extra-corporeal digestion of the cell contents. The digested contents are then withdrawn, via the stylet, by means of the pumping action of the oesophagus. The structure and location of the nerve processes observed in the odontophore of *L. leptocephala* have prompted Robertson (1974) to suggest that they may have a gustatory function, and thus facilitate the accurate location of feeding sites deep within the plant roots. Alternatively, it may be possible that the nerve processes are responsible for monitoring the release of oesophageal secretions into the host cell.

G. PHOTORECEPTORS

1. *Morphological studies*

Pigment spots have long been recognized in certain marine and freshwater nematodes; they are often associated with the oesophagus, usually paired, and vary considerably in colour. Many early investigators suggested that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these structures functioned as photoreceptors despite a report by Bastian (1866) that "they have no refracting portion answering to a cornea or lens", and a total lack of evidence as to their innervation. Rauther (1907) and Schultz (1931a) considered that the granules within the pigment spots of *Enoplus* and *Oncholaimus* were excretory products, but Chitwood and Chitwood (1950) reported that they could find no evidence to substantiate this suggestion.

Burr and Webster (1971) have examined the pigment spots of *Oncholaimus vesicarius* at the ultrastructural level; they have identified two types of pigment granule within the oesophageal muscle cells of this nematode. The dark red "eyespot" pigment granules are located only in the most anterior radial muscle cells; they have no associated nervous tissue. The only nervous structures observed in the vicinity of the "eyespots" are the amphids, and Burr and Burr (1975) have suggested that the "eyespots" may function as shading organs for proposed light-sensitive receptors in each of the adjacent amphidial sense organs (Fig. 28). The greenish coloured oesophageal pigment granules of *O. vesicarius* are located within other radial muscle cells and also within the marginal muscle cells. This pigment is not thought to have the same origin as the "eyespot" pigment and Burr and Webster have suggested that it may represent an accumulation of waste products.

The dark brown oesophageal pigment of *Enoplus communis* has been examined with the EM by Croll *et al.* (1974); it is variable both in shape and position, and is more dense in adults than in larvae. The pigment granules extend posteriorly from the concentrated "eyespots" and are, in some cases, dispersed throughout the oesophagus. In transverse sections through the oesophagus the pigment granules are located peripherally between the dilator muscles and the mitochondria. No associated nervous structure has been identified, however, and Croll *et al.* (1974) concluded that the pigment of *Enoplus* is not part of a discrete photoreceptor. It would be interesting to examine the amphidial sense organs of this nematode for possible photoreceptive terminals.

One further interesting example of cephalic pigmentation occurs in the gravid female of *Mermis subnigrescens*. A diffuse, non-granular, reddish pigment called the "chromatrope" appears in older females at the onset of oviposition; it is absent from males and young females (Cobb, 1926, 1929). Cobb (1929) found that the gravid female worm orientates itself and regulates oviposition in response to blue light, while Christie (1937) and Croll (1966a) observed that oviposition is arrested or greatly reduced in the dark, but is resumed with illumination. Ellenby (1964) used microspectrophotometric

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FIG. 28. Section showing the proximity of the amphidial sense organ (as) to the "eyespot granules" (eyg) of *Oncholaimus visicarius*. Certain of the modified cilia (mc) within the sense organ come to lie adjacent to the surface of the "eyespot" and are thought to have a photoreceptive capacity. Secretion granules, sg. (From Burr and Webster, 1971.)

FIG. 29. Transverse section showing laminated rhabdomeres (r) and oesophageal shading pigment (p) in *Chromadorina bioculata*. Lumen of oesophagus, l; radial oesophageal muscles, rm; mitochondria, m. (From Croll *et al.*, 1972.)

and histochemical techniques to identify the "chromatrope" pigment as oxyhaemoglobin. Ellenby and Smith (1966) reported that the pigment of *Mermis* is localized within large expansions of the hypodermis which extend towards the trophosome in the region of the "chromatrope". This has been confirmed at the ultrastructural level by Croll *et al.* (1974). In one specimen they found that the "chromatrope" pigment has a lattice structure with a frequency of 70 Å; this corresponds to the expected lattice for haemoglobin.

Schultz (1931b) distinguished between the pigment spots of *Enoplus* and the so-called "true ocelli with associated lenses" which he identified in *Leptosomatum, Deontostoma* and *Parasymplocostoma.* Almost all nematodes possessing ocelli are marine forms, and Weiser (1959) estimated that 40–60% of the total littoral marine nematodes have ocelli or ocellar pigment. No soil-dwelling, plant or animal parasitic nematode has ocelli and the possession of such structures tends, therefore, to be regarded as a primitive characteristic.

Schultz (1931b) reported that the ocelli of *Parasymplocostoma formosum* consist of two refractive bodies submerged in anteriorly situated pits. He described the lenses as invaginations of the superficial body cuticle behind each of which is a layer of brown granular pigment. Schultz did not believe the ocellus to be in any way connected with the oesophagus, but Chitwood and Chitwood (1950) entirely discounted this suggestion. Timm (1951) noted that two large oesophageal nuclei located a short distance behind the ocelli of *Deontostoma magnificum* are surrounded by ocellar pigment, and he suggested that these nuclei might function to control the production of pigment. Murphy (1963) reported that the paired, dorso-laterally situated ocelli of *Acanthonchus rostratus* are composed of a lensatic unit and a chromatic unit, both of which lie in a spindle-shaped pit that terminates at the body wall. In some instances the posterior extension of the pit contains an additional pigment cell, and Murphy postulated that this extension also included a nerve which extends from the ocellus back to the nerve-ring. This is one of the first reports to mention the innervation of the nematode ocellus.

The first ocelli to be examined at the ultrastructural level were those of the marine nematode *Deontostoma californicum* (Siddiqui and Viglierchio, 1970a,b), in which the paired ocelli are situated on the lateral margins of the oesophagus, each consisting of a pigment cell and a sensory cell (Fig. 30). The pigment cell, or cup, is located in the oesophagus and may be an enlarged marginal oesophageal cell. The anterior region of this cell is filled with dense pigment granules interspersed with bundles of filaments, while the posterior region is characterized by mitochondria and nerve fibres. The sensory cell is located within the pigment cup, but the two cells are separated by a fibrous connective layer. A ventro-lateral process links the proximal region of the sensory cell with the lateral cephalic nerve. The anterior region of the sensory cell is filled with stacks of concentric lamellae and thus resembles the photoreceptive rhabdomeres found in protostomes and platyhelminthes (Eakin, 1963). The morphology and innervation of this sensory cell has prompted Siddiqui and Viglierchio (1970a) to suggest that it might be a modified bipolar neuron. The ocelli of this nematode thus share three features with known photoreceptors from other phyla, (i) the presence of lamellae in the sensory

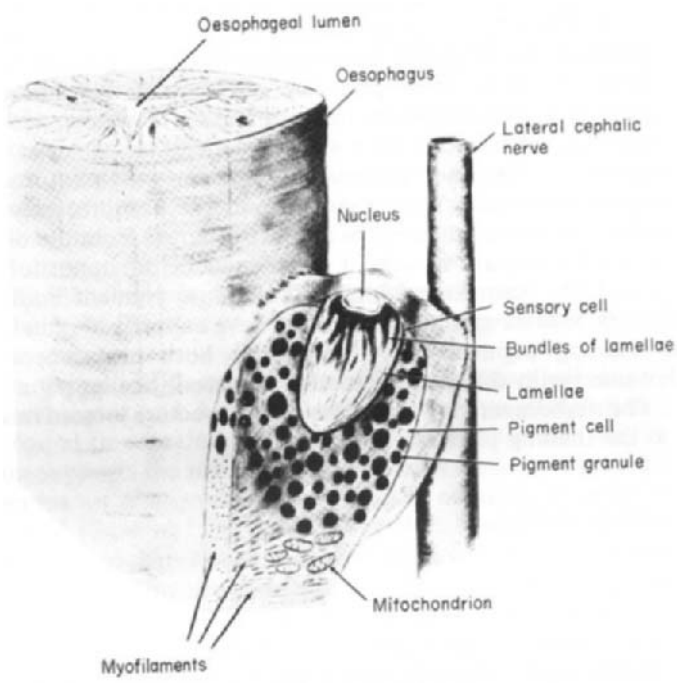


FIG. 30. Schematic diagram showing the structure and orientation of the ocellus in *Deontostoma californicum*. (From Siddiqui and Viglierchio, 1970b.)

cell, (ii) the nervous connection between the sensory cell and the central nervous system and (iii) the accumulation of shielding pigment around the sensory cell. It would seem from this study that the ocellus is the first discrete nematode sense organ which does not contain modified cilia. Siddiqui and Viglierchio (1970b) noted a marked similarity between the ocelli of *D. californicum* and those of rotifers (Eakin and Westfall, 1965) and suggested, therefore, that nematode photoreceptors belong to the rhabdomeric line of evolution. Despite the morphological evidence for the existence of photoreceptors in *Deontostoma californicum*, photoresponses have yet to be detected in this nematode. Photoreceptors present in nematodes belonging to the closely related genus *Leptosomatum* are structurally similar to those described in *D. californicum* (Croll *et al.*, 1974).

Photosensitivity has definitely been identified in the freshwater nematode *Chromadorina bioculata* (Croll, 1966b), however, and Croll *et al.* (1972, 1974) have studied the ultrastructure of the pigmented photoreceptors of this worm. The red "eyespots" of *C. bioculata* are located within the oesophagus, in the region of each dorso-lateral arm of the triradiate lumen; they contain oval and elliptical pigment granules (Fig. 29). The outer wall of the oesophagus is indented in the region of these pigment granules, and each cup-shaped invagination is occupied by a photoreceptive rhabdomere; the rhabdomeres

are thus situated outside the oesophagus (Fig. 29). Each rhabdomere consists of tightly packed bundles of membranes arranged in a series of concentric, flattened, spherical sacs (Fig. 29); the resulting lattice is perpendicular to the direction of illumination. These photoreceptive organs are surrounded by the nervous tissue which innervates the cephalic sense organs, and although no direct nervous connections have yet been identified Croll *et al.* (1972) suggest that some of these nerve axons may innervate the photoreceptors.

In the marine nematode *Araeolaimus elegans* the photoreceptive rhabdomeres and their associated pigment bodies are separate from the oesophagus (Croll *et al.*, 1974); they are situated in the pseudocoelom opposite the dorsolateral arms of the oesophageal lumen. The dense pigment bodies do not seem to contain discrete granules, but rather have an internal radial organization. The shading pigment of *A. elegans* is both ultrastructurally and spectrophotometrically different from other nematode ocellar pigments so far examined. The rhabdomere is a small laminated structure located immediately adjacent to the shading pigment.

2. *Functional considerations*

Based purely on circumstantial evidence, nematode pigment spots and ocelli have long been regarded as possible photoreceptive organs. It was not until these structures were examined with the EM that the two were clearly differentiated. Pigment spots apparently have no intimately associated sensory structures and cannot, therefore, function as discrete photoreceptors. Ocelli, however, consist of a photosensory rhabdomere innervated via the central nervous system, and shaded directionally by a closely associated pigment cell. The typical photoreceptive rhabdomere comprises a compact group of fine photoreceptor tubules (Moody, 1964), plates (Rohlich and Torok, 1961) or membraneous lamellae (Fahrenbach, 1964) derived from one cell. The laminations are thought to provide an increased photoreceptive surface (Horridge, 1964) and to contain small quantities of photosensitive pigments which are probably carotenoids (Wald, 1946). Nematode rhabdomeres clearly conform to this morphology and it seems likely that they may represent the so-called ocellar "lens" described by light microscopists.

Physiological and behavioural evidence for photosensitivity in nematodes possessing ocelli is limited. Spectrophotometric analysis of the shading pigments of *Chromadorina* sp. and *Leptosomatum* sp. indicate that these pigments are melanins (Croll *et al.*, 1974). Siddiqui and Viglierchio (1970b) also considered that the shading pigment of *Deontostoma californicum* is a melanin, while Burr and Webster (1971) suggested that the "eyespot" pigment of *Oncholaimus vesicarius* might be a lipofuschin. Such results are compatible with that fact the phenolic compounds such as melanins are found to accumulate around the sensory cells of known vertebrate and invertebrate photoreceptors, where theyare thought to aid directional sensitivity (Millot, 1957). Croll *et al.* (1972) found the area of the photoreceptor of *Chromadorina bioculata* can be stained for cholinesterase, along with a strand of tissue passing ventrally under the oesophagus; they suggest, therefore, that the photic

responses which have been clearly demonstrated in this nematode are under cholinergic control.

The "chromatrope" pigment of *Mermis* is thought to contain oxyhaemoglobin (Ellenby, 1964) and haemoglobin (Croll *et al.*, 1974), and the fact that this pigment exhibits a lattice structure at the ultrastructural level (Croll *et al.*, 1974) suggests that the haemoglobin may exist in a semi-crystalline state. Furthermore, phototaxis (Croll, 1966a) and an increase in the rate of oviposition in this nematode (Cobb, 1926; Croll, 1966a) have an action spectrum reflecting the form of the haemoglobin action spectrum. Haemoglobin or its derivatives have never been shown to be involved in photosensitivity, however, and rhabdomeric photoreceptors have not been identified in *Mermis*. The exact function of the chromatrope pigment remains, therefore, still to be resolved.

The pigment spots of *Enoplus communis*, a nematode which has no rhabdomeric photoreceptor and does not exhibit detectable photosensitivity, have also been found to contain melanins (Croll, 1966c; Croll *et al.*, 1974). This observation supports the idea that such pigment spots may possibly serve as shading organs for photoreceptive terminals in an adjacent sense organ such as the amphid (Burr and Webster, 1971; Burr and Burr, 1975). It would seem from the available data that rhabdomeric photoreceptors are likely to be the type most commonly encountered in the Nematoda, although it must be remembered that recent observations suggest the existence of possible photoreceptive cilia in the amphids of *Oncholaimus vesicarius* (Burr and Burr, 1975). More species need to be examined before we can say with any certainty that nematode photoreceptors follow the rhabdomeric line of evolution (Siddiqui and Viglierchio, 1970b). Furthermore, certain nematode species which lack discrete photoreceptors still show behavioural photoresponses to visible wavelengths (Croll, 1970). This response has been attributed to a dermal light sense (Croll, 1965, 1966d; Wilson, 1966) comparable to that found in other invertebrates (Steven, 1963). We have no clear evidence of the sensory receptors involved in this response, but nematodes are said to be abundantly supplied with free nerve endings (Hyman, 1951), and Bird (1971) has identified such endings within the cuticle of *Meloidogyne javanica* at the ultrastructural level. Furthermore, four major hypodermal commissures, the cephalids, the hemizonid, the hemizonion and the caudalids, have been identified in the hypodermis beneath the cuticle, and one of these, the hemizonid, has been shown to consist of nerve axons (Smith, 1974). Possibly, therefore, the free nerve endings, or the hypodermal commissures, or perhaps both, serve as receptors for the dermal light sense. In the absence of experimental evidence, however, this suggestion must remain purely speculative.

IV. Caudal Sense Organs

A. CAUDAL PAPILLAE

1. *Morphological studies*

Caudal or genital papillae are found principally in the male nematode, where they are grouped around the cloaca. The so-called caudal papillae of

the female worm are, in fact, the phasmids. Chitwood and Chitwood (1950) have recognized two standard arrangements of caudal papillae amongst the Phasmidia, (i) where there is a definite grouping of papillae with two to three pairs anterior to the cloaca and seven or eight pairs behind it, as in the strongyloids and rhabditoids; and (ii) where there are more serially arranged pre-cloacal papillae, but the post-cloacal papillae are not so clearly grouped, as seen in *Ascaris*. The structure of caudal papillae has not been examined in detail either at the LM or EM level. Goldschmidt (1903) reported that the genital papillae of *Ascaris* consist of raised areas of thin cuticle perforated by a canal containing from one to three nerve fibres, which are surrounded by a supporting cell. Chitwood and Chitwood (1950) noted that the fibres innervating the pre-cloacal papillae of *Ascaris* eventually reach the ventral nerve, whereas those innervating the post-cloacal papillae reach the latero-caudal nerves.

As far as I am aware the only published ultrastructural observations on caudal papillae concern the filarial worm *Dipetalonema viteae* (McLaren, 1972a) and the oxyurid nematode *Syphacea obvelata* (Dick and Wright, 1974). The adult male worm of *D. viteae* has seven pairs of caudal papillae of which three pairs are pre-cloacal, one pair is ad-cloacal and two pairs are post-cloacal. A further pair of smaller papillae is located at the tip of the tail. The outer cuticle of the nematode is much thicker in the tail region than elsewhere along the body, and large evaginations of the hypodermis extend into this thickened cuticle in the region of each papilla. The surface of each hypodermal evagination is thus covered by only a thin layer of cuticle. Longitudinal sections through the tail show that a large nerve axon penetrates through the hypodermal evagination and then takes the form of a modified cilium. For the most part the genital papillae of *D. viteae* resemble the cephalic papillae already described. There are, however, minor differences. First, the cilium is exposed to the external environment by way of a narrow channel in the surface cuticle; this channel appears star-shaped in transverse section. Second, the nerve axon innervating the cilium does not produce bulbous processes, and third, a supporting cell is present but a reticulum-containing gland cell is apparently absent. Fourth, branching lamellar structures are sometimes seen within the modified cilium (Fig. 31). Neither typical basal bodies nor basal rootlets have been observed, although in some cases a dense, spherical body is seen within the axon at the base of the cilium. The pair of papillae situated at the tip of the tail vary yet again. The modified cilium is longer than in the other caudal papillae and about half way along its length the microtubules become arranged peripherally so as to accommodate a core of dense, reticulate material. The distal tip of the cilium does not, therefore, take the form of a flattened disc as in the cephalic papillae. Furthermore, unlike any other sensory cilia in *D. viteae* these particular cilia have long, striated rootlets.

The four cloacal papillae of *S. obvelata* also contain a single nerve process surrounded by evaginated body tissue, but there is no evidence that these papillae open to the exterior. The basal zone of the cuticle becomes continuous with the cortex layer over the surface of the papilla, and Dick and Wright

(1974) have suggested that this facilitates the transmission of cuticular distortions to the underlying nerve.

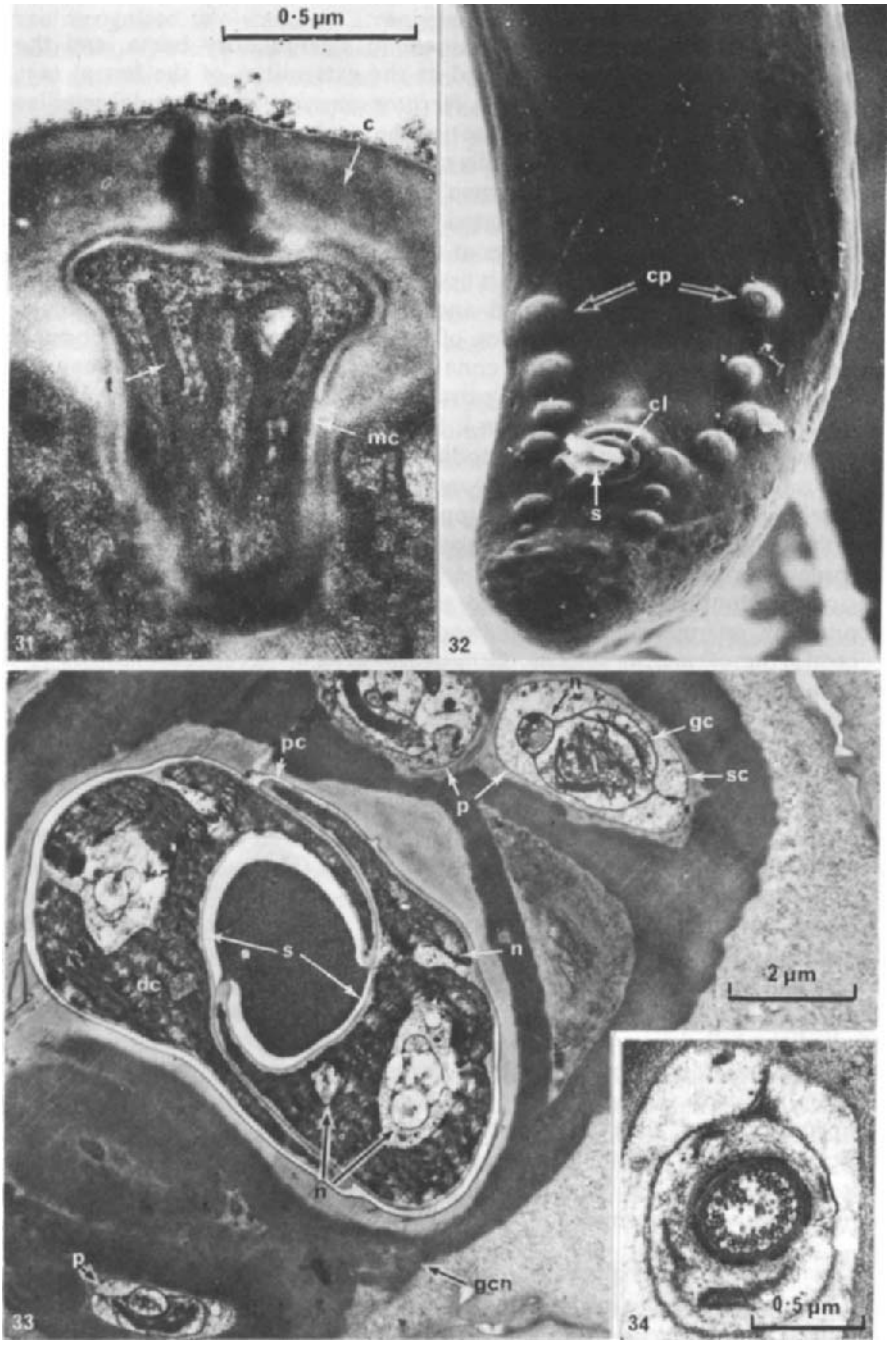
Male strongyloid nematodes terminate in a copulatory bursa, and the caudal papillae are generally situated at the extremities of the bursal rays (Chitwood and Chitwood, 1950). In *Necator americanus* the caudal papillae appear to be very similar to those in the cephalic region of the worm (McLaren, pers. obsvn.). Each papilla contains at least one and in some cases two cilia innervated by nerve axons containing numerous mitochondria. The cilia each have very long, striated rootlets. Dense, reticulate material is dispersed between the microtubules at the distal tip of the cilium.

The strongyloid bursa is not, as it first appears, a terminal structure; it is in fact lateral in position (Chitwood and Chitwood, 1950). The tail is much shortened and a posterior projection of the region around the cloaca forms a cloacal prominence or genital cone (Looss, 1905). In *Nippostrongylus brasiliensis* there seem to be two pairs of papillae (Fig. 33) located on this genital cone (McLaren, pers. obsvn.). Each papilla is innervated by a single nerve axon which gives rise to a modified cilium. The nerve axons are each partly surrounded by a small cell containing a few paired membranes, and this cell is itself surrounded by a supporting cell containing a few scattered bundles of fibres (Fig. 33). At the base of each cilium the microtubules show an ordered arrangement (Fig. 34); there are, consecutively, a ring of about 20 peripheral doublets, each joined to the ciliary membrane by a Y-shaped connection, an inner ring of singlets apparently connected to each other, and a central group of random singlets. Although not conclusive, appearances suggest that these papillae open to the exterior via a small pore in the outer cuticle.

From the few results obtained so far it would seem that the SEM could well be used to advantage for revealing the exact number and arrangement of nematode caudal papillae. Unlike many cephalic papillae, the caudal papillae seem to protrude some distance from the surface of the worm and are thus more easy to identify. The caudal papillae of the filarial worms *Dipetalonema viteae* (McLaren, 1971), *Dipetalonema spirocauda* (Helle and Blix, 1973) and *Dirofilaria immitis* (McLaren, 1971; Tulloch *et al.*, 1972) have all been examined with the SEM. The caudal papillae of *D. immitis* are the largest; each appears as a dome-shaped structure with a flattened top (Fig. 32). The normal ridges of the outer cuticle extend up the sides of the dome but not over its flattened surface. The central pore has yet to be identified. Weise (1973) has studied the tail region of *Ascaris suum* and found the male cloacal papillae to be mammiliform. The tails of both male and female worms terminate as a button-like knob. The caudal papillae of *Syphacea obvelata* are seen as cone-shaped structures, each with a cuticular nipple set on a small ring of cuticle (Dick and Wright, 1974); they have been regarded as sensitive to pressure or lateral deflection. The caudal papillae of *Ophidascaris* sp. (Sprent, 1973) appear similar to those of *S. obvelata.*

2. *Functional considerations*

McLaren (1972a) was unable to localize cholinesterase in the caudal papillae of *Dipetalonema viteae* by means of cytochemical techniques. This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result is perhaps consistent with the absence of a reticulum-containing cell in these particular sense organs. The caudal papillae are localized around the cloacal region of the male nematode, and it seems reasonable to suppose that they may assist in aligning the male cloaca with the female vulva prior to copulation. In strongyloid nematodes a copulatory bursa is used to grasp the female and enfold the vulval region, and here the papillae are situated at the extremities of the bursal rays. The caudal papillae of *D. viteae* open to the exterior by way of small pores, and may therefore function in a chemoreceptive capacity; possibly they serve as short range receptors complementing the longer range sensory capacity of the copulatory spicules.

B. SPICULES

1. *Morphological studies*

Copulatory spicules are elongate cuticular structures located in the cloacal region of the male nematode; they are usually paired but may exist singly, or be entirely absent. The spicules are extruded through the cloaca during copulation and are thought to dilate the vulva and vagina of the female worm. Two retractor and two protractor muscles are attached to the proximal end of each spicule and these control its movement through the cloacal aperture (Chitwood and Chitwood, 1950). At the LM level the spicules appear as tubular structures consisting of a central cytoplasmic core covered by sclerotized cuticle; the outer cuticular layer is continuous with the cuticular lining of the spicular pouch (Chitwood and Chitwood, 1950).

Recent transmission EM studies on the male worms of *Heterodera rostochiensis* have revealed a core of nervous tissue within the cytoplasmic central region of the spicule (Clark *et al.*, 1973). This nervous tissue appears to originate from the lateral nerve cord; it has been traced to the distal tip of the spicule where it terminates in the form of two dendritic processes. It was not stated whether these processes take the form of modified cilia. SEM has shown there to be two small pores near the tip of each spicule, and it is clear from ultra-thin sections that the dendritic processes end just beneath these pore openings. Clark *et al.* (1973) have concluded, therefore, that the spicules of these nematodes may be regarded as sensitive organs. The spicule blades of

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FIG. 31. Section showing a caudal papilla of adult male *Dipetalonema viteae*. The modified cilium (mc) contains tubular structures (t); it terminates beneath a thin layer of surface cuticle (c).

FIG. 32. Scanning electron micrograph showing the distribution of caudal papillae (cp) on the tail of adult male *Dirofilaria immitis*. The spicules (s) are seen protruding through the cloaca (cl). ×248.

FIG. 33. Transverse section through the genital cone (gen) and spicules (s) of adult male *Nippostrongylus brasiliensis*. Each of the interlocking winged spicules contains three bundles of nerve axons (n). The genital cone bears two pairs of papillae (p), and each papilla includes a nerve (n), a gland cell (gc) and a support cell (sc). Dense, hardened cuticle of spicule, dc; paler, unhardened cuticle of spicule, pc.

FIG. 34. Inset showing the complex microtubular arrangement in the cilium of a genital cone papilla.

Heterodera sp. are found to have incurved, interlocking wings, and Clark *et al.* (1973) have suggested that the tubular structure so formed, serves as an intromittent organ.

The spicules of *Heterakis gallinarum* and *Nippostrongylus brasiliensis* include several nerve axons which contain neurotubules, mitochondria, electron dense granules and vacuolated cytoplasm (Lee, 1973). Near the tip of each spicule the axons end blindly as dendrites containing closely packed neurotubules; these dendrites do not resemble the modified cilia seen in other nematode sense organs. Lee has been unable to detect a terminal pore in either species.

In transverse sections the distal region of each spicule of *Nippostrongylus brasiliensis* is roughly hemispherical in shape, with a short lateral projection extending from each end of its flattened base (McLaren, pers. obsvn.). The lateral projections of one spicule interlock with those of the other spicule and so form a central hollow tube. It is also clear from transverse sections that as the bundle of nerve axons approaches the tip of the spicule it divides into three groups. One group, comprising three or four nerve processes, is located roughly at the centre of the spicule, while the other two groups are located near to the origins of the lateral projections and each contains only one process (Fig. 33) The outer cuticular layer of the spicule is impregnated with electron-dense material which is difficult to section, and is presumably harder, therefore, than typical cuticle. The distal region of the spicule and its lateral projections are composed almost entirely of this dense, hardened cuticle; they have only a very narrow surface layer of paler, unmodified cuticle. However, in the region of each group of nerve processes the dense cuticle is interrupted by an invagination of the unmodified surface cuticle, which makes contact with the underlying nerves (Fig. 33). This arrangement may serve to bypass the physical barrier imposed by the hardened cuticle, and thus facilitate transmission of cuticular pressure or distortion to the sensory endings.

In *Necator americanus* the spicular arrangement differs somewhat, since in transverse sections the distal regions of the two spicules are seen as right-angled triangles apparently fused along their adjacent upright sides (Fig. 35) (McLaren, pers. obsvn.). Each spicule gives rise to one very long, lateral projection, the free end of which overlaps with that of its partner to form a central hollow tube. The dense hardened cuticle is restricted to the region immediately adjacent to the central cytoplasmic core of the spicule; small discrete blocks of dense cuticle are sometimes also seen within the lateral projections. A bundle of three nerve axons extends along the shaft of the spicule, but only one of these approaches the distal tip where it appears to take the form of a modified cilium (Fig. 35). At the very tip of the spicule the unhardened surface cuticle extends between the dense hardened cuticle to make contact with this cilium. A terminal pore has not yet been identified. There is some indication that another nervous structure approaches the distal tip of the spicule, but this structure is always located within the unhardened surface cuticle, and apparently does not resemble a cilium (Fig. 35).

In the filarial worm *Dipetalonema viteae* the spicules are unequal and

dissimilar, the right spicule being longer than the left and grooved (Terry *et al.*, 1961), and perhaps this longer spicule is modified for the transmission of sperm, because male gametes have been seen within the groove (McLaren, 1971). A bundle of nerve axons has been identified within the cytoplasmic core of this spicule, but only one of them approaches the distal tip (Fig. 36), where it takes the form of a modified cilium (McLaren, pers. obsvn.). The existence of terminal pores has not yet been established. Furthermore, the entire distal tip of the spicule is composed of dense hardened cuticle, and there is no evidence that the cilium makes contact with any unhardened regions of cuticle.

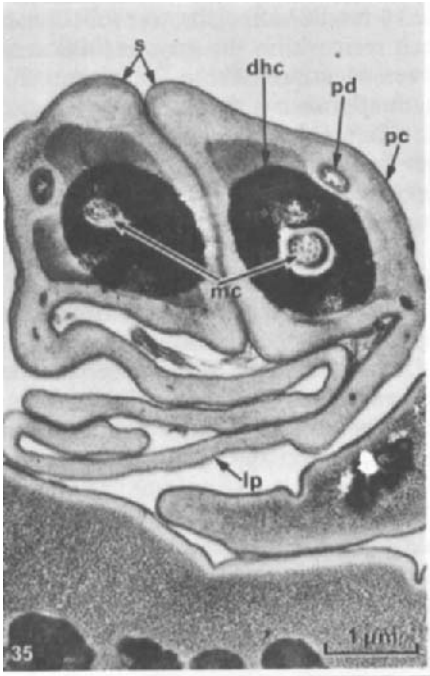
Dick and Wright (1974) have identified a nerve process within the single spicule of *Syphacea obvelata*, but the tip of this spicule appears to be solid, and there is no evidence of a terminal pore. These authors also examined the gubernaculum and accessory piece of *S. obvelata;* most of the gubernaculum lies within the body cavity, but the distal end enters the body wall cuticle and is continuous with the accessory piece. The accessory piece is hollow and contains a nerve process.

2. *Functional considerations*

Over the years a variety of functions has been attributed to nematode spicules; Schneider (1866) suggested that they might be locatory and excitatory, Looss (1905) and zur Strassen (1907) suggested that flanged spicules may come together to form a tube through which sperm are transmitted to the female worm, and more recently Chitwood and Chitwood (1950) have proposed that the spicules move back and forth during copulation so as to keep the vulva open, and to some extent assist in propelling the sperm inside. Recent ultrastructural studies have shown the morphology of the spicules to be such that all these suggestions may be valid.

Cholinesterase has been localized in the centre of the spicules of *Ascaris lumbricoides* (Lee, 1962), *Heterakis gallinarum* and *Nippostrongylus brasiliensis* (Lee, 1973). This region is now known to contain nervous tissue with sensory endings, and it therefore seems reasonable to suppose that the spicules are probably sensory structures under cholinergic control. The dendritic process within the spicules of *Heterodera* sp. end beneath small pores opening to the exterior (Clark *et al.*, 1973) and may therefore have a chemoreceptive capacity. Pores have yet to be identified in the spicules of any other nematodes examined to date, but in many cases the sensory endings are located beneath specific regions of unhardened cuticle, and may function in a mechanoreceptive capacity. On the ultrastructural evidence Lee (1973) has proposed that the spicules may function as sensory probes which first detect the female vulva and then feel their way into the vagina prior to ejaculation. Spicules not endowed with a sensory capacity could unwittingly damage the female tissues. The accessory piece of *Syphacea obvelata* is also found to contain a nerve process, and since extrusion of the spicule bends the accessory piece outwards, Dick and Wright (1974) have suggested that the accessory piece aids in the positioning of the spicule.

Sex attraction in nematodes is known to occur in several genera. Greet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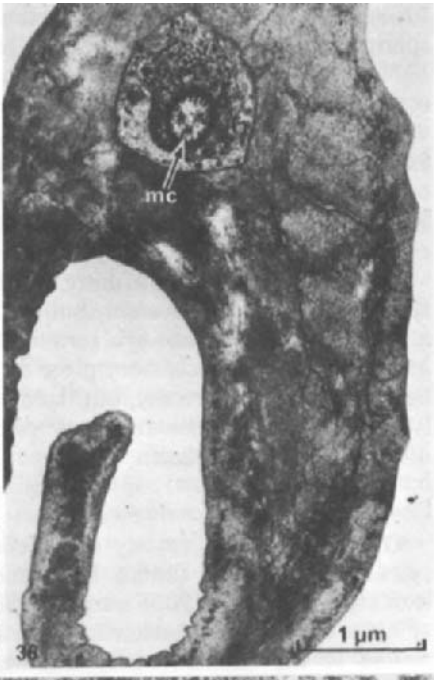
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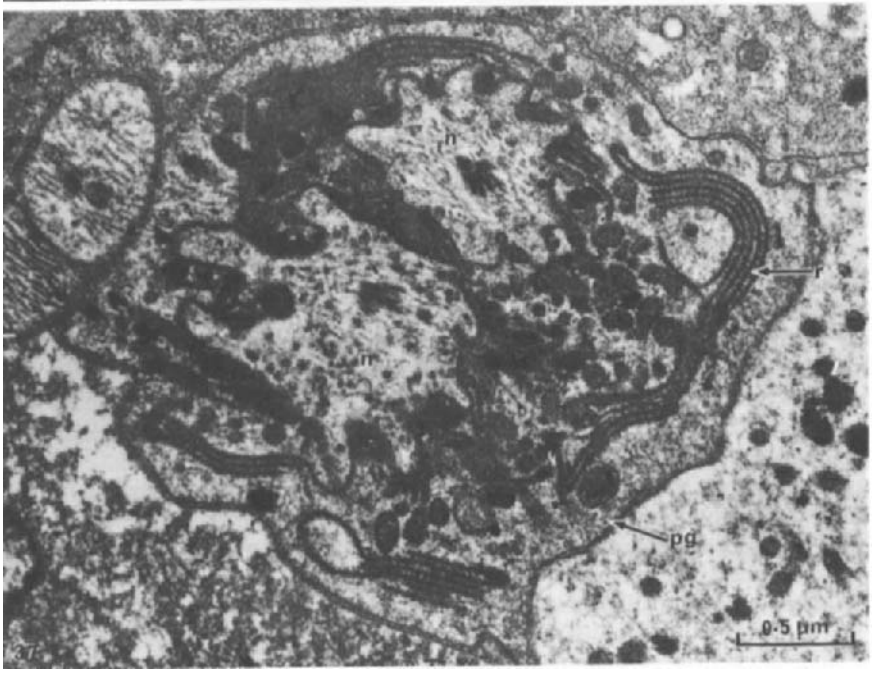
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(1964) reported that each sex of *Panagrolaimus rigidus* produces a secretion which attracts the opposite sex; he emphasized that this chemical attractant merely serves to bring the sexes together, however, and suggested that copulation may be initiated by subsequent tactile stimuli. In *Heterodera rostochiensis, H. schachtii* (Green, 1966) and *Pelodera* sp. (Jones, 1967) it is only the male nematodes which are attracted to the females. Indeed Croll (1972) observed that the sensory response of the male worm to the stimulus provided by a sex attractant overrides the endogenous feeding pacemaker. The female apparently has no corresponding receptor and continues to feed. Using laser-beam irradiation of various sites in *Panagrellus silusiae*, Samoiloff *et al.* (1973) showed that the distal end of the spicule is the probable initial receptor for mating attraction. Damage to only one of the paired spicules blocks this response, thereby indicating that the process of attraction involves orientation of the nematode so that the concentration of the attractant is equal at each spicule. Upon sensing the attractant the male worm makes rapid changes in orientation, and its extremities sweep through a maximal area (Samoiloff *et al.*, 1974); this behaviour implicates the amphidial and phasmidial sense organs in assisting orientation to the gradient of the attractant. It is clear from these latter studies that spicules probably function initially in a chemosensory capacity, and that upon careful re-examination terminal pores may well be identified above the sensory nerve endings.

C. PHASMIDIAL SENSE ORGANS AND GLANDS

1. *Morphological studies*

The phasmids are lateral paired structures located in the tail region of many free-living and parasitic nematodes, and they correspond to but are much smaller than the more anterior amphids. These structures are extremely difficult to locate at the level of the LM and it was Cobb in 1923 who proposed the term phasmid, meaning "phantom" or "ghost thing". The presence or absence of phasmids has been used as the basis of a taxonomic classification proposed by Chitwood and Chitwood (1933). However, as Goodey (1963) pointed out, phasmids have now been identified in nematode species in which they were previously considered absent, and this system of classification cannot, therefore, be regarded as entirely satisfactory.

Cobb (1923) defined the phasmids as "small lateral organs, externally pore-like, whose internal connections have yet to be determined", and he considered that they are probably glandular rather than sensory. According to Chitwood and Chitwood (1950), each phasmid consists of a phasmidial gland which opens, via a short tube, through a sub-ventral or laterally

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FIG. 35. Section showing the spicules (s) of adult male *Necator americanus*. Each spicule contains one modified cilium (mc). Dense, hardened cuticle, dc; paler, unhardened cuticle, pc; lateral projection of spicule, lp; possible accessory dendrite, pd.

FIG. 36. Section showing the grooved spicule of adult male *Dipetalonema viteae*. Modified cilium, mc.

FIG. 37. Transverse section through the phasmidial sense organ of adult female *Necator americanus*. The two nerve axons (n) lie within a phasmidial gland (pg) containing reticulum (r).

situated pore. Sensory terminals, which resemble those of the amphid, but are fewer in number, are found within the phasmidial tube. Most nematodes have a single pair of caudal phasmids, but many species of the genus *Hoplolaimus* have two large shield-like phasmids located laterally, one in the anterior and the other in the posterior part of the body (Ellington and Morgan Golden, 1971). Furthermore, Ellington and Morgan Golden have recorded multiple phasmids in paratype material of *Hoplolaimus concauda-juvencus*, and they suggest that the 15–18% incidence of multiple phasmids in male and female worms, eliminates the possibility that this phenomenon occurs only in aberrant specimens.

The phasmids have received much less attention from electron microscopists than have the amphids. Kozek (1968, 1971) reported paired ciliary channels in the caudal region of the microfilaria of *Dirofilaria immitis* which he identified as the "röten Schwanzgebilde" described from LM studies (Fülleborn, 1913); he suggested that these structures are associated with the developing phasmids. McLaren (1969, 1972b) also reported the existence of ciliated phasmidial sense organs in the microfilariae of *Dipetalonema viteae*, *D. setariosum*, *Dirofilaria immitis*, *Loa loa* and *Litomosoides carinii*. In each of these first-stage larvae the phasmidial channels are of approximately equal length, but one is usually situated slightly behind the other. Each channel is lined with cuticle and there is a thickened cuticular rim around the phasmidial pore, which is filled with a plug of electron-dense material. The single modified cilium occupying each channel is also invested with dense granular material.

The phasmids of the adult worm of *Dipetalonema viteae* are located at the tip of the tail; they are structurally very similar to the adult amphids, except that each phasmid contains only one modified cilium (McLaren, 1971, 1972a). The small phasmidial gland contains a limited reticulum, the membranes of which are orientated parallel to the shaft of the cilium; the gland is partly surrounded by a supporting cell containing bundles of fibres.

Muller *et al.* (1970) and Muller and Ellis (1973) have examined the phasmids in the first-stage larva of *Dracunculus medinensis*, finding that each phasmid includes two cilia which are apparently typical cilia rather than the modified cilia found in other nematode sense organs. Each phasmid consists of a single cell with a lumen connected to the exterior by way of a closable pore. The cell comprises an outer region filled with mitochondria and an inner region which borders on the lumen and contains endoplasmic reticulum. The reticulum gives rise to microtubules which project into the lumen of the cell; it bears very little resemblance, however, to the parallel arrays of paired membranes which constitute the reticulum seen in *Dipetalonema viteae*. The lumen of the phasmidial cell is supported by a framework of six darkly staining fibrillar strands joined at both distal and proximal ends by rings of similar fibrillar material. Clearly the phasmids of *D. medinensis* differ markedly from those of other filarial nematodes examined to date.

Recent observations on *Necator americanus* have revealed that the phasmids of both adults and fourth-stage larvae are structurally similar to those of *Dipetalonema viteae* (McLaren, pers. obsvn.). The small phasmidial gland contains a well developed reticulum composed of parallel membranes (Fig.

37), which contrasts with the elongated, granule-containing gland associated with the amphids of these hookworms. Each phasmid includes two modified cilia innervated by nerve axons. At the bases of these cilia the axons develop short bulbous processes, and in this region tight junctions are visible between the apposed plasma membranes of axons and gland (Fig. 37). The pair of cilia enter a short, cuticle-lined channel which is surrounded by a supporting cell.

The phasmidial pores of *Baylisascaris tasmaniensis* (Sprent *et al.*, 1973) and *Ophidascaris* sp. (Sprent, 1973) have been clearly revealed with the SEM but in many smaller nematodes the phasmidial pores have not been detected by this technique.

2. *Functional considerations*

The phasmids of the free-living nematodes *Rhabditis terrestris* (Stephenson, 1942) and *Pelodera strongyloides* (Poinar, 1965) and of the parasitic first-stage larva of *Dracunculus medinesis* (Muller and Ellis, 1973) have been found to enlarge when the worms are placed in hypertonic solutions; they have been suggested, therefore, to have an osmoregulatory function (Stephenson, 1942; Poinar, 1965).

Cytochemical techniques at the LM level have revealed the presence of esterases in the phasmids of *Oesophagostomum dentatum* (Ramisz, 1966), *Dracunculus medinensis* (Muller and Ellis, 1973) and *Necator americanus* (McLaren, pers. obsvn.), and acid mucopolysaccharides in the phasmids of *D. medinensis* (Muller and Ellis, 1973). At the ultrastructural level, cholinesterase has been localized in the phasmidial gland reticulum of *Dipetalonema viteae* (McLaren, 1972a). We have no information, however, as to whether or not these substances are actively secreted by the nematodes.

Paramanou (1954) proposed that nematode phasmids be divided into glandular and sensory types, the glandular type representing the more primitive function. On the limited information available it would seem that the phasmids may have both glandular and sensory functions, although it is clear from the work on *Necator americanus* that the phasmidial glands of this worm do not attain either the size or the secretory capacity achieved by the amphidial glands. The morphology of the amphidial and phasmidial sense organs is very similar except that the phasmids contain fewer sensory cilia; this may possibly indicate that the phasmids are capable of monitoring only a limited range of stimuli. The fact that the amphids and phasmids are located at the extremities of the nematode suggests that they may function to detect differences in the intensity of a given stimulus and thus help maintain the worm in a favourable environment.

D. CAUDALIDS

1. *Morphological studies*

Caudalids are hypodermal commissures located in the anal region of the nematode. They were first recorded by Rohde (1883–5) during his investigation of the nervous system in the tail of *Ascaris*, and were later described

by Chitwood and Chitwood (1950) as paired ano-lumbar commissures linking the pre-anal ganglion to the lumbar ganglia. Anderson and Das (1967) reported that the caudalids of *Stictylus macrocellus* appear to be latero-lateral commissures, which join the ventral nerves at different levels thereby creating the appearance of being "double". There have been no untra-structural observations made on these commissures, but it seems likely that they will be found to resemble the hemizonid.

E. A POSSIBLE STRETCH RECEPTOR IN THE INTESTINAL–CLOACAL JUNCTION OF *Heterakis gallinarum*

1. *Morphological studies*

The nematode intestine is a simple tube composed of a single layer of cells bearing apical microvilli, and surrounded by a basal lamina (see Bird, 1971). In some species a network of muscle fibres has been identified external to the basal lamina of the posterior intestine (Lee and Anya, 1968; Lee, 1975). The female rectum and the male cloaca are lined throughout with cuticle continuous with the external cuticle of the worm; this lining terminates where the rectum/cloaca meets the intestine. At the intestino-rectal/cloacal junction the posterior intestinal cells are surrounded by a sphincter muscle and this constitutes the intestino-rectal/cloacal valve (Chitwood and Chitwood, 1950).

Ultrastructural studies on the male nematode *Heterakis gallinarum* have shown that the intestino-cloacal junction has a most unusual morphology (Lee, 1975) (Fig. 38). The sphincter muscle is structurally similar to the somatic muscles; it consists of a myofibrillar portion which lies adjacent to the intestinal cells, and an amyofibrillar portion which extends towards the ventral nerve cord. When contracted this sphincter muscle constricts the posterior intestinal cells, and thus seals the intestinal lumen. The cells forming the posterior region of the intestine differ markedly from those in all other regions; they are seen as narrow, elongated cells which project into the lumen of the intestine, so forming a cone-shaped structure. The intestinal lumen surrounds this cone and also passes through its centre to join with the lumen of the cloaca. The cells themselves are characterized by much shorter apical microvilli, numerous microtubules within the cytoplasm, and dense granules localized in the basal region. Long zona adhaerens occur between the opposed plasma membranes of adjacent cells. In some cases the longitudinally orientated microtubules extend throughout the length of each cell and appear to be associated with both its apical and basal membranes. In other cells the microtubules are grouped in the basal region of the cell but fan out and terminate in the distal part of the cell without reaching the apical membrane.

Some cells extend through the sphincter region and project into the cloaca; their basal regions thus lie adjacent to the sphincter muscle. These cells contain microtubules and dense basal granules, but they do not bear apical microvilli (Fig. 38). A number of other cells lie between the microtubule-containing cells and the cuticular lining of the cloaca; they do possess

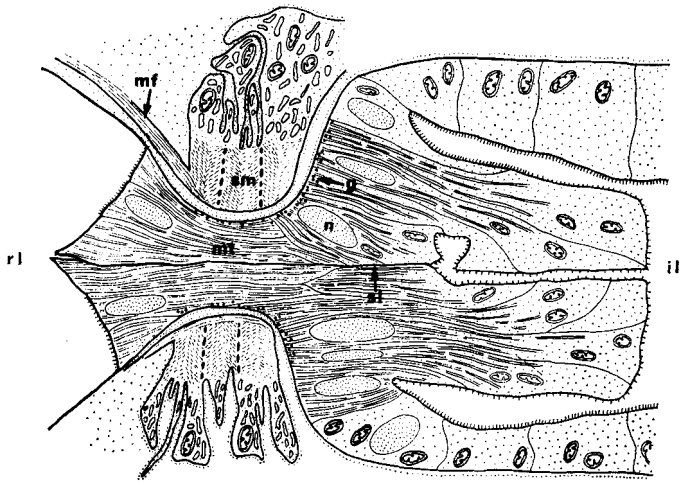

FIG. 38. Diagram of a longitudinal section through the intestinal–cloacal junction of adult male *Heterakis gallinarum* to show details of the modified intestinal cells, and of the sphincter muscle. Electron-dense granules, g; myofilaments, mf; intestinal lumen, il; microtubules, mt; nucleus, n; rectal lumen, rl; sealed lumen of intestine, sl; sphincter muscle, sm. (From Lee, 1975).

microvilli on their outer surfaces. Together these two groups of cells form a cone which projects into the lumen of the cloaca.

In one nematode the intestinal cloacal valve was fixed in the open position, and in this specimen the posterior intestinal cells were not constricted by the sphincter muscle and appeared much plumper; they also seemed to contain fewer dense, basal granules. Faeces were seen to be passing through the valve region into the cloaca of this worm.

Although these observations are restricted to the male nematode, Lee is certain that the intestino-rectal junction of female *Heterakis* shows much the same morphology.

2. *Functional considerations*

The high hydrostatic pressure of nematode pseudocoelomic fluid has been suggested to maintain orifices in the closed position. Dilator muscles have therefore been held responsible for opening these orifices, and consequently sphincter muscles have been considered unnecessary (Harris and Crofton, 1957; Crofton, 1966). Sphincter muscles have been identified, however, at the intestinal–rectal junction in all groups of the phylum (Chitwood and Chitwood, 1950), and have been said to close the intestino-rectal valve and thus prevent materials from re-entering the intestine during defaecation. More recently, Lee (1975) has suggested that as *Heterakis* does not have an anal sphincter, the intestino-cloacal sphincter is more likely to be responsible for preventing the movement of material from the intestine to the cloaca, except at defaecation. Furthermore, Lee has proposed two methods to account for the operation of this sphincter at defaecation. Firstly, the pumping action of

the oesophagus and/or the contraction of the muscle network around the intestine, might cause a build-up of pressure in the intestinal lumen which eventually overcomes the force exerted by the sphincter muscle. Release of some of the intestinal contents would result in a fall in pressure and subsequent contraction of the sphincter muscle. Alternatively, the posterior cells of the intestine, which have been found to contain microtubules and dense basal granules, might constitute a stretch receptor which can communicate directly with the sphincter muscle. Thus, when food is pumped into the intestine, the microtubules might be responsible for recording the resultant stretching and flattening of the intestinal cells. The dense basal granules, which resemble neurosecretory granules, may subsequently release neurosecretions which induce relaxation of the sphincter muscle. These neurosecretions would ultimately be destroyed, presumably by enzymatic activity, and the sphincter would close once more. If this latter hypothesis is indeed correct then the stretch receptor in the intestinal–cloacal junction would provide yet another example of a sensory-motor unit in the Nematoda.

V. Non-regional Sense Organs

A. SETAE

1. *Morphological studies*

Somatic setae are cuticular bristles which project from the surface of the nematode, generally showing a radial arrangement. Setae are found typically in marine nematodes, and when present in soil and parasitic worms they are usually very small. Villot (1875) suggested that the somatic setae of marine forms are connected to a peripheral nervous system; this was later confirmed by Croll and Maggenti (1968) from detailed LM studies on the marine nematode *Deontostoma californicum.*

The somatic setae of *D. californicum* are situated in the dorso-lateral and ventro-lateral planes (Hope, 1964) and are thought to be sensory receptors connected to the somatic nerves (Maggenti, 1964). The labial setae of *D. californicum* may be sensory and associated with the papillary nerves. According to Maggenti (1964) each of the labial setae has three components, the seta itself, a scolopoid body, and a bipolar sensory neuron. The term "scolopoid body" is borrowed from entomological terminology and means a sensory plate, apical body or receptaculum. The bipolar sensory neuron is reported to give rise to a dendrite which extends into the scolopoid body; the dendrite then gives off a filament which extends through the cuticle into the seta. Maggenti considered the somatic setae to be mechanoreceptors, and postulated that the scolopoid body might be a reduced neuron which functions to amplify the external stimulus.

More recently Croll and Smith (1974) have examined the ultrastructural morphology of somatic setae in the freshwater nematode *Chromadorina bioculata* and in the marine nematode *Enoplus communis. C. bioculata* has four rows of somatic setae, two located in the sub-dorsal and two in the sub-ventral planes. Most setae are about 7·0 μm in length, but those situated in the middle

of the body tend to be shorter. The entire surface of the seta is covered with cuticle continuous with the outermost layer of the body cuticle (Fig. 39). It is reinforced, however, with blocks of dense, hardened cuticle similar to those found in the spicules. The base of the seta is located in a socket-like depression of the body cuticle, and in this basal region the surface cuticle of the seta is not reinforced. The centre of the seta is hollow; it contains two or three modified cilia (Fig. 39) which extend through the body cuticle and into the hypodermis where they become nerve axons. Croll and Smith (1974) report that neither transmission nor scanning EM has revealed the existence of pores at the tip of the seta.

In *Enoplus communis* there are six large cephalic setae arranged in a circle just behind the stoma, and they may be paired. Each cephalic seta is located in a socket-like depression, and has much the same structure as the somatic setae of *C. bioculata*. Appearances suggest that once inside the hypodermis, the modified cilia from each pair of cephalic setae eventually converge. Croll and Smith (1974) noted that one of the cilia in the cephalic seta of *Enoplus communis* has an irregular outline when seen in longitudinal sections, and membraneous connections with the outer cuticular layer of the seta when seen in transverse sections; they suggest that these appearances may reflect a series of lateral connections between the cilium and the outer cuticle. A scolopoid body was not identified in either of these nematodes.

2. *Functional considerations*

Nematode setae have been suggested to function as organs of traction (Chitwood and Chitwood, 1950), and to have a functional relationship with their habitat (Weiser, 1959). Furthermore, in nematodes living in sand,

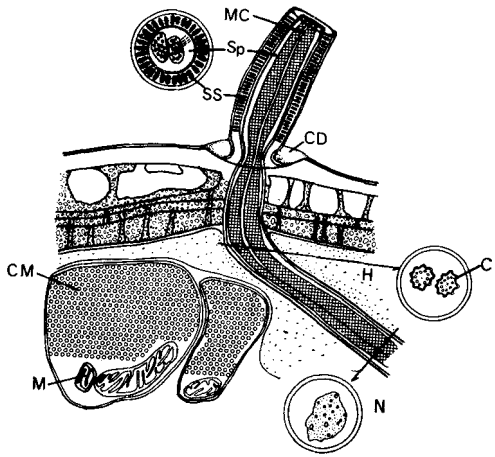

FIG. 39. Reconstruction of a somatic seta of *Chromadorina bioculata*. Cilium, C; cuticular depression, CD; contracting muscle, CM; hypodermis, H; mitochondrion, M; modified cilium, MC; nerve, N; space in seta, Sp; supporting strut, SS. (From Croll and Smith, 1975.)

secretions were apparently observed from the tips of the setae (Bütschli, 1874; Gerlach, 1953). The structural evidence now available makes it clear that somatic and cephalic setae probably function as mechanoreceptors. The setae are reinforced by hardened cuticle except where they fit into the socket-like depression of the body cuticle; it is suggested, therefore, that the setae are rigid structures capable of bending or rotating in the socket, and that the cuticular distortions so produced are transmitted to the underlying sensory cilium (Croll and Smith, 1974).

B. SENSE ORGANS ASSOCIATED WITH THE "BODY PORES" OF DORYLAIMID NEMATODES

1. *Morphological studies*

In plant parasitic nematodes belonging to the superfamily Dorylaimoidea, rows of cuticular pores occur along the body of the worm. The "body pores" occur throughout the length of the lateral cords, and are sometimes present in the dorsal and ventral fields especially in the oesophageal region (Hooper and Southey, 1973); their number and distribution are often used as a minor taxonomic parameter. The "body pores" of *Xiphinema index* and *Longidorus macrosoma* may be clearly visible after vital staining with methyl blue, michrome No. 252 (T. King, pers. comm.) (Fig. 40).

King has also studied the "body pores" of *X. index*, *L. macrosoma* and an aporcelaimid species at the ultrastructural level. He found that each "body pore" represents the external aperture of a narrow canal in the outer cuticle of the worm, and that the base of this canal is surrounded by one or more associated cells. In the aporcelaimid the associated cell is fairly large and has a glandular appearance; it contains many secretion granules, a basally situated nucleus, and usually a few large vacuoles. In *X. index*, however, the associated cell is smaller, does not contain secretion granules and does not have a glandular appearance. In both the Longidorids and the Aporcelaimid one or more modified cilia may be seen within the lumen of the associated cell, and they extend into the narrow canal in the outer cuticle. In *X. index* each cilium is innervated by a single nerve axon, and the cilium–axon junction is enveloped by the associated cell. Similar junctions have not as yet been identified in the aporecelaimid, and possibly in this case the cilia originate directly from the associated cell.

2. *Functional considerations*

A number of obscure functions have been attributed to the "body pores" of dorylaimids, but the ultrastructural evidence presented by King now indicates that each "pore" represents the external aperture of a discrete sense organ. Possibly "somatic receptors" might be a more appropriate term than "body pores". Glandular cells are associated with these sense organs in the aporcelaimid, but King has no evidence to suggest that secretions are released from these gland cells to the exterior of the worm. A superficial similarity exists between these aporcelaimid receptors and the bacillary band receptors

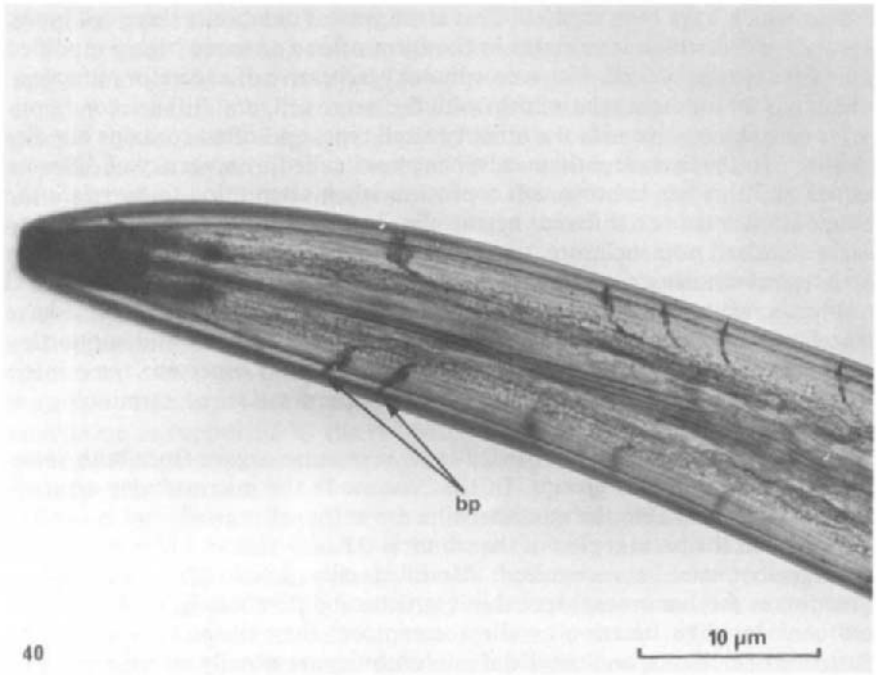

FIG. 40. Adult *Longidorus macrosoma* vitally stained with methyl blue to demonstrate "body pores" (bp). (Courtesy T. King.)

of trichuroid nematodes (Wright and Chan, 1973). However, the morphology of the apercelaimid gland cell is clearly different from that of the trichuroid hypodermal gland cell. The cells associated with the sense organs of *X. index* do not have a glandular appearance, and in some respects the somatic sense organs of this nematode resemble the isolated sensory receptors located in the ventral anterior body wall of trichuroid nematodes (Wright and Chan, 1974). On the existing evidence it seems reasonable to suppose that these dorylaimid sense organs may have a chemoreceptive capacity, but it must be emphasized that King's observations are of a preliminary nature and it is to be hoped that subsequent studies will reveal more information concerning both structure and function.

VI. CONCLUSION

The last few years have seen renewed interest in the morphology of nematode sensory organelles. Electron microscopy has played a major role in this revival and serial sectioning has, in many instances, facilitated the three-dimensional reconstruction of individual receptors. Considering the size of the phylum, the number of species examined to date is virtually negligible.

Nevertheless a basic structural arrangement is emerging in many of the sense organs which have been studied. This arrangement comprises three cell types, (i) a nerve cell which terminates in the form of one or more highly modified cilia, (ii) a non-nerve cell, with a morphology indicative of a secretory function, which has an intimate relationship with the nerve cell, and (iii) a second non-nerve cell which surrounds the other two cell types and often contains bundles of fibres. In the literature these cells have been called by a variety of different names and this has led to much confusion when attempting to correlate the observations made on different nematodes. In this review I have attempted to use a standard nomenclature, derived by assessing the available information with regard to ultrastructure, cytochemistry, *in vitro* secretion and behavioural responses, which hopefully gives some indication of possible function. I have therefore used the terms modified sensory cilium, gland cell and supporting cell respectively. It is possible that future studies will supersede these interpretations, but in the meanwhile some standardization of terminology is clearly desirable.

Modified cilia have been recorded in diverse sense organs from both vertebrate and invertebrate groups. In the Nematoda the microtubular arrangements observed within the modified cilia are extremely varied, and it is often the case that the basal region of the cilium is the only part in which an ordered arrangement can be recognized. Modified cilia which are considered to function as mechanoreceptors exhibit structural differences from those which are considered to function as chemoreceptors; their distal tips are usually flattened or bulbous, and the distal microtubules are usually interspersed with dense, reticulate material. There are some instances, however, where mechanoreceptive cilia terminate beneath a very small pore in the outer cuticle, and other instances where chemoreceptive cilia occupy the positions normally occupied by mechanoreceptive cilia. Whether these examples represent a possible evolutionary sequence or merely reflect the biology of the nematode in question has yet to be determined. It is interesting to note that multiciliate sense organs such as the amphids may be receptive to different modalities.

Relationships between sensory receptors and secretory cells have been described in other phyla, but their functional significance is not fully understood. In the Nematoda a possible feedback mechanism has recently been suggested whereby the sensory cilia of the amphid monitor and control the secretory activity of the gland cell in response to external stimuli. Such a system could be classified as a receptor–effector pair of cells, and studies on the bacillary band receptors of *Capillaria hepatica* and on the intestinal–cloacal valve receptors of *Heterakis gallinarum* suggest that this primitive arrangement may be a common phenomenon in the phylum Nematoda. In some species the gland cell of the sense organ is known to synthesize and secrete cholinesterase. The biological significance of this enzyme is as yet undetermined, but in view of the fact that other nematodes synthesize and secrete acetylcholinesterase from glands which are not associated with sensory receptors, it seems highly unlikely that the enzyme is involved in nervous transmission or chemotaxis. The release of this enzyme over the sensory cilia seriously questions the possibility that these nematodes utilize

acetylcholine as a neurotransmitter. It might be rewarding to look for other chemical transmitters in the nematode nervous system.

The typical supporting cell contains fibre bundles, isolated microtubules, and is known to assist in laying down a new cuticular lining to the ciliary channel at the time of the moult. Such features are characteristic of hypodermal tissues and it might be argued, therefore, that the supporting cell is hypodermal in origin. It is relevant to mention here that neither supporting cells nor gland cells are associated with the nervous cells of trichuroid nematodes; instead the nervous cells are ensheathed by extensions of the hypodermal tissue.

Minor variations in the three-cell structure here described, do occur in the sense organs of different nematode species; this is particularly so in the form and organization of the gland cell. These differences are probably related to functional rather than evolutionary differences between species, and they may not therefore be characteristic of particular nematode groups. This conclusion is supported by the structural changes recorded in the amphidial gland of *Necator americanus* during the development from third-stage larva to mature adult worm.

Groups of nerve axons, e.g. the hemizonid, are located beneath the surface of the nematode in regions lacking the more typical sense organs. The sensory capacity of such structures clearly needs further investigation. More information is also needed on pigment spots and ocelli before we can say whether nematode photoreceptors belong to a rhabdomeric or ciliary line of evolution. Much needs to be elucidated on the physiogical responses of individual nematode sense organs to given stimuli, but the size of these receptors makes this approach particularly difficult. A striking similarity exists between the sense organs of nematodes and arthropods, however, and it is to be hoped that useful comparisons may be drawn from this source, until such time as suitable techniques have been devised for studying the neurophysiology of these interesting helminths.

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Physiological Aspects of Reproduction in Nematodes

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I. Introduction

Nematodes are, along with the insects, the most widely distributed of the invertebrate animals. They are essentially aquatic, being always in situations where a film of water is a necessary backdrop of the environmental situation, except for the cryptobiotic species (Keilin, 1959; Ellenby, 1969). Despite this apparent ecological limitation their spread into most ecological situations has been astounding, as the oft-quoted comment of Cobb (1915) underlines (Lee, 1965).

Perhaps the most important factor in their ecological success has been the variety of reproductive strategies which they display. While these are not new or unique phenomena in the animal kingdom, the nematodes have brought a new sense of physiological economy, efficiency and adaptability to old ways. In this regard, the nematode egg-shell with its physical and chemical characteristics deserves special mention as the ultimate in the development of a self-contained, self-maintaining and resistant environmental unit.

There has been no comparable and sustained effort to study nematode reproduction in the same way that insect reproduction has been tackled (Mordue *et al.*, 1970). In the last few years, however, varied and isolated biological observations relevant to our understanding of reproduction in this group have appeared with greater regularity. The time is therefore opportune to bring these isolated facts together, at least as an incentive for a more systematic and sustained research effort to elucidate these phenomena.

There are besides good reasons why the study of reproductive and developmental physiology in nematodes should be intellectually rewarding. Nematodes show a number of biological features unique in the animal kingdom. The phenomenon of cell constancy suggests the possibility of studying particular reproductive processes throughout the period of life of the various developmental stages without the structural basis for the observed physiological changes being dramatically modified by metamorphosis or similar ontogenetic processes. This interesting possibility is already being exploited in the study of the genetic basis of behaviour. The biological basis for the regulation of cell division which serves to maintain the constancy of cell numbers in nematodes should be of interest to cancer research. The characteristic internal hydrostatic pressure of nematodes implies that internal tissues including the gonads perform their physiological function at other than normal pressures (Harris and Crofton, 1957). The biological consequences of this should also be of interest.

The determinate nature of nematode development and the phenomenon of chromatin diminution are other aspects of nematode reproductive and developmental physiology which may shed light on basic biological processes; and it is reasonable to assume that these are interrelated process. Finally, an understanding of the physiological mechanisms which underline nematode reproduction may offer us new tools for the biological control of nematode pests, especially in these days when the ecological consequences of the indiscriminate use of chemical pesticides demand, and urgently, alternative

weapons in our effort for increased food production and a healthier population.

II. Range of Reproductive Phenomena

A. General Observations

Most nematodes are bisexual organisms in which the sexes show well defined differences in morphological and anatomical characters. In some species, the sexes may differ in size and shape (*Heterodera* sp., *Meloidogyne* sp.), while in others specialized anatomical structures like the spicules (*Strongyluris brevicaudata*, *Heterakis gallinarum*) or the bursa (*Ancylostoma* sp., *Haemonchus* sp.) or the telamon may be present. Intersexes are also known in other species. However, in many plant parasitic and soil nematodes, hermaphroditic species are common (Triantaphyllou and Hirschmann, 1964), while in others, including many animal parasitic species, parthenogenesis is not unknown. Pseudogamy has also been reported from otherwise hermaphroditic and bisexual species (Triantaphyllou, 1971a). Thus, all the main types of reproductive phenomena shown in the animal kingdom have been observed in the nematodes. These are

(a) *parthenogenesis*, which may be meiotic or mitotic, and in which eggs may develop normally without the intervention of a spermatozoon;
(b) *hermaphroditism*, in which both gonads and their gametic products may be present together in the same individual nematode;
(c) *pseudogamy*, in which eggs may develop normally after activation by a spermatozoon whose role stops short of contributing genetic material to the developing embryo; and finally
(d) *syngamy* (amphimixis), in which genetic factors contributed by two individual organisms of different sexes fuse to form the zygote.

Usually, an indication of the predominant mode of reproduction in a particular nematode species may be given by the sex ratio which would normally approach one in the truly amphimictic species. Nevertheless, amphimictic species are known in which the sex ratio may be less than one, for example, *Aphelenchus avenae* (Fisher, 1972).

B. Parthenogenesis

Both types, meiotic and mitotic parthenogenesis, occur in nematodes. In *Meloidogyne hapla* and *M. graminicola*, in which the cytology of parthenogenesis has been studied (Triantaphyllou and Hirschmann, 1964), both oogenesis and spermatogenesis take place as in amphimictic species. Thus two divisions occur during maturation of the oocytes, giving rise to two polar bodies in addition to the mature oocyte which contains the haploid number of chromosomes. Activation of the mature oocyte does not appear to be triggered by a spermatozoon, although Paramonov (1962) has suggested that activation is achieved in parthenogenetic nematodes by chemical secretions emanating from the upper uterus. There is at present no evidence

to support or controvert this viewpoint, although the fact that these same secretions are known to contribute to the formation of the egg-shell (see below) may preclude such an additional role.

Despite the absence of amphimictic fertilization, the diploid chromosome number is re-established in such species in one of two ways. In some, like *M. hapla*, the second polar nucleus is not extruded from the cytoplasm of the oocyte but rather fuses with the egg pronucleus to restore diploidy (Triantaphyllou, 1966). In others, however, an endomitotic division takes place. In *Heterodera betulae* a mitotic division takes place during prophase of the first cleavage division, but this process takes place at a rate faster than that at which the formation of the first cleavage plane would take place. In consequence, a doubling of the chromosome number results, before the completion of the first cleavage division (Triantaphyllou, 1970, 1971a).

In *Pratylenchus scribneri* and *Aphelenchus avenae*, in which males are absent or very rare, reproduction is also by meiotic parthenogenesis. In these species the parthenogenetic mode of reproduction is obligatory. In many *Meloidogyne* sp. in which males are not rare, however, meiotic parthenogenesis is "facultative"; this mode of reproduction being resorted to only in situations in which males are rare. In the words of Triantaphyllou (1971a) "it ... appears that meiotic parthenogenesis prevails (in *Meloidogyne* sp.) under environmental conditions favourable for rapid development and reproduction when males are absent or rare, and amphimixis is common under adverse conditions when males are more abundant." Indeed, in the same individual, cytological observations have shown that some eggs may undergo amphimictic development after sperm penetration and others will develop by meiotic parthenogenesis (Triantaphyllou, 1966). The same pattern would seem to hold for some animal parasitic species (Zaffagnini, 1973).

Mitotic parthenogenesis is very common in the family Heteroderidae (Triantaphyllou, 1971a), in which various species of the genera *Heterodera*, *Meloidogyne* and *Meloidodera* show this mode of reproduction. In these nematodes, a regular mitotic division results in the formation of a polar nucleus and an egg pronucleus, in both of which the somatic chromosome number is present. No sperm is needed to activate the oocyte usually, although in inseminated females of *Meloidogyne javanica*, a spermatozoon may enter the oocyte at prometaphase only to degenerate therein. The egg pronucleus would thereafter undergo cleavage (Triantaphyllou, 1962). Indeed, this phenomenon is so widespread in this family that Triantaphyllou (1971a) has suggested that the occasional fertilization of eggs with unreduced chromosome number would be one way in which polyploid species may have developed in the genus.

C. HERMAPHRODITISM

Perhaps the earliest report of syngonic hermaphroditism in nematodes is that of Maupas (1900) on the rhabditid *Caenorhabditis elegans*, in which developing oocytes are found alongside sperms in individuals which are morphologically males. The protandric hermaphroditism of *C. elegans* is

shown also by many rhabditids, diplogasterids, rhabdiasoids and many aphelenchs (Triantaphyllou, 1971a). In these protandric forms, reproduction is essentially automictic and normal meiotic divisions take place by essentially the same mechanisms as in amphimictic species.

Digonic hermaphroditism is known in mononchs and tylenchs. Cobb (1918) described this in *Mononchulus ventralis*, while various species of *Helicotylenchus* manifest the condition. In these, the hermaphrodites are morphologically similar to the females but possess a special region of the gonad, the spermagonium, in which, it has been suggested, sperm are produced (Perry, 1959). However, Hirschmann and Triantaphyllou (1967) were unable to demonstrate spermatozoa in any part of this structure even in actively reproducing individuals despite its appearance as an asymmetrically off-set spermatotheca, usually located between the oviduct and the uterus in this species. In *Meloidogyne hapla*, the large spermatotheca are often found with characteristic darkly staining elements in their walls (Mulvey, 1960). These dark elements often give rise to smaller granular bodies which Mulvey interpreted as spermatozoa produced by the cells of the spermatothecal wall. However, Hirschmann and Triantaphyllou (1967) failed to detect feulgen-positive material in these bodies and consequently suggested that the granular bodies were not spermatozoa. As indicated below, the absence of feulgen-positive material at some stages of spermatogenesis in some nematodes is not, it would appear, unusual. Perhaps the ultrastructural study of these bodies may be necessary in order to determine their cytological nature and origin.

Among the animal parasitic species, *Rhabdias bufonis* and *R. fuellerboni*, which are generally regarded as protandric hermaphrodites (Nigon, 1965), are not hermaphroditic but reproduce by meiotic parthenogenesis (unpublished observations).

D. PSEUDOGAMY

In many hermaphroditic species and some bisexual species, further development of the mature oocyte is possible only after the spermatozoon has penetrated. In these hermaphroditic species, for example *Rhabditis aberrans* (Kruger, 1913) and *R. anomala* (Hertwig, 1922), it serves only to activate the egg because no fusion of the pronuclei takes place thereafter; rather the sperm pronucleus degenerates in the female cytoplasm. In many of these pseudogamous species, including the bisexual forms such as *Rhabditis maupasi* and *R. longicaudata* (Hertwig, 1922), the maturation of the oocyte involves a mitotic division in which there is usually no pairing of homologous chromosomes. *Mesorhabditis belari* differs from the other bisexual forms, however, in that synapsis takes place such that at metaphase I a reduced number of bivalents is observed. Subsequently, oocytes of the same female may develop in two alternative ways: either they undergo regular meiosis with two maturation divisions followed by amphimixis or, alternatively, they may undergo a modified meiosis in which, during anaphase I, the chromosomes in the anaphase daughter plates divide once, thus restoring the somatic

chromosome number. Consequently, no second maturation takes place and the oocyte proceeds directly to cleavage (Belar, 1923; Nigon, 1949).

E. SYNGAMY

In the great majority of bisexual nematodes, syngamy (cross-fertilization) is the rule. In these, fertilization always involves the fusion of the male and female pronuclei before further and normal development can supervene. Indeed, Bütschli's (1873) observation of the fusion of the pronuclei in *Caenorhabditis dolichura* with the consequent production of the zygote nucleus marked the beginning of the study of the cytology of fertilization in the animal kingdom. This observation represented the necessary background to the later elucidation of meiosis in *Ascaris megalocephala* (*Parascaris equorum*) (van Beneden, 1883a; Boveri, 1888) as an essential preliminary for normal development in most animals. This early work on nematodes also marked the beginning of our understanding of the continuity and role of chromosomes in reproduction and heredity, and the mechanisms which govern their activities.

Boveri (1887) observed the phenomenon of chromatin diminution (fragmentation) in *Parascaris equorum*, a process of significant importance in the reproduction of nematodes generally and one that appears to be widespread among the bisexual species (see below).

III. The Reproductive System

A. GENERAL OBSERVATIONS

In both male and female nematodes, the reproductive system generally consists of one or two tubular gonads which vary greatly in length. In parasitic species, it is generally longer than in free-living species (Bastian, 1866) and may be straight, reflexed or coiled (Chitwood and Chitwood, 1950). Seurat (1920), in his classic monograph "Histoire Naturelle des Nematodes de la Barbière", had introduced the terms amphidelphic (uteri opposed at origin), opisthodelphic (uteri directed posteriad at origin) and prodelphic (uteri directed anteriad at origin) to describe the form of the female system. These terms are now part of nematological literature. However, females with two genital tubes are also said to be didelphic (diorchic for males) while those with one tube are monodelphic (monorchic for males).

B. THE MALE SYSTEM

The male system is generally monorchic in most nematodes except in some genera such as *Meloidogyne*, *Draconema*, *Theristus* and *Bastiana*, among others, in which diorchic forms are known (Chitwood and Chitwood, 1950; Triantaphyllou and Hirschmann, 1964; Hope, 1974). It consists of three major divisions, namely, *testes* (t), *seminal vesicle* (sv) and *vas deferens* (vd) (Fig. 1(a)), although in some, four divisions are usual. The latter situation arises from the interposition of a *vas efferens* between the testes and seminal vesicle.

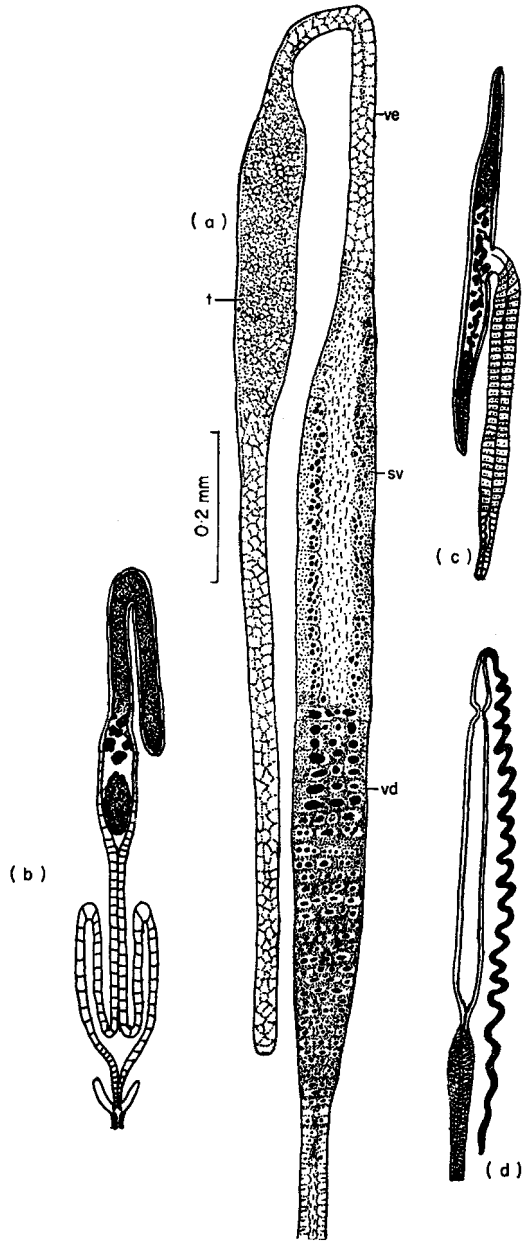


FIG. 1. Male reproductive system of various nematodes. (a) *Aspiculuris tetraptera*, (b) *Rhabditis strongyloides*, (c) *Desmolainus zeelandicus*, (d) *Trichuris suis*.

In nematodes with a telogonic testes, this structure is divisible into a germinal and growth zone, the latter usually leading into a dilated seminal vesicle (Fig. 1(b)) where fully formed sperm is usually stored. The cells of the seminal vesicle are generally of the cuboidal or columnar epithelial type (Chitwood and Chitwood, 1950; Anya, 1966a; Hope, 1974). The vas deferens is, however, the dominant portion of the male system and shows regional differentiation into sections each with characteristic secretory cells (Chitwood and Chitwood, 1950; Anya, 1966a). The cells of this layer are also often invested with a layer of muscle cells (unpublished observations.)

It has been shown that the secretory products of the different regions differ in their chemistry and perhaps in their functions (Anya, 1966a; Foor, 1974). More recently, serotonin (5-HT) has been identified as one of the secretory products. As a result, the suggestion has been made that the function of the secretions is to aid sperm ascent by initiating motility of the spermatozoa as well as contractions of the female system in a manner that aids sperm ascent (Anya, 1973a). His first suggestion has recently been given experimental support by the observation that in *Ascaris* sp. and *Brugia* sp. homogenates of the vas deferens when introduced into the male system of living nematodes initiated pseudopod formation by the sperm as well as other changes reminiscent of prefertilization spermatozoa in the female system (Foor and McMahon, 1973; Foor, 1974).

In some nematodes like *Rhabditis strongyloides*, the vas deferens may develop two large lateral pouches which may extend anteriorly on both sides (Fig. 1(b)). The cells of these pouches are generally glandular and Chitwood (1930) had suggested that these cells were responsible for the secretion of the cement substance which is often deposited on the female during copulation. Sommerville and Weinstein (1964) were unable to confirm that the cement substance deposited on females during copulation in *Nematospiroides dubius* originated from these cells.

In most nematodes there is generally a well-developed ejaculatory duct distal to the vas deferens and this always opens into the rectum to form a cloaca. Spicules may be located in an out-pocket of the dorsal wall of the cloaca while a gubernaculum, a cuticularized structure associated with the distal end of the spicule, may be found. In those nematodes which possess spicules, there is usually a pair of these structures which are often regarded as intromittent organs which serve to dilate the vulva during copulation. But many nematodes are also known which possess a single spicule. Lee (1962) and Bird (1966) had detected cholinesterase in the cytoplasmic core of the spicules of *Ascaris lumbricoides* and *Meloidogyne* sp., and Lee (1973) produced evidence indicating that the spicule is a sensory structure. In *Heterakis gallinarum* and *A. lumbricoides*, he described several nerve axons which run along the cytoplasmic core of the spicules. The dendrites from these axons ended blindly beneath the cuticular covering at the tip of the spicule. In such axons, mitochondria, vacuolated cytoplasm with pronounced electron lucid material and granules, and numerous neurotubules could be found. Consequently, Lee (1973) had suggested that the spicule may serve as a sensory probe which detects the vulval opening and modulates the

thrust into the female vagina in a manner to prevent unintended internal damage to the female system.

C. THE FEMALE SYSTEM

The female reproductive system of most nematodes consists of two paired genital tubes which show regional differentiation (Bastian, 1866; Seurat, 1920). The free-living nematodes possess genital tubes of simpler morphology than is found in their parasitic relations, in which increase in length and in the degree of muscularization of the epithelial monolayer of cells in various regions of the tubes are observed (Chitwood and Chitwood, 1950).

In these free-living forms especially, the female system consists of a ventral cleft, the *vulva*, leading to a transverse *vagina* and paired but often opposed *uteri*, which lead to the *oviducts* that join the paired ovaries to the uteri (Fig. 2(f)). The position of the vulva is variable, being anteriorly located in some nematodes such as *Oxystomina* sp. and posteriorly in others such as *Ditylenchus* sp.; in most nematodes, however, the vulva is located slightly behind the mid-body (Chitwood and Chitwood, 1950).

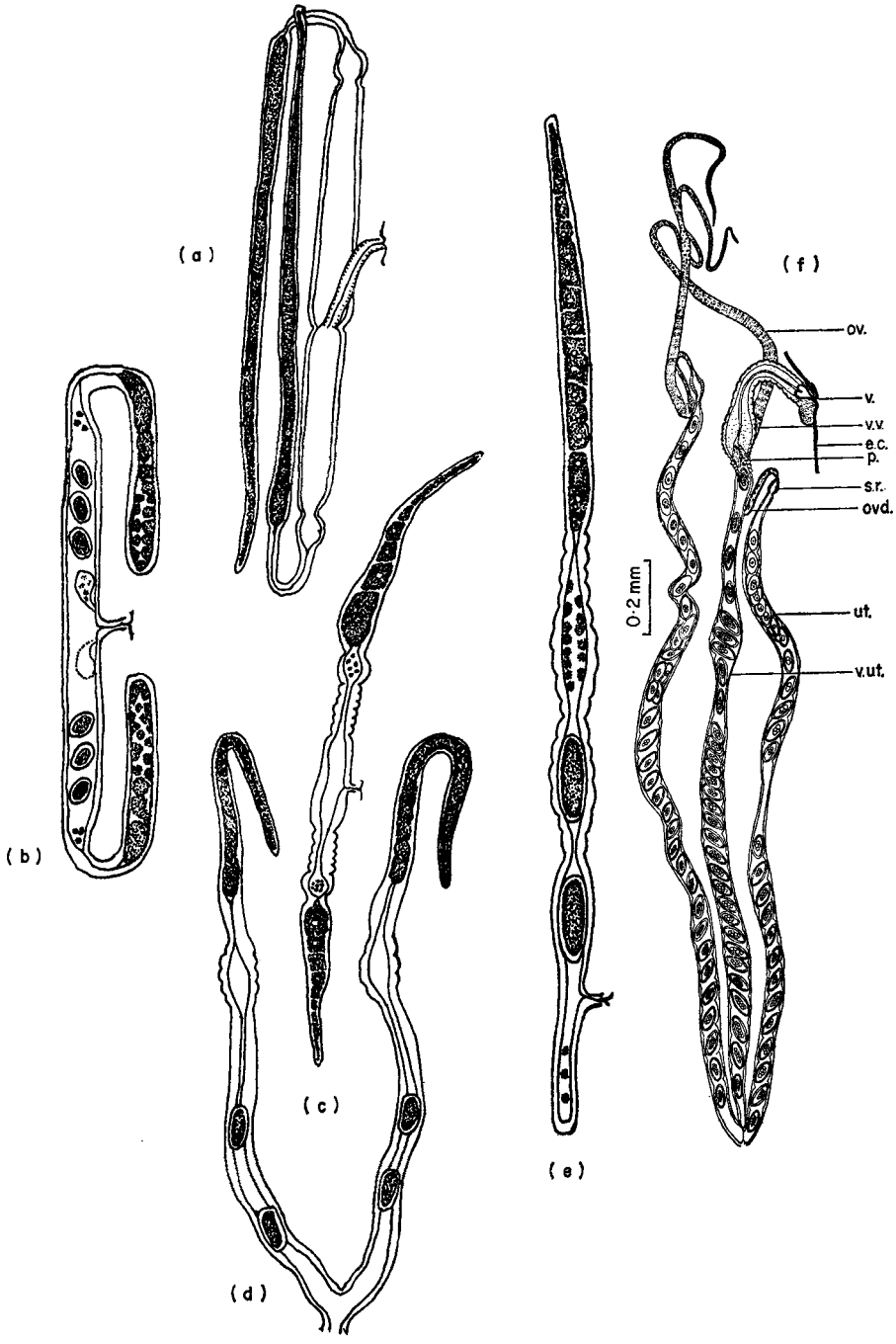
The single vagina of many nematodes is lined with cuticle continuous with the cuticle of the body surface. In the parasitic forms, with the increased production of eggs and the consequent need for the ejection of these eggs, this portion of the genital tube is greatly elongated and muscularized, and in some nematodes it is called the *ovijector* (Seurat, 1920). The vagina leads to the usually bifurcate but also muscularized uteri, (Fig. 2(d)). In some nematodes, e.g. *Aspiculuris tetraptera*, however, the heavily muscular portion of the uterus is also single and is called the *vagina vera*. In such forms, the vagina vera leads to a bifurcate portion of the uterus which then is called the *vagina uterina* (Fig. 2(f)). The latter may thus be regarded as the uterus proper. Histologically, apart from the investing muscle cells, the uterus consists of polyhedral and squamous epithelial cells (Anya, 1964b; Hope, 1974). The oviduct extends from the termination of the ovary to the beginning of the uterus and generally consists of spindle-shaped cells whose long axes are generally at right angles to the longitudinal axis of the genital tube (Hope, 1974). Moreover, the oviduct may occasionally be set off from the uterus by a conical or funnel-shaped extension of the wall as in *Anticoma* sp. and *Euchromadora* sp. (de Man, 1886), which some authors have implied may function as sphincters regulating the passage of oocytes (Rauther, 1918), or by a slight dilatation which serves as a seminal receptacle (Anya, 1964b). Fine microvilli are present on the luminal surface of the epithelial cells of the oviduct in *Aspiculuris tetraptera* (unpublished observations).

IV. THE MALE GAMETE

A. SPERMATOGENESIS

1. *General observations*

van Beneden and Julin (1884) started off the early studies of spermatogenesis in nematodes with their study on *Ascaris megalocephala* (*Parascaris equorum*).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In the intervening years, a vast amount of information was accumulated about this process in nematodes of varied systematic and ecological positions, based mainly on observations with the light microscope; see Bowen (1925), Sturtivant (1934), Nath and Singh (1956), Walton (1940, 1959) and Nath (1965).

These studies showed two predominant modes of germ cell production in the nematode testis, namely the telegonic and the hologonic. In the telogonic type of spermatogonial genesis, as observed in *Porrocaecum angusticolle* (Nath *et al.*, 1961b), *Parascaris equorum* (Favard, 1961) and *Aspiculuris tetraptera* (Anya, 1966a), germ cells are proliferated from a single but proximal and terminal cell (*Ascaris lumbricoides*) or from a nucleated syncytial mass, as in *Dipetalonema viteae* (McLaren, 1973a). In the hologonic testes as in *Dioctophyma renale* (Leuckart, 1876), *Trichinella spiralis* and *Trichuris trichiura* (Eberth, 1860), spermatogonial proliferation takes place along the whole length of the testes, either around its perimeter or at definite and limited points along the length of the testes tube (Neill and Wright, 1973). The subsequent stages of development are then seen, as in the seminiferous tubules of vertebrates—towards the lumen of the tube at each level.

These early studies had also established the basic pattern of the cell cycle in nematode spermatogenesis. Each spermatogonial cell normally goes through a phase of growth and differentiation, a phase of mitotic divisions into primary spermatocytes, and two further maturation divisions which transform the primary spermatocytes into secondary spermatocytes. The latter cells would undergo cellular reorganization and transformation through spermateleosis into spermatids; the process thus serves, as a result of morphological modifications, to transform the spermatid into the mature sperm cell. Four spermatozoa would normally result from the two maturation divisions of the primary spermatocyte (Fig. 3). This basic pattern is followed by all nematodes in which the process has been studied, including *Spirina parasitifera* (Chitwood and Chitwood, 1950), despite Cobb's earlier suggestion (1928) that, in this species, each spermatid ultimately gave rise to 128 spermatozoa through mitotic divisions. The Chitwoods' later studies (1950) had shown this interpretation to be in error.

Recent studies with the aid of the electron microscope (EM) (Table I), beginning with the now classical study of Favard (1961), while serving to confirm some of our old conceptions of the structure of nematode sperm, have also provided some vital information relevant to our understanding of the physiology of the male gamete in these organisms. These latter studies are discussed below.

2. *Fine structural changes during spermatogenesis*

The studies of Favard (1958, 1959, 1961), Lee (1971) Beams and Sekhon (1972), Neill and Wright (1973), McLaren (1973a) and Shepherd *et al.* (1973)

FIG. 2. Female reproductive system of various nematodes. (a) *Cephalobus papilliger*, (b) *Rhabditis strongyloides*, (c) *Tylenchorhynchus dubius*, (d) *Meloidogyne hapla*, (e) *Aphelenchoides ritzemabosi*, (f) *Aspiculuris tetraptera*.

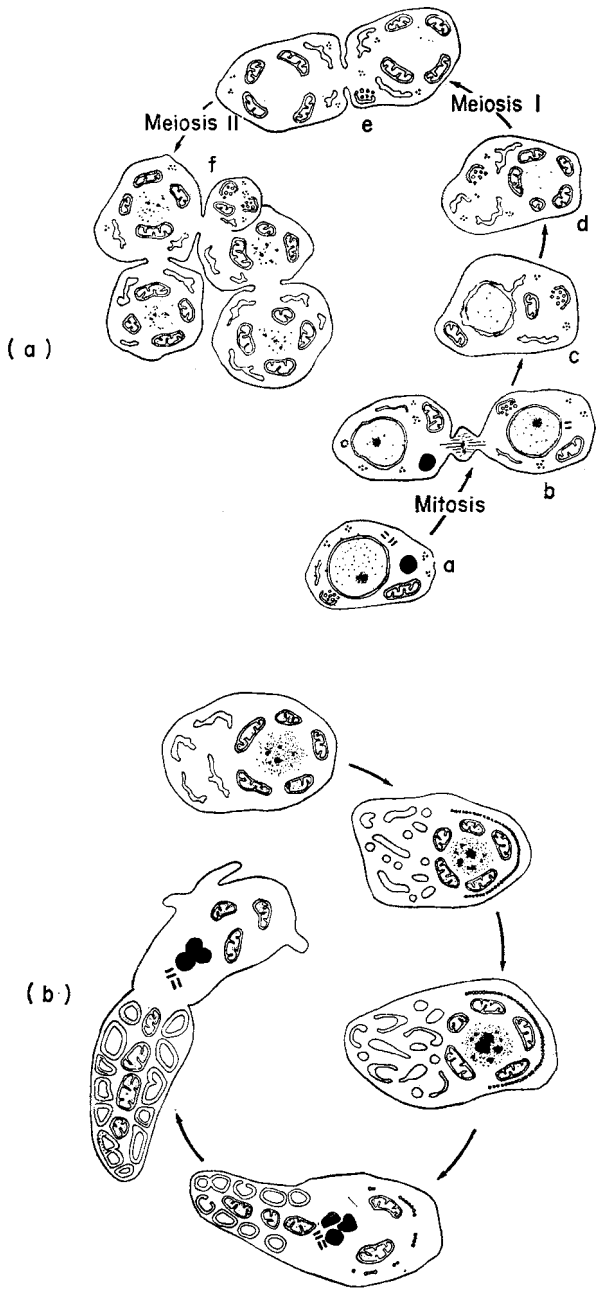


FIG. 3. Diagrammatic summary of spermatogonial cycle, spermatid differentiation and spermateleosis in (a) *Trichinella spiralis* and (b) *Capillaria hepatica* (from Neill and Wright, 1973).

TABLE I

List of nematodes for which detailed ultrastructural descriptions exist in the literature

Species	Taxonomic position	Habitat	Reference
1. *Parascaris equorum*	Ascaridae	Animal parasitic	Favard (1961)
2. *Ascaris lumbricoides*	Ascaridae	Animal parasitic	Clark *et al.* (1967, 1972); Foor (1968)
3. *Polydelphis* sp.	Ascaridae	Animal parasitic	Foor (1970)
4. *Toxocara canis*	Ascaridae	Animal parasitic	Foor (1970)
5. *Dirofilaria immitis*	Filariidae	Animal parasitic	Foor (1970); Harada *et al.* (1970); Maeda *et al.* (1970)
6. *Dipetalonema viteae*	Filariidae	Animal parasitic	McLaren (1973a); Foor *et al.* (1971)
7. *Brugia pahangi*	Filariidae	Animal parasitic	Foor (1974)
8. *Physaloptera* sp.	Spiruriidae	Animal parasitic	Foor (1970)
9. *Gnathostoma* sp.	Spiruriidae	Animal parasitic	Foor (1970)
10. *Rhabditis pellio*	Rhabditidae	Animal parasitic	Beams and Sekhon (1972)
11. *Nippostrongylus brasiliensis*	Trichostrongylidae	Animal parasitic	Jamuar (1966)
12. *Ancylostoma caninum*	Trichostrongylidae	Animal parasitic	Foor (1970)
13. *Angiostrongylus cantonensis*	Metastrongylidae	Animal parasitic	Foor (1970)
14. *Dioctophyma renale*	Dictophymoidea	Animal parasitic	Foor (1970)
15. *Aspiculuris tetraptera*	Oxyuridae	Animal parasitic	Lee and Anya (1967)
16. *Syphacia obvelata*	Oxyuridae	Animal parasitic	Dick (1971)
17. *Heterakis gallinarum*	Heterakidae	Animal parasitic	Lee (1971)
18. *Deontostoma californicum*	Enoplidae	Marine	Wright *et al.* (1973)
19. *Panagrellus silusiae*	Cephalobidae	Free-living	Pasternak and Samoiloff (1972)
20. *Heterodera rostochiensis*	Heteroderidae	Plant parasitic	Shepherd *et al.* (1973)
21. *Trichinella spiralis*	Trichinellidae	Animal parasitic	Neill and Wright (1973)
22. *Capillaria hepatica*	Trichinellidae	Animal parasitic	Neill and Wright (1973)
23. *Heterodera schachtii*	Heteroderidae	Plant parasitic	Shepherd *et al.* (1973)

on *Heterakis gallinarum, Rhabditis pellio, Capillaria hepatica, Dirofilaria viteae, Trichinella spiralis, Heterodera rostochiensis* and *Heterodera schachtii* respectively, have now provided a comprehensive picture of the cyto-physiological changes during spermatogenesis. The spermatogonia are generally spherical cells (*Heterakis gallinarum, Aspiculuris tetraptera, Trichinella spiralis*) but some may be elongate (*Dirofilaria viteae, Deontostoma californicum*) with a large nucleus showing a prominent nucleolus and nucleonema (Lee and Anya, 1967; Lee, 1971; McLaren, 1973a; Wright *et al.*, 1973). The cytoplasm of the spermatogonia show scattered mitochondria, free ribosomes, a moderately developed but smooth endoplasmic reticulum, some lipid droplets, golgi vesicles and, in some cases, microtubules (Favard, 1961). These cells undergo regular mitosis during which the daughter cells may be connected temporarily through intercellular bridges (Clark *et al.*, 1967). In some, masses of cytoplasmic material are seen between the spermatogonial cells. In those species with a central rachis, it has been suggested that these masses are part of the rachis.

The early spermatocytes are larger cells with a rather large nucleus and a dense nucleolus. The cytoplasm contains numerous polyribosomes, some endoplasmic reticulum, mitochondria and golgi vesicles. In later stages of the spermatocyte, the perinuclear cisternae widen, the outer nuclear membrane become continuous with the endoplasmic reticulum (Neill and Wright, 1973) and the nucleolus breaks down about the same time that the polyribosomes increase in number and the mitochondria migrate to the perinuclear area (Nath *et al.*, 1961b; Lee, 1971; Beams and Sekhon, 1972; McLaren, 1973a). The peripheral cell membranes in some cases show at this stage numerous outfoldings which may precede the formation of certain characteristic membrane stacks (Beams and Sekhon, 1972). Thus, this period of spermatogenesis is one of active protein synthesis (Fauré-Fremiet, 1913; Panijel, 1950; Panijel and Pasteels, 1951; Favard, 1961; McLaren, 1973a).

As the spermatocytes descend the testis in telegonic testes and towards the central lumen in the hologonic type, there is an apparent increase in the number of the golgi vesicles (Lee, 1971; McLaren, 1973a) and the small secretory granules congregate in the vicinity of the golgi complex (Favard, 1961; Foor, 1968; McLaren, 1973a). A large ovoid vacuole appears in the centre of the complex and cisternae develop in an organized fashion around this vacuole (Lee, 1971; Beams and Sekhon, 1972; McLaren, 1973a).

It is at this period that a large dense granule appears within the terminal sac of one of the cisternae, apparently synthesized from the material of the small dense secretory granules earlier formed by the endoplasmic reticulum (Lee, 1971; Beams and Sekhon, 1972; McLaren, 1973a). Around this large dense granule, other cisternae are organized into a closed but hollow cylinder within which some fibrous materials become elaborated. The whole structure, which may now be called a membranous organelle (Foor, 1970), elongates and flattens laterally, thus adopting the characteristic flattened and fibrous discoidal structure of these organelles seen in most nematode spermatozoa.

Histochemical tests on these organelles at this stage show no activity for succinate dehydrogenase or polysaccharide, nor do they react with toluidine

blue and aceto-orcein. There is, however, strong acid phosphatase activity (Lee, 1971). In the secondary spermatocytes, the major cellular changes are nuclear rather than cytoplasmic. Cytokinesis which may be incomplete takes place during this period, but the nucleus is not reorganized while the second stage of the meiotic division supervenes, giving rise finally to two more spermatids. The residual cytoplasmic lobe contains much of the new superfluous cellular organelles; some mitochondria, endoplasmic reticulum and ribosomes are usually pinched off at this stage (Monné, 1957; Anya, 1966a).

In the spermatids, the nuclear membrane is not reformed, and the mitochondria will persist in the perinuclear area while the membranous organelles move to the periphery, close to the cell membrane (Lee, 1971; Beams and Sekhon, 1972; McLaren, 1973a; Neill and Wright, 1973). This pattern of changes seems common in all nematode spermatids in which a membranous organelle is formed. Moreover, during spermateleosis, the membrane organelles generally become localized at the posterior end of the fully formed sperm, as are mitochondria now fewer in number and larger; the larger size may arise from fusion of these organelles (Lee and Anya, 1967). The anterior cytoplasmic region of the spermatozoon often contains abundant β-glycogen granules (Lee and Anya, 1967; Neill and Wright, 1973) and in some the nuclear material may be located therein. Pseudopodia are often formed in the anterior, although some pseudopodia may also be formed in the posterior, as in *Capillaria hepatica*. The fine structural changes during spermatogenesis as summarized for the spermatozoa of *Trichinella* sp. and *Capillaria* sp. (Neill and Wright, 1973) are shown in Fig. 3(a) and (b).

In some nematode spermatozoa, for example *Aspiculuris tetraptera*, spermatogenesis though basically similar to the pattern described above differs in that the elaborate cellular changes which culminate in the specific formation of the membrane organelles do not take place. In these, the development of a posterior microtubular complex is the main change observed during spermateleosis. Shepherd *et al.* (1973) have shown, however, that in *Heterodera rostochiensis* and *H. schachtii* a persistent and peripheral system of microtubules coexists with the membrane organelles; the process of formation of the latter is essentially similar to the pattern described above.

3. *A possible scheme of evolution of sperm morphology*

In the last 5 years or so, studies of the ultrastructure of nematode spermatozoa which began with Favard's (1958, 1961) observations on *Ascaris lumbricoides* and *Parascaris equorum* have suddenly increased in number as well as in the amount and quality of observed details. Detailed studies of about 23 species have become available (Table I) (Jamuar, 1966; Lee and Anya, 1967; Foor, 1968, 1970; Lee, 1971; Dick, 1971 (pers. comm. from K. A. Wright, 1973); Beams and Sekhon, 1972; Pasternak and Samoiloff, 1972; McLaren, 1973a; Neill and Wright, 1973; Wright *et al.*, 1973; Shepherd *et al.*, 1973). These studies include observations on free-living, plant parasitic and animal parasitic species, and have served to underline the variety in size, shape and details of ultrastructure observable in

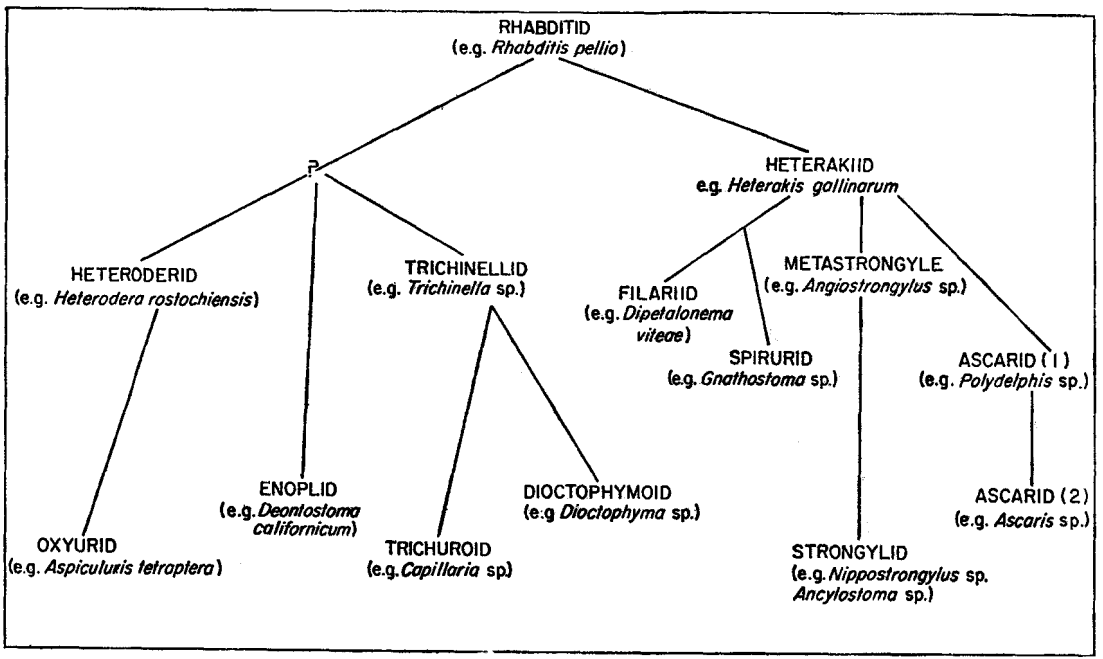


FIG. 4. A hypothetical scheme of evolution of sperm morphology in the Nematoda (see text).

nematode spermatozoa. As a result, a clearer view of what may be regarded as the basic characteristics of a nematode spermatozoon, despite the variety of types suggested in the literature, is now possible.

Foor (1970) postulated the existence of four basic types of nematode spermatozoa: the ascaroid, strongyloid, dioctophymoid and oxyuroid types. In the ascaroid type a clear anterior region can be differentiated from a posterior region in which are located numerous organelles, including a dense non-membrane-bound nucleus, surrounded by an electron dense layer, small spheres of varying density and mitochondria. In particular, certain characteristic membrane organelles containing microvillus-like elements are present usually adjacent to and often continous with the peripheral plasma membrane as well as a prominent lipid-like mass called the refringent body (Fig. 5(13)).

In the strongyloid type, a broad anterior end in which the characteristic membrane organelles are located is followed by a more filiform posterior in which the coarse and filamentous chromatin material is located (Fig. 5(11)). The mitochondria are found in the broad anterior end, while no structure similar to the refringent body has so far been observed.

In the dioctophymoid type as shown in *Dioctophyma renale*, the sperm varies between a rod-shaped form and a more rounded spherical one. There are no membrane organelles although the surface membrane is highly modified, often shows a beaded appearance, and there are no mitochondria within the cytoplasm.

In the oxyuroid *Aspiculuris tetraptera*, a broad anterior end can be distinguished from a filiform posterior end (Fig. 5(7)). In this sperm, there are no membrane organelles and a single but very large mitochondrion occupies the central portion of the filiform "tail", encompassed on either side by a complex system of microtubules. The chromatin material, which is difficult to locate in this sperm, is in the filiform "tail".

The weakness of Foor's classification lies in the fact that within each morphological type, differences are apparent in the structure of the sperm of individual species. For example, some degree of imagination is needed to accept the sperm of *Angiostrongylus cantonensis* (Fig. 5(5)) as belonging to the strongyloid type. On the other hand, the lack of a refringent body would remove it from the ascaroid group to which in size and shape it would appear to conform. In any case, not all ascaroid spermatozoa have a well developed refringent body.

Moreover, recent work in the past three years have shown that these four types are not the only morphological types possible. For example, the sperm of *Capillaria hepatica* (Fig. 5(8)) resembles in many respects the ascaroid type, but it differs in the anterior location of the nuclear material as well as in the structure of the chromatin material and in the fact that it lacks a refringent body. The presence of a tubular sheath at some stages is also indicative of a separate line of development. Foor's four types of sperm in the Nematoda can be said to show a wide divergence in their ultrastructural features especially if viewed in isolation, in which case they can be conceived as the final products of divergent lines of evolution of sperm structure in

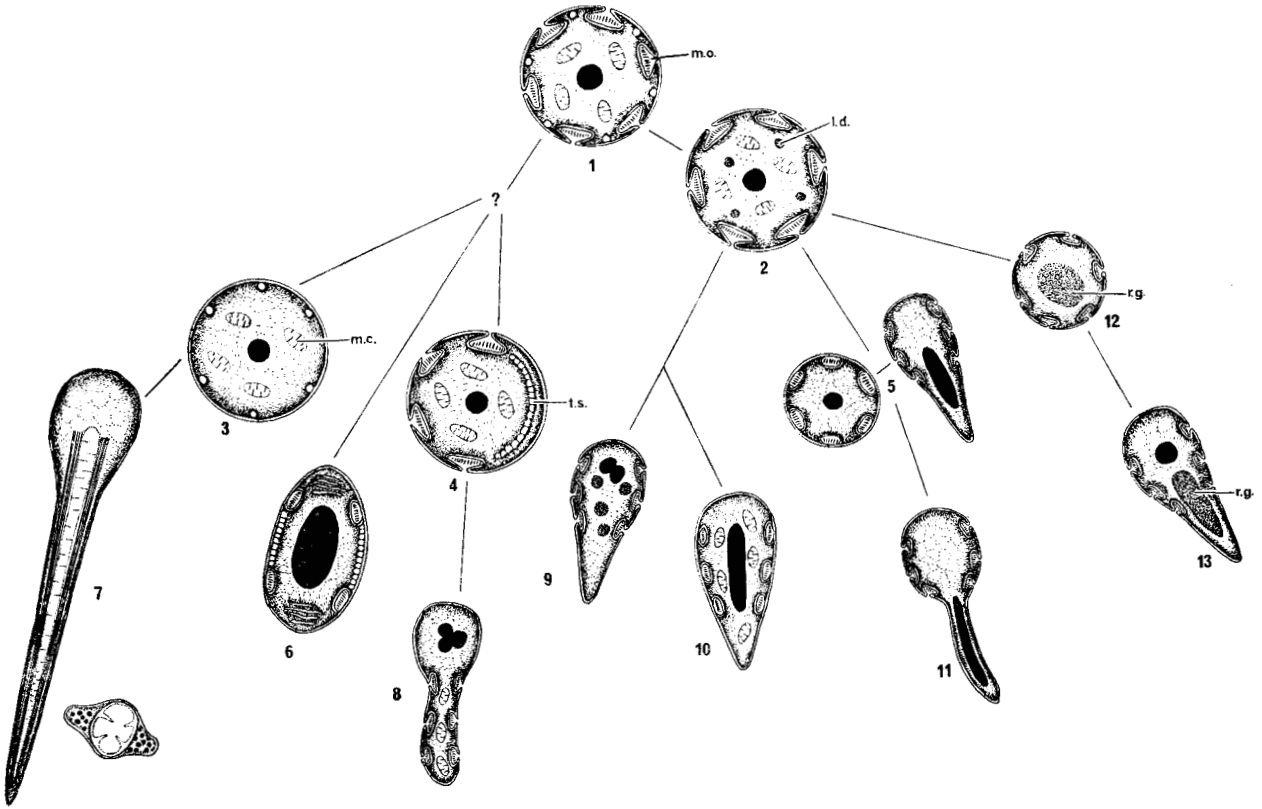


FIG. 5. Spermatozoa of various nematodes fitted to the hypothetical scheme of derivation of sperm morphology in Fig. 4. Ultrastructural details reconstructed from descriptions in the literature: (1) *Rhabditis pellio* (Beams and Sekhon, 1972); (2) *Heterakis gallinarum* (Lee, 1971); (3) *Heterodera rostochiensis* (Shepherd *et al.*, 1973); (4) *Trichinella spiralis* (Neill and Wright, 1973); (5) *Angiostrongylus* sp. (Foor, 1970); (6) *Deontostoma californicum* (Wright *et al.*, 1973); (7) *Aspiculuris tetraptera* (Lee and Anya, 1967), inset t.s. "tail" of sperm of *A. tetraptera;* (8) *Capillaria hepatica* (Neill and Wright, 1973); (9) *Dirofilaria* sp. (McLaren, 1973b); (10) *Gnathostoma* sp. (Foor, 1970); (11) *Nippostrongylus* sp., *Ancylostoma* sp. (Jamuar, 1966; Foor, 1970); (12) *Polydelphis* sp. (Foor, 1970); (13) *Ascaris* sp., *Toxocara* sp. (Favard, 1961; Foor, 1970).

nematodes; such divergences may have arisen in the first place from the interplay of different structural and functional requirements during spermiogenesis and the demands of the process of fertilization in different species.

A close examination of the ultrastructure of the 23 species thus far described will show certain cytoplasmic features as characteristic of nematode spermatozoa, although all the characters need not invariably be present in the spermatozoa of every individual species. These ultrastructural features would include the presence of

(a) the membrane organelles;
(b) the mitochondria, which would seem to have a pronounced tendency to fusion;
(c) the system of microtubules, often peripheral but occasionally organized in other ways;
(d) the capacity to elaborate reserve material, a tendency which reaches its greatest development in the sperm with lipid droplets, or a refringent body.

In addition, all nematode spermatozoa would show a lack of ciliary or flagellar structure although centrioles of a rather specialized structure have been found, and they all would possess the capability for pseudopodial formation.

Such a close observation immediately shows that the spherical sperm of *Rhabditis pellio* (Fig. 5(1)), which has membrane organelles, microtubules and mitochondria, would seem the least specialized type. It is then possible to imagine the derivation of two (but not more than three) different types of spermatozoan morphology from this generalized type by emphasizing a particular organelle/feature in the ultrastructure. In *Heterodera rostochiensis* (Fig. 5(3)) the spherical shape is still maintained, mitochondria are present, and a peripheral microtubular system is evident as well as membrane organelles. In *Trichinella spiralis*, (Fig. 5(4)) the membrane organelles are present as are mitochondria, but there is no microtubular system. Finally, in *Heterakis gallinarum* (Fig. 5(2)), the membrane organelles are present along with the mitochondria but there is no microtubular system and there is a capacity for conserving reserve materials in the form of lipids.

Other types of nematode spermatozoan morphology could be derived from these basic types, by the adoption of a non-spherical shape with distinct bipolarity in which the tendency for emphasizing particular features would be carried a stage further. For example, it is conceivable that both the strongyloid and the ascaroid types as envisaged by Foor are derivable from the heterakid spermatozoan structure. The typical strongyloid type as seen in *Ancylostoma* sp. and *Nippostrongylus brasiliensis* with its emphasized bipolarity, into a "head" and "tail", with chromatin material located in the tail and the membrane organelles in the broad anterior end, could arise from the heterakid through an intermediate stage such as is shown in *Angiostrongylus cantonensis* (Fig. 5(5)). This possibility is emphasized by the fact that the sperm of *A. cantonensis* are generally spherical in the seminal vesicle

but adopt an elongate and bipolarized morphology in the female system (cf., for example, Figs 15 and 16 of Foor, 1970).

The spermatozoa of the filariids as shown in *Dirofilaria immitis* and *Dipetalonema viteae* (Fig. 5(9)) could arise from the heterakid by the elaboration of lipid-like inclusions, as would also seem to be possible with the spirurid, *Gnathostoma* sp. (Fig. 5(10)) (Foor, 1970). On the other hand, the typical ascaroid type with its conspicuous refringent body could have arisen from the heterakid first by the incorporation of protein materials into the lipid inclusions to form the precursors of the refringent body. This scheme would suggest that the situation in *Ascaris*, *Toxocara* and *Porrocaecum*, with their conspicuous refringent body and conoid shape, may have arisen from a spherical forerunner such as in *Polydelphis* sp. (Fig. 5(12)).

The bipolarized sperm of *Capillaria hepatica* would then have arisen from a type similar to the spherical sperm of *Trichinella spiralis* (Fig. 5(4)). It is possible that the dioctophymoid type as described by Foor could also be derived from the trichinelloid by the adoption of a rod-like shape, the loss of membrane organelles and the development of a modified surface membrane. This modified cell surface consists of two parallel membranes separated by some distance in which a thin layer of dense material with a beaded appearance may often be found. The cell surface, with its dense but beaded material, cannot be homologized with microtubules. The nearest to an analogous structure would be the tubular sheath which is formed at some stage in the spermiogenesis of *Trichinella* and even more so in *Capillaria*. This peculiar surface structure precludes the direct derivation of this sperm type from the heteroderid type, in which a cortical layer of microtubules is an obvious feature. The fact that Shepherd *et al.* (1973) observed "a fine distinct, electron-dense layer, about 10 μm immediately beneath" the surface of heteroderid sperm, may nevertheless suggest homologies with the "sheath" in the peripheral region of the sperm of *Trichinella* and *Capillaria*. Thus, it is possible that both the heteroderid and trichinelloid may have been derived from a common ancestral morphological type. The sperm of *Deontostoma californicum* with its rod-like shape and membrane organelles would belong to this particular line of development.

The so-called oxyuroid type as seen in *Aspiculuris tetraptera* would be the ultimate end result of development in the line first indicated by *Heterodera rostochiensis*. In this sperm, the cortical microtubules, the tendency to fusion of the mitochondria, and the absence of membrane organelles, would underline a fundamental difference from the heterakid and trichinelloid lines of development. The scheme of derivation of morphological types in nematode spermatozoa as suggested in this review is summarized in Figs 4 and 5.

B. SPERM CYTOCHEMISTRY AND CYTOPHYSIOLOGY

1. *Cytochemistry*

Lee and Anya (1967) studied the cytochemistry of the sperm of *Aspiculuris tetraptera* while Nath *et al.* (1961a) had studied some aspects of the cyto-

chemistry of spermiogenesis in *Porrocaecum angusticolle*. The latter workers were particularly concerned with the elucidation of the chemistry and possible homology (with the vertebrate acrosome) of the prominent refringent body found in ascarid spermatozoa. Additionally, Anya (1966a) had studied the histochemistry of the seminal plasma of *A. tetraptera*.

In *Parascaris equorum*, *Ascaris lumbricoides*, *Porrocaecum angusticolle*, *Aspiculuris tetraptera*, *Dipetalonema viteae*, *Capillaria hepatica*, *Deontostoma californicum* and *Heterodera rostochiensis*, histochemical and ultrastructural studies have demonstrated glycogen reserves, usually as the β-granules (Fauré-Fremiét, 1913; Busch, 1905; Hirsch and Bretschneider, 1937; Monné, 1948; Nath *et al.*, 1961b; Lee and Anya, 1967; McLaren, 1973a; Neill and Wright, 1973; Wright *et al.*, 1973; Shepherd *et al.*, 1974). The widespread distribution of this important energy source in nematode spermatozoa suggests that it may be the main energy reserve in these cells.

Apart from glycogen, other PAS-reactive carbohydrates, including acid mucopolysaccharides (Anya, 1966a), have been found in nematode spermatozoa (Nath *et al.*, 1961a; Clark *et al.*, 1968), and Anya (1966a) had also shown the presence of glucose in the seminal plasma of *Aspiculuris tetraptera*.

The sudan dyes and oil red O have been used to study the distribution of neutral lipids in the sperm cell of some nematodes. These studies indicated the virtual absence of neutral lipids in these cells (Fauré-Fremiet, 1913; Nath *et al.*, 1961a; Anya, 1965; Lee and Anya, 1967). Phospholipids have, however, been localized in many of the sperm cell organelles (Nath *et al.*, 1961a) while Anya (1966a) identified these important lipids in some of the secretory products of the vas deferens and in the seminal plasma of *Aspiculuris tetraptera*. In ascarids, phospholipids have been found associated with the refringent body (Favard, 1958; Nath *et al.*, 1961a). Nath and co-workers also described certain inclusions which they called lipid bodies: these showed intense reactions for phospholipids. In the light of recent ultrastructural evidence and the description of Nath and his collaborators, it seems clear that some of these bodies are the same structures as the membrane organelles, which are regular structures in the sperm of ascarids and some other nematodes (Foor, 1968).

The cytochemistry of the refringent body of some nematode spermatozoa has been studied in some detail (Fauré-Fremiet, 1913; Fauré-Fremiet and Filhol, 1937). Some early workers had attempted to homologize this structure with the acrosome of vertebrate sperm (Bowen, 1925) and it was assumed by them that it contained PAS-reactive carbohydrate material, as all true acrosomes do (Clermont and Leblond, 1955). The refringent body of all nematode spermatozoa in which this has been studied is not PAS-positive; rather it consists of proteins, associated with phospholipids (Scheben, 1905; Romieu, 1911; Sturtivant, 1934; Favard, 1958). Some of the early work suggested that the refringent body contains ribonucleoproteins (Sturtivant, 1934; Fauré-Fremiet and Filhol, 1937; Pasteels, 1948; Panijel and Pasteels, 1951; Nath *et al.*, 1961a). Recent ultrastructural studies have confirmed these early observations, as it has been found that the major contribution of the refringent body to the fertilized ovum, before the fusion of the maternal and

paternal nuclear material, is to provide the material which is utilized in the formation of polyribosomes (Foor, 1970; see particularly his Fig. 27).

Various hydrolytic enzymes, notably ATPase, esterases and acid phosphatases, have been localized in the sperm of some nematodes (Lee and Anya, 1967; Clark *et al.*, 1968; Lee, 1971). In *Ascaris lumbricoides* (Clark *et al.*, 1968) and in *Heterakis gallinarum* (Lee, 1971) the acid phosphatase was associated with the membrane organelles (which Lee called alpha bodies). Lee and Lestan (1971) found succinate dehydrogenase in sperm of *H. gallinarum.* Anya (1966a) had also identified some of these hydrolytic enzymes in addition to alkaline phosphatase (in trace amounts), glucose-6-phosphatase and cholinesterases in the seminal plasma of *Aspiculuris tetraptera.* The functional significance of these enzymes in the spermatozoa is difficult to interpret. This arises from the limitation imposed on accurate visualization of sites of enzyme localization by the light microscope. There is therefore a need to repeat these studies at the ultrastructural level, where the precise localization of these enzymes on cell structures could facilitate functional interpretations.

2. *Metabolism and motility*

Although much progress has been made in the last 5–7 years on the energy metabolism of helminths, no work exists to date on the metabolism of the male gamete. The technical difficulties in the way of such studies is considerable, but the recent use of an oxygen electrode for the successful study of respiratory physiology of extremely small individual nematodes has pointed the way (Atkinson and Smith, 1973). However, recent studies of energy metabolism in nematodes has underlined the fact that, at least in the parasitic members of the group, the complete oxidation of energy-yielding carbohydrate substrates is the exception rather than the rule (Saz, 1972). In all nematodes so far studied, the predominant end products of carbohydrate metabolism, no matter the habitat or degree of dependence on environmental oxygen, are usually succinate or its derivatives such as acetate and lactate (Saz and Bueding, 1966; Ward and Schofield, 1967; Brazier and Jaffe, 1973). However, there is now accumulating evidence that two alternative pathways of energy metabolism, one leading to the formation of lactic acid through the mediation of LDH and the other leading to the formation of oxaloacetate from phosphoenolpyruvate and carbon dioxide (through the mediation of the enzyme PEP carboxykinase), may coexist in the same helminth organism. The latter pathway generally involves mitochondrial participation; it is consequently characteristic of nematodes whose energy metabolism is essentially oxidative.

As nematode spermatozoa contain considerable quantities of endogenous glycogen, it seems likely that the energy metabolism of these cells depends upon the catabolism of endogenous carbohydrate reserves rather than on exogenous substrates. This is in contrast with the situation in mammalian spermatozoa but accords with observations on other invertebrates, notably echinoderms (Mann, 1964). Whether or not phospholipids could be utilized as an alternative or supplementary source in the nematode spermatozoa

is an open question. It should be pointed out, however, that species in which intracellular phospholipids are utilized as energy sources generally have little or no glycogen reserves (Stott, 1930; Mann, 1964). It seems unlikely that the sperm cell in nematodes would show patterns of energy metabolism significantly different from the pattern in other tissues. Moreover, the paucity of mitochondria in the sperm of nematodes generally, and its complete absence in the sperm of *Dioctophyma* sp. (Foor, 1970), would suggest that energy metabolism is mainly cytoplasmic and thus glycolytic rather than oxidative.

The most important function for which energy reserves may be necessary in the sperm would be motility. There is now abundant evidence that the predominant locomotory activity in nematode spermatosoa is pseudopodial (Foor, 1968), even in species with an organelle which on structural grounds could be considered a possible effector organelle (Jamuar, 1966; Lee and Anya, 1967).

Phillipson (1969) studied the rate and mode of sperm ascent in the female reproductive system of *Nippostrongylus brasiliensis*. He found that fertilization took place in or near the seminal vesicle of the female. He calculated that the fastest moving sperm covered an average of 0·34 mm/h, or 1 mm in 2·9 h. The earliest time after copulation taken by a sperm to move up the female system was 165 min, while in 4 h all sperm had moved to the seminal vesicle. Phillipson observed that in the female, the sperm cell was more rounded (see also Foor, 1968, 1974) and apparently moved actively by its progressive attachment to successive portions of the uterine wall. Finally, he observed that by the time the sperm cell arrived in the seminal vesicle, it had decreased from a mean size of 17·5 μm to 15·1 μm. In *Ascaris*, Foor (1970) states that decrease in the size may be up to 50% of initial size. This size decrease, it could be assumed, arises from the loss of energy reserves utilized for motility.

Anya (1973a) identified the indolealkylamine 5-HT in certain membrane-bound vesicles from the male reproductive system of *Aspiculuris tetraptera*. These vesicles are usually released with the sperm during copulation, and Anya (1966a, 1973a) suggested that the release of the amine in the female system may lead to local contractions of the system, thus aiding sperm ascent. He suggested (Anya, 1973b) that the amine may also serve to stimulate anaerobic glycolysis, as is the case in molluscs (Malanga and Aiello, 1971). The failure of *in vitro* experiments aimed at the elucidation of sperm motility in nematodes (Lee and Anya, 1967; Beams and Sekhon, 1972; Neill and Wright, 1973) may arise as much from a sceptical inability to accept pseudopodial movement as the mode in nematode sperm as from the unsuitability of the experimental substratum (Abercrombie, 1961). This aspect of sperm behaviour requires greater attention.

3. *The cytophysiological significance of the membrane organelle: a hypothesis*

The mature sperm cell of animals is a biologically important but specialized cell. Its major function is to ensure the restoration of the total informational content of the zygote before development and differentiation of the embryo can resume. In the process, it also serves as the trigger stimulus for the

initiation of development. All its specialized organelles and behaviour are thus essential requisites for the successful achievement of the goal of fertilization.

In the process of fertilization, one or other of three possible ends must be served by the organelles:

(1) the metabolism of the sperm cell must be maintained at the proper level until fertilization;

(2) special mechanisms or organelles must, if necessary, be developed to aid the movement of the sperm cell to the site of fertilization;

(3) mechanisms which facilitate cell contact between the gametes and ultimate penetration by the sperm cell, are developed.

The nematode spermatozoon would, of necessity, possess organelles or specialized behaviour characteristics which serve these functions, particularly that of motility.

With very few exceptions, notably *Aspiculuris tetraptera*, most nematode spermatozoa possess the specialized membrane organelles (Favard, 1961; McLaren, 1973a). Although a mitochondrial origin was postulated for this organelle in *Nippostrongylus* and *Ascaris* (Jamuar, 1966; Foor, 1968), recent studies on *Ascaris*, *Heterakis*, *Rhabditis*, *Panagrellus* and *Dipetalonema* favour Favard's (1961) hypothesis for an endoplasmic reticulum–golgi dictyosome involvement in the formation of these structures (Clark *et al.*, 1968, 1972; Lee, 1971; Beams and Sekhon, 1972; Pasternak and Samoiloff, 1972; McLaren, 1973a). These studies have underlined the essential similarity in the cytological events which are characteristic of the formation process of these structures as observed during spermatogenesis—in the spermatocytes, spermatids and the mature sperm cell.

According to Beams and Sekhon (1972), as part of the maturation of the spermatocyte, the smooth-surfaced endoplasmic reticulum of these cells comes into intimate relationship with golgi dictyosome saccules, a dilation of the terminal saccule is observed, and a fibrous body appears within the dilation. This is the crystalline body (c-body) of Pasternak and Samoiloff (1972) or the "battonet" of Favard (1961).

Occasionally, a dense lipid-like body may be associated with the fibrous body (Lee, 1971; Beams and Sekhon, 1972; Pasternak and Samoiloff, 1972) and a mass of small tubular elements may be observed. When the fibrous body is fully formed, it will separate from the endoplasmic reticulum–dictyosome complex. In the spermatid, the fibrous bodies with their associated dictyosome-derived membranes are arranged in a circle round the circumnuclear mass of mitochondria. With the loss of the residual body in spermateleosis, there is a contraction, folding and separation of the dictyosome derived membranes from the fibrous bodies. These membranes will then reform into vesicular bodies with characteristic folds in which some secretory material is often evident; the vesicular bodies will migrate to the periphery (below the plasma membrane) and are now the characteristic membrane organelles (Lee, 1971; McLaren, 1973a).

In *Trichinella* sp., the formation process is slightly different. According to Neill and Wright (1973), in the spermatocytes cisternae develop within

the endoplasmic reticulum in which accumulations of dense materials can be discerned. These cisternae presumably contribute to the formation of characteristic tubules which are found in the cytoplasm of the early spermatid. The membrane organelles are formed from these tubules when the latter collapse on themselves to give rise to concave vesicles. The vesicles so formed may close and fuse with adjacent units, giving rise to double membrane loops in which some cytoplasmic material is often enclosed.

From these descriptions, it is clear that in the formation process of the membrane organelles two sequential "secretory" cycles are involved: in the first cycle a fibrous, presumably structural, protein material is elaborated, while in the second cycle another protein material, possibly an enzyme, is secreted, associated with PAS-reactive polysaccharide material (Clark *et al.*, 1968, 1972; Lee, 1971). The "secretory" cycles often involve the reorganization of the membrane components of the organelles, (McLaren, 1973a; Neill and Wright, 1973; Wright *et al.*, 1973).

The movement of nematode spermatozoa is predominantly pseudopodial (Lee and Anya, 1967; Phillipson, 1969; Beams and Sekhon, 1972; Neill and Wright, 1973; Foor, 1974). Fibrillar proteins are essential for the formation of pseudopodia (Allen, 1961; Garnham, 1966). Lee (1971), Pasternak and Samoiloff (1972) and Beams and Sekhon (1972) have suggested that the fibrillar proteins elaborated in the early phase of the secretory cycle of the membrane organelles in *Heterakis*, *Panagrellus* and *Rhabditis* are utilized subsequently for amoeboid movement of the sperm cell.

The later stage in the formation of the membrane organelle is characterized by the development of pronounced membrane folds (Beams and Sekhon, 1972). This would suggest that the predominant physiological function (whatever it is) performed by these organelles at this stage is advantageously enhanced by increased cell surface area. In functional physiological terms, increased cell surface is necessary in amoeboid movement, electrical impulse propagation and in nutrient transport (Allen, 1961; Davson, 1970). As the sperm cell in nematodes already contains fairly large reserves of glycogen (Anya, 1966a; Lee and Anya, 1967; Lee, 1971; Pasternak and Samoiloff, 1972; Neill and Wright, 1973; Wright *et al.*, 1973) the last function above seems unlikely. Rather, the cell membrane, especially of the posterior end of the cell, may be involved in the initiation and propagation of pseudopodial formation (Goldacre, 1961), which process often involves the propagation of electrical impulses (Bell and Jeon, 1963) and the dissolution of the posterior cell membranes (R. S. Bird, 1961).

Cell adhesion is an essential cellular characteristic for the formation of functional pseudopodia (Wenyon, 1926; Abercrombie, 1961), and surface mucopolysaccharides are essential for cellular adhesiveness. In the fully formed spermatozoa, PAS-positive material of a muco-protein nature are associated with the secretions of the membrane organelles (Nath *et al.*, 1961b; Lee, 1971) in addition to acid phosphatases (Clark *et al.*, 1968, 1972; Lee, 1971). In *Aspiculuris tetraptera*, acid mucopolysaccharides are also associated with the sperm surface (Anya, 1966a). Thus, the membrane organelle seems on functional grounds to fulfil the three essential conditions

for an organelle specialized for amoeboid movement in cells: the provision of fibrillar proteins, an intimate relationship with the functioning cell membrane, and a role in the control of cell adhesiveness. Thus, it seems reasonable to postulate that the functional significance of the membrane organelle of nematode spermatozoa lies in a specialized role in amoeboid movement. This hypothesis is given indirect support by the following observations:

(a) the need for sperm movement would exist in both the male and female systems. Consequently, in some nematodes, the membrane organelles are seen to release their substances in the male system (Foor, 1968). If a specialized role for the fusion of the gametes only were indicated as the primary function, the release of the substances of the membrane organelles would take place only in the vicinity of the mature ovum;

(b) sperm entry into the ovum is achieved by pseudopodial activity rather than an acrosomal reaction (Foor, 1968; McLaren, 1973a). Thus the problematical presence of PAS-reactive materials and hydrolytic enzymes, as in other acrosomes (Dan, 1956), which led to the postulated homology of these organelles with other invertebrate acrosomes (Clark *et al.*, 1967), becomes explicable. This postulated function for the membrane organelles would not necessarily rule out a functional role in the process of fertilization, as suggested for *D. viteae* (McLaren, 1973b).

V. The Female Gamete

A. Nuclear changes in oogenesis

Cells of the reproductive system in nematodes are derivatives of the "propagation", P cell, set aside from the somatic cells during the early cleavage divisions (Nigon, 1965). In the female reproductive system, the oogonia occupy the apical (germinal) region of the ovary and undergo regular mitotic divisions (Chitwood and Chitwood, 1950; Triantaphyllou and Hirschmann, 1966; Anya, 1964b; Lee and Lestan, 1971; McLaren, 1973b). These divisions may, as in *Ancylostoma caninum*, occur in the adult ovary (Lejambre and Georgi, 1970), while in some nematodes like *Seinura tenuicaudata* it may occur in the larval (usually fourth stage but occasionally third stage) ovaries (Hechler, 1963). In these latter cases, there may be no further divisions in the adult (Triantaphyllou, 1971b).

The daughter oogonia show the somatic (2n) chromosome number. In some nematodes, like *Anguina tritici*, the oogonial divisions appear synchronized (Triantaphyllou and Hirschmann, 1966; Triantaphyllou, 1971), while in others there appears to be no synchronization (Foor, 1970). McLaren (1973b) has suggested that the rachis may possess a functional role in the synchronization of the oogonial divisions in many nematodes.

In most animals, the chromosomes undergo characteristic and well defined changes associated with the division and development of the oogonia into the mature ovum (oocyte). The nuclear changes in nematode oogonia follow the same general pattern common to most animals, with slight modifications

at the stage of synapsis. Beyond the germinal zone, in the growth zone of the ovary, the oogonial divisions cease, and the nuclear chromatin condenses in a compact network (Hechler, 1963; Triantaphyllou, 1971b). This period of nuclear condensation is the equivalent of the synaptic pairing of homologous chromosomes observed in other animals (Swanson, 1965).

In nematodes, it is difficult to follow the various phases of synapsis such as leptotene, zygotene and pachytene. However, after this period of condensation the nuclear chromatin mass resolves into elongate and double chromatin threads. This is the early diplotene stage (Triantaphyllou and Hirschmann, 1966). It is during this period that one or more nucleoli may become prominent in the nucleus while the oocyte as a whole enters a phase of rapid growth. It increases several fold as the elaboration of cytoplasmic inclusions gets under way, and a high rate of nuclear synthesis of DNA and RNA is often observed (Triantaphyllou, 1971b). The oocytes migrate down the ovary, usually in single file as in *Aspiculuris tetraptera* (Anya, 1964b), although in nematodes with a rachis like *Ascaris lumbricoides* (Prestage, 1960) the oocytes may migrate in rows (Triantaphyllou, 1971a). During this phase of migration down the ovary the chromatin material becomes diffuse, the nuclear membrane disappears as the mature oocyte undergoes diakinesis, and tetrad bivalents appear. The oocytes at this stage (Prometaphase I) will then enter the spermatheca where fertilization often takes place. In some nematodes fertilization may take place in the upper uterus (Fairbairn, 1957). Further nuclear changes are possible only after fertilization, particularly with the penetration of the sperm which acts as a biological trigger initiating subsequent changes,

On sperm entrance, the oocyte advances to metaphase I at the same time as the process of egg-shell formation gets under way. Anaphase I and Telophase I follow rapidly and the polar nucleus is formed. The polar nucleus is extruded in amphimictic nematodes but retained close to the surface in parthenogenetic species (Triantaphyllou, 1971a). On completion of this first maturation division, the chromosomes move into Prophase II without the interphase. Metaphase II, Anaphase II and Telophase II follow successively and rapidly. A second polar nucleus is formed and extruded. The egg chromosomes will now migrate to the centre of the oocyte where the female pronucleus is formed.

B. CYTOLOGICAL CHANGES DURING OOCYTE DEVELOPMENT

Ever since the classical studies of oogenesis, fertilization and development in *Parascaris equorum* (van Beneden, 1883a; Fauré-Fremiet, 1913), the cytological features of oocyte development in nematodes have attracted much attention (Musso, 1930; Ishii and Yanagisawa, 1954; Yanagisawa, 1955; Kochhar, 1960; Favard, 1961; Anya, 1964b; Foor, 1967, 1968; Lee, 1971; McLaren, 1973b; and others).

In the early oogonia found in the germinal zone, the first cytoplasmic inclusions to appear are lipid droplets (Fauré-Fremiet, 1913; Flury, 1912; Lee, 1960; Monné, 1959; Anya, 1964a; Foor, 1967). Soon, however, large reserves of glycogen are accumulated in the developing oocyte as well as the

characteristic ascaroside esters (Fauré-Fremiet, 1913; Fairbairn, 1957). In addition, certain other characteristic inclusions described first by van Beneden (1883b) become discernible in the cytoplasm. van Beneden (1883b) described three different types of inclusions which he called homogeneous droplets, hyaline granules and refringent granules in *Parascaris equorum*. Subsequent workers confirmed the presence of these hyaline granules and the refringent granules in *Ascaris lumbricoides* (Yanagisawa, 1955; Foor, 1967), *Porrocaecum angusticolle* (Kochhar, 1960), *Aspiculuris tetraptera* (Anya, 1964b) and *Heterakis gallinarum* (Lee and Lestan, 1971), among others. These inclusions appear common in all nematodes in which the formation of the egg-shell (see below) is an essential part of the fertilization process.

The formation process, chemistry and fate of these characteristic cytoplasmic inclusions in nematode oocytes have been the subject of numerous studies (Fauré-Fremiet, 1913; Flury, 1912; Fauré-Fremiet and Filhol, 1937; Panijel and Pasteels, 1951; Fauré-Fremiet *et al.*, 1954; Yanagisawa, 1955; Kochhar, 1960; Anya, 1964b; Foor, 1967).

In *Ascaris lumbricoides*, Foor (1967) in a combined light microscope and ultrastructural study of the young oocytes showed that the refringent granules always developed near a lipid droplet. The developing refringent granule and the lipid droplet were always associated with a vesicle (possibly the phospholipid body of Kochhar); these organelles would coalesce and give rise to the fully formed refringent granule. Usually, the refringent granule showed during formation an inner dense core surrounded by a less dense and homogeneous matrix, and when fully formed it was always associated with some remnants of the endoplasmic reticulum (Foor, 1967).

In the germinal zone of *Ascaris lumbricoides*, Foor (1972) has shown that the oogonia are spherical cells which elongate and assume a radial arrangement about the rachis as they progress to the growth zone (see also Prestage, 1960). Before and during the elongation process, the young oocytes are covered by a smooth plasma membrane, possess a large nucleus, elongate mitochondria, ribosomes and some lipid droplets (Lee and Lestan, 1971; McLaren, 1973b). Oocytes at this stage show aggregations of a dense particulate material on the plasma membrane, often distributed in a net-like fashion. Infoldings of the plasma membrane take these dense particulate materials into the interior of the apical cytoplasm. Once within the cytoplasm, membrane-bound vesicles, containing copious quantities of intercellular fluid as well as the particulate material, separate off, and condense to form inclusion bodies which Foor called the dense granules, and which are perhaps the same as the hyaline granules of earlier workers. Foor (1972) observed that the inclusion bodies which result from this process of micropinocytosis fall into two size groups, an observation which may explain van Beneden's earlier description of three types of inclusions, i.e. these two types of hyaline granules (one of which he called the homogeneous droplet) and the refringent sphere.

C. BIOCHEMICAL ASPECTS OF OOGENESIS

The reproductive organs are the most important site for lipid deposition in

female *Ascaris* and perhaps in other female nematodes (Fairbairn, 1960; Lee, 1960, 1962; Anya, 1964a). Total lipids constitute about 8% of the fresh weight of the ovaries. Of this 8%, phospholipids make up 9%, while the rest consists of four classes of neutral lipids, including glycerides (65%), ascaroside esters (33%), waxes (1·5%) and sterols 0·26% (Tarr and Fairbairn, 1973a). Of the ovarian lipids, the ascaroside esters have generated the most interest ever since the studies of Flury (1912) and Fauré-Fremiet (1913) underlined their unusual nature. They are high mol. wt. glycolipids which are first elaborated in the refringent granules and later form an important constituent of the egg-shell (Fauré-Fremiet, 1913; Fouquey *et al.*, 1957, 1962). In the oocyte, the ascarosides are segregated mainly in the refringent granules and in the lipid droplets, where they exist as the acetate and propionate esters of the glycone, ascarylose, 3,6-dideoxy-L-arabinohexose (Tarr and Fairbairn, 1973b), a glycoside which has been found also in the micro-organism *Pasteurella pseudotuberculosis* (Matsuhashi *et al.*, 1964).

Fouquey *et al.* (1957, 1962) described three ascarosides A, B and C which in the ovarian tissue may be esterified at both the fatty acid (aglycone) and

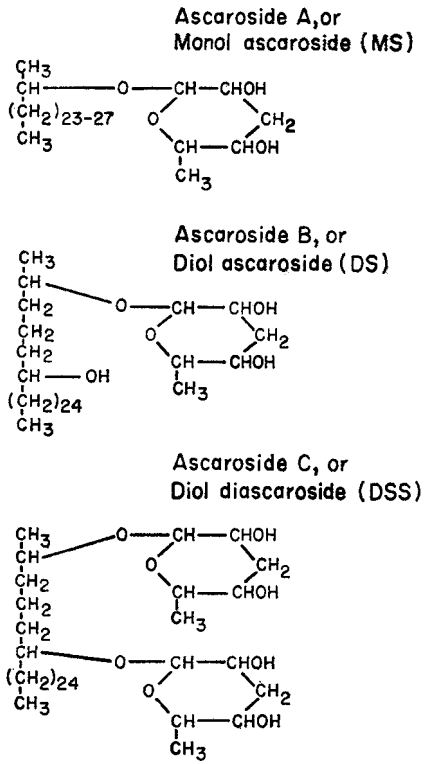


FIG. 6. Ascarosides of *Parascaris equorum* (from Tarr and Fairbairn, 1973b).

glycosidic (glycone) moieties. Tarr and Fairbairn (1973c) showed, however, that in *Ascaris suum* there are four ascarosides in the egg-shell, presumably derived from esterified precursors in the oocyte cytoplasm. These are a monol ascaroside, a diol ascaroside, a diol (acetylated) ascaroside and a diol diascaroside (Fig. 6). In *A. lumbricoides*, Tarr and Fairbairn (1973b) also found that the acid residues consisted of 95·6 mol % acetate and 4·4 mol % propionate, while in all a total of 15 esters were identified, 13 of which occured at concentrations of about 0·1 %.

Jezyk and Fairbairn (1967b) presented preliminary evidence which indicated that, as in *Pasteurella pseudotuberculosis*, the ascaroside glycone was synthesized from glucose or glucose-1-phosphate through 3,6-dideoxyhexose nucleotide-phosphate. The aglycone carbon chain, on the other hand, was synthesized apparently by condensation and decarboxylation of 2 mol of higher fatty acids by a mechanism analogous to the malonyl CoA synthesis of fatty acids. The glycone dinucleotide and the aglycones thus synthesized were then converted into the ascaroside esters. That ovarian homogenates need ATP, CoA and a reduced pyridinenucleotide before *in vitro* synthesis of these esters can take place, would seem to support the above scheme of synthesis, since these substrates are also required in the malonyl CoA pathway for fatty acid synthesis (Foor, 1967; Jezyk and Fairbairn, 1967b).

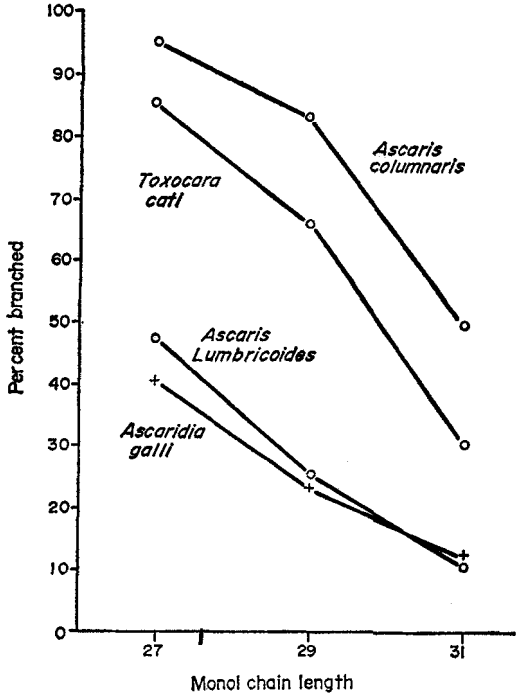


FIG. 7. Graph showing relationship between branching and length of monol chain in ascarosides of various ascarids of different sizes (from Tarr, 1973).

It is of interest in this respect to note that acetyl and propionyl groups occur almost exclusively in the ascaroside esters, while the α-methyl butyryl and α-methyl valeryl groups are found exclusively in the ovarian triglycerides and waxes, an observation which suggests the presence of highly specific kinases (Tarr and Fairbairn, 1973b).

Tarr (1973) identified free and esterified ascarosides in *Ascaris columnaris*, *Ascaridia galli*, *Toxocara cati* and the oxyurid *Passalurus* sp. He compared these with the ascarosides of *Ascaris lumbricoides* and found that ascaroside esters of all these species were identical with those of *A. lumbricoides*. As in the latter, the ascarosides were primarily acetates of identical isomeric form. He further observed that the percentages of propionate and unesterified hydroxyl groups in ovarian ascarosides, and of volatile acids in triglycerides and waxes, increased with increasing size of the parasite. Moreover, he found a consistent relation within each species between running chain length and the amount of branching (Fig. 7). Perhaps, as Tarr (1973) has suggested, unusually large nematodes may experience some limitations in the ready availability of nutrients as a result of their smaller surface area to volume ratio. Thus, to maintain the high level of egg production often characteristic of these parasitic nematodes, they have developed the capacity for substituting higher volatile acids for long chain acids in their glycerides and waxes.

In parthenogenetic species or ovoviviparous species in which the need for a thick and elaborate egg-shell does not exist, the synthetic mechanisms for the formation of ascarosides may also be absent. For example, in the rhabditid *Turbatrix aceti* and *Panagrellus redivivus*, Tarr (1973) found no ascarosides, while in an unidentified free-living *Rhabditis* sp. he found unsaponifiable lipids corresponding in mobility and colour reactions to ascarosides. It therefore seems likely that the environment in which the embryo develops, by making necessary the need for a resistant eggshell, has been in developmental terms a more significant factor in determining whether or not ascarosides are produced by a particular nematode species, than the accident of the species' taxonomic position. However, it seems likely that *T. aceti* and *P. redivivus* are here displaying biochemical adaptability rather than a loss of their genetic capacity for the synthesis of these compounds (Fairbairn, 1970).

The female reproductive organs and particularly the oocyte in all nematodes so far examined contain large quantities of glycogen (Flury, 1912; Fauré-Fremiet, 1913; Kochhar, 1960; Lee, 1960; Anya, 1964a). In addition, glucose and trehalose are present in the haemolymph (Fairbairn and Passey, 1957). It is of interest in this respect to note that in many nematodes, it is now generally accepted that fatty acids are utilized in glycogenesis (Passey and Fairbairn, 1957; Barrett *et al.*, 1970). The pathway of synthesis of trehalose from glucose would necessarily be different from that for glycogen, since the former is a disaccharide in which the glucose units are linked in $1 \rightarrow 1$ fashion rather than in the $1 \rightarrow 4$ linkage of glycogen. However, Feist *et al.* (1956) have shown that the adult reproductive tissues of *Ascaris suum* possesses the highest capacity for the synthesis of this disaccharide.

Large amounts of proteins are deposited in the oocyte of *Ascaris* (Fairbairn, 1960), and the similarity in the main events in oocyte development in nematodes generally encourages the hope that the situation would be similar to *Ascaris* in other nematodes. Much of the extensive protein synthesis which takes place during oocyte development is associated with the elaboration of protein yolk, although it now appears that some of this yolk, for example the dense granules described by Foor (1972), are produced outside the oocyte, concentrated on the external surface membrane of the oocytes (which may suggest that they are glycoproteins), and subsequently taken into the cell by micropinocytosis.

In *Ascaris* in which protein metabolism has been studied in greater detail (Pollak and Fairbairn, 1955a,b), the ovaries possess high transaminase activity which operates in the direction of protein synthesis, rather than catabolism, with pyruvate being reductively aminated to alanine. Laser (1944) showed that minced ovarian tissue of *A. lumbricoides* consumed oxygen. Pollak and Fairbairn (1955b) found that alanine, aspartic acid, lysine, valine and phenylalanine were without effect on the rate of oxygen consumption, however, which suggested that no amino acid oxidase was involved in the process. Both glutamine and glutamic acid, on the other hand, stimulated oxygen consumption, indicating the possible involvement of a glutamic acid dehydrogenase. Further study of this system by these authors confirmed the presence of endogenous dehydrogenases in ovarian tissue (Pollak and Fairbairn, 1955b).

Harris *et al.* (1972) have shown that ^{14}C-cycloleucine is actively transported *in vitro* into ovarian tissue of *Ascaris suum* in both saline and perienteric fluid. The rate of uptake was unaffected by the composition of the gas phase. The transported leucine was rapidly incorporated into protein. It should be of interest to find out whether the amino acid was transported directly into the oocyte and there incorporated into the protein yolk, or whether it was condensed on the surface membrane in the manner described by Foor (1972) and subsequently taken into the cell by micropinocytosis as the dense granules, as had been observed with *Ascaris* (Foor, 1972). There is, moreover, an unusually large concentration of free ammonia in the ovarian tissue, while glutamic acid and glutamine account for 38% of the total α-amino acid nitrogen in ovaries (Pollak and Fairbairn, 1955a). Langer (1972) has studied the glutamic acid dehydrogenase of *A. suum*. He found that this ascarid's GluDH had a relatively high affinity for NH_4^+ (2000 times greater than beef liver and 300 times greater than frog liver). This high affinity will tend to remove the toxic NH_4^+ from solution by forming glutamate, thus providing a ready source of α-amino nitrogen for amino acid synthesis via the transaminase system (Pollak and Fairbairn, 1955b). As will be seen later, the significance of the high concentrations of ammonia may also lie in its role in egg-shell formation (see below).

Of the various proteins synthesized in the female reproductive system, the haemoproteins deserve particular mention because of their strategic position in the energy metabolism of organisms. Haematin is found throughout the reproductive system of both male and female *A. lumbricoides*, while

the gametes may contain a haemoglobin whose oxygenated form may have contributed to the spectroscopic observation of a double-banded spectrum at 570 and 535 μm by Keilin (1925) and Smith and Lee (1963). In many nematodes in which haemoglobin has been detected, these proteins have been found localized usually in the body wall (muscle and cuticle) or in the perienteric fluid (Lee and Smith, 1965). *Ascaris* and perhaps other nematodes are unable to synthesize the vinyl side chain of protoporphyrins from propionic acid as other organisms do (Fairbairn, 1970). The synthesis of haemoglobin in ascarid tissues depends, therefore, upon the exogenous supply of the haem group. In the presence of an exogenous source of supply, *Ascaris* will synthesize its own haemoglobins which differ, particularly in their oxygen-binding characteristics, from host haemoglobin. The perienteric fluid haemoglobin, for example, has so great an affinity for oxygen that the partial pressure of oxygen must drop at least to 0·05 mm before appreciable deoxygenation occurs, a level of oxygen concentration which even in the near anaerobic environment of the pig small intestine is not generally attained. Thus the perienteric fluid haemoglobin is fully oxygenated for most of the time. This has led to the suggestion that the perienteric fluid haemoglobin has no role as an oxygen carrier or store but rather serves as a metabolic pool from which other haemoproteins are synthesized (Smith and Lee, 1963). The cytochromes which are so essential in cellular respiration represent one group of such haemoproteins. These haemoproteins are necessary for the energy metabolism of the eggs, which *Ascaris* and many other parasitic nematodes produce in such prolific numbers (Crofton, 1966)—*Ascaris* produces as many as $1{\cdot}6 \times 10^6$ eggs daily (Kelly and Smith, 1956). Thus, as a result of the need to ensure a supply of cytochrome molecules for every egg's respiratory requirements, the requirement for the ovarian synthesis of cytochromes would be great, since the embryonating eggs and the developing larvae of nematodes are aerobic (Fairbairn, 1960; Costello and Brown, 1962; Anya, 1966d; Barrett, 1969). Indeed, the recent observation that even adult *Ascaris* which is usually regarded as an obligatory anaerobe also possesses functional cytochromes which participate in electron transport (Cheah, 1972) gives further but indirect support to the suggestion of Smith and Lee (1963) on the metabolic role of haemoglobins in *Ascaris*.

D. VITELLOGENESIS AND OOGONIAL NUTRITION

The nematode egg is cleidoic. Consequently, the developing embryo must have all the substrates necessary for development or their precursors already in store in the mature oocyte. Such yolk is synthesized either intracellularly within the oocytes or extracellularly. In nematodes, yolk in the mature oocyte includes the glycogen reserves, lipid droplets, and the dense granules. As has already been indicated above, the glycogen, the lipid droplets and the ascaroside esters of the refringent granules are elaborated within the developing oocyte (Fairbairn, 1957; Lee, 1960; Anya, 1964b; Jezyk and Fairbairn, 1967a), while the dense granules are synthesized

extracellularly and deposited on the plasma membrane of the oocyte; the precursor material of these granules is then taken into the oocyte cytoplasm by a process of micropinocytosis (Foor, 1967).

The female reproductive organ of nematodes with its investing epithelial monolayer of cells is suspended in the body cavity and bathed by the perienteric fluid (Chitwood and Chitwood, 1950). Chemical analyses of the perienteric fluid of *Ascaris lumbricoides* and *Anisakis physeteris* indicate that this fluid is a complex mixture of proteins including free aminoacids, carbohydrates, including glucose and trehalose, fats, and inorganic ions (Hobson, 1948; Hobson *et al.*, 1952a,b; Pollak and Fairbairn, 1955a; Fairbairn, 1960; Viglierchio and Gortz, 1972). The proteins consist mainly of albumins and globulins, and in *Ascaris* and some other nematodes include traces of haemoglobin (Lee and Smith, 1965). The developing oocytes would thus find in the perienteric fluid a rich pool of metabolites for the active synthesis of proteins necessary for their full development.

In *Anisakis physeteris*, Viglierchio and Gortz (1972) identified 17 amino acids in the ovarian tissue as compared with 28 in the perienteric fluid. Obviously, the investing epithelial cell layer does exert a selective effect on the entry of amino acids into the ovarian tissue. However, the ultrastructural features of the epithelial cell do not indicate an active synthetic role (Foor, 1967; Lee and Lestan, 1971), even though McLaren (1973b) has suggested that the surface coat of spherical granular units found on the developing oocytes of *Dipetalonema viteae*, and which are obviously analogous to the precursors of the dense granules of *Ascaris* (Foor, 1967), are secreted by the epithelial cells. Indeed, Kochhar (1960) maintains that "follicular epithelium contributes lipoprotein bodies and glycogen to the oocytes. The lipoprotein bodies are essential for the production of the hyaline spheres (protein yolk)." Thus, while the perienteric fluid is the obvious source of ovarian metabolites, some part of vitellogenesis involves the ovarian epithelial cells. It is possible, however, that some of the smaller protein molecules could be synthesized in the alimentary cells, secreted into the perienteric fluid and subsequently abstracted therefrom by the epithelial cells (Foor, 1968).

In this regard, it is noteworthy that while the uptake of amino acids by ovarian tissue from the perienteric fluid has been demonstrated *in vitro*, in *Ascaris suum*, the uptake did not appear to be stimulated by exogenous glucose or by variations in the gas phase indicating that the energy required for the process may be derived from glycolytic rather than oxidative phosphorylative reactions (Harris *et al.*, 1972).

A characteristic structure of some nematode ovarian tissue is the rachis (Chitwood and Chitwood, 1950). This is a syncytial, anucleate mass of cytoplasm in which lipid droplets, glycogen, ribosomes, dense inclusions and microtubules have been found (Prestage, 1960; Foor, 1968; Lee and Lestan, 1971; McLaren, 1973b). As there are no equivalents of the follicle cells of vertebrate ovarian tissue or the nurse cells of insect and other invertebrate ovary in nematodes, it has often been suggested that the rachis may have a nutritive function in relation to the developing oocytes (Mayer,1908). Foor (1968) has drawn attention to the fact that the lack of a well defined

nucleus and organelle system in the rachis would suggest only a limited ability for synthesis of ovarian yolk or their precursors by the tissue. Nevertheless, Prestage (1960) suggested two alternative functions for the rachis, namely, to serve as a reserve store of excess materials, synthesized originally by the oocytes and sequestered by the rachis until needed (this view is supported by the observation of von Kemnitz (1912) that glycogen appeared in the rachis of *Ascaris* only after this substrate had become the chief stored product of the ovary); alternatively, it serves as an absorptive and synthetic "tank" from which nutrients may be drawn by the oocyte as and when required. In our view, neither function excludes the other. The presence of intercellular microtubules connecting the developing oocyte and the rachis as well as certain dense bodies of an apparently protein nature, with periodic repeating substructure, may suggest the greater likelihood of the second suggestion. In addition, Foor (1968) has ascribed the synchronization of oocyte development to the rachis. The balance of evidence thus seems to point to the conclusion that oogonial and oocyte nutrition with its associated vitellogenesis depends upon the abstraction of nutrients from the perienteric fluid, followed by synthesis in the epithelial cell and the oocyte itself rather than in the rachis.

VI. The Physiology of Fertilization

A. The formation of the zygote

As was indicated above, the maturation of the oocyte preparatory to the formation of the female pronucleus and subsequent union of the male and female pronuclei takes place in nematodes only after sperm penetration. Despite the enormous numbers of eggs produced, relatively few descriptions exist in the literature of the process of union of the gametes. In most nematodes this takes place in the seminal receptacle or the upper uterus of the female (Chitwood and Chitwood, 1950), where in *Aspiculuris tetraptera* the pH varies between 5 and 6 (Anya, 1964b). In *Ditylenchus destructor,* Anderson and Darling (1964) observed that preparatory to fertilization, at the stage of Prometaphase I, an oocyte moved into the spermatheca immediately after it attained the "size of a fully developed egg". This was usually 2 h after the disappearance of the nucleolus but the passage into the spermatheca took only 1 or 2 min. The actual penetration of the sperm also took 1 or 2 min, while an oocyte into which a sperm had penetrated remained in the spermatotheca for about 5 min before moving out to the quadricolumella and, thence to the uterus, the latter migration usually taking about 15–30 min.

The ultrastructural aspects of sperm formation, penetration and subsequent zygote formation have been studied in *Parascaris equorum* (Favard, 1961) *Ascaris lumbricoides* (Foor, 1968, 1970), in *Physaloptera* sp. and *Angiostrongylus* sp. (Foor, 1970), in *Dirofilaria immitis* (Maeda, 1968), *Dipetalonema viteae* (McLaren, 1973b) and *Heterakis gallinarum* (Lee and Lestan, 1971). Foor (1968) and McLaren (1967b) provided the most detailed

descriptions of the process as observed in *A. lumbricoides* and *D. viteae* respectively. Their observations as well as Foor's supplementary observations on *Physaloptera* sp. and *Angiostrongylus* sp. (Foor, 1970) suggest that the process of sperm penetration is similar in most nematodes.

The developed oocyte is delimited by an oolema, which is invested with a surface coat (Lee and Lestan 1971; McLaren, 1973b). In both *A. lumbricoides* and *D. viteae* the conical anterior end of the sperm makes first contact with the oocyte. As the spermatozoon makes contact, it comes to lie against the surface coat of the oolema, at the same time as pseudopodia are projected from its anterior end towards the oocyte. Either by undulatory movement or cytoplasmic enlargement of the pseudopodia (Foor, 1970) or as a result of enzymes liberated from the sperm membrane organelles (McLaren, 1973b), the surface coat is removed and the two gametes' membranes come to lie against each other. The membranes disappear at this point of contact and it is through the channel thus established that the sperm will be taken in, as a whole, to the cytoplasm of the oocyte. In *Heterakis gallinarum*, a second oolema is formed immediately after sperm entrance (Lee and Lestan, 1971). It is possible that the membrane organelle with its enzymes which are taken into the egg cytoplasm, contributes to this new membrane synthesis.

Foor (1970) sometimes observed the interdigitation of the membranes of the oocyte and the sperm before the complete entry of the contents of the sperm into the oocyte cytoplasm, and in *Dirofilaria immitis* he suggests that a phagocytic action of the oocyte facilitates sperm entrance. McLaren (1973b) reports that pseudopodia are not formed in *Dipetalonema viteae*, although in most other nematodes the formation of pseudopodia by the sperm is an essential part of the process of sperm contact and penetration. Indeed the spermatozoa of another filariid *Dirofilaria immitis* (Foor, 1970) form pseudopodia preparatory to penetration, as do also the sperm of *Rhabditis pellio* (Beams and Sekhon, 1972), *Syphacia obvelata* (Dick, 1971) and *Capillaria hepatica* (Neill and Wright, 1973). In many nematodes, for example *Ditylenchus destructor* (Anderson and Darling, 1964), only one sperm succeeds in gaining entrance into the oocyte, while in others polyspermy is known (McLaren 1973b). In these latter cases, only one male pronucleus unites with the female pronucleus.

Sperm entrance into the egg cytoplasm is followed by two apparently related processes, the rapid synthesis of ribosomes (Foor, 1968, 1970; McLaren, 1973b; Lee and Lestan, 1971; Beams and Sekhon, 1972) and the progressive adoption of a particulate appearance or even total disappearance of the chromatin material of the sperm (Lee and Lestan, 1971; Foor, 1968). In *Ascaris lumbricoides*, for 12 to 24 h following sperm penetration, the female pronucleus will undergo its maturation divisions at the same time as the egg-shell is being formed (Fairbairn, 1957). Ten hours after the completion of the maturation divisions, i.e. 38 h post-penetration of the sperm, the two pronuclei will then fuse (Bachofer, 1957). The first cleavage division follows a short time afterwards (Kaulenas and Fairbairn, 1966).

B. THE FORMATION OF THE EGG-SHELL

The earliest study of the process of egg-shell formation and the structure of the egg-shell in a nematode was that of Nelson (1852) on the ascarid *Toxocara cati* (*Ascaris mystax*). He recognised three layers in the egg-shell and implied in his descriptions that its formation was an endogenous process. In the intervening years, the unusual structure and properties of the nematode egg-shell ensured for it the continuing interest of generations of biologists. Much of the early studies, many of them classics of their kind, is reviewed by Christenson (1950).

1. *The structure and chemistry of the egg-shell*

The egg-shell of many nematodes is covered by a three-layered shell made up of a *lipoprotein* outer layer, a middle *chitinous* layer in which lipoproteins are also present, and the inner resistant and almost impermeable *ascaroside* layer (Blanchard, 1889; Fauré-Fremiet, 1913; Wharton 1915; Thomas, 1924; Szwejkovska, 1928; Wottge, 1937; Chitwood, 1951; Monné and Honig, 1954; Fairbairn, 1957, 1970; Anya, 1964b). In the ascaroids and the oxyuroids, the outermost lipoprotein layer may be invested with an additional and external deposit of a jelly-like material which may consist of mucoproteins as in *Ascaris lumbricoides, Parascaris equorum* and *Ascaris suum* or acid mucopolysaccharides as in *Thelastoma* sp. (Monné, 1962; Lee, 1961). In the trichuroids such as *Trichuris myocastris* and *Capillaria hepatica*, Monné and Honig (1954) suggests that the shell proper consists of a keratin-like quinone-tanned protein with disulphide (cystine) linkages, while the opercular plugs are made of a polysaccharide–protein complex which contains no chitin. Apparently, there is no chitin even in the main egg-shell of these trichuroids. It seems that different nematodes may show variations in the amount of chitin present in the egg-shell (Rogers, 1962).

Anya (1964b), using histochemical methods, identified phospholipids and proteins in all the layers of the egg-shell of *Aspiculuris tetraptera* and chitin in the middle layer of this nematode. The chitin had been identified by both the van Wissenligh test (Christenson, 1950) and by the identification of acetyl-glucosamine in hydrolysates of the egg-shell (Tracey, 1955). The presence of collagen-type proteins in the egg-shell of *A. tetraptera* was indicated by a positive reaction with van Giesen's picrofuchsin.

The most detailed study of the chemistry of the egg-shell of a nematode was that of Clarke *et al.* (1967) on *Heterodera rostochiensis*. They found that a major component of the dried egg-shell was protein (59%). On the basis of chromatographic analysis of egg-shell hydrolysates, they identified 18 amino acids (Table II) of which proline was the most abundant. Proline, with aspartic acid, glycine and serine, made up 64% by weight of the total amino acids. As a result of the small amounts of aromatic and sulphur-containing proteins and the presence of hydroxyproline, Clarke *et al.* (1967) suggested the presence of a collagen-type protein in the egg-shell of *Heterodera rostochiensis*. In addition to protein, dried egg-shells contained non-hydrolysed material (20%), chitin (9%), lipid (7%), carbohydrate (6%), polyphenol

(3%) and ash (3%). The preponderant concentration of proteins in the egg-shell was unexpected in view of the popular assumption that chitin is the principal component of nematode egg-shells (Chitwood and Chitwood, 1950).

Kreuzer (1953) and Jaskowski (1962) analysed the proteins of the different layers of the egg-shell of *Ascaris*. In the jelly-like covering of the shell proper, each of these workers identified the same amino acids as Clarke *et al.* (1967) identified in *H. rostochiensis* egg-shells, except that Jaskowski (1962) could not identify arginine in the hydrolysates of *Ascaris* material while tryptophan was also present. Neither Kreuzer (1953) nor Clarke *et al.* (1967) had found tryptophan in their material.

Kreuzer (1953) could only identify ten amino acids, namely leucine, valine, methionine, alanine, lysine, histidine, proline, aspartic acid, arginine and α-aminobutyric acid, while Jaskowski (1962) could identify only the following seven amino acids in the chitinous middle layer: cystine, aspartic acid, glycine, serine, proline, tyrosine and arginine. In the ascaroside layer,

TABLE II

Amino-acid composition of hydrolysates of the egg-shells of Heterodera rostochiensis (from Clark *et al.*, 1967)

Amino-acid	Amino-acid composition (% by wt. of total amino-acids)	
	6 h hydrolysis	24 h hydrolysis
Proline	34·2	38·3
Aspartic acid	11·4	10·9
Glycine	9·8	8·5
Serine	8·3	7·4
Glutamine	5·2	6·0
Hydroxyproline	4·7	5·2
Lysine	3·8	2·8
Tyrosine	3·1	2·1
Alanine	2·8	2·3
Cysteine	2·6	—
Arginine	2·2	1·7
Leucine	1·9	1·6
Threonine	1·9	2·7
Phenylalanine	1·8	1·5
Histidine	1·7	2·3
Valine	1·5	1·9
NH_3	1·4	0·8
Isoleucine	1·3	1·4
Methionine	0·6	2·6

Jaskowski (1962) identified the amino acids cysteine, aspartic acid, proline, tyrosine, leucine, isoleucine, tryptophan, phenylalanine and arginine.

In *Meloidogyne hapla* and *M. javanica*, Bird (1958) identified glycine, alanine, serine, threonine, valine, leucine, isoleucine, aspartic acid, glutamic acid, arginine, lysine, tyrosine, histidine, proline and hydroxyproline in the hydrolysates of the egg-sac. He also identified phenolic compounds and the enzyme polyphenol oxidase, thus indicating that the proteins of the egg-sac are tanned. Bird (1958) in fact called the egg-sac protein a tanned glycoprotein, despite this protein's obvious relationship with secreted collagens as shown by their solubility characteristics in various reagents (Anya, 1964b, 1966b). Foor (1967) re-examined the amino acid composition of the ascaroside layer of the egg of *Ascaris lumbricoides* and identified 16 amino acids including tryptophan; he found no hydroxyproline, methionine or histidine in the hydrolysates. Thus, in most respects the same amino acids as had been identified in total egg hydrolysates of *Heterodera rostochiensis* by Clarke *et al.* (1967) were also found.

Ebel and Colas (1954) claimed to have identified a proline-rich protein in the refringent granules of *Parascaris equorum* and by implication in the ascaroside layer, a result which Foor (1967) could not confirm for *Ascaris lumbricoides*. The absence of such a proline-rich residue taken alongside the absence of hydroxyproline from the hydrolysates in Foor's experiments, perhaps underlines the non-collagenous nature of the proteins of the ascaroside layer in *Ascaris*. It seems likely, nevertheless, that apart from the collagen-type proteins, other classes of proteins are also present in the egg-shell of nematodes. Indeed, Yanagisawa (1955) suggested that the inner surface of the middle chitinous layer in *A. lumbricoides* was delimited by a layer of protein.

2. *Physiological and biochemical changes during shell formation*

The process of formation of the egg-shell is an immediate result of fertilization and has been studied using the light microscope in *Parascaris equorum* (Fauré-Fremiet, 1913; Wottge, 1937), *Ascaris lumbricoides* (Musso, 1930; Ishii and Yanagisawa, 1954; Yanagisawa and Ishii, 1954; Yanagisawa, 1955), *Rhabditis terricola* (Chitwood, 1930); *Trichosomoides craussicauda* (Thomas, 1924); *Diplogaster longicauda* (Ziegler, 1895); *Aspiculuris tetraptera* (Anya, 1964b); *Trichuris leporis*, *T. ovis*, *T. myocastris* and *Capillaria longicollis* (Monné and Honig, 1954); *Toxocara leonina*, *T. cati*, *Ascaridia galli*, *Contracaecum* sp. and *Paraspidodera* sp. (Monné, 1962); *Dictyocaulus viviparus*, *D. filaria* and *Metastrongylus elongatus* (Monné, 1959); and *Porrocaecum angusticolle* (Kochhar, 1960). The descriptions of these various authors suggest an essential similarity in the main features of the process. Foor (1967) and Lee and Lestan (1971) have also studied the process in *Ascaris lumbricoides* and *Heterakis gallinarum* using the EM.

At the time of complete penetration through the oolema of the sperm of *A. lumbricoides*, a dense vitelline layer separates from the cytoplasm (Rogers, 1956; Foor, 1967), probably as a result of the shrinkage of the cytoplasm

(Lams, 1952), and the shape of the egg changes while the volume remains constant (Fairbairn, 1957). This vitelline layer which Fairbairn (1957) prefers to call a fertilization membrane (by analogy with sea urchins) thickens and becomes progressively denser as shell formation proceeds. The intervening layer between the egg cytoplasmic surface and the fertilization membrane becomes filled with a structureless, pellucid layer (Foor, 1967; Lee and Lestan, 1971). The cortical cytoplasmic matrix at this time is dense and contains many strands of rough endoplasmic reticulum. This marks the beginning of the formation of the middle chitinous layer. As there are no preformed chitin micelles in the oocyte, the synthesis of the chitin of this layer is presumed to take place from the glycogen reserves of the oocyte during this period (Fairbairn, 1957).

As an immediate response to the stimulus of sperm penetration, about one-half of the glycogen reserves of the egg is mobilized and converted into the *N*-acetylglucosamine units of chitin, utilizing, it is presumed, the rich pool of glutamine and acetate in the oocyte (Fairbairn, 1957). The elaboration of the chitinous layer is obviously associated with some degree of protein synthesis which becomes incorporated into the forming pellucid (chitinous) layer (Kaulenas and Fairbairn, 1968). The abundant rough endoplasmic reticulum of the egg cortex at this period is the most likely centre for such synthesis.

Yanagisawa (1955) and Anya (1964b) suggested that in *Ascaris lumbricoides* and *Aspiculuris tetraptera* respectively the protein moiety of the chitinous layer was derived from the pre-existent and proteinaceous hyaline granules of the oocyte. The definitive studies of the formation process in *A. lumbricoides* and *Heterakis gallinarum* have shown, however, that the hyaline granules do not contribute any material to the formation of the egg-shell (Foor, 1967; Lee and Lestan, 1971). The pellucid layer rapidly becomes dense, thickens and is quickly transformed into the chitinous layer. Anya (1964b) reported alkaline phosphatase activity in the region of the forming chitinous layer in *A. tetraptera*, although Lee and Lestan (1971) could not confirm this observation in *H. gallinarum*.

The uterine cells secrete strands of lipoprotein material which become deposited on the egg-surface to form the outermost lipoprotein layer (Anya, 1964b; Foor, 1967) at the same time as an inner ascaroside layer is being formed. The latter layer arises from the extrusion and subsequent coalescence of the ascaroside content of the refringent granules. These ascarosides account for 75% of the material of the ascaroside layer, the other 25% being protein (Foor, 1967; Jezyk and Fairbairn, 1967a). As the ascarosides exist in the refringent granules as esters (Jezyk and Fairbairn, 1967a; Tarr and Fairbairn, 1973a), these esters must first be converted into the free ascarosides before incorporation into the ascaroside layer. The conversion process must take place in the refringent granules before extrusion or in the forming layer immediately on extrusion.

The material of the refringent granules is normally seen under the EM as a dense core of particulate material embedded in a less dense but homogeneous matrix with the same consistency as the ascaroside layer

(Foor, 1967). The protein material of the ascaroside layer may be provided by the dense core material of the refringent granules, while the homogeneous matrix consists of the ascaroside esters (Foor, 1967).

Tarr and Fairbairn (1973c) showed that the timing of the conversion of esters to free ascarosides was subject to much variation in *Ascaris lumbricoides*. Nevertheless, ascaroside esters disappeared from fertilized eggs while these eggs were 3–50 mm from the oviduct, indicating the possibility that all ascaroside esters of the oocyte are converted into free ascarosides before being incorporated into the ascaroside layer (Tarr and Fairbairn, 1973c). Whether the variability in the rate of conversion of the esters into free ascarosides and subsequent incorporation into the ascaroside layer has a physiological basis is at present an open question, but Tarr and Fairbairn (1973c) found a correlation between the rate of conversion of the esters and the loss of permeability to glycerol by the fertilized eggs.

The formation of the lipoprotein layer had often been ascribed to secretion by cells of the upper uterus (Kuchenmeister, 1857; Christenson, 1950), although the first unequivocal evidence in support of this view was provided by Anya (1964b). He showed, using histochemical methods, rather high concentrations of RNA in the uterine cells immediately adjoining the spermatheca of *Aspiculuris tetraptera*, an observation which has now been confirmed by the ultrastructure of these cells as observed in *Ascaris lumbricoides*, (Foor, 1967). There is a proliferation of rough endoplasmic reticulum, golgi complexes and secretory vesicles in these cells. These were often attached to the cell surface in preparation for the extrusion of their contents, an observation which gives further support to the view of a uterine origin for this layer (Foor, 1967). Christenson (1950) suggests that these secretions lose water to the fertilized egg on adherence and in this manner will condense on the egg surface as the lipoprotein layer. Alternatively, he suggests, the condensation of the secretory material and its consequent consolidation on the egg surface may arise from a change in the pH of this region of the female tract which causes fibrillation of the protein mass. Anya (1964b) found a pH difference between the spermatheca and the upper uterus of *Aspiculuris tetraptera*, the region where such fibrillation and consolidation of the lipoprotein layer is presumed to occur in this nematode.

3. *The biological significance of the egg-shell*

The degree to which the nematode egg shell prevents or reduces the rate of passage of various substances into or out of the developing embryo is almost unique in the animal kingdom. Fat solvents cannot penetrate into the eggs of *Enterobius vermicularis* (Jacobs and Jones, 1939), while in *Ascaris lumbricoides* vital basic dyes will stain the shell proper, i.e. the lipoprotein layer and the chitinous layer, but will not penetrate into the perivitelline space, so long as the ascaroside layer has been formed and is intact (Wottge, 1937). This layer is therefore the most important permeability barrier in nematode eggs.

In *A. lumbricoides*, Passey and Fairbairn (1955, 1957) observed that decoated eggs will embryonate successfully and that the larvae remain

motile and active in 2N HCl, 2N HNO_3, 2N NaOH, 0·5 N NH_4OH, 3·3 N formaldehyde or 4N NaCl. These larvae die almost instantly, however, if released into any of these solutions by gentle application of pressure. Passey and Fairbairn (1955) also observed that the ascaroside layer will melt at 70°C and above. Under such circumstances, trehalose from the perivitelline space will leak into the incubating medium. Thus, this layer of the egg-shell not only prevents charged particles and other water-soluble compounds from getting into the egg, but ensures that substances already in the perivitelline fluid do not leak out from the embryo (Passey and Fairbairn, 1957).

The extreme impermeability of the nematode egg to water, as liquid, is indicated by many observations on different nematodes from varied ecological situations. Dropkin *et al.* (1958) showed that eggs of *Meloidogyne arenaria* developed normally in both hypotonic and hypertonic solutions. Similar observations have been made on *Ascaris lumbricoides*, *Trichostrongylus retortaeformis* and *Aspiculuris tetraptera* (Seamster, 1950; Wilson, 1958; Anya, 1966c). In none of these species was plasmolysis of the eggs observed so long as the ascaroside layer was intact.

The embryonation of eggs of nematodes is an aerobic process (Rogers, 1962; Anya 1966d; Fairbairn, 1970). Obviously, both oxygen and carbon dioxide will pass through the egg-shell of nematodes freely, as does water vapour (Seamster, 1950; Jaskowski, 1952). Indeed, it is even possible that the association of protein and chitin in the egg-shell of nematodes may, as in the cuticle of insects, ensure the unidirectional penetration of water molecules into the developing embryo (Beament, 1964). Thus, the various layers of the egg-shell prevent the entry into the environment of the developing embryo of charged particles, water and water-soluble organic compounds during embryogenesis, a period in which the precise control of environmental conditions is an essential requirement for critical morphogenetic processes (Needham, 1954).

It is of interest here to recall that the characteristic ascarosides exist as esters in the oocyte but are converted into free ascarosides before deposition in the ascaroside layer of the egg-shell. Tarr and Fairbairn (1973c) have drawn attention to the fact that while ascarosides melt at 70–80°C, the esters melt at about 40°C. Thus, the simple expedient of de-esterification of the ascarosides prior to deposition confers thermal and structural stability to the ascaroside layer, a development of obvious survival significance to the developing embryo.

It should be noted also that the acetyl esters of the ascarosides predominate in the oocyte (Tarr and Fairbairn, 1973c). As the formation of the chitinous layer, with its consequent need for *N*-acetyl groups, and the extrusion of the esterified ascaroside refringent granules (to form the ascaroside layer) are simultaneous events (Foor, 1967; Lee and Lestan, 1971), free acetate radicals are released into the egg cytoplasm at the precise moment when there is a great metabolic need for their use in the synthesis of chitin. Moreover, the active glutamic dehydrogenese (Langer, 1972) of nematode tissues, by facilitating the biosynthesis of glutamic acid (and glutamine) from NH_4^+, achieves the detoxication of this metabolic waste product.

The obvious significance of these related events lies in the conservation of essential chemical groups with consequent economy in the energy resources of the developing embryo, at a time when no extraneous source is available to it. Thus, the formation of the egg-shell of nematodes, by providing for the developing embryo a self-contained and precisely regulated environment, releases the developing egg from dependence on the adult female. In the process, the stage is set for the ecological adaptability and radiation into varied habitats which underlines the success of nematodes.

VII. Development

A. Embryonation

1. *Embryogenesis*

The early studies of development in nematodes have been reviewed by Nigon (1965) and more recently by Hope (1974). These early studies utilized mainly the large ascarids *Ascaris lumbricoides* and *Parascaris equorum* and served to draw attention to some unique features of nematode development, such as its very determinate nature, the phenomenon of chromatin diminution and the fact that nematodes show cell constancy, i.e. the number of cells in the minute larva of many nematodes is approximately the same as in the adult organism (Chitwood and Chitwood, 1950). This situation arises from the fact that the somatic cells show no further mitotic divisions once the differentiation of the somatic tissues is complete, except for the genital primordium (Bird, 1971) and the gut (Nonnenmacher-Godet and Dougherty, 1964). Moreover, during the development of nematodes, there are four moults during each of which the cuticular covering of the larva is cast off.

Interest in the determinate nature of development had led to the very detailed studies of cell lineage in *P. equorum* (Fig. 8) (Boveri, 1899). In this ascarid, the first cleavage division takes place approximately 38 h after fertilization (in the uterus), by which time the eggs have traversed the length of the uterus and are outside the female (Bachofer, 1957). This first division gives rise to two more or less equal blastomeres, generally called the S_1 "Somazellen" and P_1 "Propagationzellen" cells after the original nomenclature of Boveri (1899).

The time interval between fertilization and the first cleavage division varies in many nematodes; about 4–6 h in *Ditylenchus dipsaci* (Yuksel, 1960), 10–15 h in *Meloidogyne naasi* (Siddiqui and Taylor, 1970), and 26–35 h in *Deontostoma californicum* (Hope, 1974). The second cleavage gives rise to four cells arranged in the so-called T-shape, which in *Parascaris equorum* arises from the fact that the S_1 cell divides longitudinally while the P_1 cell divides transversely (Boveri, 1899). However, in *Ditylenchus destructor*, mitosis is delayed in one of the two cells until after the other dividing cell has divided twice, thus giving rise to a five-celled stage (Anderson and Darling, 1964); and in *Meloidogyne naasi* until it has divided once, thus forming a three-celled embryo at this stage (Fig. 9(3)).

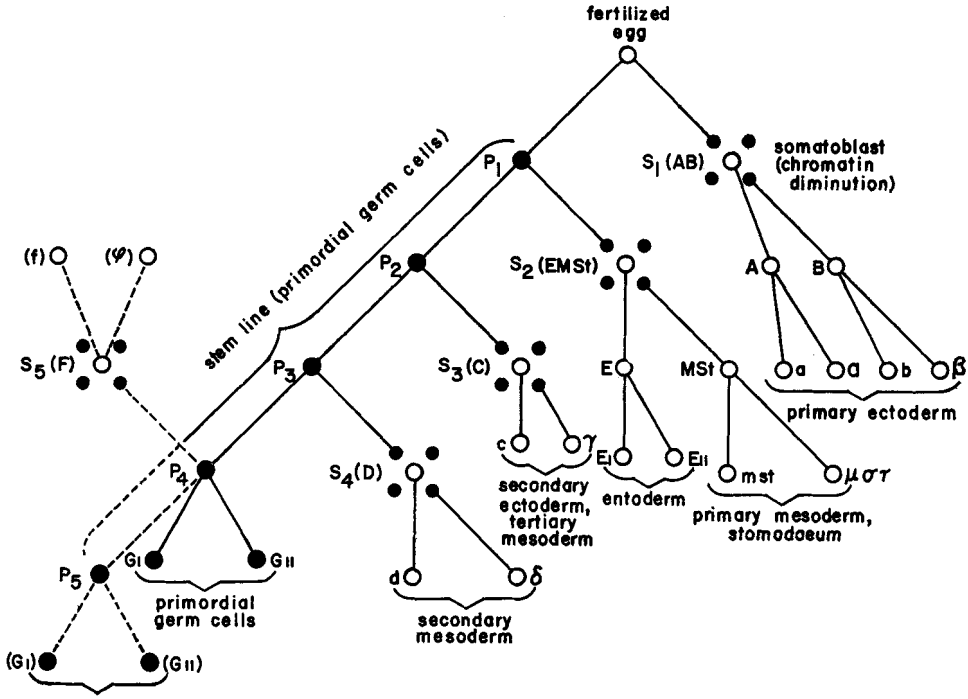
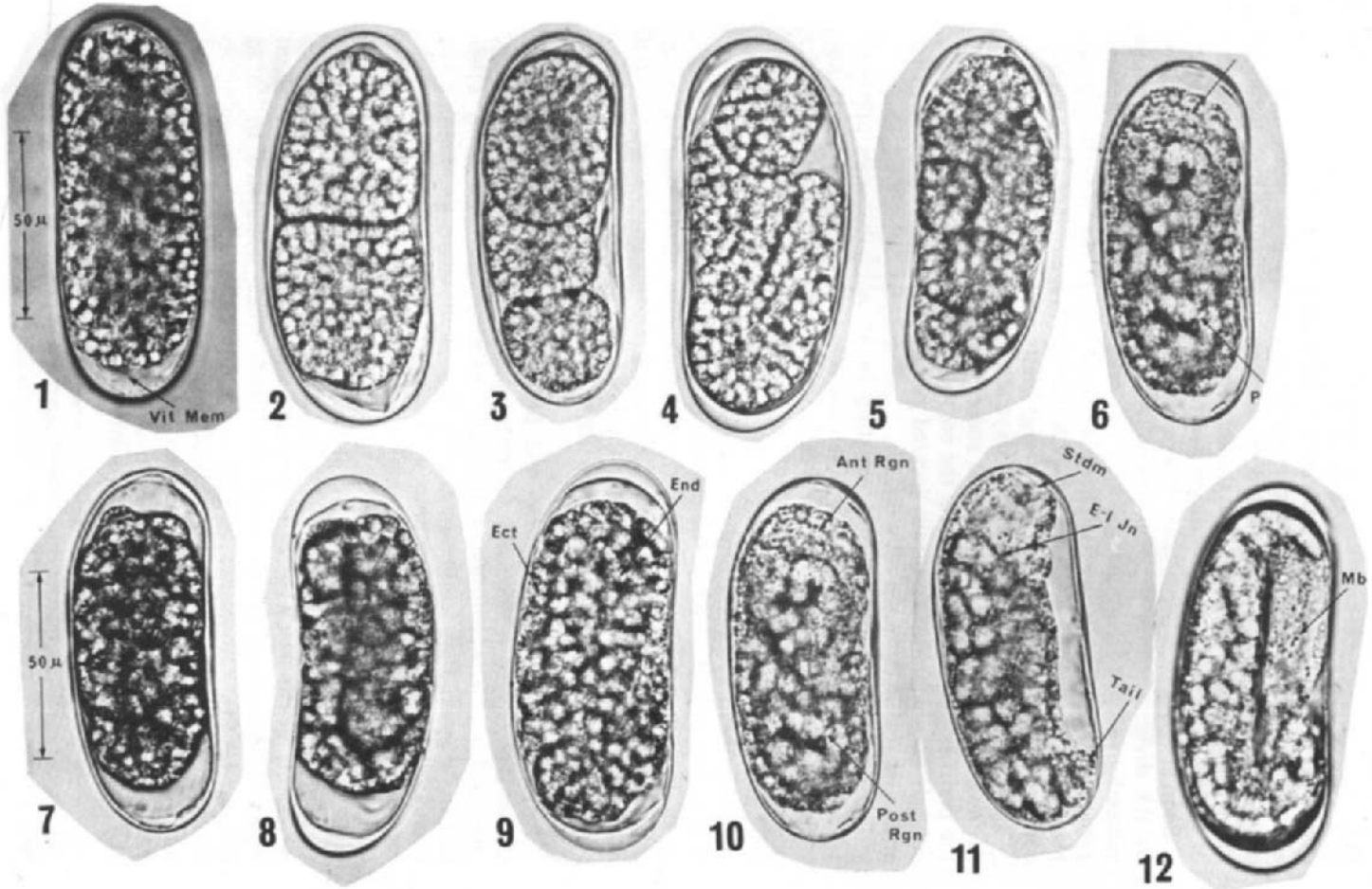


FIG. 8. Diagrammatic representation of cell lineage in *Parascaris equorum* (from Hope, 1974).

Further divisions of both the S_1 cell (which will give rise to much of the somatic tissues) and the P_1 cell (from which the gonads are derived) gives rise to a coeloblastula. Gastrulation involves then the invagination of the endoderm and the stomodaeum and converts the flattened embryo into a cylindrical body. The lineage of the cells from the first cleavage division until complete differentiation of the larval tissues as observed in *Parascaris equorum* is summarized in Fig. 8, while the general features of nematode embryogenesis based on the observations on *P. equorum* are summarized in Fig. 10.

Embryogenesis in *Meloidogyne naasi* differs in some slight respects from the situation described in *P. equorum* (Siddiqui and Taylor, 1970). In *M. naasi*, the first transversely oriented cleavage division takes place 10–15 h after fertilization and the second 12–15 h later. In the second division only the S_1 cell divides into a terminal A cell and a central B cell, as the plane of the second division is also transverse. Three cells strung out in linear fashion is the result (Fig. 9(3)); the two most anterior cells which arise become

FIG. 9. Photomicrographs of stages in embryogenesis in *Meloidogyne naasi:* (1) One-celled stage, (2) two-celled stage, (3) three-celled stage, (4) displacement of anterior cells, (5) third cleavage division, (7) blastula, (9) tissue differentiation, (11) tadpole stage, (12) fully formed 1st stage larva. (From Siddiqui and Taylor, 1970.)


50 µ
Vit Mem
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E-I Jn
Tail
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12

displaced, 8–11 h later the third division is initiated with its plane transverse and slightly oblique. This cleavage plane divides the P_1 blastomere into two cells, S_2 and P_2, while the A and B blastomeres are divided longitudinally into a group of four cells located at the anterior pole (Fig. 9(5)). The formation of this six-celled stage is generally complete 10–16 h after initiation.

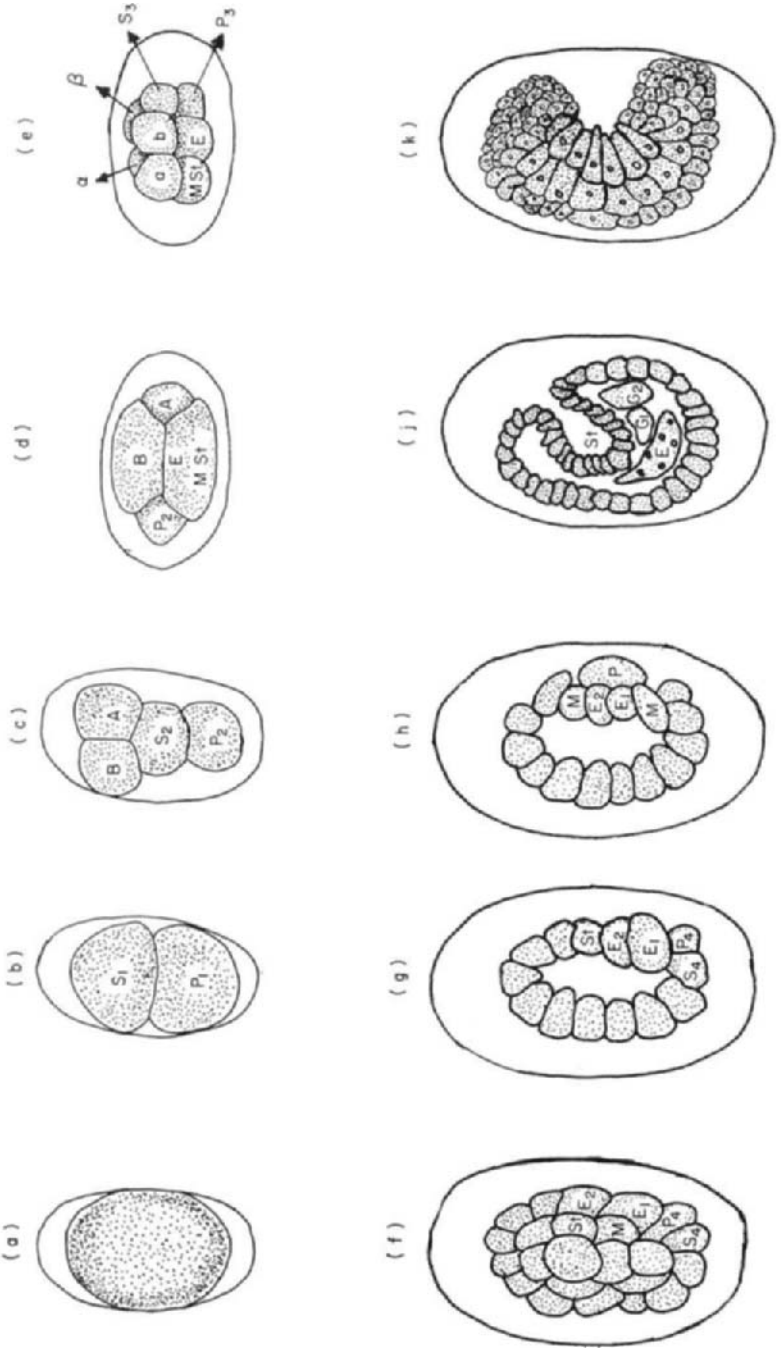
Soon afterwards, however, the embryo is converted into a 7-celled structure by the division of the P_2 blastomere. After this stage, there is a rapid succession of divisions during which the cells become very granular and apparently rotate on their axis. These processes lead to the formation of the blastula stage, which is complete 60–72 h after the first cleavage. This usually consists of 16–20 cells without an obvious blastocoele (Fig. 9(7)).

The gastrula which is formed 3½–4 days after the first cleavage division in *M. naasi* is characterized by a rapid succession of divisions particularly of the peripheral cells. This converts the embryo into a morula of large, dark, coarsely granulated and centrally located globular cells, enclosed by smaller and more lightly coloured cells.

The initiation of cellular differentiation in *M. naasi* becomes apparent 15–25 h after gastrulation occurs, when the ectoderm is clearly discernible, enclosing the endoderm. The differentiation of the stomodaeum and the oesophagus occurs 30–60 h subsequent to the initiation of cellular specialization, and is indicated first by cells of the anterior region of the now tadpole-shaped embryo becoming hyaline and developing a slight indentation (Fig. 9(11)). The posterior end of the embryo is generally slightly reflexed at this stage. First movements, indicating the differentiation of the muscles, occur in the embryo 25–60 h after the tadpole stage. In this interval the embryo becomes longer and narrower; first the head and later the whole embryo shows spasmodic movements, and this marks the end of embryogenesis.

Anya (1966d) and Nikandrow and Blake (1972), among others, have shown that oxygen is necessary for embryonation in *Aspiculuris tetraptera*, *Aphelenchoides composticola*, *Ditylenchus myceliophagus* and many other nematodes, and that temperature is an important factor determining the normality or otherwise of the process of development as shown by the subsequent history of the developed embryo. If the physiological conditions for normal embryogenesis are tampered with, it is reasonable to expect that the process of hatching or moulting of the larva, for example, might not proceed to completion, (Kisiel *et al.*, 1972). In *Aspiculuris tetraptera*, eggs which developed at 20–30°C hatched normally, but those which developed at higher temperatures (37°C) could not hatch when stimulated to do so (Anya, 1966d). Obviously, various temperature-dependent physiological changes must form an essential part of normal development in nematodes, and Wallace (1971) has demonstrated that two related but independent processes involved in embryonation, namely embryogenesis and eclosion, showed different temperature requirements. While the temperature for

FIG. 10. Diagrammatic representation of embryogenesis in *Parascaris equorum* (based on Bird, 1971).


(a)
(b)
S1
P1
(c)
A
B
S2
P2
(d)
A
B
E
MS1
P2
(e)
S3
P3
β
α
D
E
MS1
(f)
S1
E2
M
E1
P4
S4
(g)
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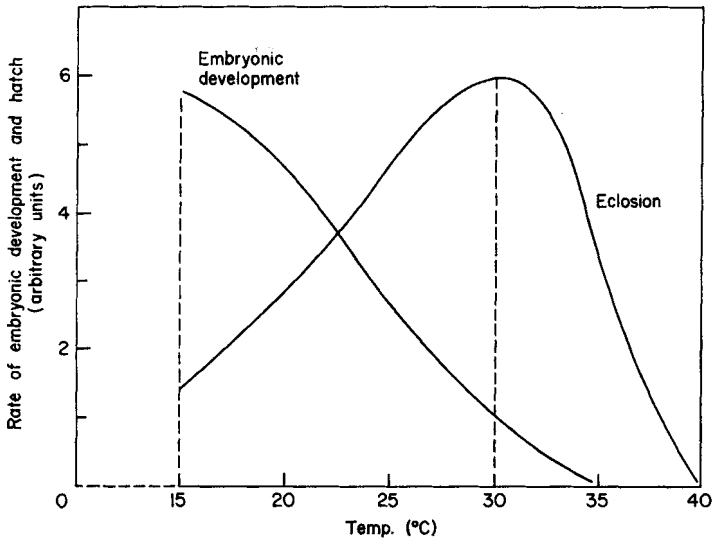


FIG. 11. Diagram summarizing the relationship between temperature and (i) embryonic development and (ii) eclosion in *Meloidogyne javanica* (from Wallace, 1971).

optimum embryonic development was about 15°C, that for eclosion was around 30°C (Fig. 11).

2. *Physiological and biochemical changes during embryogenesis*

(a) *Energy metabolism.* Early histochemical studies indicated that the mature oocyte of *Ascaris* prior to fertilization contained high concentrations of neutral lipids and some glycogen (Fauré-Fremiet, 1913; Hirsch and Bretschneider, 1937; Lee, 1960; Anya, 1964a). The careful studies of Passey and Fairbairn (1955, 1957) established the main features of the relationship between these substrates of energy metabolism and the oxygen requirements of developing *Ascaris* eggs and larvae during embryonation. During embryogenesis, the carbohydrate reserves of *Ascaris* eggs, trehalose and glucose, decrease by nearly 50% in the first 10 days. Subsequently, they are resynthesized so that by day 24 the level has gone up again to its original value; it remains more or less constant thereafter for up to 120 days (Fairbairn, 1957).

As for the triglycerides, no appreciable utilization of these substrates is obvious for the first 5 days of embryogenesis, after which there appears to be a heavy call on them until about day 20. The level also remains more or less constant thereafter for up to 120 days. Day 20 generally marks the completion of development up to the first stage larva in *Ascaris* (Passey and Fairbairn, 1957).

During the period of embryogenesis, the oxygen consumption of the developing embryo varies from an initial Q_{O_2} of 0·38 for 0-day unembryonated eggs to a minimum value of 0·24 48 h later. It then increases, reaching its highest value of 0·8 by day 10 (Passey and Fairbairn, 1955). The relationship is summarized in Fig. 12.

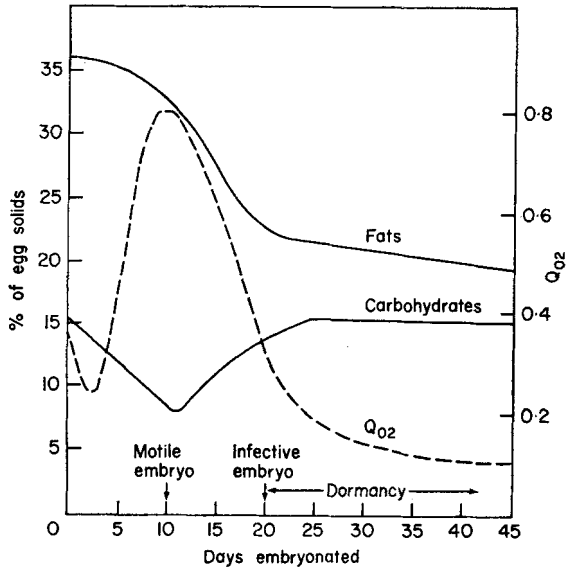


FIG. 12. Changes in Q_{O_2} and energy-yielding substrates during embryogenesis of *Ascaris lumbricoides* (from Passey and Fairbairn, 1957).

Protein does not appear to be utilized as an energy source under normal circumstances, since the value of non-protein nitrogen does not increase during the whole period of embryogenesis (Fairbairn, 1970). As the eggs of *Ascaris* like those of other nematodes are cleidoic, the non-accumulation of nitrogenous excretory products would naturally offer biological advantages of obvious significance to embryonic survival.

In *Ascaris*, Passey and Fairbairn (1955, 1957) found that respiration in developing eggs was strongly inhibited by cyanide, azide and carbon monoxide; moreover, respiration in these eggs appeared to be independent of P_{O_2}, an observation which they took to indicate the presence of a cytochrome *c*–cytochrome oxidase electron transport system in embryonating eggs. However, Costello *et al.* (1963) found that preparations of unembryonated eggs of *Ascaris* were capable of oxidizing both malate and succinate with consequent reduction of cytochrome *c*; these workers could not, however, demonstrate cytochrome oxidase activity in their preparations. This was in obvious contradistinction to their observations on preparations of embryonated eggs which also oxidize malate and succinate but possess, additionally, high cytochrome oxidase activity. They had therefore suggested that the terminal electron transport system in unembryonated eggs may be mediated through a flavin oxidase.

According to Oya *et al.* (1963) there is a 15 to 20-fold increase in cytochrome oxidase activity during embryogenesis in this ascarid, and they showed that the first traces of cytochrome oxidase activity in their preparations of developing eggs usually coincided with the period of increased respiration

by these embryos (32–64 cell stage). In other words, the onset of cellular differentiation and tissue organization was indicated by high levels of aerobiosis. However, the fact that the level of cytochrome oxidase activity is still high even in the vermiform larva in which embryogenesis is complete, has been taken to indicate that excess enzyme synthesis takes place during embryogenesis—beyond the requirements of the particular stage of development—in preparation for the next stage of development whose metabolism is also aerobic (Fairbairn, 1970).

Costello (1964) identified FAD in the unembryonated eggs of *Ascaris*, while Costello and Smith (1964) identified catalase and studied variations in the concentration of this enzyme during embryogenesis of *Ascaris*. They implied that the identification of both FAD and catalase in their preparations supports their proposition that a flavin oxidase is the terminal electron acceptor in unembryonated eggs of *Ascaris*. However, as Fairbairn (1970) has pointed out, such a proposition is negated by the observed sensitivity of the respiratory process of unembryonated eggs to cyanide, azide and carbon monoxide. The fact that the respiratory exchanges in these preparations is independent of Po_2 is also against this interpretation, since flavin electron acceptors would normally react in contrast to these observations (Bueding, 1962, 1963).

All enzymes of the TCA cycle are present in developing *Ascaris* eggs (Ward, 1970, quoted by Fairbairn, 1970). Barrett *et al.* (1970) have identified both isocitrate lyase and malate synthetase during embryogenesis in *A. lumbricoides* eggs. These observations suggest the presence of a functional glyoxylate cycle in these developing eggs (Kornberg and Krebs, 1957; Kornberg and Elsdon, 1961). Further, Ward and Fairbairn (1970) have identified the presence of all five enzymes involved in the β-oxidation of fatty acids during the embryogenesis of *Ascaris*. The appearance and concentration of these enzymes during embryogenesis closely parallelled the pattern of lipid utilization in the developing eggs. Thus, the possible enzymatic mechanism for the conversion of fatty acids to glycogen in *Ascaris* eggs, as suggested originally on the basis of gross biochemical analyses by Passey and Fairbairn (1957), has now been identified. That this pathway is functional during embryogenesis in *Ascaris* has been indicated by the work of Saz and Lescure (1969), who identified both PEP carboxykinase and glucose-1,6-diphosphatase, and the Embden-Meyerhof enzymes in embryonating *Ascaris* eggs. These enzymes are normally necessary for the conversion of C_4 carboxylic acids to glycogen (Fairbairn, 1970).

(b) *Nucleic acid metabolism.* Pasteels (1948) and Panijel and Pasteels (1951), in histochemical studies of fertilization, and Foor (1967) in an ultrastructural study of the same process, have shown that an important post-fertilization change in *Ascaris* eggs is the sudden synthesis of RNA. Kaulenas and Fairbairn (1966) showed that this newly synthesized RNA consisted mostly of polysomes unusually stable to hydrolysis by RNase. As the female pronucleus is still involved post-fertilization in its reduction divisions (see Section VI), the synthesis of this ribosomal RNA would appear to be a function of the male genome.

Kaulenas and Fairbairn (1968) found that the rate of synthesis of ribosomal RNA was greatest in those eggs of *Ascaris* located in the proximal uterus. Eggs showed a progressive decrease in the rate of RNA synthesis the nearer they were to the distal uterus. However, the relative concentration of RNA was highest in absolute terms in the distal uterus, their values being 60 mg RNA/million proximal uterine eggs and 93 mg RNA/million distal uterine eggs. Moreover, Kaulenas and Fairbairn's (1968) data indicated that while small quantities of mRNA may also be synthesized in the proximal uterus, mRNA was normally synthesized and accumulated in the egg cytoplasm as these eggs pass through the uterus. In view of the known activities of the female pronucleus at this stage (Section VI), both the rRNA and mRNA would appear to be products of the male genome only. During these early stages of embryogenesis the main protein synthetic requirements of the developing egg would be connected with egg-shell formation and cleavage. It would thus appear that while pre-existing yolk formed by the female genome may be incorporated into the egg-shell, the regulation of these specific and important developmental processes (namely egg-shell formation and cleavage) is the primary responsibility of the male genome.

It is well known that during development, somatic cells of amphimictic nematodes like *Ascaris* undergo chromatin diminution (Meyer, 1895). Tobler *et al.* (1972) have shown that the eliminated DNA does not differ in its physical characteristics (e.g. buoyant density) from nuclear, somatic or germ-line DNA, and that 27% of germ-line DNA is eliminated in the process. One half of this eliminated DNA, they showed, consists of unique nucleotide sequences. From all the evidence, they therefore concluded that there was a qualitative difference between the DNA of the somatic cells and the germ-line cells of *Ascaris*, and that an enormous amount of genetic information is lost in chromatin diminution. It has been suggested also that the phenomenon may be related, particularly, to the highly determinate nature of nematode development in a manner we do not yet understand.

There is now evidence that during embryogenesis, there may be a programmed and sequential activity of the genes in *Ascaris*. Ward and Fairbairn (1972) showed that a chitinase which was absent in 0-day eggs appeared by day 10 and reached the highest level of activity by day 20—the end of embryonic development. On the other hand, Cain and Fairbairn (1971) found that a protocollagen proline hydroxylase appeared first in the late blastula–early gastrula (day 4) of *Ascaris* and reached its maximum level by day 12. The PPH of embryonating eggs is an isoenzyme of the PPH found in adult muscles (Cain and Fairbairn, 1971).

B. POST-EMBRYONIC DEVELOPMENT

Post-embryonic development of nematodes generally involves four moults. The intermoult periods of the life cycle represent the larval stages. In some nematodes the first moult may take place while the larva is still enclosed within the egg-shell. The main morphogenetic events between the

completion of embryogenesis in nematodes and development into adults are growth, moulting and gonadogenesis. While physiological interrelationships between these processes may be presumed, there has until fairly recently been no sustained interest in these problems except the morphological and ecological studies of early workers, much of which has been summarized by Michel (1969).

Davey (1965) and Kan and Davey (1968a,b) studied the histochemistry of cuticle deposition in *Phocanema decipiens*. They showed that cytoplasmic and nucleolar RNA increases in both the hypodermis and the muscle cells during cuticle deposition, indicating that proteins synthesized in these regions are utilized in new cuticle formation. Hirschmann and Triantaphyllou (1967) found that in *Helicotylenchus dihystera* nuclear divisions in the reproductive system take place only during the periods of moulting, whereas it is only during the intermoult periods (the larval stages) that growth (by cell enlargement) takes place; this suggests some extant relationship in (*H. dihystera*) between these processes. Yuen (1965) had also observed that in *H. vulgaris* the gonad increases in size during each larval stage although no further mitotic divisions may take place. In other nematodes, however, there is continuous and gradual increase in the number and size of cells in the developing gonads throughout the intermoult periods and during moults, for example in *Ditylenchus triformis* (Hirschmann, 1962), *Ditylenchus destructor* (Anderson and Darling, 1964), *Seinura celeris* and *S. oxura* (Hechler and Taylor, 1966).

As all these morphogenetic processes involve protein synthesis, it has been assumed that experimental manipulation of the nucleic acid and/or protein synthetic mechanisms of developing nematode larvae may afford some insight into the interrelationships of morphogenetic processes (Pasternak and Samoiloff, 1970; Kisiel *et al.*, 1972). Westgarth-Taylor and Pasternak (1973) treated developing larvae of *Panagrellus silusiae* with hydroxyurea, puromycin, actidione and actinomycin-D; they observed particularly the effects of these compounds on gonad formation. By varying the dosages of hydroxyurea to which these organisms were exposed, and the time, they observed that this chemical did not alter the moulting cycles, but inhibited growth and gonadogenesis to variable degrees. Other experiments indicated that hydroxyurea inhibited RNA and protein synthesis and the last rounds of DNA replication; this latter effect was reversible. Certain abnormalities of the cuticle were also observed. Nitrosoguanidine, actinomycin-D and actidione also decreased the rate of protein synthesis, but puromycin was without effect. Westgarth-Taylor and Pasternak (1973) suggested, therefore, that growth and moulting are intimately linked phenomena and that although the physiological link between these processes and gonadogenesis could be disengaged, normal growth was essential for normal gonadogenesis. Vanfleteren and Roets (1972) studied the effect of each of ten anthelminthics on population growth of *Caenorhabditis briggsae* and *Turbatrix aceti*. They found that only those drugs (namely dithiazine iodide, pyrvinium pamoate and thiabendazole) which interfere with nutrition, metabolism or reproduction had any detectable influence on population growth. As mutant strains were developed in their

cultures, it seems likely that these drugs were more effective during the period of post-embryonic development when the epigenetic adaptability of these larvae was as its highest level (Waddington, 1940).

During post-embryonic development, many feeding nematode larvae build up extensive reserves of neutral lipids which may serve as energy substrates during the non-feeding period which precedes infection in parasitic nematodes (Croll, 1972a). During the non-feeding periods, these reserves are progressively depleted (Rogers, 1962; Barrett, 1969). In *Ascaris suum*, Magat *et al.* (1972) found that total nitrogen, carbohydrates and lipids decreased progressively from unembryonated eggs to embryonated eggs to released larvae. The suggestion has therefore often been made of a relationship between the lipid stores of a larval nematode and the larva's infective potential (Wilson, 1965; Barrett, 1969) in the parasitic species. In *Strongyloides ratti*, Barrett (1969) found that the non-feeding infective larva respires actively, catabolizing the endogenous reserves of free fatty acids and triglycerides in the process. Croll (1972b) has shown that much of the lipid is utilized in the locomotory activity associated with the larva's search for a host. Respiration in these larvae is independent of the Po_2, suggesting the presence of a cytochrome electron transport system (Barrett, 1969; Korting and Fairbairn, 1971). It is also pertinent to note that all enzymes of the β-oxidation pathway as well as those of the TCA cycle have been identified in the larvae of *S. ratti* (Korting and Fairbairn, 1971); the latter enzymes are also present in larvae of *Haemonchus contortus* (Ward and Schofield, 1967). *Ancylostoma tubaeforme* larvae can, however, survive anaerobiosis (Croll, 1972b). Although further work on the energy metabolism of developing nematode larvae is necessary, it seems possible that epigenetic adaptations of this aspect of their physiology may be the basis of the ecological success of nematodes (Chow and Pasternak, 1969).

C. HATCHING, MOULTING AND EXSHEATHMENT

Nematode larvae have to hatch to free themselves from the egg-shell, and hatched larvae often have to moult; during the latter process the old cuticle is cast off and a new one to replace it is formed. In those nematodes in which the old cuticle is retained for some time as a protective sheath for the larva, this sheath must at some stage be removed. This is referred to as *exsheathment*. In the parasitic nematodes, these processes are very important events in the life cycle and often determine the success of the parasite as an infective agent (Rogers, 1962). Consequently, the study of these processes has attracted much attention (Rogers, 1958, 1970; Sommerville, 1960; Bird, 1966; Davey, 1965; Davey and Kan, 1968; Anya, 1966c; Ozerol and Silverman, 1969, 1972a,b). The main physiological features of these processes have been adequately reviewed by Rogers and Sommerville (1963, 1968).

Rogers (1960) postulated that while a number of physical and chemical factors from the host serve as a trigger mechanism to stimulate developmental changes in the larva, culminating in its release from the egg-shell or the sheath, the enzymatic digestion of the egg-shell or the sheath is a function

of the larva itself. In the hatching of eggs of *Ascaris*, he showed that a chitinase, a lipase and a protease were released by the larvae on stimulation. Ward and Fairbairn (1972) corroborated this finding by identifying a chitinase which appeared by day 10 in the embryonating eggs of *Ascaris*, reaching the highest levels of activity by day 20 when the first stage larva is fully formed.

Rogers (1963, 1965) also produced evidence which suggested that leucine aminopeptidase (LAP) was the enzyme released by the ensheathed larva on stimulation and served to remove the protective sheath in *Haemonchus contortus* and related nematodes. Ozerol and Silverman (1969, 1972a,b) questioned the identification of LAP as the exsheathing factor in exsheathing fluid of infective larvae. Both Rogers (1970) and Ozerol and Silverman (1972a,b) have argued strongly for and against LAP as the responsible exsheathing enzyme. It seems likely, however, that the enzyme involved is a hydrolytic protease very similar in properties to LAP. It is possible that the enzyme acts on the associated mucoproteins of the gel-like matrix surrounding the collagen fibrils of the cuticle (Anya, 1966b) rather than on the collagen itself. Some of the observations of both laboratories may be reconcilable on this basis.

Rogers (1968) adduced evidence for neuroendocrine involvement in the control of these processes in nematodes. Samoiloff (1973), utilizing microbeam laser surgery, demonstrated the plausibility of the idea. Bargiel *et al.* (1970) found adrenaline and noradrenaline in adult *Ascaris suum*, while Anya (1973b) has identified 5-HT in adult *Aspiculuris tetraptera*. Rogers and Head (1972) could not identify 5-HT in infective juveniles of *Haemonchus contortus*, but noradrenaline and adrenaline were detected. When the exsheathing stimulus was applied to these larvae, the noradrenaline level increased two to nine times the values in unstimulated larvae. Rogers and Head (1972) suggested, therefore, that this amine may serve to activate the cyclic-AMP system on stimulation; this will mobilize the energy resources of the developing larva in preparation for the interrelated physiological processes involved in development. Further experimental work is obviously called for to elucidate the role of these amines in the reproductive and developmental physiology of nematodes.

VIII. Sex Differentiation

A. Cytogenetic mechanisms

In most bisexual organisms, the genetic mechanisms underlying sex determination and differentiation are well known. The genetic material of the zygote is assumed to possess two sets of genes, each of which promotes the differentiation of the developing organism either into the male or the female. Under most conditions, the relative balance of their effects is stable, thus ensuring that the particular sexual disposition of the developing organism is maintained under changing environmental situations. These sex genes may be distributed between the autosomes or, as in most organisms, concentrated in the sex chromosomes.

Whatever the pattern of distribution of the sex genes, the chromosomal basis of sex determination may be one of the following general mechanisms: XX♀-XY♂, XX♀-XO♂, ZW♀-ZZ♂, NN♀-N♂ or one of the specialized mechanisms: X♀-A♂, X♀-Y♂ and A♀-Y♂ (Triantaphyllou, 1973). The operation of these genetic mechanisms usually ensures a 1 : 1 sex ratio in the population.

It is generally presumed that nematodes being bisexual in most cases will fall into one or other of these general patterns, especially as the sex ratio of most amphimictic species is approximately 1 : 1. However, the paucity of cytological investigations covering all major groups of the Nematoda has precluded far-reaching generalizations. Nevertheless, sex determination in most nematodes so far studied appears to be of the XX♀-XO♂ type, for example in *Seinura tenuicaudata* (Hechler, 1963), *Strongyloides ratti* and *S. papillosus* (Nigon and Roman, 1952; Chang and Graham, 1957). It should be pointed out, however, that since no definite sex chromosomes have yet been identified, the XX♀-XY♂ system cannot be ruled out in some cases (Triantaphyllou, 1971a). In some parthenogenetic species, a few males may develop alongside the predominating females. The genetic basis of sex determination in such forms is not well understood, although environmental factors such as pH are important (Premvati, 1958). Little (1962) found, however, that in *Strongyloides fuellerborni* parasitic females produced two types of eggs, one of which developed into free-living males while the other developed into free-living females or inhibited larvae.

B. HOST INFLUENCE ON SEX DIFFERENTIATION

In parasitic nematodes, the host provides the definitive environment of the parasite. Perhaps the single most important attribute of this environment must be as the source of food for growth and development. The earliest indication that the amount of food available to a developing nematode parasite may influence the direction of sex differentiation in these organisms came from observations on mermithids. Cobb *et al.* (1927) and Caullery and Comas (1928) had drawn attention to the fact that the sex ratios of these nematodes varied with the degree of infestation of the host. Cobb and his co-workers observed that when only a few mermithid nematodes were recovered from their insect hosts, these were generally females, while in moderate infestations male and female nematodes were found in approximately equal numbers. In very heavy infestations, however, the sex ratio shifted in favour of the males. Christie (1929) confirmed these observations by demonstrating that in controlled experiments in which 4–5 eggs of *Mermis subnigrescens* were fed to the insect host, 92% of the nematodes recovered were females. When 20–30 eggs were fed to each insect, however, 86% of the nematodes recovered were males. These observations suggested that the nutritional status of the host's internal environment influenced sex differentiation in *M. subnigrescens*. It was suggested, therefore, that crowded conditions by creating a shortage of space and food alter the physiology and biochemistry of the insect host which affects the sex differentiation of the nematodes. Further, the

concentration of excreted or secreted substances from the nematode in the restricted space may also be important (Triantaphyllou, 1971a).

Observations on some plant parasitic nematodes have generally lent support to this view. Ellenby (1954) observed that in *Heterodera rostochiensis* more males developed on lateral roots (where presumably less food was available to developing larvae) than on primary roots on the host potato plant. Although he suggested that a differential death rate for males and females could explain his data, den Ouden (1960) showed that when potato seedlings were inoculated each with a single larval *H. rostochiensis*, absolutely more females developed than males, thus confirming that the nutritional status of the environment rather than a differential death rate accounted for the sex ratio observed. Consequently, Ross and Trudgill (1969) suggested that light infections by inducing the formation of large groups of giant cells around the attached larva (thus providing a good feeding site) ensured more food to the developing larva than is offered by the sparsely developed giant cells around the relatively crowded larvae in heavy infestations.

Although Triantaphyllou (1973) has questioned the assumption implicit in these observations, namely that environmental conditions in relation to food availability influence sex differentiation in *H. rostochiensis*, den Ouden's experiments as well as those of Trudgill (1967) clearly indicate that such an influence must exist. Nevertheless, recent reports have suggested that environmental conditions of nutritional stress often disrupt or retard the development of females more than of males, so that while the numbers of the latter tend to be constant, those of females vary widely in any given population; as a result a fluctuating death rate of the females will lead to differential sex ratios (Kerstan, 1969; Johnson and Viglierchio, 1969).

The most convincing evidence that the feeding status of some larval plant parasitic nematodes influences the direction of sex differentiation is provided by the observations of Triantaphyllou (1971b) on *Meloidodera floridensis*. In this parthenogenetic nematode, 96% of the larvae in natural populations infecting pine roots develop into females while 4% develop into males. But if larvae are kept in water in near starvation conditions, 50% of these larvae eventually develop into males. In *Meloidogyne incognita*, lowering the concentration of sucrose in the medium surrounding excised cucumber roots in which these nematodes are developing, increases the proportion of males (Triantaphyllou, 1960). Thus any condition, including the defoliation of the above-ground parts of a host plant, which diminishes the availability of organic compounds serving as food for development in *Meloidogyne* sp. may reduce the rate of development of the larvae as well as increase the proportion of males in the population (Triantaphyllou, 1973). This observation may well be general in the nematodes in which the nutritional status of the host environment influences sex differentiation.

Apart from these cases where the direct availability of food to the developing larva would appear to be the main vehicle of host influence on sex differentiation in nematodes, high infection densities of parasites and the presence of plant growth inhibitors have been suggested as other host-related factors which influence sex differentiation in favour of the male (Davide and

Triantaphyllou, 1967, 1968; Fassuliotis, 1970; Orion and Minz, 1971). In all these cases, however, it is still possible to ascribe the effects of these factors to their possible but indirect role in the reduction of available food in the parasites' environment. For example, both maleic hydrazide and morphactin, the plant growth regulators which were utilized in experiments, interfere with giant cell and syncytia formation in the host plants of the parasitic nematodes in which their influence was demonstrated. Thus, interference with host plant morphogenesis serves to reduce available food in the nematode's environment (Triantaphyllou, 1973). Indeed, Thornley and Hesling (1972) have put forward a mathematical model utilizing the data of Trudgill (1967) on sex ratio data on *Heterodera rostochiensis*. In this plant-parasitic nematode, as observed earlier, the environment apparently plays a decisive role in sex determination. However, the mechanism of environmental influence, especially nutrition, on sex is unknown, but this model implies that the mechanism of environmentally mediated sex determination might depend on the nutrient status of the host plant and the sensitivity of the target site within the larva on which the active molecules derived from the nutrients act.

C. OTHER EXTRINSIC FACTORS

In *Meloidodera incognita*, Ishibashi (1965) has shown that gamma irradiation affects the proportion of second stage larvae which develop into male nematodes. Irradiation changed the proportions of males from 1·5% to 18·3% and 27·2% at 10 krad and 20 krad respectively. The stage of development of the larvae when irradiation is carried out is important in determining the subsequent course of differentiation into male nematodes. When irradiation at a dosage of 10 krad and 20 krad was carried out on the young second stage larvae the male nematodes had two testes. This was interpreted as indicating that in the latter case, second stage larvae which would have developed into females but for the irradiation underwent sex reversal to males.

Temperature has also been shown to affect the course of sex differentiation in *Aphelenchus avenae*. Under normal circumstances, parthenogenetic strains of this nematode developed into females with an occasional male. Hansen *et al.* (1971) observed that embryonated eggs and freshly hatched larvae developed into females at 28°C and below and into males at temperatures above 31°C. At intermediate temperatures, variable sex ratios were obtained. These workers also observed that in the presence of CO_2, males developed even at temperatures lower than 28°C. The influence of temperature and CO_2 on sex differentiation was effective provided the developing larvae were exposed to these factors before the first moult. However, Evans and Fisher (1970) have shown that this temperature-dependent sex differentiation is not observed in all populations of this species, thus indicating the existence of phenotypic-based variation in respect of sex determination in this species. This conclusion is emphasized by Fisher (1972), who has found amphimictic populations of *A. avenae* in which males are found in large numbers.

Sex hormones are important in determining the direction of sex differentiation in many groups of animals, and nematodes may be expected to conform. Although few studies of this aspect of nematode reproduction have been carried out to date, it is conceivable that the sex hormones of hosts may influence sex differentiation in parasitic nematodes. In this respect the observation of Krusberg (1971), that certain nematodes are incapable of synthesizing some sterols, is suggestive of some possible lines for further exploration, because the chemical relationship of many animal hormones to sterols is well known. In summary, it is necessary to point out that environmental influence on sex differentiation seems particularly important in the parthenogenetic more than in the amphimictic species.

IX. Nutrition and Other Factors in Egg Production

Most nematodes, as far as is known, show iteroparity in the sense of Cole (1954). The total number of viable eggs produced by each individual nematode in a life span would depend on the rate of production of mature gametes, the speed and efficiency of fertilization, the rate of embryogenesis and the length of that period of the life span during which gamete production is physiologically possible.

The gamete-producing and phenological strategies adopted by various organisms are of adaptive significance (Calow, 1973), and considerations of the biotic potential of various nematode species underline this fact (Crofton, 1966). Consequently, the fecundity of a nematode would be regulated to ensure the maximum survival of the maximum number of progeny to their reproductive age, and the life history would be patterned, within the framework of ecological and phyletic constraints, to favour the survival of these progeny. Comparisons of the biotic potential of the animal parasitic *Haemonchus contortus* with that of *Neoaplectana glaseri* (Crofton, 1966) emphasizes this point.

The nutritional status of the host is known to affect the egg output of parasitic nematodes. In *Aphelenchus avenae*, Fisher (1969) showed that the rate of egg deposition was related to the rate of ingestion of food, so that when the nematodes were fasting no eggs or very few eggs were deposited. For example in *Heterodera avenae*, Banyer (1970) found that if egg-producing females were removed from the roots of host plants before the first moult of the unhatched larvae, these eggs did not develop further. In animal parasitic nematodes, faecal egg output is regulated by a "self-contained mechanism which has the effect of limiting the number of eggs produced by the whole population of worms to the same level, *irrespective of how many worms were present*" (Michel, 1969) (italics mine). From an ecological perspective, this is logical since there must be an upper limit to the level of available food in an environment, and the egg-production potential would be regulated to accord with this level (Andrewartha and Birch, 1960).

The physiological relationship between reproduction (and particularly egg production) and host nutritional status has been studied more often in plant parasitic forms (Webster, 1969). In the Heteroderidae, plant tissues

react to the presence of nematodes by the formation of giant cells and syncytia. These tissues are usually multinucleate with enlarged nuclei and a well developed endoplasmic reticulum indicating a very active metabolism. Consequently, it has been suggested that these cells are nutrient transfer cells with the nematode parasite as the nutrient sink (Jones and Northcote, 1972). In *Meloidogyne javanica* and *M. hapla*, Bird (1959) found that the demands for nutrients by these nematodes was highest when the nematodes were growing and producing large numbers of eggs. During such a period the uptake of tritium-labelled uridine increased while the DNA content of the giant cells decreased. This may indicate that the synthesis of mRNA (and protein) was going on during this period in response to the nematodes' requirements, (Nelmes *et al.*, 1973). That the pattern of availability of L-amino acids to the reproducing nematodes may be the basis of the nutritional influence on reproduction in these nematodes is indicated by experiments in which DL-methionine and DL-alanine decreased numbers of *Heterodera avenae* on oats, while DL-methionine and DL-alanine decreased numbers of *Ditylenchus dipsaci* also on oats (Prasad and Webster, 1967). Harness *et al.* (1971) had also observed that blood transfusion of anaemic hosts increased the egg output of *Haemonchus placei* and suggested that the boost to egg-production was the direct result of the improved nutritional status of the worm and host arising from transfusion.

Some inorganic ions are also known to influence the reproduction of nematodes. Sherwood (1968) found that in *Ditylenchus dipsaci* of lucerne, the level of nematode reproduction increased as the calcium concentration of the nutrient media increased. In further work, Sherwood and Huisingh (1970) found that nematode reproduction was closely correlated with the ratio of monovalent to divalent cations (Na^+ and K^+/Mg^{++} and Ca^{++}). The observed instances when temperature has had a detectable influence on aspects of nematode reproduction, it has also been suggested, arise from the assumed influence of temperature on the kinetics of transfer of essential nutrients to developing nematodes (Webster, 1969).

Evans (1970) had studied the effect of gamma irradiation on reproduction and development in *Heterodera rostochiensis*. The irradiation of second stage larvae had no apparent effect on the activity of the larvae but the F_1 generation which was derived from these irradiated larvae or from irradiated cysts produced fewer eggs per female: the number of embryonated eggs was significantly reduced. Obviously, irradiation may have tampered with gametogenesis in the adults derived from these irradiated larvae and cysts as well as with the embryogenesis of the F_1 larvae. Both factors will lead to the production of fewer eggs.

That gametogenesis, particularly oogenesis, is affected by irradiation was emphasized by experiments in which irradiated males or females of *H. rostochiensis* were mated with unirradiated individuals and the egg production of the resultant F_1 generation compared (Evans, 1970). These experiments showed that the number of embryonated eggs produced per female of the F_1 generation was significantly decreased at doses of 8 krad and above, if it was the female parent which had been irradiated; when the male parent

was irradiated, a significant reduction in numbers of eggs produced was observed at doses of 32 krad and above. In addition, Evans (1970) had found that much greater reductions in egg numbers as well as in the proportion of embryonated eggs were recorded in those experiments in which the female parent was irradiated (Fig. 13).

The availability of males *per se* and the frequency of copulation in amphimictic species may determine the level of egg production in these nematodes. In *Aphelenchus avenae*, Fisher (1972) established the pattern of egg-laying in an amphimictic species, (Fig. 14(a)). If the availability of males was restricted so that mating with reproductively mature adult females became delayed, the rate of egg-laying as well as total numbers of eggs produced was reduced (Fig. 14(b)). Fisher (1972) had suggested that so long as adequate food was available for the production of eggs, mature oocytes would be produced, but in the absence of sperm to fertilize them, some of these oocytes might regress. If males were subsequently introduced only a proportion of oocytes would remain for fertilization and deposition as eggs. Implicit in this suggestion is the assumption that each nematode species would have a finite number of eggs which, under normal circumstances, it might be expected to produce in a given life span. Available data on the average egg output of various species (Crofton, 1966) would seem to bear out this assumption: *Nippostrongylus brasiliensis* has an average daily egg output of 1000 (Phillipson, 1969), while Fisher (1972) found an average output of 134 per day in *Aphelenchus avenae*. Fisher (1972) also observed that in the presence of males the average daily egg output of parthenogenetic females of *A. avenae* was reduced (Fig. 14(c)). The physiological basis for the inhibitory

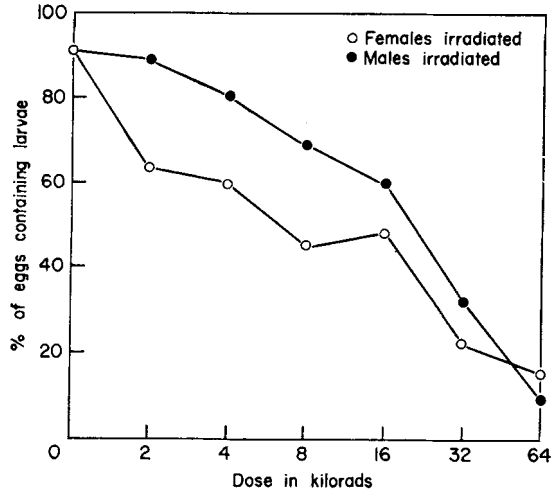


FIG. 13. The effect of gamma irradiation on egg production and embryogenesis of F_1 *Heterodera rostochiensis* when females or males were irradiated prior to mating (from Evans, 1970).

effect of males on egg production in these parthenogenetic females suggests the possibility that the regulation of egg production in nematodes may have an underlying neuroendocrine basis. It is possible that the nervous stimulation of these females which attempted copulation brought about may have inhibited the normal neuroendocrine system as it operates in parthenogenesis. Moreover, Sommerville and Weinstein (1964) also observed that two inseminated females of *Nematospiroides dubius* with mature oocytes laid no eggs when isolated for 6 days in a culture medium which otherwise supported growth and differentiation. This suggests that the process of egg deposition may depend on neurophysiological factors which could not be induced in artificial media.

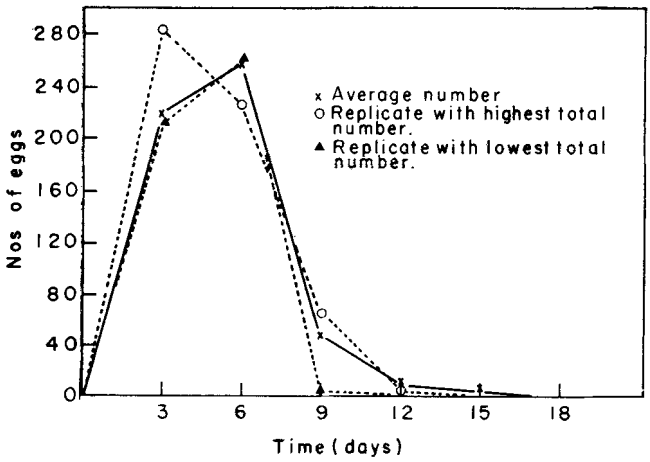
In many parasitic nematodes, the onset of immunologically mediated host resistance is usually indicated by a decreasing egg output (Chandler, 1936; Taliaferro and Sarles, 1939; Michel, 1969). The structural changes which are observed in *Nippostrongylus brasiliensis* and *Haemonchus placei* when the immune response of the host is operative have recently been described (Ogilvie and Hockey, 1968; Lee, 1969; Harness *et al.*, 1973). While Lee (1969) described direct ultrastructural changes in the gonads of *N. brasiliensis*, Harness *et al.* (1973) observed no obvious structural changes in the gonads of *H. placei*. All these workers are agreed, however, that the immune response of the host leads to the disturbance of the energy metabolism of the parasite, and this is observed as an increase in the level of deposition of lipid droplets in the intestinal and other cells. The malfunction of the intestinal cells of these nematodes would disturb the normal translocation of nutrients across the alimentary system into the pseudocoelom (Beams and King, 1972), since this is the only route for the uptake of nutrients from the environment, thus restricting nutrient supply for egg production.

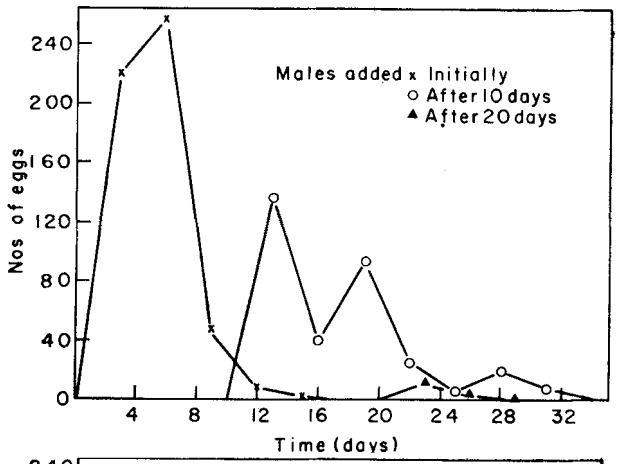
X. Behavioural Aspects of Reproduction

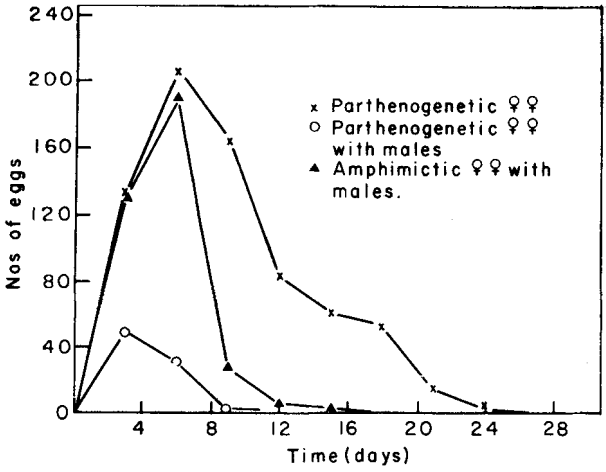
Biologically efficient reproduction in bisexual organisms depends as much on the cytological, genetic and neuroendocrine mechanisms underlying the process as on the frequency and duration of contact between the sexes which leads to copulation and ultimately to contact between the gametes. In many organisms, special behaviour patterns as well as chemically mediated strategies have often been developed to serve this end. In nematodes two relevant aspects in which there has been growing interest are the phenomenon of sexual attraction and the copulatory behaviour of adult worms.

A. SEX ATTRACTION

In *Panagrolaimus rigidus*, Greet (1964) demonstrated the phenomenon of sex attraction (for the first time) in a nematode when he observed that the individuals of a sexual population of nematodes in a tube, separated by a cellophane barrier, were randomly distributed; if the cellophane barrier separated populations of different sexes, the worms collected on each side of the cellophane barrier (Fig. 15). He suggested that the aggregation of these


Nos of eggs
280
240
200
160
120
80
40
0
3
6
9
12
15
18
Time (days)
x Average number
o Replicate with highest total number.
▲ Replicate with lowest total number.


Nos of eggs
240
200
160
120
80
40
0
4
8
12
16
20
24
28
32
Time (days)
Males added x Initially
o After 10 days
▲ After 20 days


Nos of eggs
240
200
160
120
80
40
0
4
8
12
16
20
24
28
Time (days)
x Parthenogenetic ♀♀
o Parthenogenetic ♀♀ with males
▲ Amphimictic ♀♀ with males.

worms was due to a water-soluble chemical attractant whose molecular weight was small enough to pass through a cellophane barrier. As both males and females congregated at the barrier and as homosexual attraction was not demonstrated in either sex, he suggested that both sexes must produce and are attracted by different specific substances.

Since then the phenomenon has been demonstrated in 19 other species of nematodes (Table III; Fig. 16). In *Trichinella spiralis*, *Camallanus* sp. and *Aspiculuris tetraptera* the two sexes attract one another while in all the other species it is the female which is attractive to the male.

Greet (1964), Green (1967) and Anya (1975a) suggest that the attractant substance is a water-borne chemical, while Greet *et al.* (1968) concluded, after exhaustive efforts to extract and characterise the attractant substance that there was a volatile and a non-volatile component to the substance. The question has therefore been raised as to the specificity of the responsible chemical substance. Green and Plumb (1970) found that *Heterodera rostochiensis* and *H. tabacum* which shared the same host plant (*Solanum melongena* L.) were attractive to each other, while *H. schachtii* and *H. cruciferae* were never attractive to each other even when they were occupying the same host plant. On the basis of their data on cross-specific attraction in ten *Heterodera* species, these authors recognized at least three subgeneric groups within the genus.

Samoiloff (1970) suggested that the attractant substance in *Panagrellus silusiae* is derived from the dissolved cuticular material of the moulting female. Green and Greet (1972) suggested also that the male attractant substance in *Heterodera rostochiensis* is derived from the hypodermis. However, Doncaster (1972)—quoted by Green and Greet (1972)—suggests that attraction of males by females of *H. rostochiensis* takes place when females begin to secrete from the vulva. Doncaster's view is in accord with my own observations on *Aspiculuris tetraptera*. Certain secretory cells located in the pulvilus of *A. tetraptera* seem to be the source of the male attractant in this nematode; the female attractant in this oxyurid, it was suggested, originated from the caudal glands of the male (Anya, 1975a).

Cheng and Samoiloff (1972) studied the effect of two chemical substances, hydroxyurea, a specific inhibitor of DNA synthesis, and actidione, a specific inhibitor of protein synthesis, on the production of the attractant in *Panagrellus silusiae*. They observed that female nematodes cultured from the second stage larva in a medium containing hydroxyurea did not attract untreated males; this was taken to indicate that they were not producing the attractant. But adult worms placed in hydroxyurea for 24, 48 or 72 h continued to produce attractant afterwards. On the other hand, females treated with actidione for 24, 48 or 72 h did not attract males when tested immediately

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FIG. 14. Egg production in *Aphelenchoides avenae:* (top) normal pattern of egg production in amphimictic females; (centre) pattern of egg production in amphimictic females when contact with males is delayed; (bottom) pattern of egg production in parthenogenetic species under normal circumstances compared with amphimictic females and with parthenogenetic females in the presence of males.

following actidione treatment but attracted males if tested 24 h later. Thus, on adult females, hydroxyurea had no effect, while actidione had an inhibitory effect of short duration on attractant production. Neither treatment affected the normal copulatory behaviour of the worms and treated worms copulated with themselves or with untreated adults. Thus attraction and copulation are separate events in the life of the worm and their sensory basis appears different.

Cheng and Samoiloff (1972) observed incomplete gonadogenesis in hydroxyurea-treated worms, and also found that neither hydroxyurea nor

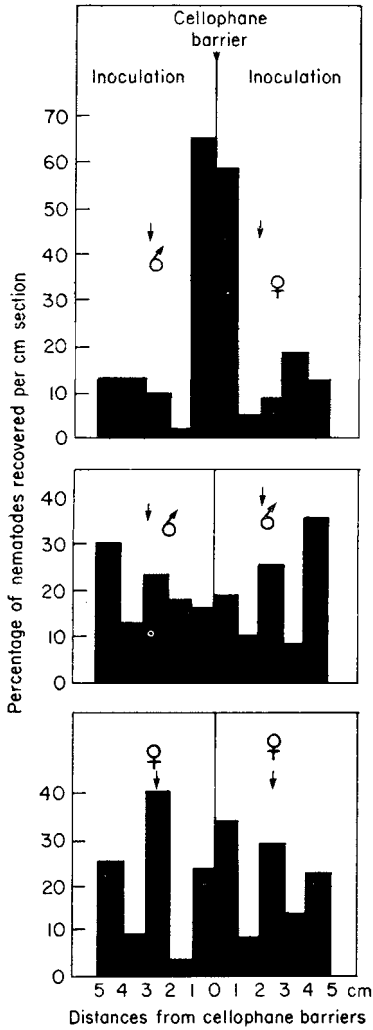

FIG. 15. Sexual attraction in *Panagrolaimus rigidus* indicating the pattern of attraction in heterosexual and homosexual populations (see text—from Greet, 1964).

actidione treatment tampered with the production of attractant if gonad development had been completed prior to the treatment. They suggested, therefore, that attractant synthesis may be under hormonal control and requires continuous protein synthesis. Preliminary efforts at chemical analysis by these workers indicated that the attractant was not a protein, because pepsin digestion had no effect on it; they concluded that it was a substituted hydrocarbon. It should be particularly rewarding to characterize these attractant substances, to enable comparisons be made with the pheromones of insects. The recent success in the extraction of hormones with identical biological properties to insect juvenile hormones from the related *Panagrellus redivivus* (Rogers, 1973) makes the possibilities for endocrinological studies of nematode reproduction even more interesting.

B. COPULATORY BEHAVIOUR

In the literature there are very few descriptions of the copulatory behaviour of nematodes. Fisher (1972) observed virgin males and virgin females of *Aphelenchus avenae*; once virgin males were introduced to virgin females, copulation commenced within 5 min. "The male would brush the ventral

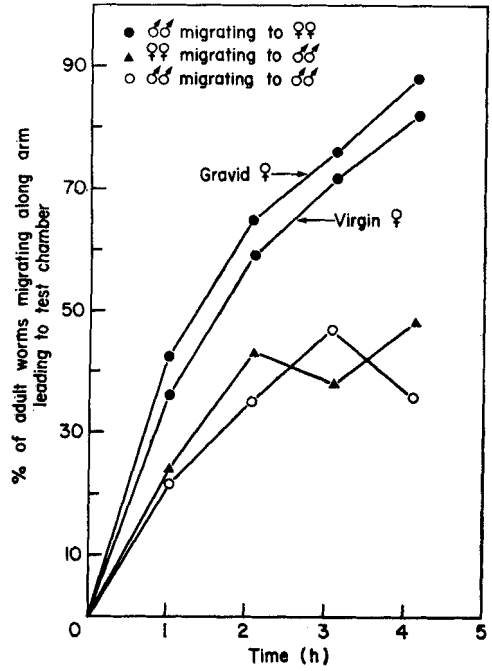

FIG. 16. Graphic illustration of rate of response to mating attractant in *Panagrellus silusiae*. Males migrate towards both gravid and virgin females. In homosexual experiments, about 45% migrate to test chamber, 45% away from test chamber and about 10% do not migrate at all (from Cheng and Samoiloff, 1972).

TABLE III

List of nematode species in which sexual attraction has been demonstrated and with the pattern of attraction displayed

Nematode species	Pattern of sex attraction displayed		Reference
	male → female	female → male	
1. *Nippostrongylus brasiliensis*	+	−	Alphey (1971); Gimenez and Roche (1972)
2. *Heterodera rostochiensis*	+	−	Green (1966); Green and Greet (1972)
3. *Heterodera schachtii*	+	−	Green (1966); Green and Greet (1972)
4. *Heterodera avenae*	+	−	Green (1966); Green and Greet (1972)
5. *Heterodera castae*	+	−	Green (1966); Green and Greet (1972)
6. *Heterodera cruciferae*	+	−	Green (1966); Green and Greet (1972)
7. *Heterodera glycines*	+	−	Green (1966); Green and Greet (1972)
8. *Heterodera goettingiana*	+	−	Green (1966); Green and Greet (1972)
9. *Heterodera mexicana*	+	−	Green (1966); Green and Greet (1972)
10. *Heterodera tabacum*	+	−	Green (1966); Green and Greet (1972)
11. *Heterodera trifolii*	+	−	Green (1966); Green and Greet (1972)
12. *Panagrellus silusiae*	+	−	Cheng and Samoiloff (1971)
13. *Trichinella spiralis*	+	+ +	Bonner and Etges (1967)
14. *Panagrolaimus rigidus*	+	+	Greet (1964)
15. *Camallanus* sp.	+ +	+	Salm and Fried (1973)
16. *Pelodera teres*	+	−	Jones (1966)
17. *Ancylostoma caninum*	+	−	Roche (1966)
18. *Cylindrocorpus longistoma*	+	−	Chin and Taylor (1969)
19. *Cylindrocorpus curzii*	+	−	Chin and Taylor (1969)
20. *Aspiculuris tetraptera*	+	+	Anya (1975a)

surface of his tail backwards and forwards against the female until, by what appeared to be chance, one of the brushing movements placed the cloaca over the vulva; the spicules were then thrust into the vulva and further movements of the male stopped." Copulation may last for up to $1\frac{1}{2}$ h, during which time a glue-like substance accumulates between the worms and may remain around the vulval region for some time.

In *Panagrolaimus rigidus* when a male touched a female there was an immediate coiling of the male's tail to form a loop into which the spicules projected (Greet, 1964). In *Aspiculuris tetraptera* the same sudden coiling of the posterior third of the male is observed as soon as a male touches a female's body (Anya, in prep.). In the loop thus formed, close observation shows that two of the male's genital papillae stand on end (Fig. 17). The loop slides along the female's body until, when opposite the vulva, there is a twisting movement of the anterior end of the male which serves to press the cloacal region of the male against the female's vulva.

In many parasitic nematodes, copulation *in vitro* is not often observed (Sommerville and Weinstein, 1964; Alphey, 1971; Anya, 1974b). This has led to the suggestion that specific features of the environment are necessary for the elicitation of normal copulatory behaviour in nematodes. Phillipson (1973) observed that in *Nippostrongylus brasiliensis* copulation between normal adults took place only if the worms were in contact with the small intestine of a rat in which the blood supply was intact. Further, he observed that there was a critical temperature requirement, the range of temperatures at which copulation usually occurred being 34·4–37·8°C (with the minimum range being restricted to 35–37·5°C).

These few descriptions in the literature of copulation leave no doubt that while sex attractants serve to bring the worms together, thigmokinetic responses are necessary for successful copulation. In this respect, the genital

FIG. 17. Scanning electron micrograph of genital papillae around the cloacal region of male *Aspiculuris tetraptera*. Note the two distal papillae, one on either side of the cloacal opening, which have been observed to "stand on end" in the presence of female *A. tetraptera* (from Anya, 1973b).

papillae found in the posterior cloacal region of many male nematodes may include different categories of sense organs, for example chemoreceptors and tactile receptors. The observation that two of these papillae in *Aspiculuris tetraptera* "stand on edge" once a female is enclosed in the posterior loop of the male preparatory to copulation is suggestive. This view is supported by the observations of Marchant (1970), who observed flaring and vibration of the male bursa of *Ancylostoma caninum* in the vicinity of a female; during copulation in the bursate nematodes, the bursa is usually wrapped around the female.

The sensory basis of copulation in the male nematode would appear to differ from the situation in the female nematode. Female nematodes continue feeding during copulation, while the male always stops feeding. Croll (1972c) has therefore suggested that in males, external stimuli relevant to copulation override endogenous pacemakers controlling feeding movements, while the absence of such external inputs in females leaves the female free to continue feeding during copulation. It seems likely, however, that in the male a posteriorly located nervous centre with local autonomy over copulatory behaviour might exist. Detailed ultrastructural studies on the relationships of the ganglia and other elements of the posterior nervous system of male nematodes should give an insight into functional relationships.

XI. Reproductive Phenomena and Parasitism

Parasitism is essentially an ecological problem (Rogers, 1962; Crofton, 1971). Consequently, whatever physiological and functional relationships are found in a host–parasite relationship must be explicable in an ecological perspective.

The total environment of a parasite involves considerations of factors in three different and to some degree characteristic environments: the environment external to the host, the *macroenvironment;* the environment within the host, but external to the parasite, the *microenvironment;* and the internal environment of the parasite, which for want of a better name we may call the *infra-environment.* Changes in any of these may be expected to have consequences in the host–parasite relationship.

In any discussion of this relationship, the nature of the interdependence of factors operating in each of the environments which make up the total environment of the parasite, and the final result of the operation of these factors in the maintenance of the population of the host and of the parasite in a biologically stable state of equilibrium, are relevant.

The host as a living system abstracts nutrients from the external environment for its basal metabolism, growth and reproduction, as would the parasite. Thus, in a gross overview of the energy resource allocation function in the host or in the parasite, it is valid to consider reproduction as catabolic (Calow, 1973). When a parasite is introduced into the host, the needs of the parasite now superimposed on the needs of the host—all of which are to be satisfied from the external environment—would lead to a readjustment in energy resource allocation, such that the overall catabolic

element in the energy equation of the host will increase but in a way which takes account of the hosts' and the parasites' needs, in a regulated manner. In other words, it is valid to expect a feedback relationship between the total energy resources in a given host–parasite environment, the individual needs of the host and parasite, and the proportions of the resources which they utilize for particular ends.

It is within this overall framework of constraining energy resource allocation by the host that the parasite has to make its own adjustments, particularly with regard to what may be allocated to its basal metabolism, growth and reproduction. In conditions of restricted availability of energy resources, it is reasonable to expect that the needs of reproduction in the parasite could be sacrificed in favour of the more immediate needs (of the individual parasite) for basal maintenance and growth. However, as the distribution of a parasite population is discontinuous and the parasite population is an overdispersed one (Andrewartha and Birch, 1960; Crofton, 1971), the situation poses a fundamental problem for the survival of the population. In particular, the question does arise of what reproductive strategies may be considered viable options for the parasite. For example, the long-term need of the parasite population for survival as opposed to the individual parasite's immediate needs, sets a minimum level beyond which the needs of reproduction for resources must be met *even in conditions of scarcity*. Thus, once again, it may be expected that in a given host–parasite system, there would be a feedback relationship between nutrient conditions in the environment, the utilizable energy resource levels, and the requirements of the host and parasite populations. The more successfully adapted a host–parasite system, the greater the degree and efficiency of control exercised over the energy resources, always in a manner that optimizes the survival of the host and parasite populations. As the need for feedback and control in a living system or group of interrelated systems increases, so does the number of functional parts required to exercise regulation (Lwoff, 1965). In other words, the degree of development of tissues and the general level of differentiation is related to functional requirements.

In nematodes, parasitism has generally led to the development of more elaborate reproductive systems and the production of large numbers of eggs, without the basic anatomical relationships of the system being drastically changed (Chitwood and Chitwood, 1950). Obviously, this development could have arisen in the first place in the nematode parasite's effort to elaborate sensitive and efficient feedback control to regulate the production of gametes and ultimately infective agents (Calow, 1973), especially as they are iteroparous.

The fact that the location of nematode parasites within their hosts is usually in regions where essential nutrients are easily available, for example the gut of vertebrates, further ensures that high levels of egg production, beyond the basic requirements for maintaining the population, can be achieved. The coincidence of these two factors, efficient control of gamete and infective agent production, and easy availability of nutrient resources, has combined to guarantee the astounding success of nematodes as parasites

without recourse to any specialized morphology. And in the parthenogenetic species, the limited capacity for amphimictic reproduction which is retained ensures the maintenance of a desirable degree of variation and consequently of adaptive capability in these species.

XII. Summary

A general survey of the range of reproductive phenomena in the Nematoda indicates that all the main cytogenetic mechanisms observed in the animal kingdom are apparently represented in this phylum. In the pursuit of reproductive efficiency, there has been an elaboration of the reproductive systems, particularly in the parasitic members, such that greater histological and cytological differentiation has engendered precise and perhaps unique physiological mechanisms which ensure successful fertilization and development of the embryo. In particular, the physiological and biochemical mechanisms involved in the formation of the egg-shell while conserving resources, lead to the emergence of unique structural properties in the egg-shell, providing for the developing embryo a self-contained and precisely regulated environment. It is suggested that the great success of nematodes as parasites is derived mainly from their ability to maintain reproductive efficiency with little morphological specialization, while retaining at the same time some capability for biological variation in ontogeny.

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SHORT REVIEWS

Supplementing Contributions of Previous Volumes

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The Epidemiology and Control of some Nematode Infections in Grazing Animals

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I. Introduction

This short review, which covers the 6 years from 1968 to 1974, does not attempt to be comprehensive. Only those topics are dealt with in which the writer considers that new ideas or developments are of particular interest.

When the subject was reviewed in Volume 7 of "Advances in Parasitology", it was beginning to be realized that, in temperate climates at least, the important nematode parasites of grazing animals complete very few generations each year and that some are monocyclic. The period since 1968 has been one of consolidation. Appropriate methods of investigation have been more widely used and a good understanding has been achieved of the

epidemiology of a number of infections. There has also been some progress, in that control measures with a clearly defined and relevant aim now promise to replace the haphazard use of anthelmintics and practices depending on the cumulative effect of many unconnected and insignificant factors.

II. Methods of Investigation

There has been considerable progress during the past 15 years in the study of the epidemiology of helminth infections of grazing animals, and it may be asked whether this was due to the development of a new approach or of new techniques and whether these could be employed in elucidating other problems.

Most early work was of a detailed nature and concerned with small portions of the parasite's life history. No means existed of relating the knowledge so gained to the whole problem, which appeared to be of almost infinite complexity. In an attempt to understand the approach used by a number of workers in successful investigations in helminth epidemiology, Michel (1971a) came to the conclusion that it was necessary to assume that the epidemiology of any helminth infection was dominated by a very few elements, so that for practical purposes all the rest could be disregarded. If this were done the problem became sufficiently simple to be soluble.

These dominant elements could be identified by means of observations and experiments in situations in which the life-history could be completed. The life-history might be seen in terms of the flow of individuals through a sequence of arbitrarily defined populations. These populations were connected by processes which could be studied by monitoring the populations which they connected. It was neither practicable nor necessary to monitor every possible population, but the fewer the populations monitored, the larger and more complex were the processes by which they were connected. For example, a full flow diagram for a trichostrongylid infection might be written as follows: adult worms → eggs → larvated eggs → first-stage larvae → second-stage larvae → third-stage larvae in faeces → third-stage larvae on herbage → larvae ingested by host → parasitic third-stage larvae → early fourth-stage larvae → late fourth-stage larvae → immature fifth-stage → adult worms. Apart from technical limitations the choice of populations, and hence of the processes to be studied, should favour those which can be isolated within an experimental situation, for every population is controlled by two processes, one of recruitment and one of depletion. A simplified flow diagram might therefore be: adult worms → eggs → infective larvae on herbage → early fourth-stage larvae → late fourth-stage → adult worms.

The dominant processes are those which are the most variable. To this extent Michel and Ollerenshaw (1963) were wrong in emphasizing those phases of the life history at which there was the greatest loss.

Investigations in which appropriately selected populations are monitored make it possible to find quite specific answers to questions of the following form: When did the animals acquire the disease-producing infection? Where did they acquire it? Why were they susceptible? By which animals was the

pasture contaminated? When was it contaminated? Why did a large infestation result? A clear picture can be built up of the sequence of events leading up to an outbreak of disease.

Bradley (1972) has pointed out that while formerly, investigators tended to enquire how parasites contrived to survive, a more profitable approach is to investigate how their numbers are kept within bounds. This would provide a clue to the most eligible strategy of control.

While these techniques are demonstrably successful and are coming to be more widely employed, some workers still rely on inadequate or unsuitable parameters. Tongson and Balediata (1972), for example, having observed faecal egg counts of a group of calves in the Philippines (and found them to conform to the stereotyped pattern described by Michel (1969a,b) and therefore unlikely to reflect worm numbers), used these data as the basis of ingenious epidemiological speculation. Similarly Neumann and Kirsch (1970), having examined great numbers of faeces samples from calves in Schleswig-Holstein, deduced an unorthodox picture of the epidemiology of parasitic gastro-enteritis.

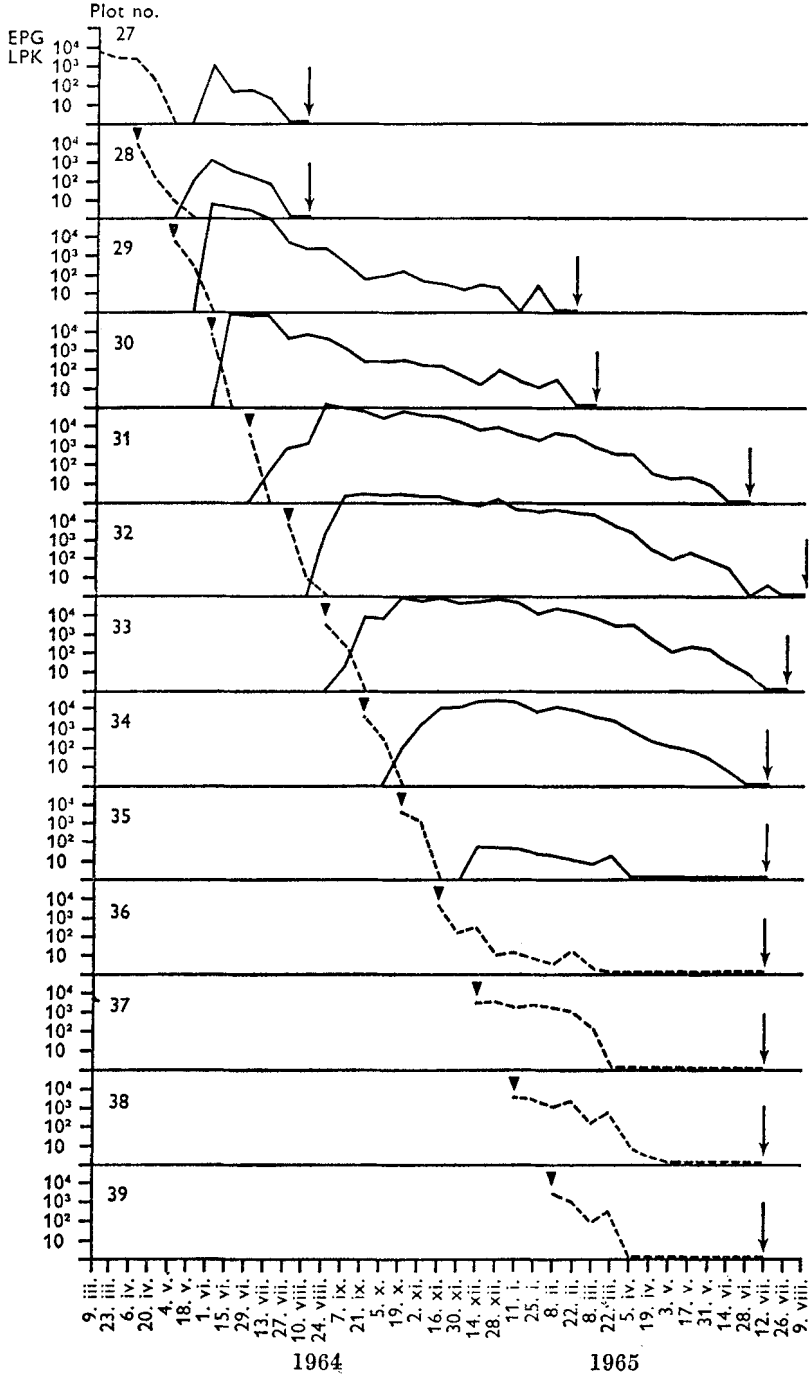
III. Free-living Stages

A. Studies on the bionomics of eggs and larvae

The aims of work on the free-living stages have become clearer and more realistic. The thinking behind much early work was that a knowledge of the reaction of free-living stages would make it possible to predict not only the geographic distribution of different species but also to identify years of high incidence or actual occasions when a hazard of disease was present.

Apart from the fact that the underlying epidemiological assumptions may not always have been warranted, this approach encounters fundamental difficulties. The results of laboratory studies on the reactions of eggs or larvae to temperature, humidity or other factors, while they may be of considerable theoretical interest and may, like those of Waller and Donald (1970, 1972), illuminate limited questions, cannot be related to the field where conditions to which the worms are actually exposed cannot readily be measured.

Accordingly a trend developed in the 1950s towards experiments in which infected faeces were exposed to situations simulating field conditions. Grass grown in boxes or small plots of pasture was contaminated, and samples of faeces and herbage examined periodically. Valuable information of a rather general kind could be derived from these studies and work of the same kind is still being done. Goldberg (1968, 1970) made a number of observations on cattle nematodes of mixed species, and Williams and Bilkovich (1971) showed that in Louisiana development of *Ostertagia ostertagi* in cattle faeces could proceed throughout the year but that in summer, presumably owing to dry conditions, migration of larvae from the faeces, and their survival, were depressed. Ogbourne (1972), in experiments of the same type, found in England that eggs of *Trichonema* spp. and of *Strongylus* spp. passed in the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faeces of horses during the winter failed to reach the infective stage, that the faeces at no time of year dried out quickly enough for development to the infective stage to be hindered and that the migration of larvae from the faeces was greatly influenced by rainfall.

But this kind of work and the more intensive but basically similar studies of Andersen *et al.* (1970) and of Levine and Andersen (1973) cannot overcome the difficulty that the meteorological data which are available do not measure the conditions to which the larvae are exposed. Attempts to relate the reactions of larvae to macrometeorological conditions have not met with success (Levine, 1963). Andersen *et al.* (1970) therefore stress the need to make measurements of the microclimate in their experiments. But it is difficult to see, if the reactions of larvae cannot be related to meteorological data that are available, how progress is to be made by relating them to measurements which are not generally available and which, if specially made, would have a very local validity.

The attempt to obtain a detailed understanding of the effect of climatic factors on worm eggs and larvae in field conditions encounters further difficulties. Firstly, the experiments are necessarily made in prevailing weather conditions, and since they extend over a period of time, the sequence of conditions in which any one result was obtained is unique. Secondly, the process studied is complex. Andersen *et al.* (1970) recognized seven separate steps: (1) survival of the undeveloped egg, (2) development of the egg to the pre-hatch stage, (3) hatching, (4) development of larvae to the infective stage, (5) migration of infective larvae onto the herbage, (6) survival of the infective stage and (7) infection of the definitive host. But an even larger number of valid processes could be identified. Moreover there is considerable individual variation in the rate at which eggs proceed through these stages. Therefore overlapping processes are studied in a sequence of conditions unique to the experiment, and it is open to question whether it would be possible to draw valid conclusions as to the effect of microclimate on any component process.

Experimenters have reacted to the difficulties in two ways. Some have attempted some separation of component processes in the design of their experiments, and like Goldberg (1970) or Andersen *et al.* (1970) have exposed faeces containing infective larvae in order to study migration and survival only. Others, like Levine (1963) and Levine and Andersen (1973), use a single expression to summarize the reaction of the worms. This "infective potential" or "transmission potential" measures the area subtended by the curve of larval population on the herbage following the deposition of faeces on one occasion, and is of limited practical usefulness.

FIG. 1. The development of eggs of *Trichostrongylus colubriformis* and the survival of infective larvae on experimental plots. The broad arrows indicate the date when faeces were spread on each plot; the long arrows indicate when observations ceased on each plot. The broken line shows the number of eggs found in the faeces, the solid line the number of third-stage larvae recovered from the herbage. (From Gibson and Everett, 1967.)

It may be asked whether the entire line of thought is not approaching a dead end.

Transmissibility is only one element in the epidemiology of helminthiasis and not always a crucial one. The aim of many investigators is now to build up, by similar experimental techniques, more empirical knowledge on the course of herbage infestations in an average year. To this end, new experimental plots are contaminated at monthly or preferably at fortnightly intervals for a period of years and frequent samples of faeces and of herbage are examined. This approach has been used by Gibson and Everett (1967, 1972), Boag and Thomas (1970) and Pacenowsky *et al.* (1971). An example is shown in Fig. 1. Given a little application, or the use of a computer, it is possible from such a family of curves to calculate the pattern of herbage infestation likely to result from any particular pattern of egg output. Pacenowsky *et al.* (1971) have worked out from data obtained in a single year, the extent to which eggs of *Cooperia oncophora* deposited in different months contribute, respectively, to the herbage infestation during the grazing season and to the overwintering infestation (see Fig. 2).

This approach also has its limitations. It provides a picture only of the pattern in an average year and in a single location. It is not possible to extrapolate to other places or to years of abnormal climate. Nor does it yield anything beyond a pattern, giving no indication of absolute levels. Further, the conditions in which these experiments are conducted are necessarily a little artificial and commonly the manner in which the faeces are distributed and the quantity applied affect the microclimate. The practical problems involved in maintaining a continuous supply of infected faeces of a constant egg count for a period of years are also considerable, the more so since, if several species are to be compared, they must be exposed at the same time. It can be argued that an experimental design in which infected sheep or cattle graze a series of paddocks might give more meaningful results without demanding greater resources.

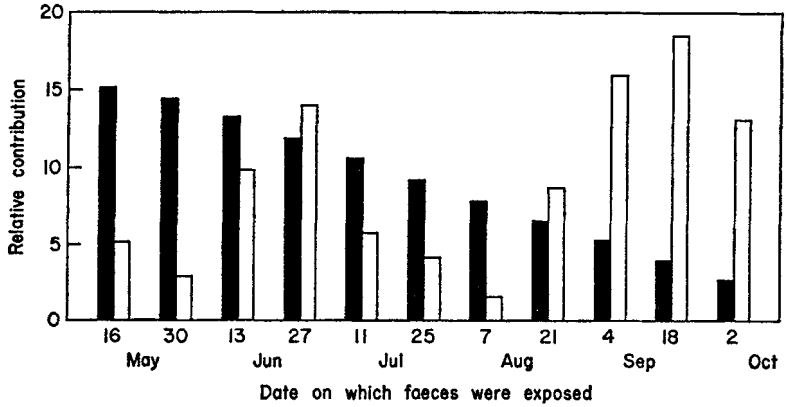

FIG. 2. The relative contribution made to the herbage infestation during the grazing season (black columns) and to the overwintered infestation (white columns) by infected faeces deposited on the dates indicated. (Drawn from data published by Pacenowsky *et al.*, 1971.)

Barger *et al.* (1972) used a much bolder approach. They constructed a computer simulation program, not on the basis of experimentation, but making drastic though not unreasonable assumptions regarding the reactions of *Haemonchus contortus* to temperature and moisture. The course of herbage infestations calculated from the faecal egg counts of sheep on an experimental pasture corresponded closely with that actually observed, but Barger *et al.* very prudently avoided the conclusion that their assumptions were therefore confirmed.

An equally bold approach is that whereby methods have been developed of forecasting the incidence of disease or the timing of some helminthological event from meteorological data. The subject was reviewed by Ollerenshaw and Smith (1969) but a few general comments seem appropriate here.

In most cases the procedure by which these forecasts are devized is entirely empirical. By a process of trial and error a formula is derived from seemingly relevant meteorological parameters which gives a good correlation with past records of incidence or of the date of the event in question. There seems to be a tendency, however, to test the formula against the very records from which it was derived. If a formula based on the records of 10 years gives reliable results in 3 years out of the next 5, its value is upheld on the spurious grounds that it has been misleading in only 2 years out of 15.

An example of the empirical approach in its most extreme form is a method suggested by Thomas (1974) for predicting the date on which larvae of the gastro-intestinal nematodes of sheep appear on the herbage in summer. According to Boag and Thomas (1973) this event is of rather regular timing in different years, and Thomas (1974) attributes the simultaneous appearance on the herbage of larvae due to pasture contamination in several months to an increasing rate of development as the temperature rises through spring and early summer. Nonetheless, the basis of this forecast is the expectation that the rise in herbage infestation will occur when the total of 6 h "wet" periods after 15th April reaches 100. This basis is so much at variance with the views of its author as to the underlying mechanism, that doubts must arise concerning the validity of the method.

This is less so in the case of the forecast by Ollerenshaw and Smith (1966) concerning the incidence of nematodiriasis in lambs, or in that of the related forecast by Smith and Thomas (1972) concerning the date on which infective larvae of *Nematodirus battus* are likely to appear on the herbage.

Even if a forecast is reliable and can be issued sufficiently early for action to be taken, a more important question, perhaps, is whether it can play any useful part in the control of disease. This matter is discussed in a later section.

B. THE TRANSPORT OF LARVAE

It is now generally accepted that eradication of the nematodes of grazing animals is not feasible. Spedding (1969), with a long experience of maintaining a "helminth-free" area for experimental purposes, draws a distinction between the eradication of helminthic disease, which he regards as possible, and the eradication of helminths, which is not. But it is in this context that uncommon

routes for the transport of larvae have been considered. There have been no recent observations resembling those of Enigk and Düwel (1962), who traced the distance travelled by larvae of *Dictyocaulus viviparus* in the water of ditches, or of Bizzell and Ciordia (1965) who, following the observations of Robinson (1962) on the dispersal of *D. viviparus* by fungal gunnery, showed that *Cooperia punctata* and *Trichostrongylus colubriformis* could be transported in the same way.

Jacobs *et al.* (1968) and Tod *et al.* (1971), however, have shown that, like *Oesophagostomum* spp. of pigs, *Ostertagia ostertagi* can be carried twined round the legs of psychodid flies. Another interesting question arises from the observation of Jacobs *et al.* (1971) and El Rafaii (1962) that *Oesophagostomum* spp. of pigs can use not only rodents but also insects as paratenic hosts. The possibility that the survival of larvae of *Oesophagostomum* spp. of ruminants might be extended through the agency of an insect acting in this way, is potentially interesting.

IV. Parasitic Stages

A. Regulation of populations in the host

It is a convenient device to regard the various manifestations of host resistance and other phenomena influencing populations in the host as based on separate mechanisms. Almost every new development tends to vindicate this approach but it is not yet widely accepted. Many authors lump together a number of phenomena under the heading "immunity" and separate others, chosen just as arbitrarily, as being of different causation. Thus, Jones and Ogilvie (1971) regard the expulsion of *Nippostrongylus brasiliensis* from the rat as an expression of "protective immunity", while Kelly (1973) has doubts regarding the status of regulatory mechanisms described by Michel (1970) on the grounds that they operate in animals which are not refractory to the establishment of new infection.

Yet the most important of these mechanisms, namely a loss of worms (Michel, 1963, 1970) by which populations (of *Ostertagia ostertagi*) are maintained at a level proportional to the rate at which new infection is acquired, shows points of similarity with the expulsion of *Nippostrongylus*, a phenomenon now commonly called "self-cure". Jarrett *et al.* (1968a) showed that the course of other than very small infections of *N. brasiliensis* consisted first of a period in which there was no loss of the worms that had initially become established, and that there was then, from day 11 to day 18, a logarithmic decrease in worm numbers until a residual burden was reached which persisted for a considerable time. It appears from the work of Armour *et al.* (1966), Michel (1973) and Malczewski (1971) that populations of *Ostertagia circumcincta*, *O. ostertagi* and *Haemonchus contortus* respectively follow much the same course although on a very much longer time scale. Whether the underlying mechanism is really the same remains to be determined.

Meanwhile there has been an interesting development concerning the causes of self-cure as seen in the field, particularly in infections of *H. contortus*. As described by Gordon (1948) in New South Wales, this phenomenon occurred simultaneously in different flocks and different groups of sheep and followed a period of rain sufficient to cause grass growth to be resumed. Gordon suggested that some factor associated with grass growth might be the essential stimulus, but in the light of the results of Stewart (1950) and others (that the administration of infective larvae to sheep could induce the elimination of the worms they were already carrying) it was assumed that this was invariably the cause and that the factor envisaged by Gordon was nothing other than a new wave of infective larvae.

Recent work by Allonby and Urquhart (1973) in Kenya has reopened the question. They showed that the self-cure of infections of *H. contortus* occurred at the same time (a few days after a period of rain at the end of the dry season) in naturally and experimentally infected sheep no matter whether they were running on infected or on worm-free pasture.

B. ARTIFICIALLY INDUCED IMMUNITY

The success of the vaccine which is now widely used against lungworm infection in cattle depends on this circumstance: that natural populations are limited by the rapid acquisition by the host of a resistance to the establishment of worms. Nonetheless, the search continues for vaccines even where this is not the case and where a product useful in practice is therefore not likely to result.

A number of lines of thought appear in this work. Bürger and Pfeiffer (1969) used larvae attenuated by X-irradiation in an attempt to vaccinate calves against *O. ostertagi* and *C. oncophora*. Scott *et al.* (1971) tried to vaccinate lambs against *H. contortus* by means of a preparation of metabolic antigens produced from worms grown *in vitro*. Denham (1969) used a similar vaccine against *Trichostrongylus colubriformis* but with the addition of an adjuvant. Herlich *et al.* (1973) tried to protect calves against *Oesophagostomum radiatum* by injecting extracts of killed worms. Allen *et al.* (1970) used larvae of a strain of *H. contortus* isolated from pronghorn antelope and found to be of low pathogenicity to sheep. Subsequently they terminated these immunizing infections by anthelmintic treatment.

In none of these trials was the immunity achieved more than very slight, and at present there are no grounds for modifying the view that where immunity does not play an important part in limiting naturally occurring populations, vaccination is not likely to prove a fruitful approach to control.

C. IMMUNOLOGICAL INCOMPETENCE

Immunological incompetence in its broadest terms was reviewed by Urquhart (1970) and Kelly (1973).

The finding by Manton *et al.* (1962) and Urquhart *et al.* (1966) that young lambs could not be immunized against *H. contortus* while older lambs could,

or rather that a very much greater antigenic stimulus was needed in young lambs (Christie and Brambell, 1966), is now a fairly common experience. For example, similar findings with *Nematodirus helvetianus* in calves, *Trichostrongylus axei* in lambs and *Nematospiroides dubius* in mice have been described by Ross (1970), Smith (1973) and Cypess *et al.* (1973). Gibson and Parfitt (1972) have shown that with groups of lambs from 8 to 36 weeks old, the older the lamb when first infected with *T. colubriformis*, the sooner the infection was terminated.

Particular interest has centred on the great persistence of worms in hosts infected soon after birth. Kassai and Aitken (1967) had shown that very young rats infected with small numbers of *Nippostrongylus brasiliensis* failed both to become resistant to challenge and to expel the initial infection. Further infection given at a more advanced age did elicit a resistance to challenge (Kassai and Szepes, 1968) but the initial infection would persist, especially if the host had been continuously exposed to infection in the meantime (Kassai, 1967; Jarrett, 1971). Jarrett *et al.* (1968b) demonstrated that the failure of baby rats to respond to infection with *N. brasiliensis* depended in some measure on how many larvae they were given. If the initial worm burden exceeded a critical size, a loss of worms did occur, but the residual population which persisted into adult life was only slightly smaller than the critical burden. Jenkins and Phillipson (1970), however, working with slightly older rats, found that if the initial infection was given in small daily doses, a very large persistent burden could be built up.

Clearly, if similar phenomena were demonstrable in the nematode infections of grazing animals, and lambs or calves exposed to infection at a very early age retained large numbers of worms for an uncommonly long time, the epidemiological implications might not be without importance. But no observations on the subject have been reported.

The question of whether and in what circumstances immune exhaustion can be shown to occur in nematode infections also remains unresolved. The results of Dineen and Wagland (1966) and Wagland and Dineen (1967) indicated that sheep infected with larvae of *H. contortus* on a number of occasions were more resistant to challenge if the immunizing infection had first been removed by anthelmintic treatment. But Donald *et al.* (1969), in broadly similar experiments, failed to reproduce the phenomenon. Further work is awaited.

The interaction between anthelmintic treatment and the development of an acquired resistance is more often the subject of speculation than of experiment. Apart from the suggestion that continued infection may lead to immune exhaustion, the usual assumption is that the periodic removal of worms must have the effect of retarding the development of resistance. Indeed, Ciordia (1969) considers that once a beast has been given anthelmintic treatment, its resistance is so much reduced that treatment must be repeated at intervals. Gibson *et al.* (1970), however, have demonstrated that anthelmintic treatment must be given very frequently indeed before there is any reduction in the rate at which a resistance to *Trichostrongylus colubriformis* is acquired.

D. REACTIONS OF DIFFERENT HOSTS

Some mention must be made of the epidemiological effects of differences in the reaction of different hosts to a common parasite. Perhaps the earliest example to come to light was the case of *Dictyocaulus arnfieldi* in horse and donkey (Wetzel and Enigk, 1938). In the horse, infections are highly pathogenic but short lived; in the donkey, large infections cause negligible symptoms and persist for a long time. When hosts of either species graze alone, there is little clinical dictyocauliasis and the level of infection in horses and on the pastures that they graze is very much lower than in the donkeys and on their pastures. When the two species graze together there is frequent lungworm disease among the horses which are exposed to heavy infestations produced by the donkeys. The subject has recently become topical and is discussed by Enigk and Weingärtner (1973).

Very similar circumstances appear to be the cause of heavy mortality among moose in Canada due to *Pneumostrongylus tenuis*, which has occurred where the territory of moose overlaps with that of white tailed deer in which this worm is of low pathogenicity (Anderson, 1964, 1970). A similar example is provided by *Elaephora schneideri*, which is almost without pathogenicity to mule deer and white tailed deer but is highly pathogenic to the American elk. Disease occurs in elk where deer are also present but not where they are absent (Hibler *et al.* 1968).

Occurrences of this kind may become increasingly common with the growth of wildlife parks and other collections where ungulates of widely different origins graze together. There is some circumstantial evidence of such a relationship between European bison, Sika deer and *Haemonchus contortus*.

Varietal differences within host species may also give rise to this phenomenon. Wiltshire horn sheep, for example, appear to be singularly tolerant of *Dictyocaulus filaria* infection. Where sheep of this breed grazed together with Clun Forest sheep, there was an uncommonly high incidence of lungworm disease among the Cluns. This was very greatly reduced when the Wiltshire Horns were removed (Rose and Michel, unpubl. obsvns, 1957).

Within a single breed there may be considerable individual variation in susceptibility (see for example Downey, 1973; Gordon, 1973) and it is possible that animals of the kind regarded by Michel (1963) as inherently incapable of normal responses to helminth infection might on occasion play a significant role as a source of pasture contamination.

E. ARRESTED DEVELOPMENT

Recent work on arrested development of nematodes has been reviewed in Volume 12 of "Advances in Parasitology", to which the reader is referred.

An interruption at an early parasitic stage may occur facultatively in the development of nearly all nematodes and serves, in one way or another, to synchronize their activity with events in the host or the outside environment. In the case of several trichostrongylids, seasonal factors are of importance, the receipt of appropriate signals by the free-living stages apparently inducing

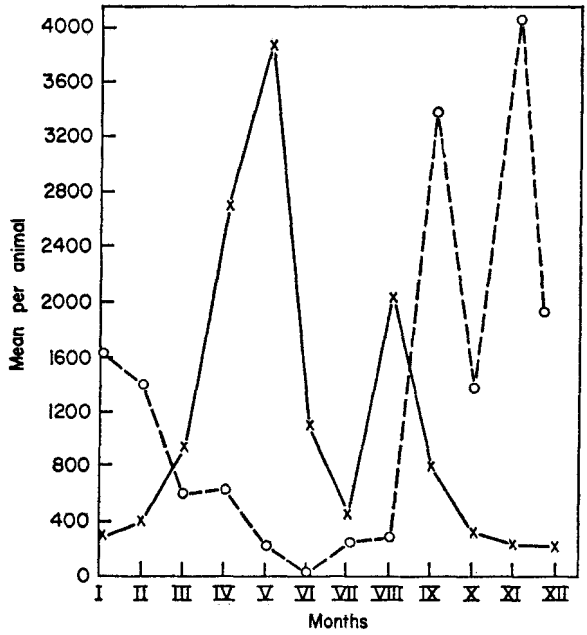

FIG. 3. The annual fluctuations in the worm burdens of cattle, 12–18 months old, showing adult worms × – – – × – – – × and larvae ○ – – – ○ – – – ○. (From Malczewski, 1970.)

a state resembling diapause at an early parasitic stage. In temperate climates, larvae picked up from the pasture in autumn and early winter tend to be arrested and the development of most is resumed in the spring. A number of studies have therefore shown worm burdens in winter to be partly, largely or entirely composed of arrested forms, while populations in summer consist predominantly of adult worms (Bessonov, 1967; Malczewski, 1970; Connan, 1971; Reid and Armour, 1972; Ayalew *et al.*, 1973). An example is shown in Fig. 3.

Haemonchus contortus represents an extreme case, the infection being carried on from one grazing season to the next entirely in the form of arrested early fourth-stage larvae, their development being resumed during a short period in the spring. Ayalew and Gibbs (1971) in Canada have commented on the very short season during which the life cycle can be completed without interruption, and in North-east England, Waller and Thomas (1974) have found that the period during which larvae picked up from the pasture can develop directly to maturity is so short that for this reason alone, *H. contortus* must be virtually monocyclic. Nonetheless, LeJambre and Ractliffe (1971), for reasons to be discussed in a later section, still believe that at Ithaca in upstate New York, this very species can complete several generations each year.

F. THE POST-PARTURIENT RISE

The connection between arrested development and the increase in worm egg output seen in many host–nematode systems at or following parturition, and which has come to be called the post-parturient, periparturient or lactation rise, is largely incidental. The matter was discussed in some detail by Michel (1974). Because this rise could be demonstrated in ewes that had been housed for several months before lambing, it was rightly deduced that arrested worms that had resumed their development were involved. But it was also assumed, unjustifiably, that the post-parturient rise was solely and invariably due to arrested worms which had been prompted to resume their development by events associated with parturition or more particularly with lactation, for the work of Jacobs (1966), Connan (1968a) and Brunsdon and Vlassoff (1971) had shown that the rise could be suppressed by prematurely weaning the young.

It has since been shown by Connan (1968b) and by O'Sullivan and Donald (1970) that, in sheep at least, the post-parturient rise may also be due to recently acquired worms, and there is some evidence also that egg output per female worm may be increased. Meanwhile, Connan (1970) and Dineen and Kelly (1972) have demonstrated that *Nippostrongylus brasiliensis* is not expelled from lactating rats. Some writers therefore refer to the phenomenon as the "post-parturient relaxation of resistance" (Gordon, 1973), and envisage that endocrine events associated with lactation lead to a general loss of resistance mechanisms which, in unbred animals, prevent the establishment of newly acquired worms, prevent arrested worms from developing and limit the fecundity of such adult worms as are present. (For a robust exposition of this viewpoint, see Kelly 1973.)

Evidence presented by Blitz and Gibbs (1971), Cvetković *et al.* (1971) and others suggests, however, that the resumed development, of *Haemonchus contortus* at least, occurs independently of parturition. Michel (1974) has proposed a simple explanation which, he claims, can account adequately for all the observed facts. According to this theory, the resumed development of arrested worms occurs at a particular time of year in all animals whether pregnant, lactating or empty, but the subsequent fate of the worms is affected by parturition or lactation. Like populations of *Ostertagia ostertagi*, those of *H. contortus* and other trichostrongylids are turned over rapidly, the average life span of adult worms being short (Michel, 1963; Donald *et al.*, 1964; Dineen and Wagland, 1966; Whitlock *et al.*, 1972). It is necessary to postulate only that the loss of worms is suspended during lactation. In the dry or barren ewe, newly acquired worms that become established, or arrested worms that have resumed their development, have a very short life, so that only few adult worms are present at any time and of these only few remain long enough to reach an appreciable rate of egg laying; whereas in the lactating animal, the adult worms persist and large numbers accumulate and grow to full maturity. At the end of lactation, or a little earlier, the normal loss of worms is resumed and worm numbers rapidly decline to low levels.

This theory accounts for certain facts: that worms taking part in the post-

parturient rise may be either newly acquired from the pasture or arrested forms that have resumed their development; that a rise in egg count, though to a lesser extent, may occur in empty animals in the spring; that a post-parturient rise may occur in ewes lambing in the autumn (though presumably only if they are exposed to infection at the time); and that little or no rise occurs in unbred animals in autumn. It also explains the findings of Cvetković *et al.* (1971), Brunsdon (1967) and Salisbury and Arundel (1970) that the post-parturient rise may be reduced or absent if ewes lamb appreciably before or after an optimum time. The close relationship seen by Crofton (1954) and a few other workers between the date of lambing of individual ewes and the peak of their faecal egg count, suggests either that the resumed development of the worms involved was spread over a considerable period or that the rise was due to worms newly picked up from the pasture, or both.

While, in sheep, the chief significance of the post-parturient rise is that it provides an important source of infection for the lambs, a number of workers (e.g. Connan, 1973) believe that the clinical effect on the ewe cannot be neglected. Apart from reports of clinical disease in housed ewes due to the resumed development of *H. contortus* (Gibbs, 1964) and *Ostertagia circumcincta* (Reid and Armour, 1973), a loss of production has been demonstrated by Leaning *et al.* (1970) in anthelmintic trials in ewes.

V. Parasitic Gastro-enteritis in Sheep

A. Population Growth

When the subject of parasitic gastro-enteritis in sheep was reviewed in Volume 7 of "Advances in Parasitology", the central issue appeared to be whether disease-producing infections were built up by exponential population growth through several generations, as held by Crofton (1955, 1963), or whether, as argued by the present writer, a single generation was involved, the eggs passed by the ewes during the post-parturient rise being the source of almost the whole of the worm burden that caused disease in the lambs. The question has now been largely settled and Boag and Thomas (1973) state categorically that "the increase in faecal egg count . . . is clearly not logarithmic and in this climatic area" (NE England) "there is no evidence for the occurrence of six or seven successive generations of parasites causing an exponential increase to high population levels as suggested by Crofton (1963)". Donald (1969) also has pointed out that in Australia, free-living development of several species of trichostrongylid nematodes takes far longer than was formerly thought.

LeJambre and Ractliffe (1971), however, claim that in upstate New York *H. contortus* completes several generations in the season. They base this contention on the finding that there is a seasonal shift in the proportion of different sub-types of females with linguiform vulval flaps, those without cuticular inflations becoming relatively more numerous as the grazing season advances than those with these inflations. This shift appears to be of regular occurrence in natural populations (Le Jambre and Whitlock, 1968; Slocombe,

1973), and in experimental conditions, LeJambre and Whitlock (1968) were able to demonstrate that the proportion depended on when the worms were picked up from the pasture. They regard this as evidence of the occurrence of a succession of generations, but it is clear that even if the presence or absence of cuticular inflations is genetically determined, which it may not be, then their results need mean no more than that the larvae of one morph survive better on the pasture than those of the other, so that the proportions within a single generation would change with the passage of time.

Progress in work on parasitic gastro-enteritis in sheep has been in three main directions. The essentially seasonal nature of the phenomenon of arrested development has been recognized and seasonal changes in the structure of populations studied. The nature of the post-parturient rise is better understood. The epidemiology of parasitic gastro-enteritis in ewes and lambs in Britain has been worked out in detail and this has led to clearer thinking on the design of control measures.

B. THE SUCCESSION OF DOMINANT SPECIES

As the grazing season progresses, the relative numbers of worms of different species present in lambs changes. Crofton (1957) attempted to explain this in terms of the generation interval and relative fecundity of each species. Since the basis on which these calculations were made (that the generation interval was near its theoretical minimum for several months of the year) can no longer be regarded as valid, a new explanation is needed.

Brunsdon (1970), who observed a succession of species in post mortem worm counts of lambs in New Zealand, sought the cause in differences in the resistance of the host and the spontaneous elimination of the infection. Boag and Thomas (1971) thought that the succession was occasioned by "the extent to which some species overwinter on the pasture while others must be perpetuated by the ewe", a suggestion that might explain some of the observations of Fabiyi (1973).

Gibson and Everett (1971a) contaminated a series of paddocks by running on each, lambs experimentally infected with a different species, and found that the first appearance of larvae as well as the peak of herbage infestation was in the usual order: *O. circumcincta*, *H. contortus*, *Trichostrongylus* spp. They therefore explained the succession in terms of the rates of free-living development of different species.

It seems probable that the explanation ultimately accepted will contain elements of all three theories.

C. EPIDEMIOLOGY OF PARASITIC GASTRO-ENTERITIS IN EWES AND LAMBS

Boag and Thomas (1971) confirmed that the worm eggs passed by ewes in the course of the post-parturient rise were the source of nearly all the worms that were the cause of disease in their lambs. Where ewes and lambs ran on initially clean ground, the patterns of egg output and herbage infestation were exactly as described by Heath and Michel (1969), the eggs passed by the

ewes appearing as infective larvae on the herbage rather suddenly at the end of June or in July. According to the studies of Gevrey (1969) and Reid and Armour (1972) the peak of herbage infestation may occur a month or so later than this.

The high herbage infestation due to contamination of the pasture by the ewes gives rise to large worm burdens in the lambs and a great output of worm eggs in their faeces. By continuing their observations longer than had Heath and Michel (1969), Boag and Thomas (1971) and Gibson and Everett (1973) showed that this in turn gave rise to a second and rather lower peak of herbage infestation in the autumn. (See Fig. 4). This might not represent as frequent

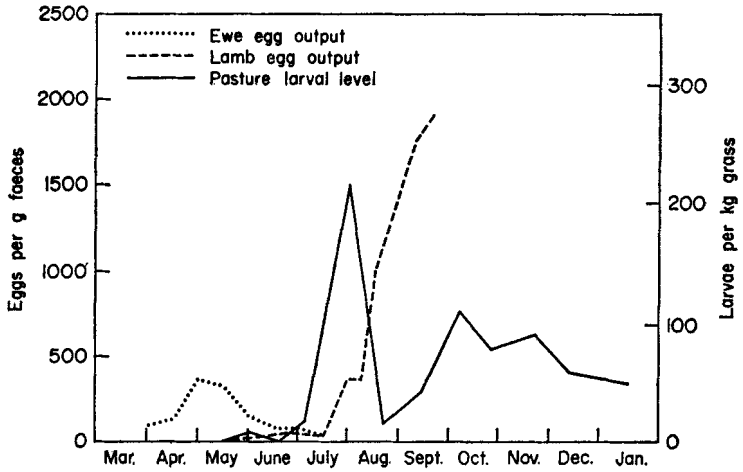

FIG. 4. The pattern of egg output and herbage infestation on an initially clean pasture grazed by ewes and lambs, showing a first peak of herbage infestation in July due to eggs passed by the ewes and a second peak in October/November due to eggs passed by the lambs. (From Boag and Thomas, 1971.)

a cause of disease in the lambs as the July peak but it is likely to be the chief source of infection in the following year, surviving either on the pasture or in the host in the form of arrested larvae. In New Zealand, Vlassoff (1973) has also described two peaks of herbage infestation, due apparently to eggs passed by ewes and by lambs respectively, but in his case the second peak was considerably greater than the first.

If there is an overwintered infestation on the pasture, the lambs will become a source of worm eggs much sooner and will make a significant contribution to the disease-producing infestation. Thomas and Boag (1972) analysed the relative parts played by the overwintering pasture infestation and the post-parturient rise in an experiment comparing four situations in which these factors were either present or absent. It was shown that larvae developing from the eggs passed by the lambs in June appeared as infective larvae on the herbage at exactly the same time as those passed by the ewes.

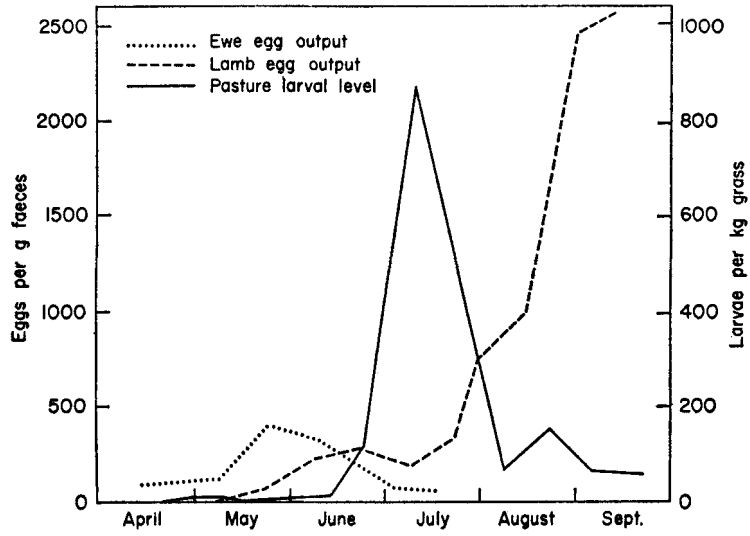

FIG. 5. The pattern of egg output by ewes, of herbage infestation and of egg output by lambs on a pasture on which there is no overwintered infestation of gastro-intestinal nematodes. (From Thomas and Boag, 1972.)

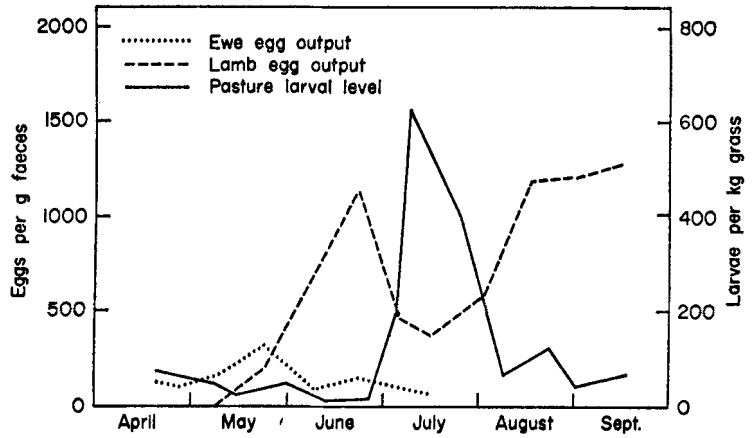

FIG. 6. The pattern of egg output by ewes, of herbage infestation and of egg output by lambs on a pasture carrying an overwintered infestation of gastro-intestinal nematodes. (From Thomas and Boag, 1972.)

The egg output of the lambs showed two peaks, one due to the overwintering pasture infestation and the other to the July peak. The second peak of egg output was lower, relative to the herbage infestation to which it was referable, than the first, and lower than that seen in lambs that had not been exposed

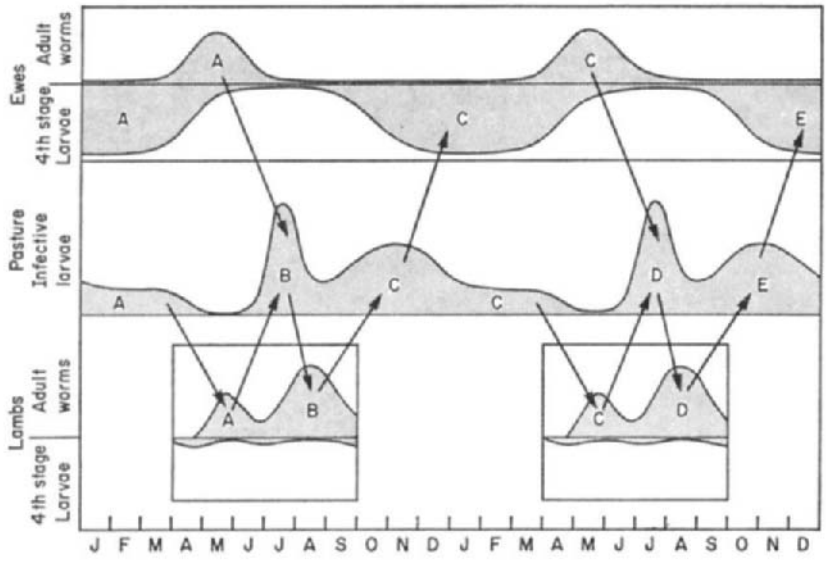

FIG. 7. Generations of *Ostertagia* spp. in ewes and lambs and on the pasture grazed by them.

to the overwintered herbage infestation. These two patterns, on initially clean pasture and on pasture carrying an overwintered infestation, are shown in Figs 5 and 6. Gibson and Everett (1973), who simulated both autumn contamination of the pasture and that due to the post-parturient rise by spreading infected faeces on their paddocks by hand, obtained broadly similar results.

In a further experiment, carried out when the overwintering pasture infestation was uncommonly large, Thomas and Boag (1973) demonstrated not only that the growth of the lambs could be affected directly by the overwintered infestation, but also that in these circumstances the post-parturient rise could not be materially reduced by anthelmintic treatment of the ewes.

In Fig. 7 an attempt has been made to analyse the generations of *Ostertagia circumcincta* completed over a 2 year period in ewes and lambs remaining on the same pasture.

D. THE CONTROL OF PARASITIC GASTRO-ENTERITIS IN LAMBS

In Britain, two strategies for the control of parasitic gastro-enteritis in lambs are considered. Either egg output by the ewes is suppressed by anthelmintic treatment, given either just before or shortly after lambing, or the lambs are moved in late June or during July from the pasture which they and the ewes have grazed up to that time.

Boag and Thomas (1973) summarized the problem as follows: "For April lambing flocks" (in NE England) "the normal 14 to 16 week suckling period

is just sufficiently long to permit the transmission of the major wave of infection from ewe to lamb before separation, and weaning unfortunately tends to coincide with maturation of the lamb worm burden. This explains he strategic importance of measures aimed at controlling either the deposition of contamination by the ewes or its acquisition by the lambs." It also focuses attention on the date of lambing.

It is now recognized that the choice between these two strategies must depend on whether the ewes and lambs initially graze a clean pasture or one on which there is an overwintered infestation.

Working with ewes and lambs on an initially clean pasture, Boag and Thomas (1973) have studied the relative benefits from dosing ewes at lambing, lambs at weaning, or from moving the lambs to clean pasture at weaning. They examined the effect of these measures separately and in all combinations and found, not surprisingly, that provided the lambs were moved at weaning it mattered little whether they or the ewes had been dosed. But if the lambs remained on the same pasture after they were weaned, dosing the ewes gave a far greater measure of control than dosing the lambs.

Dosing ewes either before or shortly after lambing has been widely advocated (Leiper, 1951; Nunns *et al.*, 1965; Brunsdon, 1966; Herweijer, 1969), and Reid (1973) regarded it as the standard method of control against which the costs and benefits of other procedures could be judged. Not only have doubts been cast on the efficacy of such treatment, for example by Arundel and Ford (1969), especially against *Ostertagia* spp. (Sewell, 1973), but the procedure is clearly inapplicable on infested pasture where the lambs become a source of contamination and contribute materially to the disease-producing infestation, and where the post-parturient rise is partly or largely due to newly acquired worms and cannot be suppressed (Arundel, 1971). In these circumstances, reliance must be put on moving the lambs to another pasture before a dangerous infestation is present on the herbage.

On the basis of 4 years' observations, Thomas and Boag (1973) concluded that the date on which the disease-producing infestation appeared on the herbage was fairly constant and pointed to the practical significance of this.

Gibson and Everett (1968a, 1971b) have continued their experiments on the effect of dosing lambs and moving them to clean pasture in mid July. They found that moving the lambs had a marked effect in preventing loss and that lambs which were dosed when they were moved showed marginally better weight gains than those that were moved but not dosed. It is probable that in these lambs which were moved when the disease-producing generation of worms was already present, this difference was due to the removal of worms able to do damage rather than to a reduction or postponement of the contamination of the clean pasture. In a later experiment on the same theme, Gibson and Everett (1973) advanced the date of moving to clean pasture to late June.

Almost inevitably the date on which lambs are moved to clean pasture will also be the date on which they are weaned. A number of studies made in widely different conditions were concerned with the effect of the date of weaning on worm burdens of lambs. An extreme case was reported by

Cameron and Gibbs (1966) in Quebec, who showed that lambs weaned before they were turned out in late spring and which therefore never grazed with the ewes, were much less heavily parasitized than those weaned later. In New South Wales, Lewis *et al.* (1972) found that lambs weaned at 3 or 6 weeks old had smaller worm burdens than those weaned at 12 weeks, and Levine *et al.* (1960) in Illinois have shown that lambs weaned on 1st May were less heavily parasitized than those weaned later. But the advantage of early weaning in terms of lower worm burdens is offset, as demonstrated by Southcott and Corbett (1966) and by Bizzell *et al.* (1964), by the effects of poorer nutrition.

It may be, since the nematode hazard arises at a particular point in the calendar rather than at a given interval after lambing, that earlier lambing might therefore be advantageous. This question has been studied by a number of workers. Thus, Southcott *et al.* (1972) in New South Wales, compared the worm burdens of lambs born in winter, spring and summer, and found that spring born calves had the largest worm burdens at weaning. Knight *et al.* (1973) in Nebraska obtained similar results. In the Eastern Transvaal, Thomas (1967) showed that lambs born at the end of the rainy season remained almost worm free for several months, and in Lesotho, Fitzsimmons (1971) commented on the advantage of lambing at this time. But as conditions that promote grass growth and those that favour the transmission of trichostrongylid infection tend to be the same, problems of management may need to be solved if the lambs are to be born at such a time that they may be weaned before the transmission of a large infection can occur.

In Britain, the necessary adjustments to the farming system may not be too difficult, but a more serious difficulty may arise because very early lambs are more likely to encounter a dangerous overwintering infestation. In some years the overwintered infestation can affect the growth even of April-born lambs, and Thomas and Boag (1973) therefore suggest that if ewes and lambs graze an infested pasture in the spring, they should be dosed and moved to a silage aftermath in May. The reasoning behind this advice is not easy to follow. Presumably, in the situation that Thomas and Boag are considering, no clean pasture is available in April. Their advice therefore depends on the assumption that taking a cut of silage from a pasture materially decreases the concentration of larvae in the herbage. This has not been demonstrated and on theoretical grounds it is not very likely. Moreover, silage aftermaths are unlikely to be available until late in May by which time, according to Thomas and Boag, the overwintering infestation would have reached a harmless level.

It is clearly desirable that ewes and early lambs should not graze pastures carrying a heavy overwintered infestation. Where lambs are moved at weaning it will be the pastures to which they were moved and which they grazed in the second half of the season that will be liable to carry a heavy infestation in the following spring. A system in which ewes graze the same group of pastures throughout the year while the lambs are weaned onto aftermaths would avoid damage from the overwintered infestation. Such a system would, however, entail a hazard of nematodiriasis, the control of

which demands that the lambs should not graze pasture grazed by the previous year's lambs during the first half of the grazing season. One possible solution might lie in alternating the pastures which lambs graze before and after weaning, annually, and dosing the lambs when they are moved. But this hypothetical situation, in which no pasture that is relatively free from infestations of both *Nematodirus* spp. and other trichostrongylids is available in early spring, is likely to be rare. Where sheep are not the only class of stock, no difficulty should arise and many systems of management can be devised. Among these, the annual alternation of sheep and cattle on different parts of the farm, which has been studied by Helle (1971), not only simplifies the control of parasitic gastro-enteritis in calves but also provides effectively clean pasture for ewes and lambs.

Southcott (1971) has expressed the view that helminth control may be more important in fat lamb production than in the rearing of replacements for the breeding flock. In Britain this may not be so. It is the aim to have fat lambs ready for market in July and if this is achieved they are away before parasitic gastro-enteritis becomes a problem. But it is questioned by many flockmasters whether elaborate or costly control measures are justified for lambs that are not fat in July and which cannot be advantageously sold in the autumn when prices are low. Such lambs, if they are not to be excessively heavy at Christmas, need to grow at only a modest rate. In the case of replacements for the ewe flock, on the other hand, especially where the best lambs are put to the tup, sustained and rapid growth is desirable.

A number of practices which were formerly believed to have relevance to the control of nematode infection continue to be revealed as ineffective. Just as Levine and Clark (1961), Gibson and Everett (1968b) and Smeal *et al.* (1969) have shown that rotational grazing is without effect on burdens of gastro-intestinal nematodes, so Donald (1969) contends that in Australia the practice of spelling pastures contributes nothing to the control of trichostrongylid infection. Jordan and Marten (1970) have compared rotational grazing of ewes and lambs with forward creep grazing (Dickson, 1959) but could demonstrate no difference in either live weight gain or worm burdens.

Less emphasis has been put in recent years on tactical drenching (the practice advocated where arid conditions limit the transmission of worms), giving anthelmintic treatment after an appreciable fall of rain. Fitzsimmons (1971), however, advocates this in Lesotho.

VI. Parasitic Gastro-enteritis in Cattle

A. Experiments with *Ostertagia ostertagi*

The factors which determine the size of populations of *O. ostertagi* and which were discussed in Volume 7 of "Advances in Parasitology" have been further studied. The conclusion that the chief mechanism was a loss of adult worms at a constant rate, which tended to maintain the worm burden at a level proportional to the rate of new infection, was called in question by the

results of Anderson *et al.* (1969). These workers found that the worm burden of calves that remained on a pasture until clinical ostertagiasis appeared, were equal to the total of the worm burdens of an unbroken sequence of tracer calves which had grazed the pasture for short periods. They deduced that the worms that were the cause of disease had been accumulated throughout the spring and early summer. A closer examination of the data shows, however, that the "permanent" calves that were autopsied were the most severely affected, while the tracers represented a random sample. If this is taken into account the results do not provide evidence of a sustained accumulation of worms.

Michel *et al.* (1973a) have shown that cattle exposed to regular infection gradually acquire a resistance to the establishment of *O. ostertagi.* In calves infected with 1000 larvae daily for 250 days, only one-twentieth as many larvae become established as in uninfected controls. This resistance, together with a rapid turnover of worms, can entirely account for the course of experimental infections. This is illustrated in Figs 8 and 9.

It is believed that in the field, cattle in their second grazing year have a considerable resistance to the establishment of *O. ostertagi,* and there is some evidence that the worm population is turned over more rapidly in older cattle than in calves. Smith and Archibald (1968a), while demonstrating that yearling cattle were refractory to infection with *C. oncophora* at the stage of their second grazing season, found them to be still susceptible to *O. ostertagi.* The susceptibility of yearling and adult cattle is a subject of more than academic interest, partly because some procedures for the control of parasitic gastro-enteritis in calves tend to expose older cattle to heavy infestations and partly because outbreaks of ostertagiasis in adult cattle, usually in recently calved heifers, are increasingly reported (Hotson, 1967; Wedderburn, 1970).

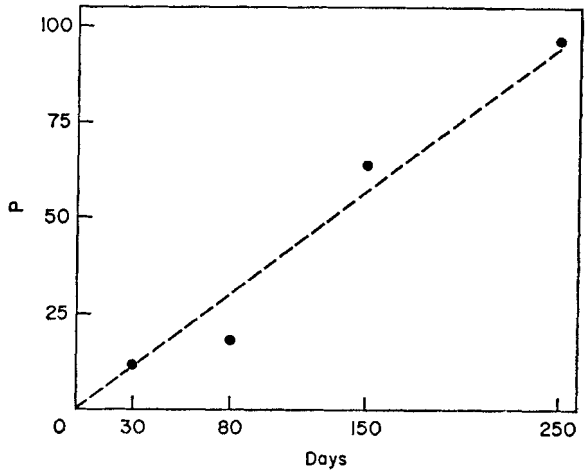

FIG. 8. Resistance to the establishment of *Ostertagia ostertagi* (P) of calves receiving 1000 larvae daily. (Drawn from data published by Michel *et al.*, 1973a.)

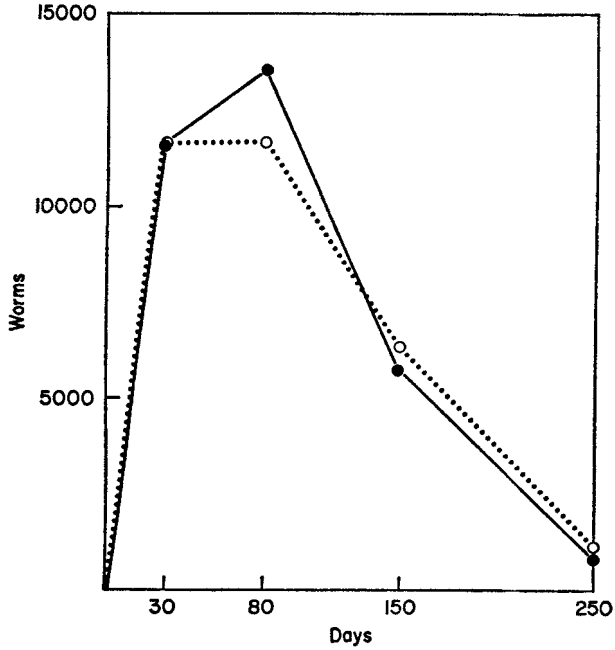

FIG. 9. The worm burden of calves receiving 1000 larvae of *Ostertagia ostertagi* daily (continuous line), and calculated values (broken line) based on a mean life of 28 days and the increasing resistance to the establishment of worms shown in Fig. 8. (From Michel *et al.*, 1973a.)

In this connection, Michel, Lancaster and Hong (unpubl. obsvns, 1974) have found that in experimentally infected Friesian heifers there was a loss of resistance about the time of parturition, and that severe disease could result in down-calving heifers from a rate of infection that was without effect on unbred control heifers.

B. INFESTATIONS ON THE HERBAGE

The seasonal pattern of infestations of *O. ostertagi* and *C. oncophora* has been further studied by Michel (1969d) and Michel *et al.* (1970) in Britain, Kloosterman (1971) in Holland, Brunsdon (1972) in New Zealand and Downey (1973) in Ireland, all with closely similar results. The factors underlying this seasonal pattern are also better understood. Rose (1970) examined the causes of the sudden increase in the herbage infestation in July and identified the following factors: (a) development to the infective stage is slower early in the season than later; (b) the faecal egg count of calves turned out in late April tends to reach its peak in June; (c) wet weather is needed for the emergence of larvae from the faeces; (d) time is needed for the larvae to migrate far enough from the faeces to be available; (e) fewer of

the eggs passed early in the season reach the infective stage. Rose also concluded that September was effectively the end of the season during which new herbage infestations could be created, eggs passed after the end of that month perishing before they could reach the infective stage.

Meanwhile, Michel *et al.* (1970), on the basis of a field study, concluded that the time taken from contamination of the pasture to the first appearance of infective larvae in herbage samples decreased from 3 months for fields contaminated in March to 2 weeks for fields contaminated in July, and then gradually increased again. They confirmed that the increase in pasture infestation in July was sufficiently regular to serve as a basis for control measures. Armour (1970), Kloosterman (1971), Brunsdon (1972) and Downey (1973) are also of this opinion, and in New South Wales, Smeal (cited by Hotson, 1974) also bases a system of control on the occurrence of a predictable pattern.

Michel (1971a) believes that a population of larvae on the herbage should be visualized as being in a state of dynamic equilibrium. If it is measured as a concentration per unit weight of herbage (and this is the most meaningful way to express an infestation), the most important cause of depletion is the diluting effect of herbage growth. Recruitment to the population is by the emergence of larvae from the faeces which act as a reservoir (a view shared by Anderson, 1971 and Hotson, 1974). The annual pattern can be explained in terms of these two processes. For reasons already discussed, emergence of larvae from the faeces begins rather abruptly in July, but grass growth is rapid and in an average year the effect of dilution soon comes to balance emergence, and the infestation remains fairly constant into the autumn. In winter, grass growth is negligible but the release of larvae may be speeded by the disintegration of the faeces. Indeed the highest levels of herbage infestation are encountered in the winter. In spring, grass growth is resumed and there is considerable mortality among the larvae, and because the faecal reservoir is entirely depleted, the population very rapidly declines.

There are slight departures from this pattern in very wet and in very dry summers. If July is very wet a larger proportion than usual of the larvae emerges from the faeces and the herbage infestation rises to a high level, but because few remain in the reservoir, levels on the herbage in autumn and winter tend to be low. If summer and early autumn are uncommonly dry, there is little emergence until the return of wet weather. Accordingly, infestations on the herbage in late autumn and winter tend to be very high. Armour (1970) and Ollerenshaw and Smith (1969) suggest that there is a high incidence of winter ostertagiasis in the spring following a dry summer and also believe that in such a spring, herbage infestations may remain at a potentially dangerous level for longer than usual.

In almost any year outbreaks of disease due to the overwintering pasture infestation may occur, in Britain, in calves turned out before the middle of April, but on occasion pastures may remain dangerously infested until the middle of May. Taylor *et al.* (1973) have described two outbreaks in Ireland in calves turned out on 9th and 15th April respectively. In one case susceptible calves which grazed the pasture from 2nd to 16th May became severely affected.

Where winters are longer and colder, the survival of heavy infestations on the herbage appears to be common. In Canada, Smith and Archibald (1969), Smith (1972) and Slocombe (1974) have demonstrated that larvae of *Ostertagia* spp. and of *Cooperia* spp. persist well through the winter, and Smith and Archibald (1968b) have shown clinical parasitic gastro-enteritis to occur in calves within a very few weeks of their being turned out in June. In Norway, Tharaldsen (1970) has found overwintering infestations of *O. ostertagi* and *C. oncophora* to be the cause of disease in calves turned out at the end of May. It appears that where winters are long and the grazing season short, each generation of the worms occurs in a different crop of calves.

C. WINTER OSTERTAGIASIS

Progress in the study of winter ostertagiasis or, as it is not infrequently called, ostertagiasis type II, was reviewed by Michel (1974), who discussed the evidence that the primary cause of arrested development of *O. ostertagi* was the receipt by the free-living stages of signals from the environment. Temperature plays a part, the larvae being more rapidly conditioned when stored at low temperature than at higher (Wright *et al.*, 1973). Michel *et al.* (1975) have also shown that a sudden decrease in storage temperature rapidly increases the proportion of larvae of both *O. ostertagi* and *C. oncophora* that are arrested. These phenomena parallel those described in infections of *Obeliscoides cuniculi* in rabbits by Fernando *et al.* (1971) and Hutchinson *et al.* (1972).

The changes produced in the larvae are reversible. After prolonged storage, even if there is no change in conditions, the larvae lose their aptitude for arrested development. This reversal is hastened by an increase in temperature (Michel *et al.*, 1974, 1975).

The factors that govern the resumed development of arrested worms in the host are still obscure. It is evident that adult worms that are lost or removed are promptly replaced by the development of arrested forms. A burden of adult worms, in an animal also carrying arrested larvae but not exposed to new infection, is therefore turned over, a loss of adults being balanced by the development of small numbers of arrested larvae. The burden of arrested forms can be reduced a little more rapidly by the frequent removal of adults by means of anthelmintic treatment (Michel, 1971b). This implies the operation of a feedback mechanism of some kind; but it is not easy to visualize the mechanisms involved in a situation in which the number of adult worms is determined by the rate at which arrested worms resume their development, while this in turn is controlled by the number of adults present.

It is becoming clear, however, that this regulatory mechanism has only a small effect on large burdens of arrested worms. It appears that in the spring almost the entire burden of naturally acquired arrested *O. ostertagi* develops within a fortnight (Michel 1974), but it is not known whether development always occurs over so short a period nor by what it is occasioned. Bruce and Armour (1974) observed the development of arrested worms in calves infected with experimentally conditioned larvae to ensue $3\frac{1}{2}$ months

after infection, but unpublished observations by Michel, Lancaster and Hong (1974) indicated that arrested worms persisted longer in cattle that were infected with experimentally conditioned larvae in autumn than in cattle infected with comparable larvae in summer. This suggests that development may be triggered by some seasonal signal transmitted by the host, possibly via the endocrine system. The almost simultaneous development of arrested *Ostertagia* could occur (a) after a fixed time from infection of the host, (b) at a certain time after environmental conditioning of the larvae, or (c) at a particular time of year. The question is open to experimental proof and should be fully elucidated within the next few years.

It also remains to determine whether outbreaks of winter ostertagiasis are an inevitable outcome of the presence, in winter, of large burdens of arrested *O. ostertagi* or, to use Scottish terminology, whether pre-type II ostertagiasis invariably leads to clinical type II ostertagiasis. Certainly, there can be no clinical winter ostertagiasis if there is not a sufficient burden of arrested fourth-stage larvae, but the occurrence of disease must depend also on whether large numbers resume their development simultaneously and what is their subsequent fate.

Arrested development of trichostrongylid and metastrongylid nematodes of grazing animals may be seen as an adaptation to aid survival through an unfavourable season. The short adult life of *O. ostertagi* and the failure, in temperate climates, of eggs passed in winter to reach the infective stage, effectively select against worms reaching maturity at this time, for they leave no progeny. As shown by Armour *et al.* (1969), there is considerable variation within and between populations in the ability of the worms to respond to environmental conditioning, and the results of Michel *et al.* (1973b) suggest that populations can change rapidly in response to selection. It is not surprising, therefore, that where hot dry summers lead to the destruction of free-living stages, it is worms picked up by the host in the spring that are arrested. This appears to be the case in parts at least of Australia (Hotson, 1967; Anon, 1973; Smeal, cited by Kelly, 1973).

In many of these cases it was recently calved heifers that were affected. Wedderburn (1970) has described a similar case from New Zealand, and the older literature contains records from many parts of the world of outbreaks of ostertagiasis in recently calved heifers, in most of which records there is circumstantial evidence that the development of arrested larvae was the cause.

Resumed development of arrested *O. ostertagi* appears also to be the cause of loss in feedlots. It is coming to be recognized that anthelmintic treatment of cattle in the feedlot is frequently ineffective and that cattle should be free of worms before they get there (Ames *et al.*, 1969). But as arrested *O. ostertagi* are not susceptible to anthelmintics (Reid *et al.*, 1968; Armour, 1970), the pre-conditioning treatment recommended by Herrick (1967) and others may not be effective even if, as suggested by Gordon (1973), it is repeated several times. Adult worms removed by anthelmintic treatment are promptly replaced by the development of an equivalent number of arrested larvae. Since very large burdens of arrested worms may be associated with

modest numbers of adults, even repeated anthelmintic treatment may make little impact on a large burden of arrested larvae. Douglas and Baker (1968) have therefore suggested continuous medication throughout the period that the cattle are in the feedlot.

Large burdens of arrested worms unquestionably represent a hazard, and Gordon (1973) has discussed the question of whether it is preferable to do nothing or to attempt to trigger development so that the resulting adults can be removed by anthelmintic treatment. As yet, no means of triggering development has been found. Host resistance does not appear to play more than a very minor role and immuno-suppressants do not stimulate development (Pritchard *et al.*, 1974).

The solution to the ostertagiasis problem in feedlots lies in the management of the store cattle on their farms of origin and not, as Douglas and Baker (1968) suggest, in the feedlot. It may be that in the U.S.A. the basic problem is that while the feedlots require a steady intake of stores throughout the year, nearly all the calves are spring born. Calves must therefore be stored on the farms where they were reared, and it is during this phase that they acquire large burdens of arrested *Ostertagia.* Vegors (1958) and Ciordia *et al.* (1971, 1972) have published interesting observations on large burdens of arrested *O. ostertagi* in store cattle in Georgia which had been wintered on specially sown temporary pastures. But whether these burdens were acquired on the winter pastures as Ciordia *et al.* suggest or whether, as seems possible, on the summer pastures after weaning, remains to be determined. This could readily be done by the methods discussed in an earlier section of this review and simple preventive measures designed.

In Britain and northern Europe, routine anthelmintic treatment at the end of the first grazing season is frequently advocated and fairly widely practised. Reports by van Adrichem (1970) and Cornwell *et al.* (1973a,b) claim to demonstrate a measureable benefit from this practice, but it remains very doubtful whether the treatment of thrifty cattle at yarding can be justified.

D. CONTROL OF PARASITIC GASTRO-ENTERITIS IN CATTLE

The suggestions of Michel (1967 and Michel and Lancaster (1970) on the control of parasitic gastro-enteritis in calves have gained a fairly wide acceptance. Meadowcroft and Yule (1972) confirmed that moving calves to aftermaths in mid-July led to better live-weight gains but they pointed to practical difficulties where semi-intensive beef was the only livestock enterprise. In New Zealand, Brunsdon (1972) has found the practise of dosing and moving calves in mid-January to give good control of gastro-intestinal nematodes, and it is recommended by Khouri *et al.* (1969). Downey (1973) regards the method as applicable to Irish conditions. Eckert (1972) in Switzerland favours dosing and moving calves twice, in June and again in August, but does not appear to have demonstrated experimentally that this is more effective than a single move in July.

It is probable that an excessive emphasis was put on this single method

of control. Control measures can be classified into three categories. First are practices by which the contamination of pasture is prevented, usually by letting hand-reared calves graze new leys or other clean pasture during the first half of the grazing season. The possibility of reducing the contamination of the pasture by anthelmintic treatment during the first half of the grazing season has been investigated by Bürger *et al.* (1970) without encouraging result. Second are procedures in which the contamination of the pasture is not prevented but the calves are removed from the contaminated pasture before it becomes infective. Either the calves are moved twice, once in mid-July, generally to aftermath, and once in August, or a single move to aftermath in July is accompanied by anthelmintic treatment to reduce the contamination of the aftermath. In control measures of the third kind contamination of the pasture is reduced by dilution. In conditions of even moderately intensive grazing, where all the herbage grown is consumed by the stock, there is an almost constant relationship between the weight of herbage grown and the quantity of faeces with which it is contaminated. Accordingly, reduction in the average worm egg content of the faeces proportionately reduces the likelihood that heavy herbage infestations will occur. This reduction may be achieved by grazing helminthologically inert stock together with the calves. A proposal by Leaver (1970) for the intensive rearing of dairy replacements by a modification of the Ruakura system (McMeekan, 1947) belongs to this category. But while in the Ruakura system the calves graze rotationally ahead of the dairy herd so that the infected faeces that they pass are diluted by at least a factor of 12, Leaver's calves graze ahead of an equal number of heifers achieving a dilution of only one in three.

The same process of dilution accounts for the common observation that the single suckled calf is not affected by parasitic gastro-enteritis while running with its dam (Winks, 1968). A study by Michel *et al.* (1972) showed that the worm egg output of the single suckled calf was no less than that of the hand-reared calf, but on the pastures grazed by the calves and their dams, large herbage infestations did not arise because the egg output of the cows, which constitute a very large part of the grazing force, was very low, even during the course of a small periparturient rise. But if the calves graze together in the absence of the cows, which they normally do after they are weaned, dangerous infestations can arise on the herbage. This hazard is restricted, however, to autumn born calves (which are weaned in summer) and may be avoided by not allowing the calves to remain for more than three or four weeks on the pasture onto which they were weaned.

Some advice on the control of parasitic gastro-enteritis is still on strongly traditional lines. Georgi *et al.* (1972), for example, in a serious case of "primary gastro-intestinal strongylosis", advised that replacement stock should be reared indoors to the age of at least 1 year and that yearlings be grazed at a low stocking density. Enigk (1970) advocates regular and frequent change of pasture (rotationally, it is to be presumed) and integration of grazing and conservation. Ciordia (1969) recommends rotational grazing although he has not been able to demonstrate the practice to affect worm burdens. Cornwell and Jones (1971) claim to have shown that routine monthly anthelmintic

treatment in the second half of the grazing season is economically advantageous. Neumann and Kirsch (1968) believe that prophylactic dosing should be timed with the benefit of scientific guidance based on the frequent examination of faeces samples. Laudren and Raynaud (1973) think that treatment should be given after periods of optimal free-living development as determined according to the criteria of Levine (1963).

VII. Parasitic Bronchitis in Cattle

The past six years have not seen any startling developments in the study of parasitic bronchitis, but a number of aspects of epidemiology and of control have been worked out in greater detail.

Gupta and Gibbs (1969) examined lungs of young cattle from a slaughterhouse in Quebec province and found that while in calves the number of lungworms showed a single peak in late summer or autumn, in yearlings there was, in addition, a second peak in the spring. This spring peak is not reflected in the seasonal pattern of faecal larval counts described by Hendriksen (1967) in Denmark, but this anomaly may be due to his use of samples submitted to confirm diagnoses of clinical lungworm disease. Gupta and Gibbs (1970) demonstrated that while the overwinter survival of larvae on the pasture was highly precarious, carrier animals played an important role. Moreover, calves which stopped passing lungworm larvae in their faeces in the winter began to do so again in the spring (see Fig. 10). In similar observations

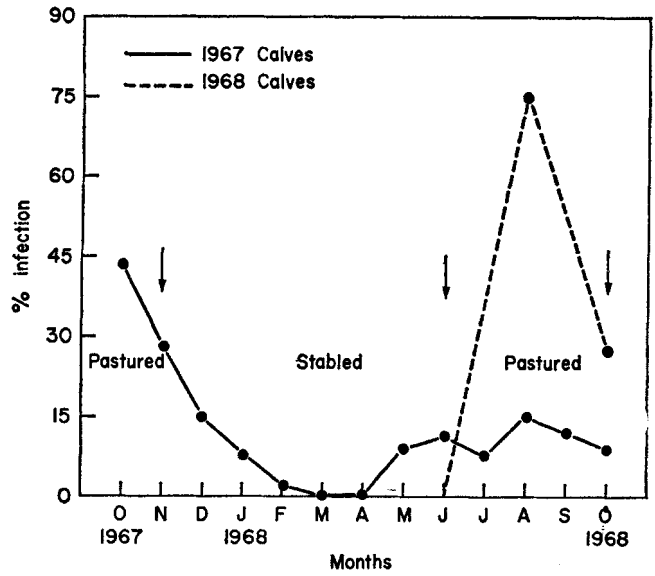

FIG. 10. The incidence of calves passing larvae of *Dictyocaulus viviparus* in their faeces, showing the disappearance of patent infections in the winter and their reappearance in the spring before the animals go to pasture. (From Gupta and Gibbs, 1970.)

Supperer and Pfeiffer (1971) were able to demonstrate that this resumed output of larvae was not prevented by treating the calves, during the winter, with an anthelmintic which removed adult worms but was without effect on immature stages. From these observations and those of Pfeiffer (1971) it is clear that arrested worms in the host which develop to maturity in the spring represent the most important means of overwinter survival.

Düwel (1971) sees the events that lead to an outbreak of disease as the infection, from an overwintering pasture infestation or from carrier animals, of a proportion of the calves, and the creation by these of a disease-producing infestation on the herbage. Eckert (1972) visualizes the increase in populations on the pasture and in the calves as a rather more gradual process which leads to disease when particularly favourable conditions for transmission occur in July or later. But the rather difficult critical experimentation needed to elucidate the relationship between developing host resistance and the challenge that the calves meet, and to identify the generations of the parasite involved, does not appear to have been attempted.

Equally neglected is the probable interaction between lungworm disease and parasitic gastro-enteritis. That intercurrent infections of stomach and intestinal worms adversely affect resistance to lungworms has long been suspected and is hinted at by Düwel (1971).

Pouplard (1968) discussed three approaches to the control of dictyocauliasis: (1) Isolation of calves on entirely clean pastures which will however leave them susceptible; (2) vaccination; (3) rotational grazing. On this third technique, which is very much his own, Pouplard offers some mature observations, noting that, in the system which he advocates herbage infestations arise which are dangerous to susceptible calves that are added later but which are without effect on calves that have grazed since the beginning of the season. It therefore appears that in so far as Pouplard's rotational system is successful in controlling lungworm disease, it is by reducing the potential rate of increase in the challenge to which the calves are exposed.

Michel (1969c), in listing three approaches to control, omitted rotational grazing but included "vigilance and anthelmintic treatment" on the basis that if treatment is given promptly as soon as the first symptoms appear, no further steps need be taken because the calves should by this time be almost refractory to further infection. A report by McCulloch *et al.* (1968) is sometimes quoted as evidence that calves treated for husk may relapse if left on infested pasture, but it is not clear to what extent this conclusion drawn from uncontrolled observations may have been related to parasitic gastro-enteritis.

Unquestionably, vaccination with larvae attenuated by X-irradiation has become accepted as the most eligible method of control where the hazard is high. Poynter *et al.* (1970), reviewing 10 years' experiences with the commercial vaccine, extolled its merits and analysed causes of breakdown. Among these are an adverse effect on immunity to husk of debilitating diseases, especially other forms of parasitism and pneumonia, and faulty grazing practices, i.e. the sudden exposure of vaccinated calves to heavy infestations built up by unvaccinated animals. Like Blindow (1966), Poynter

et al. (1970) note that vaccinated calves frequently pass lungworm larvae in their faeces, but they consider this to be, if anything, an advantage in that the resulting infestations help to reinforce immunity. This point is also made by Eckert (1972). Clearly, vaccination could have no part in any policy aiming at the eradication of lungworms. It is therefore surprising that Swiss cantons have been given the power to compel the vaccination of calves at the public expense.

VIII. Opinions on the Control of Nematodes

The view was expressed in Volume 7 of "Advances in Parasitology" that methods used for the control of nematodes should, in each case, be based on the factors which restrain the increase of parasite populations in the field, that these measures should have quite specific objectives, and that practices having a small effect could be regarded as irrelevant. The alternative view that control may be achieved by the cumulative action of many small factors and practices of limited effect is still advanced by a number of authors. Eckert (1972), in discussing the control of dictyocauliasis, stresses a number of practices such as land drainage, the provision of hygienic drinking troughs and the avoidance of heavy stocking, the effect or relevance of which might well be questioned but which he sees as reinforcing other measures.

The standpoint, which is also taken by Kirsch (1969), that almost anything that might be harmful to parasites must be worthwhile, occasionally leads to contradictions. Thus Enigk (1972a,b) advocates the use of elaborate installations to render slurry or dung entirely helminth-free, but suggests grazing practices which do not exclude infection from other sources and which, indeed, assume it to be present.

According to Gordon (1973) the aim of control measures (against gastro-intestinal nematodes) is to "detect, delay and deter", to slow down the process of population increase so that a resistance may develop before large worm burdens are present. But this position, which is shared by Luffau (1973), is not applicable to the common species of stomach and intestinal nematodes. There is, however, some substance in the warning by Gibson (1973), that control measures should not so effectively withhold young animals from access to infection as unduly to delay the development of an acquired resistance.

There are also differences of opinion on the design and implementation of control measures and their integration into farming systems. A number of workers believe that, ideally, a preoccupation with worms as such should be limited to those engaged in research and development, and that the adviser should offer the farmer a choice of *ready-made* management systems which, among their multifarious advantages, ensure a freedom from the hazard of helminthiasis and which can be operated without any knowledge of parasitology. In contrast, Gordon (1973) and others consider not only that control measures should be *made to measure* for each farm but also that they must contain a tactical element. This means that the adviser, be

he veterinarian or agronomist, must make an "appraisal of the situation" by means of an "epidemiological excursion" and that the farmer must continuously make such appraisals and act accordingly.

The conflict between the consistent use of proven systems of management and the tactical use of expedients enters also into the use of forecasts and other aids to scientifically guided opportunism which are designed to take the place of continuous assessments by the farmer. This approach may be questioned both on the score of feasibility and on more fundamental grounds. For example, Ross and Woodley (1968) issue warnings of the need to take measures against nematodiriasis in Northern Ireland on the basis of the fortnightly examination of faeces samples from a number of flocks. But when it is considered that infections of *Nematodirus* spp. can exert their damaging effect before they are patent and that, in England at least, herbage infestations may rise very rapidly, it is evident that the success of these warnings would depend, among other things, on a body of experience indicating that in the area covered the increase in the herbage infestation is always slow and of constant form.

Ross and Woodley (1968) use a similar basis for advice on the control of parasitic gastro-enteritis in calves. "Warnings of development of infection in the spring and late summer are based on the analysis of several thousand routine faeces samples received at the laboratories from veterinary surgeons. Whenever rises in the strongyle faeces counts are observed, warnings are issued." In view of the stereotyped pattern of egg counts due to *O. ostertagi* in calves, it is open to question how meaningful such warnings can be. Neumann and Kirsch (1968) operate a similar system of monitoring faecal egg counts from individual farms in Schleswig-Holstein and recommend anthelmintic treatment whenever the mean egg count of a group of calves exceeds 300/g. Stampa and Linde (1972) in South Africa believe that monitoring the faecal egg output of sheep would be a valuable aid to control, but they question whether such counts give a sufficiently accurate estimate of worm burdens for the purpose.

The various forecasts of helminthic disease made on the basis of meterorological data have the same object as schemes of monitoring. In the words of Ollerenshaw and Smith (1969) they "allow the farmer a greater degree of flexibility with regard to the management of stock and the utilisation of his pasture. Limitations of husbandry practices and control of disease are made only when there is real need. In this way more efficient control is achieved at less cost". But this argument may be fallacious. If the action to be taken in response to a warning or forecast consists, for example, of a change of pasture, then it is necessary to have the alternative pasture available every year, even in those when it is not needed. This implies an under-utilization of resources. It is plainly more efficient to follow the same procedure every year even though some other management might have been equally successful in some years.

That measures for the control of helminthiasis must necessarily conflict with agricultural objectives, as is commonly held (Gordon, 1973; Eckert, 1972), is likely to be true only where systems of management have been

devised without regard to the hazard of parasitism. As Spedding (1969) has pointed out, cooperation between specialists of different disciplines is essential.

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The Immunology of Schistosomiasis

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I. Introduction

We began our original review (Smithers and Terry, 1969a) by stating that "the overall pattern of immunity in schistosomiasis remains obscure, full of apparent anomalies and unanswered questions". We are glad to report that at least some of the anomalies have been cleared up and at least some of the questions answered. This has been largely due to the intelligent application of modern immunological and biochemical techniques to the problem, a process aided by advances in basic immunology and the increasing interest shown by immunologists in the problems of anti-parasite immunity.

Problems solved have, of course, been replaced by new problems, but these are being tackled at a different level of sophistication from the rough and ready experimentation of 5 or 6 years ago. The ultimate goal of this work, successful vaccination against schistosomes, is yet to be achieved; but there is a general feeling among workers in the field that, given industry, intelligence, financial support and a modicum of good luck, this aim may be realized within the next few years.

II. Innate Immunity

The only developments relating to innate immunity since our last review (Smithers and Terry, 1969a) refer to the death of cercariae in the skin.

Clegg and Smithers (1968) showed that the skin of some laboratory hosts is a major barrier to cercariae of *S. mansoni*. They recovered schistosomules from the skin of animals within a short time after cercarial penetration and determined their viability by a dye-exclusion test. Up to 50% of the cercariae which enter the skin of rats die shortly afterwards, whereas about 30% die in mouse skin and only 10% in hamster skin. The deaths in the skin occur within the epidermis. After 15 min many of the cercariae have penetrated the Malpighian layer and entered the dermis where few deaths occur.

Using the same technique as Clegg and Smithers (1968), Ghandour and Webbe (1973) studied the factors affecting the death of *S. mansoni* cercariae in the skin of mice. They demonstrated that the age of the cercariae after emergence from the snail is directly related to the number which die in the skin. In mouse skin, approximately 30% of 2 h old cercariae die, but as the cercariae age, the percentage dying rises steadily and after 8 and 24 h mortality is almost doubled and trebled respectively. Glycogen is reduced to about a quarter of the initial value in *S. mansoni* cercariae which have been swimming in water for 18 h (Bruce *et al.*, 1969), and there is a reduced level of glycogen in the cercariae of *Austrobilharzia terrigalensis* which die in bird skin compared with those which survive (Rai and Clegg, 1968). These findings suggest that a low level of energy reserves may be at least partly responsible for the increased mortality of aged cercariae in the skin.

Direct evidence that host age resistance is correlated with cercarial death in the skin is also provided by Ghandour and Webbe (1973). They assayed the number of cercarial deaths in the skin of mice of different ages and showed up to about 1 month old, the age of the mouse directly affects the proportion of cercariae which die. In 2 day old mice, the level of mortality in the skin is less than one third the level found in adult mouse skin. During the first month of life, the number of cercariae dying in the skin rises steadily and reaches the adult level when the mouse is 28–35 days old. They showed clearly that the higher worm recovery in young mice compared with that in older mice was attributable to the lower level of mortality in the skin of younger mice. Lewert and Mandlowitz (1963) believe that age resistance in mice is due to the skin of older mice being more resistant to cercariae than the skin of young mice. It seems likely, therefore, that the death of cercariae in the epidermis is due to exhaustion of their energy reserves during the period of intense activity in the early phase of penetration. As the time spent in the active non-feeding cercarial stage is increased, energy reserves are depleted and fewer cercariae are able to penetrate the Malpighian layer to reach the dermis. Similarly, more energy is required to penetrate the denser skin of older mice compared with the less resistant skin of very young mice, and consequently fewer cercariae survive when penetrating the skin of older animals.

III. Acquired Immunity

A. Concomitant immunity

The concept of concomitant immunity in schistosomiasis was first introduced by Smithers and Terry (1969b). It describes a situation in which

the host is resistant to reinfection but cannot at the same time rid itself of an established parasite population. The concept was based on the observation that established adult *S. mansoni*, derived from an initial infection, persisted long after resistance had developed to a challenge infection (Smithers and Terry, 1965). Support for the concept has developed from studies of *S. mansoni* in the baboon (Taylor *et al.*, 1973a), *S. mansoni* in the mouse (Sher *et al.*, 1974a) and *S. haematobium* in the baboon (Webbe and James, 1973).

The slow development of immunity in monkeys and mice points to the adult worm as a major immunogenic stimulus (Smithers and Terry, 1965; Sher *et al.*, 1974a). Direct evidence of this was provided by Smithers and Terry (1967) who transferred established adult worms surgically into the portal systems of rhesus monkeys. These monkeys, completely without experience of migrating juveniles, strongly resisted cercarial challenge, but at the same time failed to reject the transferred adults. Thus there appears to be an interesting and seemingly paradoxical situation in which adult worms are exempt from the immune response they provoke.

Concomitant immunity has the obvious biological advantage of preventing overcrowding of the parasites in the host. Worms derived from early infections, in conjunction with host immunity, may create a barrier to the continual reinfection which would otherwise threaten the long-term survival of both host and parasite.

It was of major interest to determine whether concomitant immunity operates in human schistosomiasis, and preliminary studies in this area by McCullough and Bradley (1973) have now been reported. These authors performed repeated egg counts on urine collected from a group of school-children at the same time of year for 3 years. The results were surprisingly clear cut. There was a remarkable relative stability of egg output; those children with a high output of eggs in the first year were still at the top of the group in the final year, and those with low egg counts remained low. (However, the absolute values of egg output were more variable, and the authors caution against over-interpretation of the data.)

As the transmission of *S. haematobium* in the area was known to have been maintained throughout the study, the data suggest two possibilities. Either there is a homeostatic control of individual infection, in which new schistosomes penetrate at a rate similar to the loss of old worm pairs; or the schistosomes already present in the schoolchildren have a long life and a steady egg laying rate, and due to some immune process, there is a failure of new worms to establish themselves. McCullough and Bradley consider the first possibility biologically unlikely and they consider that the results provide suggestive evidence for the existence of concomitant immunity to *S. haematobium* in man.

The overall view of Bradley and McCullough (1973) of *S. haematobium* epidemiology is one of progressive infection in the first decade, development of substantial concomitant immunity, a slow decrease in egg output over the next 20 years, reversion of some people to the uninfected state and decay of their immunity, and a steady state of loss of worm pairs and reinfection among older people.

Concomitant immunity represents then a balance between developing host immunity and the evasion of this immunity by the parasite. In order to appreciate this balance and, hopefully, to be able to tilt it in favour of the host, it is essential to gain a detailed knowledge of both the immune mechanisms and the evasion mechanisms. Progress in these two overlapping areas of study is summarized in the next two sections.

B. MECHANISMS OF IMMUNITY

Five years ago it would not have been possible to discuss the mechanism of immunity to schistosomiasis beyond the evidence offered from histological studies (e.g. Lichtenberg and Ritchie, 1961) and the negative passive transfer experiments (Warren *et al.*, 1972). Two reasons which encumbered progress in this area were the lack of a convenient small experimental animal model in which immunity could be easily measured (although the rhesus monkey develops a strong immunity to reinfection it has certain disadvantages as a laboratory animal), and the failure to adapt the *in vitro* culture of schistosomes to immunological studies. Both these neglected approaches are now in use and as a result encouraging signs of progress are evident.

1. *Immunity in mice and rats*

One difficulty which is encountered in studying acquired immunity to schistosomiasis is measurement of host resistance. Antibody or cell mediated activities have not so far been correlated with protection, and resistance to reinfection can therefore be assayed only by following the fate of a challenge infection. This is generally achieved by recovering the adult worms of a challenge infection by perfusion from the liver several weeks after the challenge has been given. The major disadvantage of this technique is the long period required for the parasite of the challenge to mature.

A more rapid assay of immunity, suitable for small laboratory animals, has been developed recently and is based on the recovery of schistosomules of a challenge as they traverse the lungs (Perez *et al.*, 1974; Sher *et al.*, 1974a). In mice and rats, schistosomules of *S. mansoni* can be recovered from the lungs from day 3 onwards, with peak numbers on day 4 or 5 (Clegg, 1965). About 20% of a challenge can be recovered from the lungs of normal mice and 10% from normal rats 5 days after exposure. In animals which have developed immunity there is a considerable drop in the number of schistosomules recovered from the lungs after challenge, indicating that in immune animals a large proportion of invading schistosomules are eliminated in the lungs or at an earlier point in their migration pathway.

The advanatage of the lung recovery assay is that the results become known soon after the challenge has been given and therefore they are applicable to the condition of the host at the time of challenge and not several weeks later. Figure 1 illustrates the development of acquired immunity in mice and rats following a primary infection, as measured by the lung recovery technique. Groups of animals were challenged at regular intervals after an initial infection and the number of schistosomules recovered from

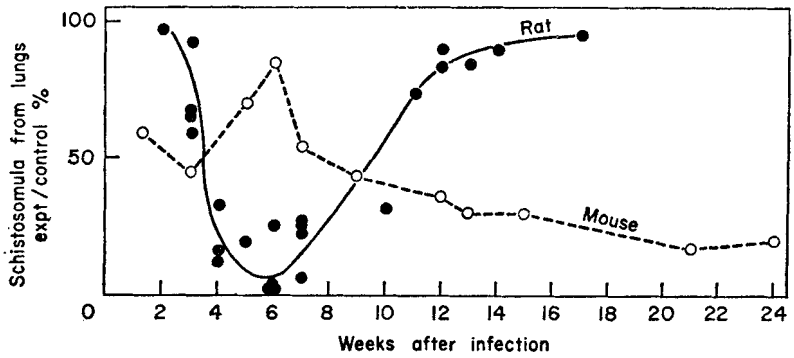

FIG. 1. The development of protective immunity to *S. mansoni* in rats and mice, following a primary infection. Groups of animals were challenged with cercariae at intervals following a primary infection and immunity to the challenge was assayed by the lung recovery technique. The lower the recovery from the lungs, the greater the degree of immunity. (After Perez *et al.*, 1974 and Sher *et al.*, 1974a.)

the lungs on day 4 or 5 was compared with the number recovered from control animals. It can be seen that immunity to reinfection follows a different pattern in the two hosts. In the rat, immunity can be demonstrated at week 3; it reaches a peak between weeks 6 and 7 and then declines to zero (Perez *et al.*, 1974). This decline in immunity may be explained by the fact that most worms from this host are eliminated between weeks 4 and 8 and it is possible that a continual antigenic stimulus is needed to maintain immunity at a high enough level to enable a challenge infection to be destroyed at or before the lung stage. When the lung recovery technique shows immunity to be declining, hepatic perfusion demonstrates that immunity to reinfection is partially retained, indicating that at this stage the challenge is killed after the first 5 days.

In the mouse, the decreases in the recovery of schistosome challenges from the lungs occur in two separate stages following a primary infection (Sher *et al.*, 1974a): a first stage, which arises between 1 and 4 weeks after exposure, and a second stage appearing at 7 weeks and onwards. Resistance as judged by the recovery of the adult parasites is present only during the second stage of decreased lung recovery; the first stage reflects a delay in the migration of the parasite and this effect can be passively transferred to normal mice with a serum fraction which contains mainly IgG_2 antibodies (Sher *et al.*, 1975).

The mechanism of true protective immunity in mice remains unresolved. About half the level of protection in mice which have been infected for 12–15 weeks can be transferred to normal mice with 0·3–0·5 ml of serum. This result is achieved whether immunity is measured by the lung recovery assay or by adult perfusion (Fig. 2). This result strongly suggests that humoral antibody is involved in the immune mechanism; attempts to identify the antibody are not yet complete, but it appears to have different characteristics from the antibody responsible for the delay in migration (Sher *et al.*, 1975).

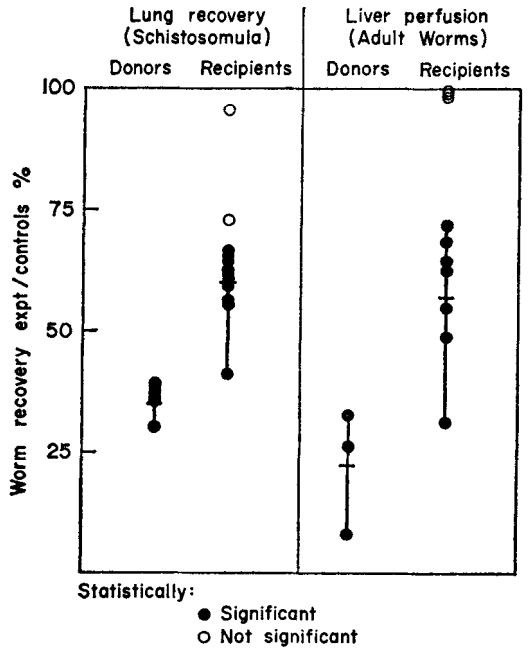

FIG. 2. Passive transfer of immunity to *S. mansoni* in mice with serum from immune donors. The figure compares worm recovery after challenge of recipient mice with that of the donors from which the sera were obtained. (After Sher *et al.*, 1975.)

Partial immunity can be similarly transferred with serum in rats. In this host, however, there is clear evidence for the involvement of a non-sensitized cell in the effector mechanism of immunity (Perez, 1974). The lung recovery technique has been used to show that immunity after an initial infection is severely depressed when the rats are subjected to 500 rad whole body irradiation a few hours before challenge. When irradiation is given 24 h after challenge, immunity is less affected; when given 48 h after challenge there is no effect on immunity. The effects of irradiation on immunity can be reversed by the intravenous transfusion of approximately $1{\cdot}5 \times 10^8$ bone marrow cells from syngeneic normal donors.

2. *Immunity in hamsters*

The mechanism of protective immunity in hamsters has been studied in a different way by Smith *et al.* (1975). The lung recovery assay demonstrates that hamsters with a small primary infection of *S. mansoni* develop a high level of immunity to reinfection which reaches a plateau about 6 weeks after the initial infection. Immune hamsters show an acute inflammatory reaction in the lungs as schistosomules of a challenge infection reach this organ. When inflammation in the lungs of normal hamsters is stimulated non-specifically by, for example, an intravenous injection of dead *E. coli* 3 days after

challenge, then the animals are able to destroy most of the migrating schistosomules.

Vaccination of hamsters with particulate schistosome antigen has shown that the destruction of the schistosomules is not dependent on anti-schistosome antibody alone; although antibody induces lung inflammation possibly by attaching to migrating schistosomules, the schistosomules are not destroyed. However, when vaccinated hamsters are given a relatively small dose of dead *E. coli* 3 days after infection, a high level of protection is obtained. Antibody alone, or low levels of dead *E. coli* alone, do not protect, but the two together act synergistically.

The interpretation of this evidence is that anti-schistosome antibody mediates sequestration of inflammatory cells in the lungs, but these cells need to be activated in some way before they can kill schistosomules. In these experiments activation was provided by the foreign protein and the stimulus is clearly non-specific. In the naturally immune hamster it is suggested that circulating complexes of schistosome antigen with specific antibody may be effective in stimulating phagocytosis of the inflammatory cells in the lungs.

3. *Immunity in primates*

It has long been established that rhesus monkeys are able to develop a strong resistance against reinfection with *S. mansoni* and *S. japonicum*. More recently it was shown that baboons develop a partial resistance to reinfection with *S. mansoni* (Damian *et al.*, 1974; Taylor *et al.*, 1973a) and a strong resistance to *S. haematobium* (Webbe and James, 1973), although it is probable that resistance in the baboon takes longer to develop than in the rhesus monkey.

The rhesus monkey is noted among the primates for its ability to destroy the majority of an adult worm population a few weeks after a heavy infection. Cheever and Powers (1972) have made a careful study of worm numbers and egg production in rhesus monkeys. They found that during the first 12 weeks the number of eggs per worm pair passed in the faeces and retained in the tissues is the same for monkeys exposed to 100 or 600 cercariae. Between weeks 12 and 27, two-thirds of the worms die in monkeys exposed to 600 cercariae and the number of eggs per worm pair passed in the faeces decreases. None of these changes occurred in monkeys exposed to 100 cercariae and in these animals the number of eggs passed in the faeces increases after the 12th week. A recent report by Taylor *et al.* (1973a) has suggested that baboons heavily infected with *S. mansoni* or with a hybrid of *S. mattheei* and *S. mansoni* may also eliminate their adult worms a few weeks after infection.

It is tempting to assume that spontaneous cure is based on an immunological mechanism. Adult elimination in rhesus monkeys appears to be related to the size of the initial infection and therefore the degree of antigenic stimulus and perhaps the destruction of adult worms depends on the level of immunity induced. Although Cheever and Powers (1969) report that fewer adult worms die in splenectomized monkeys than in intact animals, there have been few other studies dealing with the mechanism of spontaneous cure and the role of immunity in this phenomenon is not yet established.

In view of the fact that a strong acquired immunity develops in rhesus monkeys, it is surprising that, unlike the rat and the mouse, passive transfer with serum alone cannot be demonstrated in this host (Smithers, 1975). Furthermore, ALS, cyclophosphamide and total body irradiation have failed to alter resistance to a challenge infection. As a result, there is little indication of the nature of the mechanisms involved in immunity in monkeys (Maddison *et al.*, 1971).

In a preliminary report, however, Maddison *et al.* (1973 and personal communication) claim to have transferred delayed hypersensitivity to *S. mansoni* in rhesus monkeys by transfer factor, i.e. the dialysable or, in their work, also the non-dialysable extracts of leucocytes from immune monkeys which had been re-exposed to infection once, twice or three times. Delayed hypersensitivity was assessed by skin biopsy and histology; it was not always evident in recipients tested 48 h after the first injection of transfer factor, but most monkeys were positive on retesting 3–4 weeks later. No detectable antibody was induced in this way and there was no cross-specificity between tuberculin and *S. mansoni* transfer factor systems. Monkeys injected with *S. mansoni* transfer factor were challenged with cercariae; some of the monkeys also received immune serum just before challenge (day −1) and again on day 12. Those monkeys receiving both dialysable factor and serum from immune donors had a significantly lower mean worm burden than did untreated controls or the animals which received either immune serum or transfer factor alone. However, the *S. mansoni* worm burden of animals receiving a combination of normal transfer factor and normal serum fell between those of the untreated controls and the recipients of transfer factor and serum from immune donors. Maddison *et al.* believe these results to suggest that both cellular and humoral mechanisms are required for immunity.

In human infections, delayed hypersensitivity to intradermal injections of adult or cercarial antigens has been demonstrated (Moriearty and Lewert, 1974a). The low sensitivity of the delayed skin test makes it of limited value in diagnosis and the test cannot be correlated with the severity of the infection or clinical state of the disease. From the results of an epidemiological investigation of intradermal responses in endemic areas of Uganda, Moriearty and Lewert (1974b) suggest that positive delayed skin tests may be related to the frequency of past exposures to schistosome infections and is a response which might be associated with resistance.

4. In vitro *studies of immunity*

Attempts are now being made to analyse the mechanism of acquired immunity by studying the effect of immune serum and cells on schistosomules in culture. Clegg and Smithers (1972) were the first to demonstrate that young schistosomules of *S. mansoni* are killed within 4 days when cultured with serum from monkeys which have been exposed to *S. mansoni*. This lethal activity is due to an antibody of the IgG class and is dependent for its effect on labile factors in fresh normal serum, probably components of the complement system.

Similar *in vitro* assays have been used to demonstrate complement

dependent lethal activity in sera from infected rats, baboons, rabbits and man (Perez *et al.*, 1974; Smith and Webbe, 1974; Capron *et al.*, 1974). A simplified *in vitro* culture system was used by Murrell and Clay (1972) to detect complement dependent lethal antibody in sera from rats, rabbits and monkeys exposed to *S. mansoni.*

Dean *et al.* (1974) found that a rapid lethal effect on schistosomules in culture could be produced by the combined action of an IgG antibody from infected rats and normal rat neutrophils. Again, this opsonizing antibody is dependent on heat labile factors in normal serum, probably complement, and damage to the worm is due to the release of lytic enzymes from the neutrophils.

The surface of the schistosomule is damaged in the presence of lethal antibodies and complement (McLaren *et al.*, 1975); lethal antibody can be absorbed from immune serum with preparations of the surface membranes of adult *S. mansoni*, and preparations of adult worms containing surface and other membranes induce high levels of lethal antibody when injected into rats (Sher *et al.*, 1974b). It follows that lethal antibody reacts with an antigen present at the worm's surface and this reaction activates complement to produce a biochemical lesion on the surface membrane.

Can the presence of lethal antibody or opsonizing antibody be correlated with acquired resistance? An antibody produced by an immune host, which is directed against antigens on the schistosome surface and which kills young schistosomes in culture, would be expected to participate in protective immunity. However, the induction of high levels of lethal antibody in rats after immunization with worm membrane preparations, does not induce resistance against cercarial challenge (Sher *et al.*, 1974b). Furthermore, although the maximum titre of lethal antibody in rats is reached 6–7 weeks after an initial infection and the appearance corresponds with the development of peak immunity as measured by the lung recovery technique, immunity subsequently declines almost to zero by week 12, while lethal antibody titres remain high throughout this period (Perez *et al.*, 1974). A similar time course is seen for the rat opsonizing antibody of Dean *et al.* (1974).

Further evidence against the direct involvement of complement dependent lethal antibody in protective immunity comes from Smith and Webbe (in preparation). They worked with *S. haematobium* and *S. mansoni* infections in hamsters and demonstrated a complement dependent lethal activity only in the homologous system. There is no cross-reaction of the lethal activity induced by *S. mansoni* infection against *S. haematobium* schistosomules and vice versa. On the other hand, an infection of one species will induce protective immunity against the other.

One must therefore conclude that at least in rats and hamsters, if complement dependent lethal antibody has a role in acquired immunity, it must be one of cooperation with other components of the immune system.

A series of independent studies concerning antibodies which are not complement dependent but which cooperate with host cells to damage schistosomules *in vitro*, have recently been described. Butterworth *et al.* (1974) have examined the effect of inactivated serum from human subjects

infected with *S. mansoni* on schistosomules of *S. mansoni in vitro*; they have utilized the chromium release technique for assaying damage to the parasite. Their observations indicate that schistosomules can be damaged *in vitro* in the presence of inactivated sera from infected patients, provided normal human peripheral leucocytes are present in the culture; damage does not occur in sera from uninfected control subjects and peripheral leucocytes. The relevance of these observations to protective immunity cannot at the moment be established.

An opsonizing antibody which is independent of lytic complement has recently been demonstrated in the sera of rats immune to *S. mansoni* (Perez, 1974). Inactivated serum is used to sensitize schistosomules for 3 h. The parasites are then washed and incubated in foetal calf serum and Earle's lactalbumin containing 2×10^6 peritoneal exudate cells from normal rats. Twenty-four hours later, a high proportion of the schistosomules are covered with cells, and although the schistosomules are not dead, electron microscopy reveals that their tegument is damaged. This system works equally well if the cells instead of the schistosomules are first sensitized with inactivated serum and are then placed together with schistosomules in foetal calf serum.

The titre of this complement independent opsonizing antibody correlates well with immunity in the rat as measured by the lung recovery technique, i.e. titres are clearly decreasing 12 weeks after an initial infection. Fractionation of immune rat serum on a Sephadex QAE column has shown that opsonizing activity is found in the second peak, whereas the complement dependent lethal antibody is concentrated mainly in the first peak. It may be significant that only the second peak from the QAE column transfers passive immunity to normal rats.

Capron (1974) has also reported that normal inbred Fisher rat macrophages incubated in the serum of immune rats are strongly adherent to *S. mansoni* schistosomules *in vitro*. Adherence was markedly inhibited when the serum was heated at 56°C for 3 h and was not restored by addition of fresh guinea pig serum, suggesting that the antibody itself was thermolabile. Adherence was completely inhibited by treating the serum with mercaptoethanol. These properties of thermolability and mercaptoethanol sensitivity are characteristic of IgE antibodies, and Capron *et al.* (1975) have now reported elegant experiments which strongly suggest that this cytophilic opsonizing antibody is indeed IgE. Absorption of the immune rat serum with goat anti-rat IgE serum, specific for the ϵ chain, led to a marked decrease in macrophage adherence; absorption of the sera with anti-rat IgG, IgM and IgA was without effect. Adherence was also markedly reduced when the immune serum was absorbed with goat anti-rat IgE linked to cyanogen bromide-activated Sepharose.

These findings may be of great significance. The association of specific anti-schistosome IgE antibodies with infection has been reported by many workers, but it has been difficult to determine any role for these antibodies in protective immunity (Smithers and Terry, 1969a). If IgE antibodies are concerned in promoting macrophage adherence to schistosomules, and if this *in vitro* phenomenon can be shown to reflect protective immunity, then

clearly vaccination procedures should aim to stimulate this particular arm of the immune response. This finding may also have relevance to other helminthoses which are characterized by high levels of IgE antibodies, and perhaps also in the general field of reagin-mediated allergies.

C. HOST ANTIGENS

In our previous review we discussed the evidence available at that time for the existence of antigens shared between schistosomes and their hosts, generally referred to as host antigens. Such shared antigens had been demonstrated by Damian (1962, 1964, 1967) and Capron *et al.* (1965). These workers subscribed to the view, originally put forward by Sprent (1959), that such antigens were of parasite origin and had evolved under selection pressures to resemble those of the host, thus reducing the overall immunogenicity of the parasite and favouring its survival. Capron *et al.* (1968) elaborated the basic hypothesis to account for parasites such as schistosomes which can parasitize a variety of antigenically distinct hosts; they suggested that schistosomes might have the genetic capacity to synthesize antigens of several host types and the capability of turning on the correct genes for the particular species being parasitized.

Smithers *et al.* (1969) originally studied host antigens in an *in vivo* system involving the transfer of worms from donor to recipient species, the recipients being immunized against donor tissues, particularly erythrocytes. Based on their findings, they put forward an alternative hypothesis for the origin of host antigens: that these antigens were truly of host origin but were somehow acquired by the parasite during its migration. It was also suggested that the presence or absence of such acquired antigens might explain the phenomenon of concomitant immunity. Cioli and Neis (1972) have also demonstrated the existence of host antigens on the surface of schistosomes by means of a mouse–hamster transfer system. Work carried out over the past 5 years has centred around two topics; the nature and origin of these antigens, and their role, if any, in protecting the parasite against host immunity.

There now seems to be incontrovertible evidence that certain of these shared antigens are of parasite origin. Damian *et al.* (1973), Damian (1974) and Kemp and Damian (1974) have described an antigenic determinant on the surface of *S. mansoni* which cross-reacts with mouse α_2 macroglobulin (α_2M). This antigen is present on the surface of worms grown in rhesus monkeys as well as those grown in mice. Since primate α_2M does not cross-react with mouse α_2M, this determinant must be of parasite origin. Damian *et al.* (1973) suggest that this antigen has evolved as a result of long contact between parasite and host but, as Clegg (1974) has pointed out, it is hard to understand why the antigen should resemble a mouse rather than a primate determinant, when *S. mansoni* is mainly a parasite of man.

Even more direct evidence for parasite synthesis of shared antigens has been provided by Bout *et al.* (1974). Using labelled amino acid precursors they have obtained the synthesis by schistosomes *in vitro* of a protein which

cross-reacts with hamster liver proteins. The schistosomes studied were obtained from hamsters; it will be of great interest to see whether schistosomes grown in mice synthesize the same determinant or others, perhaps cross-reacting with mouse liver proteins.

There is now equally convincing evidence that other shared antigens are of host origin and are acquired by the parasite. Clegg *et al.* (1971a), Dean (1974) and Goldring (in prep.) have demonstrated that schistosomules grown *in vitro* in human blood develop surface antigens which show human blood group specificity of the ABO type. The antigens expressed relate to the specificities of the erythrocytes present in the culture. Thus, the experiments of Goldring (in prep.) showed that worms grown with group A cells express A antigens; those grown with B cells express B antigens; those grown with AB cells express A and B antigens; those grown with AB cells express A and B antigens; and those grown with O cells express H antigen. Neither Dean (1974) nor Goldring (in prep.) have been able to show the acquisition of other blood group antigens such as the rhesus antigens and the MN antigens. Goldring, Kusel and Clegg (pers. comm.) have studied the transfer of tritium-labelled molecules from red cells to schistosomes. The terminal galactose and acetylgalactosamine groups of the surface glycolipids and glyco-proteins of human AB erythrocytes were labelled with tritium by the method of Gahmberg and Hakomori (1973). Young schistosomules were cultured in a medium containing these labelled cells for 3 days, carefully separated from the erythrocytes, solubilized in sodium dodecyl sulphate and electrophoresed on polyacrylamide gel. Comparing the gel profiles of the schistosomules with the erythrocytes, it was apparent that a transfer of labelled molecules had taken place. The transferred molecules were not large proteins, but their identity and hence their role as host antigens has yet to be established.

It seems, then, that the so-called host antigens may be derived from two sources; some, which appear to be proteins, are synthesized by the parasite, whilst others, which may be surface glycolipids, are acquired from the host. The question now to be answered is: do these host antigens serve in any way to protect the schistosome from the immune response?

It must be admitted that although there is much circumstantial evidence for protection by host antigens we have as yet no direct evidence. If the phenomenon of concomitant immunity is correctly explained by the hypothesis that the host antigens on the surface membrane of adult worms protect them against the immune response of the host, it follows that the developing stages of the parasite, which are susceptible to immune attack, should lack host antigen. Initial transfer experiments (Clegg *et al.*, 1971b) suggested that 15 day schistosomules had a full complement of host antigens, 7 day old schistosomules had less, and cercariae were without host antigens. Furthermore, Clegg and Smithers (1972) showed that 3 h schistosomules are killed *in vitro* by a complement dependent antibody in hyperimmune monkey serum, while 4 day worms are protected in some way against the effect of immune serum. Using more sensitive techniques and electron microscopy, McLaren *et al.* (1975) have demonstrated the presence of mouse erythrocyte

antigens on schistosomules grown in mice for 4 days, but failed to find them on newly penetrated 3 h schistosomules. Conversely, immunoglobulins from rhesus monkeys hyperimmune to schistosomes, failed to bind to the surface of 4 day parasites but readily attached to 3 h schistosomules.

If it could be demonstrated conclusively that antibody damage *in vitro* is a true reflection of protective immunity *in vivo*, the hypothesis concerning the role of host antigens would be considerably strengthened. Unfortunately, the several *in vitro* demonstrations of antibody induced damage to young schistosomules cannot as yet be correlated with the protection mechanism *in vivo*.

There remains the further argument that the appearance of host antigens and the loss of susceptibility of the schistosomules *in vitro* are unrelated, being merely coincidental in time. In this context, the preliminary report of Dean and Sell (1974) is of interest. These workers have described an *in vitro* method for killing schistosomules with immune guinea pig serum and polymorphonuclear leucocytes. It is claimed that whereas young schistosomules are susceptible, older ones are not, even when grown in a chemically defined medium, free of host antigens which could be adsorbed by the parasite. Before this finding can be properly evaluated it will be necessary to show that the schistosomules are behaving reasonably normally and expressing surface antigens during their culture in the chemically defined medium.

In summary, antigens shared by host and parasite have been conclusively demonstrated in schistosomes; these antigens may be of either parasite or host origin; and, in spite of much circumstantial evidence, it has yet to be shown conclusively that they serve to protect the parasite from immune attack. It is still, however, a reasonable working hypothesis (Terry and Smithers, 1975) that while host antigens of parasite origin may serve to reduce the overall immunogenicity of the parasite, it is those antigens of host origin, which attaching to the parasite surface, complete the immunological disguise of the adult worm and protect it from the immune attack.

IV. Parasite Antigens

A. Surface and secretory antigens

There is no accepted serological or *in vitro* test which correlates with protective immunity in man or experimental animals. Consequently, it is difficult to develop an experimental system for identifying and characterizing those schistosome antigens which are responsible for inducing protective immunity (the protective antigens). At best, only very poor protection can be induced by vaccination with dead parasite antigen; protective immunity only develops after the host has experienced a living infection, and this may imply that the stimulation of immunity is due to some metabolic secretion involving the release of protective antigens.

There is evidence from studies with both the adult worm (Hockley and Smithers, 1970) and the schistosomule (McLaren *et al.*, 1975) that the

effector mechanism of the immune response is directed against the schistosome surface. Antigens on the surface might therefore act as targets for the host's immune response, and surface antigens which are secreted by the schistosome could be responsible for inducing protection. Accordingly, Kusel *et al.* (1975) have been using radioactive techniques to study the surface membrane turnover of schistosomes and the release of membrane antigens into culture.

S. mansoni adult worms are labelled after 24 h in culture in a medium containing ^{3}H leucine. On transfer to a non-radioactive medium they exhibit a progressive loss of labelled proteins from the membrane, while new proteins are inserted into the membrane. The half-life of the schistosome surface membrane is about 30 h.

As the radioactivity of the schistosome surface membrane decreases, there is a corresponding increase in the TCA precipitable radioactivity in the culture medium. Since about half of this labelled material complexes specifically with serum from rhesus monkeys which have developed immunity following infection, it follows that these macromolecules are recognized by the host as foreign antigens. These complexes can be isolated from the medium by co-precipitation with rabbit anti-monkey IgG, and their molecular weight can be determined by electrophoresis in SDS polyacrylamide gel. About one-third of the culture antigens isolated in this way were shown to have been derived from the worm membrane by first absorbing the immune monkey serum with non-radioactive isolated schistosome membrane. At least six of these "membrane culture antigens" were shown to have molecular weights identical to antigens in the schistosomule's surface. It is these antigens which are the most likely candidates for inducing protective immunity.

B. CIRCULATING ANTIGENS

The serological demonstration of antibodies specific to schistosome antigens is a valuable tool in diagnosis and epidemiological studies. With the possible exception of the circumoval precipitin test (Shoeb *et al.*, 1967; Rifaat *et al.*, 1969), none of the tests has so far demonstrated antibodies that correlate with the presence of an active infection, with the size of the worm burden, or with successful drug treatment. A reliable serological test which would indicate the presence and the number of living schistosomes in the host would be of immense value to clinicians, epidemiologists and experimental workers. If antigenic material, excreted or secreted by schistosomes, could be assayed in the blood, then theoretically such an assay would provide a quantitative test which would relate to the presence of living worms.

An experimental model for studying this problem was devised by Berggren and Weller (1967) and Gold *et al.* (1969). They used gel diffusion techniques and serum from rabbits immunized with homogenates of adult *S. mansoni* to show the presence of schistosome antigen in the plasma of massively infected mice and hamsters. A direct relationship appears to exist between the number of worms in the host and the amount of antigen circulating in the blood. The antigen could be isolated from adult worm extract and it was

found to be negatively charged, heat stable, dialysable, and to have a molecular weight of less than 10 000. The antigen was also found in low concentrations in the urine of some hamsters.

Okabe and Akusawa (1971) report what they believe to be a similar antigen from the urine of rabbits infected with *S. japonicum*. This antigen was detected by gel diffusion using an antiserum from rabbits immunized against homogenates of *S. japonicum*. The antigen is Anthrone positive, partially dialysable and partially heat stable.

Nash *et al.* (1974) confirm the presence of a strongly negatively charged, heat stable circulating antigen in mice heavily infected with *S. mansoni*. They showed that this antigen was not species-specific: serum from rabbits immunized with a TCA soluble-chloroform extract of adult *S. mansoni* gave one precipitation line against supernatants of *S. haematobium*, *S. japonicum*, *S. mansoni* and plasma from mice infected with *S. mansoni*. Hamsters infected with *S. haematobium* produced antibodies against the antigen, as did three of eight patients studied in Washington. Nash *et al.* estimated the molecular weight of the antigen to be greater than 100 000, a difference from the results of Gold *et al.* which could not be explained. The failure of proteolytic enzymes to destroy the antigen, the TCA solubility and resistance to chloroform and its destruction by periodate, strongly indicate that the antigen is a polysaccharide.

Clearly, antigens of schistosome origin may be detected in the urine or plasma of infected hosts, including man, and quantitative techniques for assaying the antigen might be developed. Further research designed to produce a test which relates to the presence and intensity of infection should be encouraged.

V. The Schistosome Granuloma

Previous work has shown that after sensitization to schistosome eggs given intraperitoneally, mice exhibit an accelerated and augmented granuloma formation to eggs introduced into the lungs by intravenous injection. This reaction is specific in cross sensitization experiments with *Ascaris* eggs (a high degree of specificity was later shown among the three human schistosome species: Warren and Domingo, 1970a) and sensitization could be transferred between histocompatible mice with lymph node or spleen cells but not with serum (Warren *et al.*, 1967). These results strongly suggested that the schistosome egg granuloma is essentially a manifestation of delayed hypersensitivity.

Studies with a series of immunosuppressive measures provided confirmation of a cell mediated type of immunological response. Procedures which primarily inhibit cell-mediated immunity, e.g. neonatal thymectomy (Domingo and Warren, 1967), anti-lymphocyte serum (Domingo and Warren, 1968), anti-macrophage serum (Boros and Warren, 1971a) and advanced Hodgkin's-like tumours (Warren, 1969), almost eliminate granuloma formation, whilst measures which tend to suppress antibody formation, e.g. chronic X-irradiation (Perrotto and Warren, 1969) and Friend virus leukaemia (Warren, 1969), have no effect whatsoever.

The sensitization of mice to produce an accelerated granulomatous response can only be achieved with antigens associated with the schistosome egg. Warren and Domingo (1970b) exposed mice to large numbers of irradiated cercariae; or they infected mice with either all male or all female worms; or they injected killed 4 week old worms intraperitoneally into mice. All three procedures exposed mice to schistosome antigens but not to antigens specifically associated with the egg. In the treated mice, granuloma formation after an intravenous challenge of eggs was similar to that in the control unsensitized mice, thus demonstrating that sensitization did not occur in the absence of the schistosome egg.

Boros and Warren (1970) went on to analyse the components of the eggs of *S. mansoni* for their sensitizing activity. They showed that the sensitizing property could diffuse out of intact eggs and that it was almost solely confined to a soluble material which escaped when the eggs hatched. The soluble egg antigen (SEA) was isolated from a homogenate of eggs by taking the supernatant after spinning at 100 000 *g* for 2 h. Its sensitizing activity was destroyed by trypsin and ribonuclease, but not by deoxyribonuclease, and it was labile at 56°C.

Between 6 μg and 9 μg of SEA were sufficient to sensitize a mouse. Eight protein bands were demonstrated in SEA and partial purification of the active antigen was possible by preparative electrophoresis on Pevikon block. The most interesting property of SEA was its ability to induce a delayed cellular response in the absence of humoral antibody. When mice were sensitized by an intraperitoneal injection and challenged homologously 1 or 2 weeks later by a foot-pad infection of 10 μg of SEA, the animals developed a delayed foot-pad swelling caused by an infiltration of mononuclear cells which reached a peak at 24 h. At the time of challenge, a sensitive haemagglutination technique failed to detect the presence of humoral antibodies to SEA.

The elicitation of delayed hypersensitivity in mice by SEA has been confirmed by Colley (1971), who found that cultures of lymph node cells from mice infected with *S. mansoni* undergo a vigorous blastogenic reaction in response to SEA, and by Dunsford *et al.* (1974) who showed that SEA will inhibit the migration of macrophages from mice sensitized with SEA.

A new experimental model which involves the adsorption of antigens onto carrier particles has been used to study granuloma formation. This technique was reported simultaneously by Boros and Warren (1971b) and Lichtenberg *et al.* (1971); bentonite particles, roughly the size of schistosome eggs, were coated with SEA or with *Mycobacterium tuberculosis* antigen and injected intravenously into sensitized mice. An augmented granulomatous reaction developed around the coated particles in homologously sensitized mice, but there was no cross sensitization between the SEA and the *Mycobacterium* antigens. Bovine-serum-albumin-coated particles did not induce granulomata in bovine-serum-albumin-sensitized mice.

Further work with this model has given support to the hypothesis that both delayed hypersensitivity and antigen sequestration are involved in granuloma formation. Dunsford *et al.* (1974) showed that whilst SEA gave

a delayed dermal response in the footpad of sensitized mice lasting 2–4 days, SEA adsorbed onto bentonite particles or latex beads elicited a true granulomatous response in the sensitized mouse lung. Thus the cellular response to the same antigen in the same animal, differed according to whether it was immediately diffused or was gradually released from a carrier particle. Apparently the release of SEA from carrier particles is sufficient to elicit a tissue reaction which peaks between days 2 and 4. (The peak reaction of an anamnestic schistosome egg granuloma is reached on day 8, a difference which is possibly due to a more rapid release of antigen from the carrier particles than from eggs.) The response around the carrier particles continued for at least 16 days with bentonite and 8 days with the latex beads, the duration being proportional to the antigen uptake by the particle. Although SEA release from particles was complete within 1 h *in vitro*, immunofluorescent studies showed that antigen persisted in living granulomas for at least 24 h. Dunsford *et al.* believe that granulomas are formed in response to sustained release of antigen from a depot state. Under these conditions antigen which would otherwise be toxic to the host is sequestered *in situ* by host phagocytes, and this cellular immune response appears to be important in enabling the host to handle the schistosome infection.

On the other hand, the granulomatous reaction of the host to the schistosome egg is the major factor in the development of hepatosplenic disease (Warren, 1968). It therefore seems logical to ask whether chronic schistosomiasis robbed of the host's granulomatous reaction to egg antigens might not be a much more benign disease. Henson *et al.* (1972) have produced a partial inhibition of hepatic granuloma in mice infected with *S. mansoni* with the anti-inflammatory drug propriomazine, and found that the mean survival time of the treated mice was increased by about 10 days compared with untreated infected mice.

Other work has shown, however, that when granuloma formation is completely inhibited, the death of mice infected with *S. mansoni* is accelerated. Complete inhibition of granuloma formation was attained by Fine *et al.* (1973) and Buchanan *et al.* (1973) in T-cell depleted mice. Such hosts failed to develop cellular hypersensitivity to SEA as manifested by lymphocyte blastogenesis and delayed skin reactivity; further, they failed to develop reaginic antibody to SEA although agglutinating antibody, and antibodies, mediating early dermal responses to SEA, were unaltered. In such mice, egg induced granulomas were not seen, but the ova were surrounded by a zone of liquefactive necrosis containing cell debris. The authors think it possible that the host, being incapable of mounting a cell mediated immune response which normally circumscribes the egg, is left defenceless against the enzymatic activities and phospholipids of the egg; alternatively, in the absence of cell-mediated reactivity, the formation of immune complexes and their subsequent interaction with complement, produces tissue damage. Far from having a prolonged survival or more benign course as might reasonably have been postulated considering the crucial role of the granuloma in the pathology of the disease, the mortality of the T-cell depleted mice was accelerated.

Detailed comparative studies of the lesions caused by schistosome eggs have been made by several workers. All agree that the nature of the granuloma is dependent on both the host species and the parasite species. In general, however, the evolution of lesions elicited by the eggs of *S. japonicum* and *S. mansoni* in various hosts is similar.

Hsu *et al.* (1969) have described five stages in the development of the granuloma: (1) *the non-reactive or weakly negative stage* describes the lesion around living immature eggs; (2) *the exudative stage* is formed around mature eggs and is characterized by an accumulation of neutrophils which arrange themselves in a palisade among the acidophilic PAS-positive radiating filaments which originate from the egg shell periphery; (3) *the exudative–productive stage* is manifested by a granulomatous transformation, including an arrangement of epithelioid cells in a palisade around the egg and the formation of giant cells; (4) *the productive stage* is seen when the miracidium shows signs of degeneration and when the egg is surrounded by giant cells outside of which are layers of epithelioid cells, histiocytes, fibroblasts and fibrocytes, and finally a periphery of lymphocytes, plasma cells and neutrophils; (5) *the involutional stage* is seen when giant cells invade and destroy the egg, the layers of epithelioid cells become thinner, fibroblasts become more prominent and there is a formation of collagen fibres.

Apart from minor modifications these five stages hold true for both *S. mansoni* and *S. japonicum* eggs in all hosts studied. (Hsu *et al.*, 1972a). In poorly susceptible hosts, however, viz. the rat and guinea pig, immature schistosome eggs elicit lesions of the *productive* type rather than lesions of the *non-reactive* or *weakly negative* type (Hsu *et al.*, 1973).

Although Warren and Domingo (1970a) believe that the inflammatory reaction around *S. japonicum* eggs in mice resembles that which forms around inert plastic beads, there is some disagreement about this. Lichtenberg *et al.* (1973) believe that the pathological changes due to eggs of *S. japonicum* are more severe than those of other species, and Hsu *et al.* (1972a) state that the neutrophil chemotaxis which is seen during the *exudative* stage with *S. japonicum* is stronger than that with *S. mansoni*. Hsu *et al.* (1973) also believe that the size of granuloma in susceptible hosts is larger than that in less susceptible hosts like the rat and guinea pig, although this result is not altogether in agreement with Akpom *et al.* (1970), who showed that hamsters have smaller granulomas than guinea pigs or rats.

It is assumed by Hsu *et al.* (1972b) that the inflammatory cells which form the granulomas are attracted to the egg by the secretions of the miracidial cephalic glands, the *exudative* stage being absent in granuloma caused by immature eggs in which the cephalic glands have not yet formed.

VI. Conclusions

At the end of our 1969 review we listed a number of topics where research was urgently needed. Because of much effort expended on these topics, we now have a clearer understanding of many aspects of the complex host–parasite relationships found in schistosome infections.

Concomitant immunity has been shown to operate in a number of experimental systems, and there are encouraging reports that it may operate in human infections. Many more careful epidemiological studies, in different areas of the world, are required before its existence can be fully accepted. A good deal of circumstantial evidence implicates host antigens as playing a role in concomitant immunity, but more direct evidence is needed to show that they indeed serve to protect the mature parasite from immune attack.

The use of small animals, particularly those where syngeneic strains are available, and *in vitro* cultivation, have increased our understanding of the immune mechanisms. In particular, a number of *in vitro* systems have been described in which antibodies and cells combine to damage and to kill young schistosomules. It is essential to determine whether the differences described for these several systems truly reflect a multiplicity of host responses, or merely represent differences in technical approach to a single system. The relevance of *in vitro* observations to the responses of the intact animal, and those of man, should always be kept in mind.

Much work has concerned the immunopathology of the disease, particularly granuloma formation. Although the granuloma contributes significantly to the pathology, it does seem to protect the host liver from the toxic secretion of the egg. Reduction in granuloma size, without affecting its protective function, would seem to be a desirable aim.

The eventual aim of all this work, immunological control of schistosomiasis, is yet to be achieved. A number of approaches are currently under investigation, including the use of live cercariae and schistosomules, and the use of more or less purified antigens. We believe that one or other of these approaches will eventually be successful, but that this success will probably depend on our gaining a greater understanding of the detailed mechanisms of immunity and the devices which enable the parasite to evade this immunity.

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