

AN  
INTRODUCTION  
TO  
NEMATOTOLOGY

J. R. CHRISTIE  
*Editor*



ALBERT HASSALL

Born in Woolwich, Kent, England. M. R. C. V. S., Royal College of Veterinary Surgeons London, 1886. Inspector, 1887-1905; Assistant in Zoology, 1905-1910; Assistant Zoologist, 1910-1922; Associate Zoologist, 1922-1924; Zoologist, 1924-1925; Senior Zoologist and Assistant Chief of Zoological Division, 1925-1932; Collaborator, 1934- ; Bureau of Animal Industry, U. S. Department of Agriculture.

Zoologist, bibliographer, author of numerous papers in parasitology, compiler of Index Catalogue of Medical and Veterinary Zoology published in collaboration with Stiles. Nematologists' best friend and severest critic.

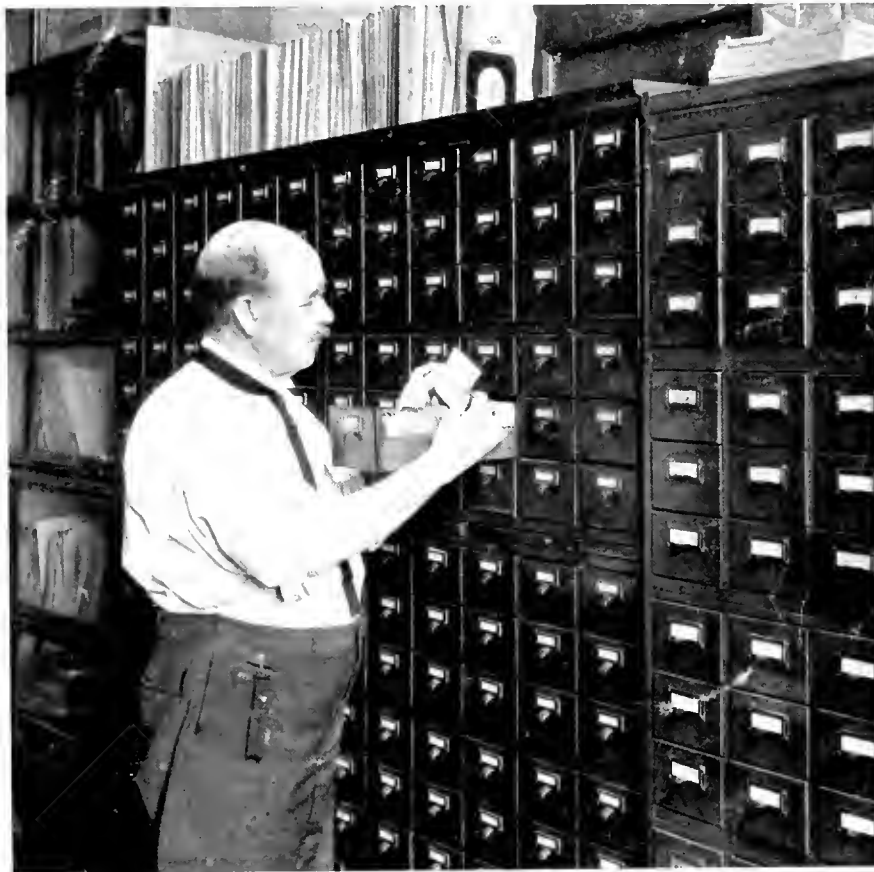
QL .  
391.N  
C 57



AN  
INTRODUCTION  
TO  
NEMATOTOLOGY

J. R. CHRISTIE

*Editor*



ALBERT HASSALL

Born in Woolwich, Kent, England. M. R. C. V. S., Royal College of Veterinary Surgeons London, 1886. Inspector, 1887-1905; Assistant in Zoology, 1905-1910; Assistant Zoologist, 1910-1922; Associate Zoologist, 1922-1924; Zoologist, 1924-1925; Senior Zoologist and Assistant Chief of Zoological Division, 1925-1932; Collaborator, 1934- ; Bureau of Animal Industry, U. S. Department of Agriculture.

Zoologist, bibliographer, author of numerous papers in parasitology, compiler of Index Catalogue of Medical and Veterinary Zoology published in collaboration with Stiles. Nematologists' best friend and severest critic.

QL  
391.N  
C 57





## PREFACE

In the preparation of Section II, Parts II and III, it has been necessary to deviate from the arrangement proposed in the original announcement. During the course of preparation it became evident that the treatment of the subject, as developed by the various authors, would result in more pages than can be saddle stitched into one cover. It also became evident that it would be impossible to complete some of the chapters dealing with free-living and plant-parasitic nematodes until long after most of the others were ready for printing. Hence it seemed advisable to so change the arrangement of the chapters as to permit the immediate publication of completed manuscripts even though it was necessary to modify what might seem the most logical sequence.

The various authors are responsible for the facts presented and the opinions expressed in the parts accredited to them with the one exception of taxonomic nomenclature, for which Dr. B. G. Chitwood assumes responsibility.

To the contributors I wish to express my appreciation and thanks for their sympathetic cooperation as well as for the excellence of their contributions and the care and thoroughness with which these were prepared.

J. R. CHRISTIE.



## CHAPTER IV LIFE HISTORY. GENERAL DISCUSSION

B. G. CHITWOOD

The development of nematodes in its simplest form is direct, or not marked by a metamorphosis such as occurs in the insects. In general the newly hatched nematode resembles the adult in all gross morphologic characters with the exception of the reproductive system and secondary sexual characters. The various growth stages, except the adult stage, are terminated by molts (or ecdyses), the number of molts being four, the number of stages five. Internal changes do not occur to any marked extent in the simplest form of life history. We should, therefore, speak of the stages previous to the adult as nymphs, if a terminology were used similar to that employed in the Arthropoda, but usage has made larva, as applied to such stages, the accepted term.

The number of molts occurring in the course of development is common for nearly all nematodes, and it appears to be the generalized or primitive number for the class. Development may be outlined as follows:

- Egg
- First stage (larva)  
(molt)
- Second stage (larva)  
(molt)
- Third stage (larva)  
(molt)
- Fourth stage (larva)  
(molt)
- Fifth stage (adult)

Correlated with mode of life, various adaptations or modifications have taken place in the life history, these adaptations having arisen through the need for food and a means of dissemination. With free-living nematodes, living either upon decaying matter or preying upon other microscopic organisms, these factors seem to have played a smaller part than with those living as parasites.

Probably need for dissemination was the earlier influence; at any rate, it has caused the simplest modifications of life history. The action of this factor on some free-living nematodes is evidenced by the occurrence of a persistent stage, the cuticle of one of the larval molts being retained as a protective sheath or "cyst." It is not uncommon for such species to have two types of larva, environmental conditions determining whether or not larvae will be of the persistent type. The significance of these persistent larvae is indicated by their negatively geotropic tendencies, for they crawl to the highest surface available and "standing" on their tails swing about, catching upon any moving object. The climax of this type of development is found in species where an encysted stage on some arthropod (*Rhabditis coarctata*, see cover page, see I, part 1), or annelid is obligatory before the adult stage can be reached.

The need for obtaining food plays a much more striking role, being evidenced by all conceivable degrees of parasitism both on and in plants and animals. In the group of "herbivores" life cycles may be of numerous types, depending on whether the nematodes are "grazers," passing from host to host, or sedentary forms, entering the host and there undergoing all or most of the stages of development. Life histories may be further modified by the factor of dissemination and the growth habits of the host. Among the parasites of animals the factors of dissemination and nourishment also play their roles. We have forms that are parasitic only during a particular larval stage, the third, which, incidentally, is usually sheathed or "encysted." Certain parasites of annelids (i.e., *Rhabditis pollia*) pass the third stage in the nephridia of their host and can only develop to adults in the decomposing tissues of their host. Other species (mermithids) enter their hosts either as eggs or larvae and develop to preadults (fourth stage) within the body cavity, finally leaving their host before maturing. In such instances the nourishment necessary for the entire life cycle is obtained from the host and stored during the parasitic stages.

The type of life history in parasitic nematodes being entirely correlated with the degree of parasitism, we find, with more advanced parasitism, more complicated life cycles and more morphologic changes taking place during the course of development. Seurat (1916; 1920), recognizing this, proposed a terminology for the different types of life cycles based on the mode of development.

Some forms have an alternation of generations, one generation being free living, the other parasitic. This type of life

cycle is termed *heterogenous*. In such forms we find free living adults giving rise to larvae which enter the host and develop to parasitic adults. These larvae may or may not be ensheathed (third stage), i.e., the cuticle of one or more larval molts retained though separated from the body. The stage ready to enter the host is termed the *infective* stage. Nematodes with no alteration of generations are termed *monogenous*. These are by far the most common.

Parasites of animals may also be classified according to the number of hosts necessary for completion of the life cycle. Species in which there is a single host are termed *monoxenous*, those in which there are two or more hosts, *heteroxenous*.

Some nematodes have both free-living and parasitic stages, the free-living stages being larvae, the parasitic stages late larvae and adults. In these we find young larval stages (the first and second) feeding upon bacteria and similar organic matter, the third stage usually ensheathed or persistent, this commonly being the infective stage. Upon entering the host these species develop through the fourth stage to the sexually mature adult.

A further development of parasitism is indicated by the absence of the free-living stages. Eggs of the parasite pass out of the host and, outside, undergo only embryonic development within the egg shell. In such instances the egg shell is often covered by a protein layer (p. 178), and the embryo often contains more yolk than forms in which the eggs hatch before entering the host. With such a completely parasitic mode of existence, the factor of dissemination again becomes manifest and we find still other modifications in the life cycle.

Some nematode parasites of vertebrates pass through larval stages in invertebrates, this course of development being either obligatory or facultative; still others undergo larval development in other vertebrates, such development usually being obligatory, rarely facultative. The host in which such a parasite develops to infectivity is termed the *intermediate* or *secondary host* while that in which it develops to sexual maturity is called the *terminal, definitive* or *primary host*. Sometimes the intermediate host is eaten by another animal (secondary intermediate host) in which the parasite can continue its existence but cannot reach maturity. When this second animal is, in turn, eaten by the primary host the life cycle is completed. If the parasite neither feeds nor undergoes growth within an animal, that host is termed a *transport host*. This type of intermediate host serves chiefly as a means of dissemination and is facultative rather than obligatory.

We have attempted to extend Seurat's outline to include all nematodes. With the recent and extensive increase in information on the life histories of vertebrate parasites it has become very difficult to adjust Seurat's classification to the many variations in life cycles. For example it is hardly proper to speak of a form as being heteroxenous when the use of an intermediate host is facultative (*Dictyoceanlus flaria*) yet other nematodes in the same taxonomic group may be truly heteroxenous requiring an intermediate host (*Metastrongylus elongatus*). In general one can say that the Spiruroidea, Filarioidea, Camallanoidea, Draecunuloidea and Dioctophymatoidea are heteroxenous. The Strongyloidea, Trichostrongyloidea and Oxyuroidea are monoxenous while the Metastrongyloidea and Ascaridoidea contain forms with both monoxenous and heteroxenous life cycles. Some exceptional forms do not fit into any part of the classification, *Neoaplectana glasceri* (Rhabditoidea) and *Probstmayria vivipara* (Oxyuroidea) reproduce through several consecutive generations within the host. Some strains of a *Strongyloides* species may reproduce without an alternation of generations while other strains of the same species may be predominantly heterogenetic. The difficulty in fitting life histories into a well defined classification appears to be due to the adaptation of each species to its host which entails a means of dissemination suitable to the host's environment and habits.

A large assembly of nematodes have been found in more or less close association with vertebrates or invertebrates. Some of these merely use the "host" as a means of transportation (*Rhabditis coarctata* which may pass an encysted stage on the surface of dung beetles). Such nematodes are not considered parasitic unless they actually penetrate the host. Some well known free-living nematodes have been reported also existing under parasitic conditions. Thus *Rhabditis strongyloides* has been repeatedly taken, in the larval stage, from diseased skin of dogs and *Diploscapter coronata* from the hydrochloric acid stomachs of human beings. Yet these forms are free-living

nematodes and it would not be proper to classify them otherwise. If it happens that they are adaptable to unusual environments it is but an evidence of the nature of the group to which they belong.

Because of the numerous difficulties and inconsistencies apparent in any classification of nematode life histories, each of the authors has followed the system which seemed most logical to himself. Thus, the nematode parasites of invertebrates are grouped according to the manner and site of parasitism, beginning with the semiparasitic forms that mature at the death of their host and feed upon the carcass, then taking up the intestinal parasites and finally the parasites of the body cavity. Most of the invertebrate parasites belong to the Rhabditoidea and Tylenchoidea in which groups parasitism has arisen so many times and adaptations are so numerous that life cycles have little in common with systematics. The vertebrate parasites are taken up according to their systematic position since the large groups show some consistency within themselves and distinct trends are apparent.

For those who desire an outline after the manner of Seurat, we have revised his system to include groups with which he did not deal. The classification is entirely artificial. Nematodes are divided into the Vagantia or wanderers and the Parasitica. The Vagantia includes members of the Rhabditoidea, Tylenchoidea, Monhysterina, Chromadorina, Eupoilina and Dorylaimoidea. Some representatives of most, if not all, of these groups have been found in more or less close association as semiparasites or parasites of plants or animals but the groups are basically free living. The only known modification in the life history of such free living forms is the existence of a persistent stage. Thus far, this stage is known only in terrestrial and semiterrestrial forms.

The Parasitica is subdivided into Phytoparasitica and Zooparasitica. All the known nematode parasites of plants belong to the Tylenchoidea though certain members of the Rhabditoidea and Dorylaimoidea are commonly found in close association with plants. In the Zooparasitica the heteroxenous group consists exclusively of parasites of vertebrates including all members of the order Spirurida, the suborder Diectophymatina, and representatives of the Trichuroidea, Ascaridoidea and Metastrongyloidea. Those monoxenous nematodes in which the adult is wholly or partially free living belong to the Rhabditoidea, Tylenchoidea and Mermithoidea and are all parasites of invertebrates. The monoxenous nematodes in which the adult is wholly parasitic include the Strongyloidea, Trichostrongyloidea, Oxyuroidea and representatives of the Rhabditoidea, Metastrongyloidea, Ascaridoidea and Monhysterioidea. One commonly thinks of the groups with this type of life cycle as vertebrate parasites yet *Ncoaplectana*, and *Cephalobium microbivorum* are rhabditoid parasites of invertebrates, the Thelastomatidae (*Leidyema*, *Pseudonynnons*), Rhigonematidae and Ransomnematinæ are oxyuroid parasites of invertebrates while *Longibucca*, *Rhabdias*, and *Strongyloides* are rhabditoid parasites of vertebrates and *Odontobius* is the lone monhysterid parasite of vertebrates.

#### CLASSIFICATION OF NEMATODES ACCORDING TO LIFE HISTORY\*

##### I. Vagantia (Free-living nematodes).

###### 1. Without persistent stage.

###### Enoplidae

- (1) *Enoplus communis* (Marine)

###### 2. With persistent stage.

###### Rhabditidae

- (1) *Rhabditis strongyloides* (Soil, sometimes causing dermatitis in dogs).  
 (2) *Rhabditis coarctata* (Dung, encysting on dung beetles).

##### II. Parasitica (Nematodes deriving nourishment from their host).

###### 1. Phytoparasitica (Nematode parasites of plants).

###### A. Vagrant parasites. More or less migratory, often feed externally, do not permanently localize in part of plant.

###### Tylenchidae

- (1) *Criconeoides mutabile*—*Tagetes crecta* (External, roots).  
 (2) *Pratylenchus pratensis*—Cowpea (Internal, roots).  
 (3) *Aphelenchoides ritzema-bosi* — Chrysanthemums (Leaf and bud).  
 (4) *Ditylenchus dipsaci*—Narcissus, onions, clover (Stem, leaf, and bulb).

###### B. Semivagrant parasites. (Localize during definite period of life history.)

###### Tylenchidae

- (1) *Anguina tritici*—wheat (Stem and seed).

###### C. Sedentary parasites. (Female does not migrate after maturity.)

###### Tylenchidae

- (1) *Heterodera marioni*—Tomatoes, potatoes, tobacco (Roots and tubers).  
 (2) *Heterodera schachtii* — Sugar beets, potatoes (Roots and tubers).  
 (3) *Tylenchulus semipneustrans*—Citrus plants (Roots).  
 (4) *Rotylenchulus reniformis*—Cowpea (Roots).

##### 2. Zooparasitica (Nematode parasites of animals).

###### A. Monoxenous (Only 1 animal host in life cycle).

###### AA. Adult stage wholly or partially free-living.

###### a. Only larval stages parasitic or semiparasitic.

###### aa. Feed in adult stage usually on carcass of host.

###### Rhabditidae

- (1) *Rhabditis pellio*—Earthworms (Nephridia).

###### Diplogasteridae

- (2) *Pristionchus acrivora*—Termites (Head).

- (3) *Alloionema appendiculatum*—*Limax ater* (Foot, alternation of generations reported).

###### Steinernematidae

- (4) *Ncoaplectana bibionis*—flies (Intestine).

###### bb. Do not feed in adult stage.

###### Mermithidae

- (1) *Agamermis decaudata*—Grasshoppers (Body cavity).

- (2) *Mermis subnigrescens*—Grasshoppers (Body cavity).

- (3) *Allonermis myrmecophila* — *Lasius* spp. (Body cavity).

###### Allantonematidae

- (4) *Chondronema passali*—*Popilius interruptus* (Body cavity).

###### Tetradonematidae

- (5) *Tetradonema plicans*—*Sciara coprophila* (Body cavity).

###### b. Adult stage partially parasitic, partially free living.

###### aa. Monogenetic (Without alternation of generations).

###### Allantonematidae

- (1) *Allantonema mirabile*—*Hylobius abietis* (Body cavity).

- (2) *Tylenchinema oscinella* — Frit-fly (Body cavity).

- (3) *Howardula benigna*—Cucumber beetle (Body cavity).

- (4) *Scatocma wülkeri*—*Scatopsi fuscipes* (Body cavity; sometimes reproduces several generations in host).

- (5) *Aphelenchulus diplogaster*—*Ips typographus* (Body cavity).

- (6) *Parasitylenchus dispar*—*Ips typographus* (Body cavity).

- (7) *Sphaerularia bombi* — *Bombus terrestris* (Body cavity).

- (8) *Tripilus gibbosus*—*Cecidomyia pini* (Body cavity).

###### bb. Heterogenetic (With alternation of generations).

###### Allantonematidae

- (1) *Fergusonia curriei*—One generation in plant, *Eucalyptus macrorrhyncha* (Leaf and flower) other in fly *Fergusonia nicholsonia* (Body cavity).

- (2) *Heterotylenchus aberrans*—One generation bisexual, other parthenogenetic, both in body cavity *Hylemyia antiqua*.

###### BB. Adult stage wholly parasitic.

###### a. Heterogenetic (Free-living generation sometimes suppressed).

###### Strongyloidae

- (1) *Strongyloides stercoralis*—Man (Small intestine).

###### Rhabdiasidae

- (2) *Rhabdias bufonis*—*Bufo americanus* (Lung).

###### b. Monogenetic.

###### aa. Reproduce in the host.

###### Atractidae

- (1) *Probstmayria vivipara*—Equines (Intestine).

###### Steinernematidae

- (2) *Ncoaplectana glaseri*—Japanese beetle (Body cavity).

###### Cylindrogasteridae

- (3) *Longibucca lasiura* — *Lasiurus borealis* (Small intestine).

###### Diplogasteridae

- (4) *Cephalobium microbivorum*—*Gryllus assim-*

\*In this outline no attempt is made to supply all hosts or to include all nematode life histories. Only examples are given.



- us* (Intestine).  
 Monhysteridae  
 (5) *Odontobius ceti*—Whale (Baleen).  
 (6) *Monhystera cambari*—Crawfish (Gills).  
 (7) *Tripylidium carnicolam t. carnicinus lateralis* (Gills).  
 Myenechidae  
 (8) *Myenechus batelhou*—Leeches (Muscle & connective tissue).  
 bb. Do not reproduce in host.  
 aaa. First three larval stages free living.  
 Ancylostomatidae  
 (1) *Ancylostoma duodenale*—Man (Small intestine).  
 Trichostrongylidae  
 (2) *Haemonchus contortus*—Sheep (Abomasum).  
 (3) *Oswaldoeruzia filiformis*—Amphibians (Intestine).  
 Syngamidae  
 (4) *Syngamus trachea*—Poultry (Bronchi or trachea) [Invertebrate, annelid, mollusc or insect transport host facultative].  
 (5) *Ollulanus tricuspis*—Cats (Stomach). [1st moult in parent worm.]  
 Metastrongylidae  
 (6) *Dictyocaulus filaria*—Sheep (Bronchi). [Annelid transport host facultative; 1st 2 larval stages do not feed.]  
 Cosmoecereidae  
 (7) *Cosmoecereoides dukae*—Amphibians and snails (Intestine).  
 hbb. Eggs infective to host.  
 Thelastomatidae  
 (1) *Leidyneema appendiculatum*—*Periplaneta americana* (Intestine).  
 (2) *Pseudonymus spirotheca*—*Hydrophilus piceus* (Intestine).  
 Oxyuridae  
 (3) *Enterobius vermicularis*—Man (Appendix, caecum).  
 (4) *Oxyuris equi*—Equines (Colon).  
 Heterakidae  
 (5) *Heterakis gallinae*—Poultry (Intestine).  
 (6) *Ascaridia galli*—Poultry (Intestine).  
 Ascarididae  
 (7) *Ascaris lumbricoides*—Man (Intestine).  
 Trichuridae  
 (8) *Capillaria columbae*—Pigeons (Small intestine).  
 (9) *Trichuris trichiura*—Man (Caecum).  
**B Heteroxenous (Two or more animal hosts in life cycle).**  
 a. Eggs infective to intermediate host.  
 Metastrongylidae  
 (1) *Metastrongylus elongatus*—Earthworms—Swine (Lung).  
 Heterakidae  
 (2) *Subulura brumpti*—Various insects—Poultry (Caecum).  
 Ascarididae  
 (3) *Raphidascaris canadensis*—*Erygon* nymphs—Minnows—*Esox lucius* (Intestine). 2 intermediate hosts, mandatory.  
 Thelaziidae  
 (4) *Gongylonema pulchrum*—Beetles, roaches—Pig, sheep, deer (Esophagus and mouth).  
 (5) *Spirocerca lupi*—Dung beetles—Dog (Esophagus).  
 (6) *Ascarops strongylina*—Dung beetles—Swine (Stomach).  
 (7) *Physoccephalus scrofae*—Dung beetles—Swine (Stomach).  
 Spiruridae  
 (8) *Tetrameres crami*—Amphipods—Duck (Proventriculus).  
 Acuariaeidae  
 (9) *Chetosporium hamulosa*—Grasshoppers—Poultry (Gizzard).  
 (10) *Echinuria acicula*—Cladocera (*Daphnia*)—Duck (Fore and mid gut).  
 (11) *Dispharurus spiralis*—Isopods—Poultry (Esophagus and crop).  
 Gnathostomatidae  
 (12) *Hartertia gallinarum*—Termites—Poultry (Small intestine).  
 Trichuridae  
 (13) *Capillaria annulata*—Annelid transport host obligatory—Chickens (Crop).  
 Cystoosidae  
 (14) *Cystoopsis acipenseri*—Amphipods—Sturgeons (Skin).  
 Eustrongylidae  
 (15) *Eustrongylides ignotus*?—Crustacean—*Fundulus diaphanus*—*Aedea herodias* (Gizzard).  
 Dioctophymatidae  
 (16) *Dioctophyma renale*?—Crustacean?—fish—Man, dogs, mink (Kidney).  
 b. Larvae infective to intermediate host.  
 aa. Enter final host per os.  
 Draconculidae  
 (1) *Draconculus medinensis*—Cyclops—Man (Under skin).  
 Philometridae  
 (2) *Philometra nodulosa*—Cyclops—*Catostomus commersonii* (Lip).  
 (3) *Philometra fujimotoi*—Cyclops—*Ophicephalus argus* (Fin).  
 Camallanidae  
 (4) *Camallanus sweeti*—Cyclops—*Ophicephalus gachua* (Intestine). Second intermediate host, small fish? obligatory.  
 Pseudaliidae  
 (5) *Muellerius capillaris*—Molluscs—Sheep and goats (Lung).  
 Spiruridae  
 (6) *Habronema muscae*—*Musca domestica*—Equines (Stomach).  
 (7) *Habronema microstoma*—*Stomoxys* spp.—Equines (Stomach).  
 (8) *Draschia megastoma*—*Musca domestica*—Equines (Stomach).  
 Gnathostomatidae  
 (9) *Spiraxys contorta*—Cyclops—Minnows—Turtles (Stomach). Second intermediate host not mandatory.  
 (10) *Gnathostoma spinigerum*—Cyclops—Fish or snakes—Felidae (Stomach). Second intermediate host mandatory.  
 Ascarididae  
 (11) *Contracaecum spiculigerum*—Minnows—Carnivorous fish—Cormorant (Proventriculus). Second intermediate host mandatory.  
 (12) *Raphidascaris canadensis*—*Erygon* nymphs—Minnows—*Esox lucius* (Intestine). Second intermediate host? mandatory.  
 Trichinellidae  
 (13) *Trichinella spiralis*—Rat, pig, man (Intestine). Hosts serve both as intermediate and final host.  
 bb. Enter final host through skin.  
 Dipetalonematidae  
 (1) *Wuchereria bancrofti*—Mosquitoes—Man (Lymphatic system).  
 (2) *Onchoecera volvulus*—*Simulium damnosum*—Man (Subcutaneous).  
 (3) *Onchoecera cervicalis*—*Calicoides nebulosus*—Equines (Cervical ligament).  
 (4) *Dirofilaria immitis*—Mosquitoes—Dogs (Heart).

# CHAPTER V

## LIFE HISTORY (ZOOPARASITICA)

### Parasites of Invertebrates

J. R. CHRISTIE, U. S. Horticultural Station, Beltsville, Md.

#### Introduction

There are many different types of association between nematodes and other invertebrates and it is difficult to draw a line between what should and what should not be regarded as parasitism. Most of the nematodes that live within the bodies of invertebrates are customarily referred to as parasites though there is little evidence that some of them interfere materially with the well-being of their "hosts." We know very little, however, about the effects of these nematodes on the animals that harbor them unless the manifestations are pronounced and obvious. The only feasible procedure is to regard as eligible for inclusion in this chapter all nematodes that regularly spend part of the life cycle within the bodies of invertebrates regardless of the precise character of the association. Species for which vertebrates serve as definitive hosts and invertebrates only as intermediate hosts are dealt with in the following chapter.

In general the parasites of invertebrates and those of vertebrates are not found in the same phylogenetic groups and in those cases where both belong to the same group the vertebrates involved are almost always amphibians and reptiles. However, the Thelastomatidae and the Oxyuridae have very close affinities.

Arthropods, annelids and mollusks are the invertebrates most commonly parasitized by nematodes though scattered cases have been reported where other invertebrates, even nematodes themselves, serve as hosts. There are surprisingly few records of marine invertebrates harboring nematodes and most of these apparently deal with cases where the association is erratic or accidental or where some vertebrate serves as definitive host.

Included among the nematodes harbored by invertebrates are species where a parasitic mode of life is only now being acquired and others where it is of great antiquity. There is great diversity in the types of life cycles and to simplify discussion and facilitate comparison the nematodes are divided into three groups.

The first of these groups is made up of nematodes that are more or less closely related to free-living species and in the life cycles we often find a combination of saprophagous and "parasitic" habits. In one line of evolutionary development the nematodes live and reproduce in the carcass of the "host," to the death of which they may or may not have contributed. Life cycles are simple, perhaps the most outstanding feature being the frequent occurrence of dauer larvae,\* a characteristic that has been carried over from a free-living to a parasitic mode of life. Another line of evolutionary development seems to have culminated in a life cycle where the nematode may pass through one or more free-living generations, then gain entrance to the host and pass through one or more parasitic generations.

The second group comprises those nematodes, not included in the first group, that inhabit the alimentary tract. Life cycles, so far as known, are simple. With perhaps an occasional exception (i. g., *Cephalobium microbitorum*), only the egg stage occurs outside the host, a characteristic shared by very few species in the other two groups.

The third group includes the body-cavity and tissue parasites. In contrast to the first group, these nematodes are highly specialized, obligate parasites and, in contrast to the second group, they pass, at the most, only a transitory period in the alimentary tract of the host. Five families are included in this group. The Drilonematidae and Mylenchidae have received little attention and our knowledge regarding life cycles is very meager. The Tetradenematidae, Mermithidae and Allantonematidae have been somewhat more adequately studied. The nematodes belonging to these three families have been parasites for a very long time and many of them have complicated life cycles that are highly adapted to individual requirements. Of

the various factors that have influenced these life cycles, two stand out as being of great importance.

One of these factors is the necessity for the infective stage to reach and gain entrance to the host. This, of course, is a requisite in the life cycle of every parasite but for the allantonematids and mermithids there are certain restricting conditions with which many of the others do not have to contend, at least not to an equal extent. Some of the hosts are insects that develop in seasonal cycles and where the total life span of the individual may be only a few months. It is frequently necessary that the parasite enter when the host is in a particular stage and this stage may be available only at restricted times of the year. As a result the life cycles of many of these parasites have become closely correlated with the life cycles of their respective hosts.

The other factor is the ability of the nematode to take food only during restricted periods. The fact that for many of these parasites the free-living stage may be of considerable duration and that during this period the nematodes take no food, but, nevertheless, pass through important phases of the life cycle, has had a profound effect on development. In many cases the larval mermithid, during a comparatively short period of parasitic life, must make a phenomenal growth and store sufficient nutrient materials to carry the adult through its relatively long, free-living period of sexual activity and reproduction. The larval allantonematid that develops to maturity outside the host after only a very brief period of parasitic life, must exercise the strictest economy in the utilization of its limited supply of stored nutrients. Since, as a rule, only the female again becomes parasitic, the male must produce and mature its spermatozoa though the production and maturation of the eggs by the female is postponed. There can be little or no increase in body size during this free-living period, hence the adult, impregnated female, after entering a new host, undergoes a period of rapid growth. In the Sphaerulariinae a prolapsus of the uterus has resulted through the inability of the small, underdeveloped body of the young female to keep pace with the rapidly growing reproductive organs.

#### Novitious Parasites and Semiparasites

Among these nematodes two lines of evolutionary development seem to stand out more or less distinctly though it is obviously improbable that they account for the origin of all the different types of parasitism or semiparasitism encountered in this heterogeneous group.

One line of evolutionary development appears to have been initiated when certain saprophagous nematodes utilized other invertebrates, frequently saprophagous insects, as vehicles for transportation. These "hitch-hikers," first seeking protection from desiccation in crevices on the external surface, eventually entered the bodies of their "hosts." In the life histories of species representing an intermediate step in this line of development, larval nematodes, after gaining entrance to the body of the "host" and becoming established therein, do not at once grow to maturity and reproduce but remain in a more or less quiescent condition. These larvae do not appear to interfere materially with the life processes of the animal that harbors them but when the animal dies from other causes the nematodes immediately resume development and reproduce in the carcass.

In some cases, however, this type of relationship has evolved to a point where it is no longer passive but where the nematodes are an important factor in bringing about the death of the animal whose body they enter. Even though present in small numbers, some species of *Neoaplectana* are said to kill their insect hosts in a very short time.

The parasitic or semiparasitic relationship between these nematodes and their respective "hosts" is not always obligatory. Johnson (1913) concluded that entrance into the body of an earthworm is not necessary in the life cycle of *Rhabditis maupasi* but if larvae, during their sojourn in the soil, find suitable decaying organic matter they will develop and reproduce therein. Neither is *Pristionchus acrivora* dependent on entrance into a termite or some other insect to complete its development as it has been found reproducing in a number of different habitats including decaying plant tissues. *Neoaplectana glaseri*, on the other hand, appears to be an obligate parasite that, in nature, develops only after entering the living body of its insect host.

\*The term *dauer larva* is used in this text to designate a larva, in a particular stage of development, that is especially adapted to withstand adverse conditions and, when a dauer stage is not obligatory, that differs from a larva of the same stage that develops when conditions are favorable and food is abundant. The term is not new, having been used by Fuchs and others with approximately this same meaning and, while not of classic origin, it is short, expressive, appropriate and useful. Dauer larvae are of common occurrence in the Rhabditidae and Diplogasteridae and are more characteristic of free-living than of parasitic species, hence the term is not synonymous with "infective larva."

Most of these nematodes are bisexual and females produce fertile eggs only after copulation. Males are usually somewhat less numerous than females, reach maturity a little quicker, and do not live quite so long. According to Johnson, females of *Rhabditis maupasi* usually, though not always, reproduce without males. In many species of this group a female may be oviparous when young but toward the end of life some of the last eggs produced may be retained and hatch in the uterus. The resulting larvae may not escape through the vulva but undergo part of their development within the mother nematode, consuming her internal organs and converting her into a brood sac. Incidentally, this same mode of reproduction is characteristic of many free living species of *Diplogaster*, *Rhabditis* and related genera.

The second line of evolutionary development referred to above may have been initiated when, during periods of adversity, certain saprophagous nematodes, seeking refuge and succor, entered and temporarily dwelt within the bodies of other invertebrates. In the case of nematodes in this category parasitism apparently does not ordinarily result in the death of the host nor are the parasites able to live in a decaying carcass. Usually these nematodes either inhabit the alimentary tract of the host (e. g., *Angiostoma limacis*) or are associated with its reproductive organs (e. g., "*Angiostoma helcis*"). For at least one species (i. e., *Alloionema appendiculata*) an alternation of one or more parasitic generations with one or more free living generations has become a more or less regular procedure.

*RHABDITIS MAUPASI* Caullery and Scurat, 1919 (Syn. *R. pelloi* Bütschli, 1873; not Schneider, 1866). Larvae of *Rhabditis maupasi* are found in the nephridia and coelom of living earthworms. For *Lumbricus terrestris* L. the incidence of infection is frequently very high and at least several and perhaps many other species harbor these nematodes more or less frequently.

Larvae are found near the nephridiopore in the dilated, muscular termination or "bladder" of the nephridial tube. Often nearly every tube is inhabited, the number of worms in each varying from 2 or 3 to 12 or more. Also larvae may occasionally be found in the seminal vesicles. When in these above mentioned locations larvae are in an active condition and not ensheathed. Johnson concluded that these inhabitants of the nephridia are not necessarily confined to this location throughout the life of the earthworm but may move out into the soil and later go back through the nephridiopores into the same or a different earthworm.

Larvae occur also in the coelom and these are usually ensheathed and inactive (Fig. 165C). Occasionally a larva may be embedded in the muscles of the body wall or encysted on a septum. Frequently several larvae are embedded in a brown, oval body composed of cysts of the sporozoan, *Monocystis*, and various earthworm tissues. Such bodies are most common at the posterior end of the coelom.

There is no evidence that the presence of these larval nematodes is detrimental to the annelid. So long as the earthworm is alive the nematodes remain in a larval stage but when the earthworm dies they quickly grow to adults (Fig. 165 A & B) and reproduce in the carcass. Otter (1933) concluded that a female lives from 7 to 10 days after reaching maturity and lays from 150 to 300 eggs. Males, in his opinion, live about a third as long as females. No doubt several generations occur before the food supply is exhausted though Johnson was uncertain on this point. After the body of the earthworm is consumed large numbers of larvae move out into the soil where they live awaiting the opportunity to enter another earthworm. Larvae from the soil are said to be in the same stage as those from the nephridia, but what this stage is has not been stated.

With regard to the method of entering the earthworm, Johnson writes: "Those that enter by the nephridiopores take up their position in the terminal, bladder-like part of the nephridia. Those that use the spermiducal apertures travel up the vasa deferentia and occupy the seminal vesicles. Lastly, those that pass in by the dorsal pores and the oviducal apertures find themselves in the coelom, where, being attacked by the amoebocytes, they encyst. These encysted larvae coated with amoebocytes are worked backward by the movement of the worm till they come to rest in the tail end of the worm, where, together with other foreign bodies, such as cysts of *Monocystis* and discarded setae, and with masses of dead brown-colored amoebocytes, they are compressed and cemented into the brown bodies which are found there."

According to Keilin (1925) the accumulation of foreign bodies in the posterior segment of an earthworm may induce the development of a stricture that will sever this distended terminal portion from the rest of the body. The detached portion then decomposes and in this manner *R. maupasi* and other coelomic parasites of the earthworm may be liberated.

Males of *R. maupasi* are much fewer in number than females. Although Johnson did not observe copulation, his rearing ex-

periments lead him to conclude that most females are hermaphroditic but that occasionally females occur that are able to reproduce only after being fertilized by males. Otter, who observed copulation and agrees, in the main, with Johnson, writes that *R. maupasi* "may thus be considered to be one of those species of *Rhabditis* in which hermaphroditism is in a very early stage, and in which functional males, females, and hermaphroditic females, exist side by side in fluctuating proportions."

*PRISTIONCHUS AERIVORA* (Cobb, 1916), was first found by Merrill and Ford in the heads of termites, *Leucotermes lucifugus* Rossi,\* collected near Manhattan, Kansas. Under natural conditions the nematodes varied from 0 to about 75 per insect. After experimental termites had been kept for 4 days in soil heavily infested with *P. aerivora*, the average number of nematodes per insect was 46.6 while termites used as controls averaged about 3 nematodes per insect. How the nematodes enter or why, in living termites, they are found only in the head are points that have not been determined. The parasites do not reach maturity in living hosts but when the termites are heavily infected they become sluggish and die, whereupon the nematodes reproduce in the carcass. Hence, in this instance, the relationship is not purely passive.

Merrill and Ford were able to rear this nematode in water cultures with various substances supplied for food, preferably the macerated bodies of insects. Eggs hatched in about 18 hours and the adult stage (Fig. 165 J) was reached in about 2 days. The complete life cycle from egg to egg required about 4 to 5 days but after beginning to lay eggs an adult female usually lived for 12 to 13 days. During a period of 13 days one female, while under observation, copulated with 7 males and deposited 317 fertile eggs and 14 infertile eggs. Males were somewhat less numerous than females. They lived for about 19 days and one male, while under observation, copulated with 10 different females.

Toward the end of life a female becomes sluggish and eggs are not extruded but hatch in the uterus. While the resulting larvae may sometimes escape through the vulva they usually remain in the mother nematode, feeding on her internal organs.

Since Merrill and Ford's investigations nematodes identified as *P. aerivora* have been reported from various other habitats. They have been found in other termites, usually located in the head while the insect is alive. They have been found in dead pupae of the corn ear worm, *Heliothis armigera* (Hübner), and in dead pupae of the rose leaf beetle, *Nodonota puncticollis* (Say). They have been found in grasshopper egg masses where they were reported to have been destroying the eggs. On several occasions they have been found in decaying plant tissues. However, the populations from these different habitats may represent different strains or, perhaps, even different, though closely related, species.

The peculiar habit of swallowing air, to which this nematode owes its specific name, is shared by several species of *Diplogaster* and *Rhabditis*. When mounted in water on a microscope slide, one of these nematodes may place its head against the surface of an entrapped air bubble and air can be seen as it passes down the esophagus to the anterior end of the intestine where it is quickly absorbed. According to Cobb (1915) some of these nematodes can ingest their own volume of air in the course of an hour or two. The swallowing of air is accomplished by the usual rhythmic muscular movements of the esophagus. During the first muscular movement a small bubble of air passes quickly from the mouth to the median pseudobulb where it stops. At the next muscular movement the bubble passes on into the intestine while another simultaneously passes from the mouth to the median pseudobulb. This may continue uninterrupted for a considerable period of time.

*NEOAPLECTANA BIBIONIS* Bovien, 1937, was studied by Bovien (1937) who found it in Denmark associated with the dipterous insects *Bibio ferrugineus* (L.), *B. hortulans* (L.) and *Dilophus vulgaris* Meig.

An interesting and significant point in the life cycle of this nematode is the occurrence of dauer larvae (Fig. 165 G). These, according to Bovien, are in the third stage. A dauer stage is not obligatory but occurs only when environmental conditions are unfavorable to enable the nematode to persist through periods of adversity. Dauer larvae are relatively sluggish and are usually enclosed in a partly separated cuticle though this may be lost before the end of the dauer stage. These larvae are easily distinguished from third-stage larvae that develop under favorable conditions being slender and differing in other morphological details. Bovien found dauer larvae clinging to the surface of adult flies and being transported by them.

The various host insects become infected by swallowing these dauer larvae which, on reaching the alimentary tract, remain

\*Regarded by Snyder, according to Van Zwaluwenburg (1928, p. 9), as either *Reticulitermes tibialis* Banks or *R. claripennis* Banks.

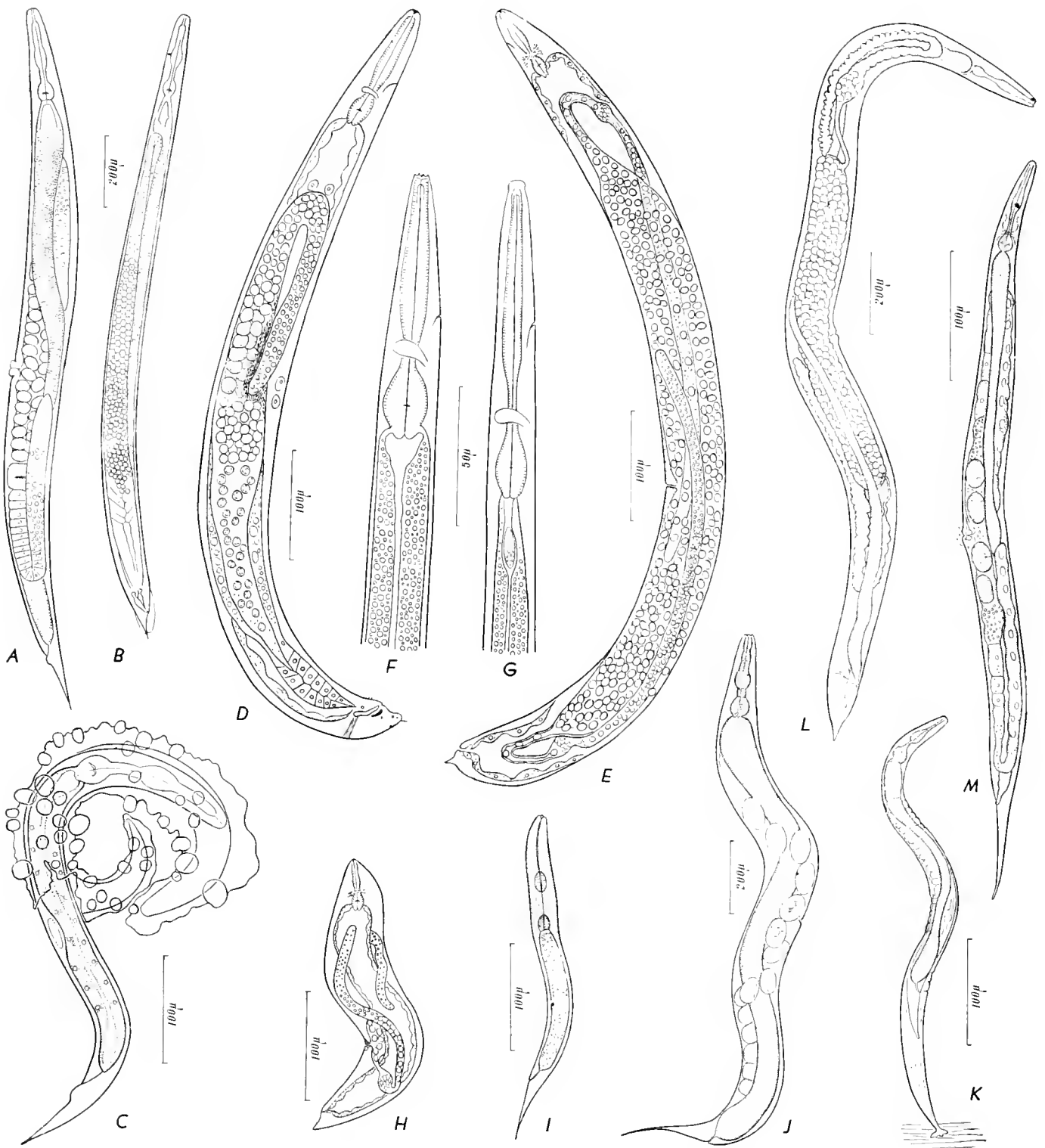


Fig. 165. NOVIIOUS PARASITES AND SEMIPARASITES

A-C—*Rhabditis manpasi* (A—Adult female; B—Adult male; C—Larva escaping from "cyst"). D-H—*Neoaplectana bibionis* (D—Adult male; E—Adult female; F—Larva that developed under favorable conditions; G—Dauer larva of same stage as F; H—Pigmy female). I-J—*Pristionchus aerivora* (I—Newly hatched larva; J—Adult female). K—

*Diplogaster lobiata*, dauer larva. L & M—*Alloionema appendiculatum* (L—Adult female of parasitic generation; M—Adult female of free-living generation). A-C, after Johnson, 1913; D-H, after Bovien, 1937; I-K, after Merrill and Ford, 1916; L & M, after Claus, 1868.

unchanged, apparently having no adverse effect on the insect. When eventually the insect dies, presumably from other causes, the larvae move into its tissues and proceed in development, passing through several generations and quickly building up a large population. When the carcass has been consumed young nematodes move out into the soil and develop into dauer larvae.

Although flies become infected while in the larval stage, if the nematodes, on reaching the intestine, are wholly innocuous, many of the insects must carry their infection on into the adult stage. Boyien is not very lucid on this point but he mentions finding nematodes in living fly pupae, on one occasion in the body cavity.

Boyien concluded that development from egg to egg laying female requires about 4 days. A young female is oviparous but some of the last eggs laid by an old female hatch within the uterus. Each female usually produces somewhat in excess of 200 eggs.

It is not strictly necessary that *N. bibionis* enter living insects as Boyien was able to rear several generations on dead insects of different species if fresh cadavers were periodically provided. Several generations could sometimes be reared on egg albumen.

Gravid females (Fig. 165 E) usually attain a length of up to 5 mm, but Boyien reports finding mature, reproducing females that failed to reach a length of 1 mm. (Fig. 165 H), perhaps due to some nutritional deficiency. Between these dwarfs and females of maximum stature numerous intermediate sizes were found.

*NEOAPLECTANA GLASERI* Steiner, 1929, was first found in dead larvae of the Japanese beetle, *Popillia japonica* Newm., collected in New Jersey and is best known as a parasite of this insect. However it has been demonstrated that this nematode will infect larvae of the European corn borer, *Pyrausta nubilalis* (Hüb.) and coleopterous larvae belonging to at least nine genera including the white fringed beetle, *Pantomorus leucoloma* (Boh.).

The life history of *N. glaseri* has been investigated by Glaser (1932) and by Glaser, McCoy and Girth (1940). The following account is based on their results that were secured, in part by using Japanese beetle larvae as experimental hosts and in part by rearing on culture media. It is believed that the behavior of this nematode is not materially different whether growing on culture media or in the various susceptible insect hosts.

Japanese beetle grubs acquire their infection by ingesting third stage, infective larvae of the parasite. On reaching the alimentary tract the larvae immediately develop to maturity and copulate. A female will not produce offspring unless fertilized by a male. The female is ovoviviparous, eggs hatching within the uterus. Larvae may remain within the uterus and move about for a considerable period but eventually pass out through the vulva one at a time. If a female dies before all larvae are born those remaining may undergo partial development within the dead body. Each normal sized female produces a total of about 15 offspring and a generation under optimum conditions requires about 5 to 7 days. By the time the first generation of offspring have matured the insect is usually dead whereupon its entire body is invaded. The nematodes usually pass through two more generations consuming the carcass and leaving only a sac formed by the skin and head capsule and filled with a thin fluid swarming with larval parasites. In a few cases Glaser was able to infect newly killed beetle grubs but he concluded that the nematodes do not enter and multiply as readily in cadavers as in living insects.

With regard to the virulence of this parasite, Glaser, McCoy and Girth (1940) write that "occasionally an insect host becomes parasitized very lightly, so that only one nematode becomes successfully established. This individual may be of either sex, and while it frequently (if not always) causes the death of the host, there is no reproduction."

McCoy, Girth and Glaser (1938) report that exceptionally large females of *N. glaseri* are occasionally found in beetle larvae though never on cultures. Such individuals may develop an enormous number of eggs, one giant female producing 1,420 larvae. When offsprings of a giant are reared to maturity on cultures only normal sized females are obtained. McCoy, Girth and Glaser concluded that fecundation at a late period in development and abundant food are factors contributing to the production of these giant females.

So long as conditions are favorable and abundant food is available, the life cycle of *N. glaseri*, according to Glaser, McCoy and Girth (1940), is completed in three molts the third stage being omitted. When conditions are unfavorable, as when the carcass of the beetle larva has been consumed and food is exhausted, the young parasites develop into third stage, dauer larvae. At the end of the second stage growth ceases, the alimentary tract is emptied, and, as a result of certain morphological changes, the body becomes more slender. The second molted

cuticle is retained hence the dauer larva is ensheathed though the sheath is not very tenacious and may soon be lost. These dauer larvae escape into the soil where they are able to persist, in a more or less active condition, for at least 2½ years.

Glaser and his coworkers have reared this nematode successfully on Petri dish plates of veal infusion agar flooded with living yeast, on potato culture medium, and on veal pulp medium. These investigators found that "distinct cultural characteristics occur in nematodes from different insect cadavers. . . . There is a slow decline in fecundity of the cultured nematodes, some 'strains' dying out after 5 or 6 transfers, while others continue to yield good cultures after 20 or more transfers." If beetle larvae are infected with nematodes from cultures that are dying out and several generations are passed in the natural host, the nematodes can again be reared successfully on cultures, the length of time before the cultures again die out depending, to some extent, on the number of generations passed in beetle larvae.

*ALLOIONEMA APPENDICULATUM* Schneider, 1859, has on several occasions been found within the bodies of slugs. Schneider found it originally in *Arion ater* (L.) and Claus (1896), who investigated its life history, secured his material from the same host. The life cycle of this nematode appears to represent a somewhat different line of evolutionary development than the life cycles already discussed. According to Claus (1896), one or more free living generations alternate with one or more parasitic generations, both males and females (Fig. 165 L & M) developing in each instance. Individuals of the parasitic generations leave the host just before reaching maturity by boring their way out through the foot. On reaching the exterior they mature, copulate and produce progeny that usually develop as free-living individuals. Maupas (1899) found that larvae of the free-living generation undergo the usual four molts and reach maturity in about 3½ days.

A regular alternation of a free-living with a parasitic generation does not necessarily follow, however, as there may be several consecutive free living or several consecutive parasitic generations. There are usually consecutive free-living generations as long as conditions are favorable but when conditions become unfavorable the nematodes "encyst" and these "encysted" larvae will continue development only when taken into the body of a slug. According to Maupas, "encysted" larvae that fail to gain entrance to a slug become exhausted and die in about 4 months. Precisely how the nematodes enter the slugs and whether or not, in event of consecutive parasitic generations, females mature without leaving the host, are points that seem to need further elucidation.

Claus found certain morphological differences between corresponding stages of the two generations. Adults of the parasitic generations are much larger than adults of the free-living generations and parasitic larvae, in the later stages of development, are said to possess two long, ribbon-like, caudal appendages not present on free-living larvae of the corresponding stage.

**OTHER SPECIES.** *Diplogaster labiata* Cobb (in Merrill and Ford, 1916) was found in the elm borer, *Saperda tridentata* Oliv., collected near Manhattan, Kansas. This nematode reproduces in the intestine of the living, adult borer and may accumulate in sufficient numbers to rupture the gut and kill the insect. Infected female beetles are usually sterile. When reared on cultures, Merrill and Ford (1916) found that eggs hatched in from 30 to 32 hours and the nematodes matured in 7 to 10 days. Oviposition began from 2 to 4 hours after copulation and lasted for about 2 days with an average output of seven eggs per female. Only a few individuals were seen copulating a second time. Apparently dauer larvae (Fig. 165 K) develop when conditions are unfavorable.

*Neoaplectana affinis*, Boyien, 1937, was found in Denmark where it infects larvae of the same insects that harbor *Neoaplectana bibionis*, i.e., *Bibio ferruginatus*, *B. hortulans* and *Dilophus vulgaris*. These two nematodes were differentiated morphologically by Boyien (1937) only on the basis of males and dauer larvae, the life cycles and behavior of the two being almost identical. Boyien made one observation, however, that deserves mention. When in the intestine of any of its three natural hosts mentioned above, *N. affinis* remained in the dauer stage and was apparently innocuous so long as the insect remained alive. When two larvae of a beetle, *Telophorus* sp., were experimentally infected, they became moribund in a few days and dissection revealed several adult and preadult nematodes in the body cavity of each beetle. This observation suggests that whether or not *N. affinis* remains passively in the intestines depends on the insect involved.

A mode of life on the border line between saprophagous and parasitic is characteristic of other nematodes, probably of a considerable number. Other species of *Neoaplectana* are known to exist but life cycles have not been investigated. *Steinernema*

*kraussei* (Steiner, 1923), found in the intestine of the wasp, *Cephalcia abietis* (L.), is so closely related to the genus *Neoplectana* that a similar mode of life is suggested but verifying information is lacking.

Among the rather numerous and diverse nematodes that have been reported from snails and slugs are representatives of the Angiostomatidae and Cosmocercidae, two families that include also parasites of Amphibia. The four species mentioned below will serve as examples but very little information is available about life cycles. *Angiostoma limacis* belongs to the Angiostomatidae while the other three, according to Chitwood and Chitwood (1937), probably belong to the Cosmocercidae.

*Angiostoma limacis* Dujardin, 1845, has, on at least two occasions, been found in the intestine of *Arion ater* (L.) (Syn. *Limax rufa*) where, apparently, it reaches maturity. Chitwood and Chitwood (1937) report finding a very closely related species in the intestine of a salamander, *Plethodon cinereus*.

*Ascaroides limacis* Barthélemy, 1858, was found in eggs of *Deroceras agrestis* var. *cineracea* Moq. Tand. (Syn. *Limax griseus*), each infected egg containing one to four larval parasites. Barthélemy (1856) determined that the nematodes were already present when the eggs were deposited. Apparently the adult of this parasite has not yet been studied.

"*Angiostoma*" *helicis* Conte and Bonnet, 1903, was secured by its discoverers from the slug, *Helix aspersa* (Müll.), where it occurred in the genital organs, especially the oviducts and seminal vesicle, but not elsewhere in the body. Conte and Bonnet (1903) concluded that the parasite is passed from host to host during copulation.

*Trionchonema rusticum* Kreis, 1932, was secured from the land snail, *Polygyra espicola* Bland. Presumably this parasite is an inhabitant of the alimentary tract though the location within the host was not specified. Kreis (1932) refers to the development of a "filariform" larva and suggests the possibility "that there is still another stage of development, perhaps a rhabditiform larva, which could not be found and which may perhaps be free-living."

### Parasites of the Alimentary Tract

All nematodes belonging to the families Thelastomatidae and Rhigonematidae and to the subfamily Ransonnematinae are parasites of the alimentary tract and one finds an occasional species of the family Diplogasteridae that has acquired this mode of life.

The thelastomatids are parasites of insects and myriapods and scattered through the literature are descriptions of between 60 and 70 species but usually not much other information. However, studies by Gale (1878), Dobrovolny and Ackert (1934), and others indicate that most of these species probably have about the same type of life cycle and that it is comparatively simple. Eggs pass out of the host with the feces. Eggs do not hatch in the intestine to reinfect the same host but must first undergo some development on the outside to reach an infective stage. The various arthropod hosts acquire their parasites by swallowing these infective eggs.

In the genus *Pseudonynnus*, the species of which are parasites of aquatic beetles, the egg is provided with two entangling appendages, the so-called spiral filament (Fig. 135 R, p. 176) which, presumably, enables the egg to hang on aquatic vegetation thus increasing its chance of being ingested. From two to four eggs of *Binema binema* and *B. ornata* (Fig. 166G) are enclosed in an outer capsule or case of loose texture formed, apparently, by the entangling and anastomosing of polar filaments. The purpose of this adaptation is obscure.

The Rhigonematidae and Ransonnematinae are small groups with only a few species each. It seems probable that life cycles of these nematodes are not materially different from the type of life cycle characteristic of many thelastomatids though, admittedly, such a statement is wholly conjectural.

*CEPHALOBIVUM MICROBIVORUM* Cobb, 1920, a member of the Diplogasteridae, inhabits the intestine of the black field cricket, *Gryllus assimilis* (Fab.), where it may occur in numbers up to 30 or more. Infected crickets have been collected in Virginia and Kansas. In the region of Manhattan, Kansas, according to Ackert and Wadley (1921), there are two races of this insect each having one brood a year. One race matures during April and May and overwinters in the nymph stage while the other race matures during August and September and overwinters in the egg stage. These investigators found that in autumn over 85 per cent of the adults of the latter generation were infected, the incidence being somewhat higher in female (about 90 percent) than in male crickets (about 70 percent).

Eggs of *C. microbivorum* are usually deposited in a four-cell stage and pass out of the host with the feces. Ackert and Wadley concluded that probably eggs hatch after being voided and that a cricket becomes infected by ingesting larval nematodes

perhaps after these have undergone a brief period of free-living development. The two races of crickets provide the parasite with suitable hosts throughout most of the year and, no doubt, some of the nematodes pass the cold season in overwintering nymphs. The presence of this nematode has no obvious effect on the well-being of the cricket.

*LEIDYNEMA APPENDICULATUM* (Leidy, 1850) Chitwood, 1932. —The life history of *Leidynema appendiculatum*, which was investigated by Dobrovolny and Ackert (1934), is probably more or less typical of many thelastomatids and will serve as an example of the family. This nematode is a parasite of the cockroaches, *Blatta orientalis* (L.) and *Periplaneta americana* (L.). Out of 259 individuals of *P. americana* collected by Dobrovolny and Ackert at Manhattan, Kansas, 90 harbored this parasite in numbers of from 1 to 36 per host.

The egg, deposited in a one to a four-cell stage, passes out of the insect with the feces. After extrusion it undergoes a short period of development and a tadpole-like larva (Fig. 166 A) is formed. The larva is at first motile, wiggling and squirming about, but becomes inactive as the infective stage (Fig. 166 B) is reached. Dobrovolny and Ackert found that at 37° C. eggs reach this infective stage in 3 to 7 days and

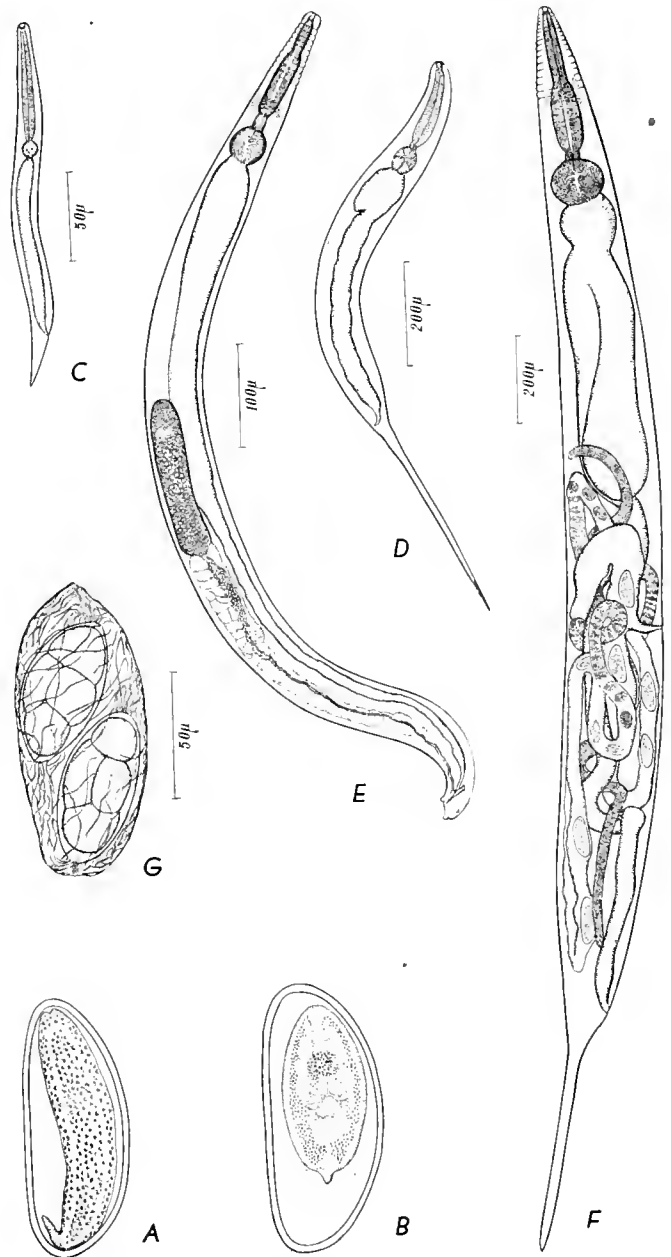


Fig. 166. PARASITES OF THE INTESTINE

A-F—*Leidynema appendiculatum* (A—Egg with active embryo; B—Egg with larva in resting stage; C—An early stage larva, presumably second stage; D—Larval female showing intestinal diverticulum beginning to form; E—Adult male; F—Adult female). G—*Binema ornata*, egg capsule. A-F, after Dobrovolny and Ackert, 1934.

that eggs containing motile larvae are not infective. Alicata (1934) found that the larva of *Blatticola blattae*, a closely related thelastomatid, molts in the egg before reaching the infective stage and while Dobrovoly and Aekert do not mention the matter their figures indicate that *L. appendiculatum* undergoes a similar molt. If kept moist at room temperature and in subdued light infective eggs remain viable for a considerable time but are killed by prolonged exposure to direct sunlight. Infective eggs are ingested by the insects and hatch in the posterior part of the midgut.

Dobrovoly and Aekert kept heavily infected cockroaches in captivity for more than a year and saw no evidence that the insects were markedly affected by the parasites.

### Body Cavity and Tissue Parasites

This group includes what are, perhaps, our oldest parasitic nematodes in the sense that their progenitors were the first to assume a parasitic mode of life and through the ages they have become very highly adapted to this way of living. Some of the allantonematids have become almost incredibly specialized in morphology, behavior and host-parasite relationships and among them are to be found some of the most unusual nematodes known.

#### MYENCHIDAE

This is a small and comparatively little known group of nematodes that are parasites of amphibians and leeches. The systematic position of the family is somewhat questionable but investigators who have studied the group regard it as probably related to the Tylenchidae. Both sexes are characterized by a medium sized stylet without basal swellings and by a peculiar, sucker-like organ situated on the mid-ventral surface about one-fifth of the distance from head to tail, this latter presumably marking the position of the excretory pore. Two species have been reported from leeches.

MYENCHUS BOTHRYOPHORUS Schuberg, 1904, was found in Germany parasitizing the leech, *Eropobdella octoculata* (L.) (Syn. *Nephele vulgaris* (Müller) Moq. Tand.). Different stages of the nematode, including sexually mature individuals (Fig. 167 A & B), occurred in the connective tissues and larvae were found within the muscle cells (Fig. 167 C). Adults were also found in the cocoons of the leech. All the details of the life cycle are not known with certainty but Schuberg and Schröder (1904) concluded that larvae undergo the first part of their development within the muscle cells, then leave this location and enter the connective tissues where they continue development to sexual maturity. From this point on the life cycle is apparently continued outside the host, presumably in the cocoons. Schuberg and Schröder suggest that the nematodes reach the cocoons either by penetrating into the gonads and passing out with the reproduction products or by penetrating directly through the body wall and entering the cocoon while this structure still encompasses the body of the leech. The fact that the parasites are frequently found in the connective tissues immediately underlying the epidermis of the leech seems to make the latter alternative all the more probable. Schuberg and Schröder concluded that the females lay their eggs within the cocoons and that the resulting larvae infect the young leeches. How the parasite enters the host has not been determined.

MYENCHUS BOTELHOI Pereira, 1931, is a parasite of the leech, *Limnobdella brasiliensis* Pinto, and was found and studied in Brazil. According to Pereira (1931), infected leeches harbored the nematode in all stages of development. The epididymus was a favored location but the parasite was found in other connective tissues though rarely in the muscles and never in the alimentary tract. Apparently the worms occurred between but not within the cells. The outstanding point of interest regarding this nematode is the fact that Pereira found it regularly within the spermatophores of the leech. It would appear, therefore, that the parasite enters the spermatophores at some time during their formation or passage out of the leech and uses them as a vehicle for transmission from host to host.

#### DRILONEMATIDAE

This is a small family of about a dozen genera that are either monotypic or contain only a few species each. These nematodes are parasites of earthworms and occur in the coelomic cavities, in or associated with the reproductive organs or embedded in the muscles. Many of the species are characterized by large, sometimes almost sucker-like, phasmids and some of the species by large cephalic hooks. Very little is known about life cycles.

DICELIS FILARIA Dujardin, 1845.—Of the specimens of *Lumbricus rubellus* Hoff., collected by Wülker (1926) in Germany near Frankfurt a. M., about 25 percent harbored this parasite

(Fig. 167 E & F) but other species of earthworms collected in the same region were not infected. The usual number of nematodes per host was 6 to 8 with a maximum of 22, females generally outnumbering males. The parasites occurred in the body cavity of the host in the region of the reproductive organs but not in the nephridia.

The covering of the egg (Fig. 167 D) is thick with a rough outer surface indicating that the shell proper is probably covered by an external coat and suggesting that the egg is equipped to resist adverse conditions and persist in the soil for a considerable period. Eggs are laid in the body cavity of the host but do not continue development in this location. Wülker did not find larval stages either in earthworms or in surrounding soil and was unable to follow the life cycle. It is not known how eggs are expelled from the host, or in what stage, the parasites enter. Wülker demonstrated that if the earthworm dies these nematodes are unable to reproduce in the egg mass but perish with the host.

#### TETRADONEMATIDAE AND MERMITHIDAE

To the family Tetradonematidae there have, as yet, been assigned only two species, *Tetradonema plicans* and *Aproctonema entomophagum*. These, essentially, are primitive mermithids and must be included in any general consideration of life cycles in this group.

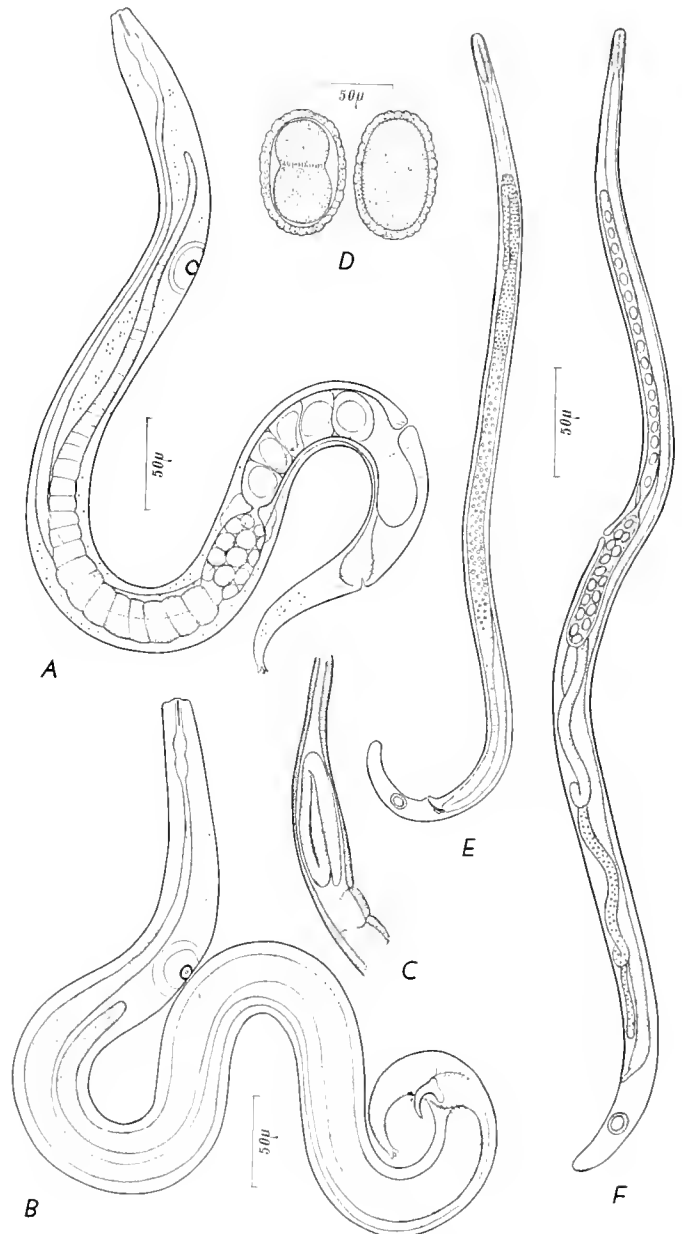


Fig. 167. MYENCHIDAE AND DRILONEMATIDAE

A-C—*Myenchus bothryophorus* (A—Adult female; B—Adult male; C—Larva in muscle cell). D-F—*Dicelis filaria* (D—Eggs; E—Adult female; F—Adult male). A-C, after Schuberg and Schröder, 1904; D-F, after Wülker, 1926.

The mermithids are preeminently insect parasites although crustaceans, spiders, snails, and some other invertebrates are included among their hosts. Most of our knowledge regarding life cycles and habits has been derived from a study of species that infect insects and the following discussion has, of necessity, been written with these hosts in mind.

Eggs may hatch outside the host and larvae reach the body cavity of the young insect by penetrating its body wall or eggs may be ingested and larvae reach the body cavity by penetrating the wall of the gut. In the former type of life cycle there is a tendency for larvae to enter while their hosts are young and for each host to harbor a small number of parasites. In the latter type of life cycle the chances of the host becoming infected are likely to increase with its age and food consumption and the number of parasites per host is likely to be greater.

*Tetradonema plicans*, after reaching the body cavity of its dipterous host, develops to maturity, copulates and lays its eggs as an internal parasite. This is a simpler and probably a more primitive life cycle than that known for any mermithid. Most mermithids, after completing growth, force their way out of the host and are free living during the adult stage. For *Agamermis decaudata*, *Mermis subnigrescens*, and probably some other species, the free living period is of two years' duration and during it the worms undergo their last molt, copulate, and females lay their eggs. *Aproctonema entomophagum* develops to maturity and copulates within its host but females emerge to lay eggs, while *Paramermis contorta* undergoes its final molt within the host but emerges before copulation. For both these species the free-living stage is of very short duration and these life cycles seem to represent intermediate steps between the life cycle of *Tetradonema plicans* and that of such species as *Agamermis decaudata*.

If a species enters its host by penetrating the body wall the posterior portion of the larva is often modified to serve as a propelling organ. In some cases, as for example species of *Agamermis*, this modified posterior portion, which may constitute as much as four-fifths of the total body length, is detached during the act of penetration and remains on the outside. In other species this modified portion is relatively shorter and persists to form a horn-like appendage at the posterior terminus of the fully grown larva.

In most mermithids, especially those having an adult, free-living stage of considerable duration, the intestine grows rapidly during parasitic development until it fills nearly all the space in the body not occupied by other organs. This modified intestine, filled with reserve nutrient materials and frequently referred to as the "fat body," is largely responsible for the opaqueness of the fully grown larva. The adult becomes increasingly transparent as these stored nutrients are consumed and life ends when they are exhausted.

Most mermithids are represented by both sexes but the sex ratio is subject to a good deal of variation, not only as between different species but in the same species. Males of *Amphimermis zumushi* and of *Agamermis decaudata* considerably outnumber females while males of *Mermis nigrescens* and *M. subnigrescens* are rarely found. The sex ratio of some species is influenced by environmental conditions during parasitic development. One or a few parasites per host results in a preponderance of the larvae developing into females while a large number of parasites per host results in all, or nearly all, developing into males. Convincing data demonstrating this environmental influence on sex ratios have been presented by Caullery and Comas (1928) for *Paramermis contorta*, by Christie (1929) for *Mermis subnigrescens*, and by Kaburaki and Iyatomi (1933) for *Amphimermis zumushi*. There is evidence suggesting that some other species behave in a similar manner.

Functional females that possess such male characters as caudal papillae, male copulatory muscles, and even rudimentary spicules have been reported from numerous species. It seems probable that there is some correlation, as yet not understood, between the influence of environment on sex and the occurrence of these so-called "intersexes."

Females of *Ucramermis* sp. (parasite of the ant, *Phidole pallidula*) and of *Agamermis decaudata* lay eggs only after copulation. Females of *Allomermis myrmecophila* and of *Mermis subnigrescens* produce viable eggs in the absence of males though individuals of the latter species have been observed in copula.

The presence of mermithid parasites affects insects in various ways: development of the gonads, especially the ovaries, is usually suppressed resulting in sterility; wing muscles are sometimes weakly developed reducing ability to fly; internal fat deposits are largely consumed; development of the body as a whole may be retarded and metamorphosis delayed; and infected individuals may be sluggish or, in the case of ants, have a voracious appetite. As a rule external morphological characters are not appreciably modified but there are exceptions, that of

ants being the most outstanding. The emergence of the parasite usually results in the death of the host.

Numerous species of ants are rather commonly infected with mermithids. Males, females, workers and soldiers have been reported as harboring these parasites and there is wide variation in the effects of the mermithids on the external anatomy of the hosts. In some instances infected ants show little recognizable difference from normal individuals of the same sex or caste, except, perhaps, a somewhat more distended gaster and slight variations in color. This seems frequently to be the case with infected males but sometimes, according to Gösswald (1930) and Vandel (1934), infected females, workers or soldiers are not materially modified. In some instances, on the other hand, the external anatomy is greatly modified (Fig. 169 C G) and infected ants are not identical to any normal caste but show female, worker and soldier characters in varying degrees. Such individuals are called *intercastes*.

In the genus *Lasius* infected females resemble normal females but are easily recognized, at least in many instances, by a smaller head, shorter wings, and a somewhat more distended gaster. Intercastes of this type have been designated *mermithogynes*.

In the genus *Phidole*, Wheeler (1928) found a variety of different intercastes with mixtures of soldier, worker and female characters. He recognized five more or less distinct types based on the degree of resemblance to one or another of these three normal castes. In all these types the resemblance was more especially to workers and soldiers and for these intercastes Wheeler proposed the term *mermithergates*.

To Vandel (1930), working with *Phidole pallidula*, the situation was somewhat simpler as he was able to recognize only two types of intercastes. One type showed no very pronounced difference from normal workers except a somewhat more distended gaster. The other type he believed to be modified soldiers and for these he proposed the term *mermithostratiotes* reserving the term *mermithergates* for those intercastes where resemblance to workers predominates.

Gösswald (1930) found young mermithid larvae in ants at various times of the year and concluded that there may be considerable variation in the time when these insects acquire their parasites. Although mermithids have been found in larval ants, only a few such cases have been reported, and Vandel concluded that the infection is usually acquired during or just prior to the pupal stage. Based on the size and development of larval mermithids from young ants, Gösswald concluded that the parasites may be acquired when the immature insects are in different stages of development and that the stage when the parasites are acquired determines, in a large measure, the degree to which the adult host will be modified.

How ants acquire these parasites is a question that has aroused considerable interest but stimulated little actual investigation. Gösswald (1930) conducted infection experiments with *Lasius alienus* and used eggs of what was, presumably, *Allomermis myrmecophila*. His results indicate that the ant acquires this parasite by ingesting the eggs. As ant-infecting mermithids belong to several genera (*Agamermis*, *Ucramermis*, *Allomermis*, etc.) life cycles and behavior undoubtedly differ and all may not necessarily enter the host at the same time or in the same manner. It would be surprising if an ant became infected with a species of *Agamermis* by ingesting its eggs.

*TETRADONEMA Plicans* Cobb, 1919, is a parasite of the dipterous insect, *Sciara coprophila* Lint. It has been found in only one collection of these insects made by Hungerford (1919) at Manhattan, Kansas, in which every individual was infected. It occurred in larval, pupal, and adult flies each insect harboring from 2 to 20 parasites with an average of about 10, the number of males slightly exceeding the number of females. *T. plicans* passes its adult stage and lays eggs within its host, differing in this respect from any mermithid of which the life history is known.

How the insects acquire their infection has not been determined. Eggs (Fig. 168 G) secured by Hungerford from around females dissected out of fly maggots hatched in a few hours when placed in water and the larvae that emerged seemed to be identical with the youngest larvae found within the insects. These larvae were of two types, a slender type about 125 $\mu$  long with a curved caudal end and a plumper type about 90 $\mu$  long. This difference, presumably, is sexual dimorphism. Hungerford found eggs of the parasite in the digestive tract of small *Sciara* larvae and concluded that eggs are probably swallowed and nematode larvae, after hatching, penetrate through the wall of the gut into the body cavity. He noted, however, that "the older maggots are much less susceptible to infestation than the younger ones" and he figures the tail of the adult parasite with a horn like projection which suggests that the larva has a caudal propelling organ, two characteristics that one is inclined



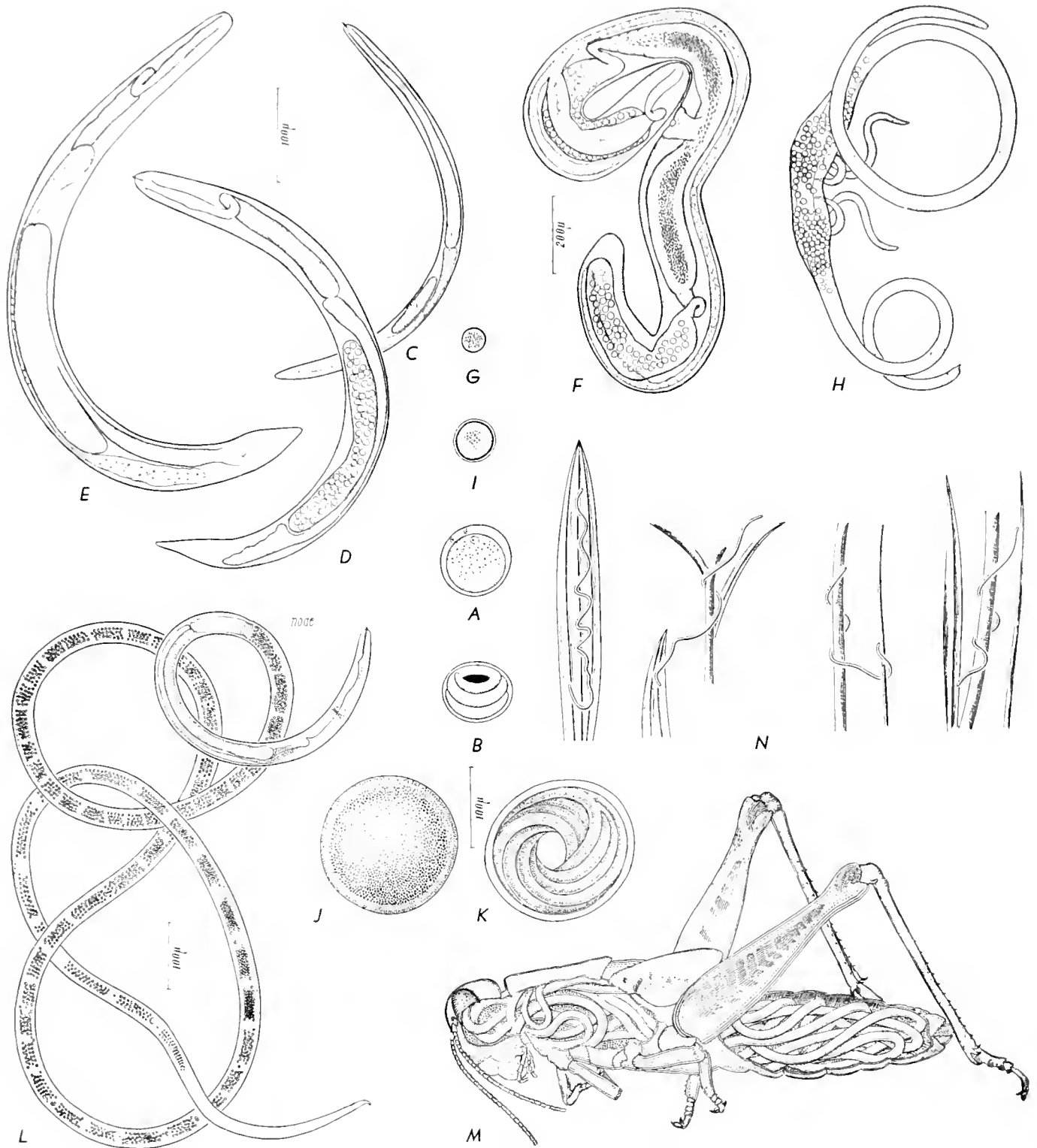


Fig. 165. TETRADONEMATIDAE AND MERMITHIDAE

A-F—*Ipnotanema entanophagum*: A—Fertilized egg; B—Egg containing ovic larva; C—Very young larval female; D—Older larval female; E—Larval male; F—Spermatized female). G & H—*Tetradonema phycus*: G—Egg; H—Egg-laying female with males attached). I—*Allomerus wemicaphala* egg. J-M—*Aguormis decudata*: J—Newly deposited egg; K—Egg containing ovic larva; L—Infective, preparasitic

larva; M—Grasshopper nymph containing one fully grown parasitic female). N—*Mermis subnigriceps*, females depositing eggs on vegetation. (All eggs, A, B, G, I, J, & K drawn to same scale). A-F, after Kellin and Robinson, 1933; C & F, drawn from compressed specimens; G & H after Hungerford 1919; I, after Crawley and Baylis, 1921; J, K & M, after Christie, 1936; L, after Christie, 1929; N, after Christie, 1937.

to associate with a species that enters its host by penetrating the body wall.

After arriving in the body cavity of the host the larval nematodes grow rapidly and development is usually so timed that females copulate and deposit eggs before the fly pupates. Adult flies were found that contained egg-laying female nematodes and also small larvae that approximated the size usually reached after a few days of parasitic life. Hungerford believed these small individuals had been arrested in development by the growth and maturity of the other worms.

Eggs pass through the vulva and are retained within the separated but unshed cuticle of the final molt which, near the middle region of the body, becomes distended to form a more or less spindle-shaped egg capsule (Fig. 168 H). Eggs are not normally discharged from this capsule prior to the death of the female. This final molt of the female is the only one mentioned by Hungerford. How the males circumvent this encompassing cuticle and effect ecdysis is not explained. The host insect is eventually killed and the body disintegrates to set free a residual mass of nematode eggs.

Hungerford found that the internal fat deposits of infected fly larvae were largely consumed leaving the body much more transparent than that of a normal individual. Most infected fly larvae died before pupating but where the infection was acquired late or the parasites were few in number the fly might endeavor to pupate. Many such pupae died being little more than nematode-filled shells but some succeeded in casting off the larval skins. The emerging, infected adults were able to fly and differed very little in appearance from normal individuals but they lacked functional reproductive organs.

*APROCTONEMA ENTOMOPHAGUM* Keilin, 1917, was found in England where it is a parasite of the dipterous insect, *Sciara pullula* Winn., the larval stages of which inhabit decaying wood. The morphology and life history of the nematode are discussed in a paper by Keilin and Robinson (1933) upon which the following account is based. It will be noted that the host of this parasite belongs to the same genus as the host of *Tetradonema plicans* and the two nematodes have many points in common.

Each infected larval fly usually harbors several females of *A. entomophagum* (Fig. 168 F) and a varying number of smaller males (Fig. 168 E). Mention is made of one larval fly that contained 2 females and 10 males. The parasites reach maturity in the body cavity of the host and copulate whereupon the males die and the females emerge forcing their way out of the host in the manner of most mermithids. Egg laying begins almost immediately after emergence. Each female deposits somewhat over 200 eggs and when egg laying is completed the female dies. Hence only the female has a free-living, post-parasitic stage and it is of very short duration.

The egg (Fig. 168 A) is laid before cleavage but develops rapidly and in a few days contains a coiled larva (Fig. 168 B) that molts before hatching. There seems little reason to doubt that the larval mermithids enter the young fly larvae by penetrating the body wall though actual penetration was not observed.

If infection occurs late in the development of the fly larva the parasites may be carried through the pupal stage and infected adult female flies were found though not infected adult males. The parasites delay the metamorphosis of the insects and infected adult female flies lack functional reproductive organs.

*PARAMERMIS CONTORTA* (Linstow, 1889) Kohn, 1913, is one of the aquatic mermithids of which there are a considerable number. It is a parasite of *Chironomus* larvae and was discovered and studied in Europe. Each host usually harbors one parasite but sometimes two to three or more. The sex ratio, as reported by different investigators, varies a great deal but in most cases females have considerably outnumbered males.

According to Kohn (1905), *P. contorta* molts before leaving its host. This, undoubtedly, is the last molt and the uteri are already filled with eggs. The parasite may issue through the anus or force its way directly through the body wall, the majority emerging just before their insect hosts would normally pupate. The worms settle into the mud at the bottom of the pool and copulation soon takes place to be followed immediately by egg laying. According to Comas (1927), the uteri are emptied and egg laying completed in 4 or 5 days whereupon the female dies.

Eggs are laid before cleavage but develop immediately and hatch in the course of a few weeks. The mermithid larvae swim in the water and seek young *Chironomus* larvae which they enter by penetrating the body wall. Comas states that these mermithid larvae do not appear capable of living long in water and, if unable to find and enter a host, will die in a few hours. Comas recounts that if a mermithid larva attempts to penetrate between the more posterior abdominal segments of its prospective host, the *Chironomus* larva may reach back and

with its mandibles pull the nematode away or bite it in two. If penetration is attempted nearer the middle of the body the insect will be unable to reach the nematode and penetration is more likely to take place.

*ALLOMERMIS MYRMECOPHILIA* (Crawley and Baylis, 1921) Steiner, 1924, was named and described by Baylis and its life history was studied by Crawley (Crawley and Baylis, 1921). The specimens were from two species of ants collected in England, *Lasius alienus* (Först), and *L. flavus* (F.) and a third ant, *L. niger* (L.), was reported as a host. Observations on a mermithid identified as this species and secured from the same ants were made in Germany by Gösswald (1929; 1930).

After completing its parasitic development this mermithid, according to Crawley, emerges from the ant, sometimes through the anus and sometimes between two of the ventral plates of the gaster, whereupon it enters the soil. As with many other mermithids, emergence apparently occurs over a considerable period during summer and autumn; Baylis mentions specimens that emerged during July. Crawley first saw eggs in the uteri of experimentally reared females on December 5. Egg laying begins before completion of the final molt and many eggs are retained within the separated but unshed cuticle after the manner of *Tetradonema plicans*. As mention is made of four experimental females that had molted by November 20, one might infer that two molts take place after emergence but Crawley and Baylis are not explicit on this point. Some of Gösswald's (1930) ant-infesting mermithids molted twice after emergence but presumably these were not *A. myrmecophila*. By actual count Crawley found that one cast cuticle contained 6,560 eggs and another 5,900 eggs. Oviposition continues after the cuticle is cast off and probably at least as many more eggs are laid making a total egg output of 12,000 or more. Eggs (Fig. 168 I) are embedded in a "gelatinous" matrix that causes them to collect in masses around the vulva or sometimes to be extruded in the form of a ribbon. Crawley and Baylis failed to find males of this mermithid and Gösswald demonstrated that females develop and lay viable eggs without copulation.

Crawley believed that ants become infected while in the larval stage and Gösswald's infection experiments seem to indicate that eggs of the parasite are ingested. Crawley and Baylis reported finding only mermithogynes which, when present in a colony, rarely exceeded the normal females in number and usually were much fewer. One series of colonies showed an average proportion of about 1 to 12. Gösswald found infected males and workers of *Lasius alienus* and *L. flavus* and one infected male of *L. niger*. Each infected ant usually harbors one mermithid though sometimes as many as three.

Infected males and workers, according to Gösswald, show, at the most, only very slight external differences from normal ants. The ovaries and wing muscles of mermithogynes fail to develop normally, according to Crawley and Baylis, but, except for a marked reduction in the size of the wings and a more distended gaster, the external characters show no pronounced difference from those of normal females (Fig. 169 C & D).

*HEXAMERMIS* sp. This unidentified species of the genus *Hexameris* is a parasite of the ant, *Pheidole pallidula* (Nyl.), and its life history was studied in France by Vandel (1934). Most individuals complete parasitic development by late summer or autumn and emerge from the ant by forcing their way out through the anus. They do not remain in the ant galleries but penetrate a short distance into surrounding soil where they occupy small cavities. The final molt occurs about a month after emergence and is followed, within the next month, by copulation and egg laying. One of Vandel's experimental females had begun to lay eggs by December 23 and was still laying eggs on March 15. Females exhaust their reserve nutrient materials, stop laying eggs, and die by the end of March or soon thereafter. Hence there is one generation each season with no post-parasitic individuals in the soil during late spring and early summer.

The infected individuals of this ant are mermithergates and mermithostratiotes and, with at most very few exceptions, each harbors one parasite. It is not known how the parasite enters the host. Vandel concluded that the infection is acquired either immediately prior to, or during the pupal stage. The location where eggs are laid, the small number of parasites per host, and the vestigial caudal appendage of the adult is circumstantial evidence suggesting that the larva penetrates the body wall of the young ant.

Copulation is necessary in the reproduction of this mermithid. Experimental females reared in the absence of males by Vandel failed to lay eggs. These females lost their opaque appearance very slowly and some lived for from 22 to 33 months after emergence whereas females that were allowed to copulate and that laid eggs lost their opaque appearance much more quickly and lived for only about 5 months after emergence.

*AGAMERMIS DECAUDATA* Cobb, Steiner and Christie, 1923, oc-

ours in the north central and northeastern United States where it is a common parasite of grasshoppers including both Acrididae and Tettigoniidae. It sometimes infects crickets (Gryllidae) and has been found, occasionally, in leaf hoppers and beetles. The life history of this mermithid was studied by Christie (1936) upon whose work the following account is based and which applies to the soil and climatic conditions of northeastern Virginia.

The free living stages of this nematode occupy small cavities in the soil usually from 5 to 15 cm. below the surface (probably deeper in sandy or loose soil). When inhabited by adults each cavity, almost without exception, contains one female and several males, generally two or three, sometimes as many as eight, coiled and intertwined to form a "knot." Copulation is necessary and females reared in the absence of males fail to lay eggs. Egg laying begins about the first of July, continues until interrupted by the advent of cold weather, and eggs (Fig. 168 J & K) accumulate over the surface of the soil cavities and over the parent nematodes. For the most part eggs laid during a given summer do not hatch until the following spring. Cleavage and embryonic development take place after deposition and the first molt occurs within the egg shell.

At the time of hatching the second stage larva is immediately infective. The body, which shows a high degree of organization and development, is divided into two parts by the *node* (Fig. 168 L). In the anterior part, which constitutes about one fifth of the total length, one finds most of the organs common to nematodes including esophagus and esophageal glands, intestine, nerve ring, and excretory pore. The posterior part of the body serves as a propelling and food storage organ and contains a row of cylindrical cells, probably modified intestinal cells. An anus is apparently lacking.

During late fall and winter a female is surrounded by her total egg output of the season. Egg counts on six females made during the winter showed the total number of eggs present to vary from 2,625 to 6,530. As will be noted later, a female lays eggs during two summers hence these figures represent roughly about half the total egg output.

Although some larvae may begin to emerge from the eggs fairly early in spring, a greater part of them hatch during a short period at about the middle to the latter part of June. The species of grasshoppers that most commonly serve as hosts (*Melanoplus femur-rubrum* and *Conocephalus brevipennis* (Scudder) in northeastern Virginia) also hatch at about this time. The larval nematodes migrate to the surface of the soil and climb grass and other low vegetation when it is wet with dew or rain. They seek newly hatched grasshopper nymphs and enter their body cavity by penetrating the body wall. Penetration takes place under the edges of the pronotum, between the abdominal segments, or at other places where the chitinous covering is thin. Penetration is effected by the use of the stylet probably aided by the dissolving action of a chitin solvent secreted by one or more of the most anterior esophageal glands.

After the anterior end is inserted into the host the body of the larva breaks at the node and the postnodal portion is left on the outside. If the body fails to break, as occasionally happens, the postnodal part undergoes no development in the host but remains as a vestigial appendage that eventually sloughs off. The *nodal scar* (Fig. 93, p. 89) persists throughout the parasitic stage as convincing evidence that no molt takes place during this period. The number of parasites per host is usually one (Fig. 168 M), sometimes two, rarely three or more.

Once inside the body cavity of the host the parasite undergoes a period of phenomenal growth accompanied by pronounced morphological changes. The stychoocytes (see p. 92) are a conspicuous anatomical feature of larvae that have been in the host from 4 to 10 days (Fig. 93, p. 89). As the body increases rapidly in length it becomes filled by the intestine, in fact intestinal tissue eventually fills all available space not occupied by other organs even growing past the base of the esophagus and extending into the neck region. Apparently this modified intestine performs no digestive function but serves as a reservoir for nutrient materials. Males remain in the host for from 1 to 1½ months and females from 2 to 3 months. The mermithids emerge head foremost forcing their way through the body wall between the segments, fall to the surface of the ground, and enter the soil.

During the first winter in the soil males and females remain isolated each individual forming a separate "knot." The final molt takes place the following spring about the latter part of June and at this time males seek the females. It will be noted that only two molts have been observed. Egg laying begins soon after the final molt, usually about the first of July, and continues until interrupted by cold weather. The following spring a year old female begins laying eggs slightly earlier than one that has just molted. By the end of the second sum-

mer of egg laying the reserve food has become exhausted and the transparency of the body is in sharp contrast to its opaqueness at the time of emergence from the host. Most females probably fail to survive a third winter in the soil. Information regarding the longevity of males is not very satisfactory but it seems probable that they live for about the same length of time as females.

*A. decaudata* causes no noticeable change in the external anatomy of grasshoppers. Infected individuals sometimes have distended abdomens and are likely to appear sluggish, adults being incapable of sustained flight. The most pronounced effect of this parasite is on the gonads of the host (Fig. 169 A & B). It is doubtful if infected female grasshoppers are capable of laying eggs as the ovaries are always greatly reduced in size. The effect on the testes is less pronounced and infected male grasshoppers have been observed *in copula*. The emergence of the parasite invariably results in the death of the host.

*MERMIS SUBNIGRESCENS* Cobb, 1936, appears to be strictly a grasshopper parasite. It occurs in the United States over about the same range as *Agamermis decaudata* where it has been found infecting nine different species of grasshoppers including both Acrididae and Tettigoniidae. Several other species have

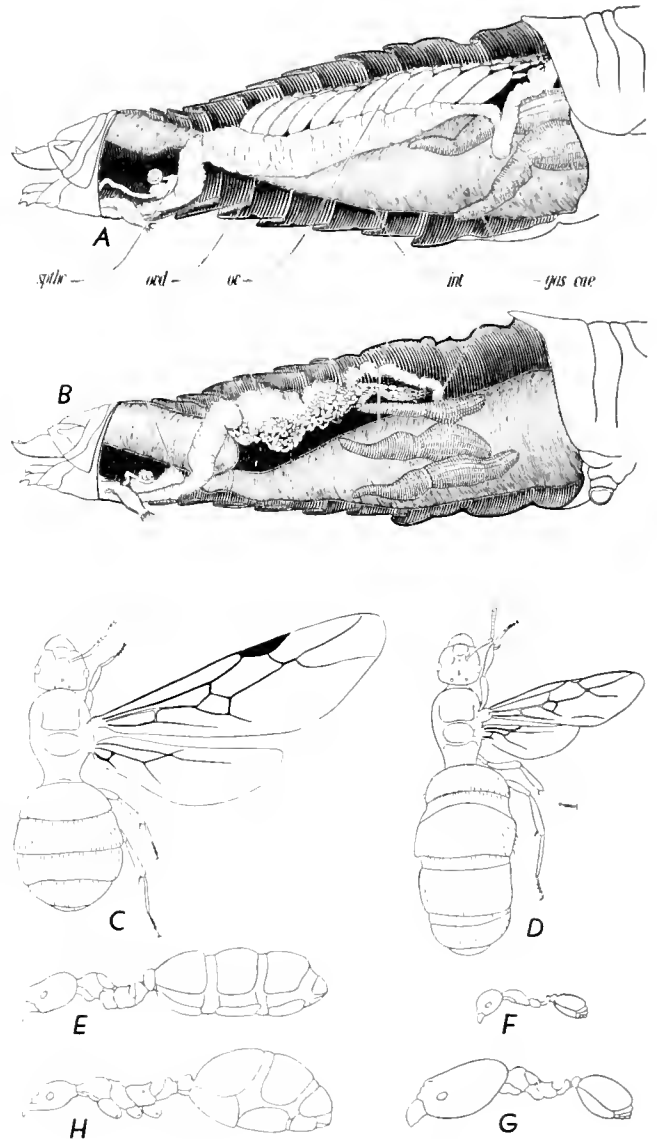


Fig. 169. EFFECTS OF MERMITHIDS ON THEIR HOSTS

A & B—Dissections of adult female grasshoppers, *Melanoplus femur-rubrum*, showing reproductive organs (A—Normal grasshopper; B—Grasshopper parasitized by *Agamermis decaudata*). *gasc*, gastric caeca; *int*, intestine; *ov*, ovary; *ovd*, oviduct; *sph*, spermatheca. C & D—Females of the ant, *Lasius alienus* (C—Normal female; D—Female parasitized by *Allomermis mermithophyla*, i.e., a mermithogyne). E-G—The ant, *Pheidole absurda* (E—Individual parasitized by a mermithid, i.e., a mermithergate; F—Normal worker; G—Normal soldier). H—The ant, *Pheidole gaudii*, a mermithergate. A & B, after Christie, 1936; C & D, after Crawley and Baylis, 1921; E-G, from Wheeler, 1928; after Emery; H, after Wheeler, 1928.

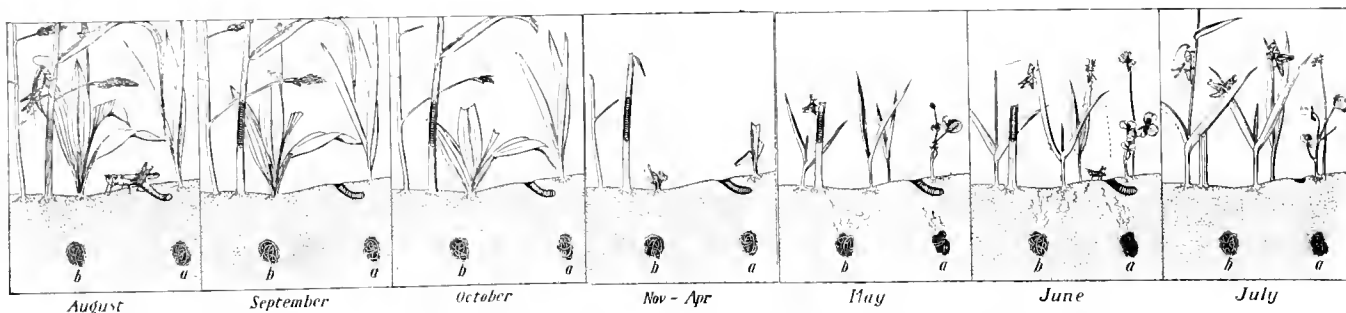


Fig. 170a. LIFE CYCLE OF *AGAMERMIS DECAUDATA*

Diagram illustrating 12-month period, August to July, inclusive. *a*, "knot" composed of one female and several males that emerged from hosts 2 years previous to beginning of period represented. *b*, "knot" composed of one female and several males that emerged from hosts 1 year previous to period represented. Mermithids that would emerge during September and October of period represented are omitted for simplicity. Female in "knot" *a* has, by October, completed its second summer of egg laying and dies during ensuing winter but the accumulated eggs, deposited during previous summer, hatch May to June. Female in "knot" *b* has, by October, completed its first summer of egg laying and the accumulated eggs hatch May to June while the second summer of egg laying is begun during May.

been experimentally infected. Attempts to infect other insects including crickets (*Gryllidae*), mole crickets (*Gryllotalpinae*), and larvae of several species of *Lepidoptera* have been unsuccessful. The following account of the life history is based on investigations by Christie (1937) conducted, for the most part, in Massachusetts.

Grasshoppers become infected with *M. subnigriseens* by swallowing the eggs. In order to bring this about the egg-laying habits of this nematode are radically different from those of *Agamermis decaudata*, otherwise the two life cycles are somewhat similar. Eggs of *M. subnigriseens* are never laid in the soil. Gravid females climb low vegetation on which they lay their eggs (Fig. 168 N) and to which the eggs cling by means of the entangling appendages or bissi.

The egg, when deposited, contains a fully developed infective larva (Fig. 140 B, p. 181). The shell proper is protected by an outer covering that is divided into two cup-like halves by a groove at the equator (Fig. 140 A, p. 181). At each pole there is a raised or thickened area formed by the attachment of the entangling appendages (Fig. 139, p. 181). The outer covering breaks apart along the groove at the equator and the two cup-like halves are easily removed. In the shell proper there are two opposite areas at the equator where the color is lighter than elsewhere and these areas are partly dissolved by the digestive action of the host thus facilitating the escape of the larva. Both the outer covering and the shell proper contain brown pigment, presumably to protect the larva from the action of sunlight. Eggs deposited on foliage remain viable throughout the summer. When eggs were kept experimentally in a moist chamber some remained viable for a year.

When an egg reaches the alimentary tract of its host the outer covering has usually been rubbed off. The two opposite areas of the shell at the equator gradually become clearer and begin to protrude until they appear as colorless hemispherical projections (Fig. 140 C, p. 181) that finally rupture and provide openings for the escape of the larva. The larva itself does not appear to aid in its own liberation. When first freed it is rather sluggish but soon becomes active, penetrates the wall of the gut and enters the body cavity. Penetration through the intestinal wall is aided by the stylet which is rhythmically protruded.

From 1 to 5 parasites per host is the number most frequently encountered but there is great variation and grasshoppers harboring 100 or more parasites of widely different ages are not uncommon in some localities. As a nymph grows older and its food consumption increases, its chance of becoming infected is correspondingly greater. The sex ratio of *M. subnigriseens* is influenced by the number of parasites per host. When a grasshopper harbors a large number, all develop into males but when a grasshopper harbors only 1 or 2 these usually develop into females (Christie, 1929).

The parasitic development of *M. subnigriseens* is essentially the same as that of *Agamermis decaudata*. There is the same rapid increase in size and the same extensive proliferation of intestinal tissue. Males remain in the host from 4 to 6 weeks and females from 8 to 10 weeks. At the end of this time the parasites force their way through the body wall of the host and enter the soil. When a grasshopper harbors parasites of different ages, all that are too immature to escape and survive in the soil perish with the host when the older ones emerge.

Postparasitic individuals of *M. subnigriseens* are found in the soil down to about 60 cm., the majority occurring from 15 to 45 cm. below the surface. They usually remain isolated and one rarely finds a "knot" composed of a female and one or more males as is characteristic of *Agamermis decaudata*. Most individuals emerge from the host during summer and autumn and molt the following April. This is the final molt and the only one that has been observed. Copulation may take place and has been seen on several occasions but copulation is not necessary as females reared in the absence of males produce viable eggs. By July females begin to exhibit a brownish color due to accumulating eggs and by September they are nearly black except for a short region at each extremity of the body. At this time most of the eggs are viable but they are not laid until the following spring. Before ovipositing, a gravid female 85 mm. long contains about 14,000 eggs.

Egg laying usually begins in May and may continue throughout July or even into August, depending on weather conditions. Eggs are laid during rain and should the early summer months be dry egg laying will be delayed. Gravid females climb grass and other low vegetation over which they constantly move while eggs are being laid. If rain continues egg deposition goes on throughout the day but if the rain stops and the foliage becomes dry females coil up, fall to the surface of the ground and enter the soil, presumably to resume egg laying during the next rain.

It is not known how long females live after the uteri are emptied of eggs but by this time their stored food is nearly exhausted and it seems highly improbable that they are able to survive a third winter or to develop more eggs. However, if prevented from coming to the surface to deposit eggs they are able to survive a third winter and to lay eggs the following spring. Females that normally would have deposited eggs in 1932 were buried in containers and prevented from coming to the surface (Christie, 1937). When examined during May, 1933, many of these females were alive, in good condition, and filled with eggs. There was no evidence that eggs had been deposited, although these females promptly began laying eggs when brought to the surface and placed in the light.

Apparently eggs are not laid at night. Egg laying is controlled, at least in part, by light stimuli. When an ovipositing female is placed in the dark, egg laying promptly stops, but is resumed just as promptly when the female is again placed in the light. The head of the adult female is colored with areas of reddish-brown pigment which, presumably, is an organ for light perception. The male, which never comes to the surface, lacks this pigment.

*Mermis subnigriseens* has about the same effects on its host as does *Agamermis decaudata*. These effects are suppression of the gonads, especially the ovaries, and death of the host when the parasite emerges. With *M. subnigriseens* the effect on the gonads of the host is much more variable than with *A. decaudata* due to variations in the number of parasites per host and the time the parasites are acquired.

#### ALLANTONEMATIDAE

The Allantonematidae is a group of insect parasites that are closely related to the preeminently plant-infecting Tylenchidae. The species that have been studied and named probably constitute but a small part of the number that exist but in nearly every instance where the life cycle is known it follows the same general plan and differs from that found in any other group of nematodes.

Adult gravid females occupy the body cavity (haemocoel) of the insect, frequently in small numbers, often one per host. Here larvae accumulate and develop to a certain stage, molting at least once (probably twice in most species); then they escape from the host either by entering the alimentary tract and passing out through the anus or by entering the female reproductive system and passing out through the genital aperture. Most species infect both males and females of their host insect. In some cases the only known way by which larvae are able to

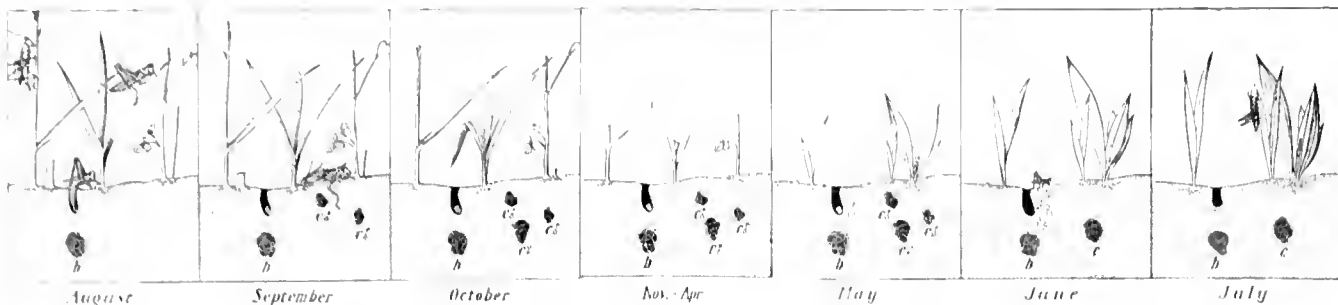


Fig. 170b. LIFE CYCLE OF *AGIMERMIS DECAUDATA*—Continued

Diagram illustrating ensuing 12-month period, August to July, inclusive. *c*, nematodes emerging from hosts September to October. Female and males remain in separate cavities until latter part of May when males seek female, copulation takes place, and, during June, the female begins its first summer of egg laying. Female in "knot" *b* has, by October, completed its second summer of egg laying and dies during the ensuing winter but the accumulated eggs hatch May to June. (While the above diagram is essentially correct for a majority of individuals where grasshoppers serve as hosts, the various life-cycle changes are actually spread out over somewhat greater periods of time than the diagram indicates. A few eggs hatch before May and June. A few preparasitic larvae enter hosts as early as April and as late as August and the time of emergence is correspondingly affected. A few individuals, especially males, emerge from hosts at least as early as July.)

leave the host is via the female reproductive system and the fate of larvae that inhabit the body cavity of male insects is not yet fully understood.

A free living stage is passed wherever the host insect undergoes its early development and during this period the nematodes molt at least once (as a rule probably twice) and become adults. In the adult male the stylet is usually either absent or weakly developed and the esophageal glands are inconspicuous and apparently lacking. In the preparasitic adult female the stylet is usually well developed and at least one of the esophageal glands is large and conspicuous. Exceptions to these morphological differences between the sexes are usually correlated with deviations from the more typical life cycle. The apparent absence of esophageal glands and the somewhat more rapid development of the genital primordium in the male usually make it possible to distinguish sex at an early stage sometimes while a larva is still within the egg.

The ovary of the adult preparasitic female is small and composed of only a few cells the extent of its development differing somewhat with different species. When copulation takes place the uterus is packed with small, more or less spherical spermatozoa. After copulation males usually die and impregnated females enter their respective hosts, usually by penetrating the body wall while the insect is still in the larval stage. The fact that in most species only the female possesses an effective stylet and at least one well developed esophageal gland has been regarded as evidence that these structures function in connection with penetration into the host. There is little reason to doubt that the stylet is employed for this purpose. It has been suggested that penetration is further facilitated by a secretion of the esophageal glands which may serve as a chitin solvent. The validity of this suggestion does not rest entirely on morphological evidence for Bovien (1932) demonstrated that *Scatocma wilkeri* does, in fact, exude a rather copious secretion through the stylet at the time of penetration.

The free-living stage is usually of short duration. There is no evidence that the nematodes feed during this period (with the exception of *Fergusobia currici*) and larvae, at the time they leave the host, are at least nearly as large as young adults. However, after entering a new host, the female undergoes a very great increase in size. The fully grown gravid female of most species is curved ventrad and assumes a form usually referred to as "sausage-shaped." There are exceptions, however, and, for example, *Allantomea mirabilis* is oval while in many species of *Aphelenchulus* the body is bent dorsal with the vulva on the outside of the curve.

Some species deposit eggs in the body cavity of the host but in many species eggs hatch before deposition and the uterus becomes distended with developing eggs and larvae that gradually fill the greater part of the body and push the ovary into the anterior end. As a rule larvae eventually pass through the vulva into the body cavity of the host. There is a tendency for the other internal organs of the female to degenerate, the extent of this degeneration differing in different species.

In most species the rapid increase in the size of the female after becoming parasitic provides space for the reproductive organs. In one group, the Sphaerulariinae, adequate space for the developing reproductive organs is not provided by a corre-

sponding increase in body size. The uterus of *Sphaerularia bombi* is everted through the vulva and the entire reproductive system develops outside the body proper. This prolapsed uterus increases enormously in size and the body proper remains attached to one end as a vestigial and apparently functionless structure. *Tripinus gibbosus* (Syn. *Atractocuma gibbosum*) represents an intermediate stage in the evolutionary development of this peculiar adaptation and the size of the body and of the prolapsed uterus is less disproportionate. In both these species the life cycle, so far as known, is essentially the same as that of most allantonematids.

There are, nevertheless, several deviations from this typical life cycle. Young adult males, as well as young adult females, of *Parasitlenchus dispar typographi* enter the body cavity of their host insect where they are found in large numbers, while neither adult males nor adult females of *Chondronema passali* become parasitic, only larval stages being found in the host insect. *Chondronema passali* enters its host, not as young adults, but as young larvae, probably by being ingested.

Two species of this family have heterogeneous life cycles. There is interpolated into the life cycle of *Heterotylenchus aberrans* a parasitic, parthenogenetic generation and into the life cycle of *Fergusobia currici* several, consecutive, "free-living," parthenogenetic generations. The parthenogenetic females of *Fergusobia currici* occur, associated with their "host" insect, in plant galls where they feed on plant cells and are, in fact, plant parasites. In each of these heterogeneous species, however, the gamogenetic generation still follows the typical allantonematid plan of development.

*TYLENCHINEMA OSCINELLAE* Goodey, 1930, is a body-cavity parasite of the frit fly, *Oscinella frit*. (L.). The life history of this nematode was studied in England by Goodey (1930, 1931).

The frit-fly has three generations a year. Eggs are laid on small oat plants generally during May and fly larvae penetrate the shoots, destroying the central tissues. This is the first or stem generation. Adult flies appear by mid July and deposit eggs on the panicles of oats where the larvae attack the tissues of the inflorescence. This is the second or panicle generation. Adult flies again appear during August or early September and lay eggs on various species of wild grasses. This is the third or grass generation; also it is the overwintering generation and winter is passed in the larval stage. The life cycle of the nematode is, of necessity, closely correlated with that of the frit-fly and like it, undergoes three generations a year (Fig. 175).

Infected flies harbor usually one, sometimes two or three, more rarely four to eight, adult female nematodes that give birth to living young. Eggs pass into the uterus of the mother nematode where they undergo development. As more and more eggs are produced the uterus becomes distended, pushing the ovary into the anterior end and finally occupying most of the space within the body. Larvae (Fig. 171 A & C), escaping from the egg membranes, pack the posterior end of the uterus and finally pass through the vulva into the body cavity of the host. Here they accumulate and continue development. Goodey observed one molt that takes place when a larva is about 460 $\mu$  long and which he believed to be the second suspecting that the first molt takes place while the larva is still within the uterus of the mother. The gonads undergo considerable development and show differences that make it possible to distinguish sex. The wall of the intestine becomes well stocked with reserve food globules.

After attaining a size nearly as large as free-living adults, the larvae escape from the host. To accomplish this they penetrate the food reservoir of the fly's digestive system from which they migrate through the intestine to the rectum and are ejected through the anus. With regard to this escape of larvae, Goodey writes as follows: "Parasitized flies of both sexes, having failed to develop their sex cells, fly about and instead of taking part in the normal process of reproduction are able only to deposit larvae of the nematode parasite. Normal females go to oat panicles and there lay eggs; similarly, the parasitized flies responding to the same urge of the life-cycle rhythm also fly

to oat panicles, but, instead of eggs, deposit larvae of the nematode parasite. These find their way, possibly in response to some chemotactic stimulus, into the plant tissues surrounding the fly larvae." In this environment the nematode larvae continue development and two final molts take place, the last cuticle separating while the larva is still within the cuticle of the preceding molt.

Larvae that Goodey removed from the gut of infected flies and kept in tap water completed their final molt in about 41 hours. Males remained alive for about 14 days and females for about 29 days but copulation did not take place while the worms remained in water. In nature copulation follows the final molt and the uterus of the female is distended with spermatozoa. The preparasitic female (Fig. 171 F & G) has a well developed stylet with basal swellings and a large dorsal esophageal gland. These structures are inconspicuous or lacking in the male. (Fig. 171 E).

The male does not again become parasitic but the impregnated, precocious female enters the body cavity of a frit-fly larva. Goodey did not actually observe the entrance but assumed, no doubt correctly, that it is accomplished by penetrating the body wall. The incidence of infection is about the same for male and female flies except possibly in the grass or

overwintering generation, where Goodey found that about two-thirds of the infected flies were females.

After becoming parasitic the female nematode increases very greatly in size and is about fully grown when the host emerges from its pupal case. The body has assumed the characteristic "sausage shape" and the ovary has completed its development (Fig. 115 J, p. 136). The stylet is retained and Goodey believes that probably the parasite continues to take food via the alimentary canal.

*Tylenchinema oscinellae* produces no noticeable effect on the external characters of its host but it prevents the normal development of the gonads and both male and female flies are sterilized. Occasionally, however, parasitized flies of both sexes develop normal sex organs and when this happens the parasite fails to undergo normal development. In regard to this Goodey (1931) writes: "In the great majority of cases the worm manages to get the upper hand and grows to sexual maturity within the host, but occasionally the fly, during its final metamorphosis, is able, by some means, to build up its gonads in the normal manner. When this happens the worm fails to grow, remains non-functional and becomes degenerate. . . . These relationships may possibly be explained on the supposition that the worm secretes or excretes something, perhaps from the intesti-

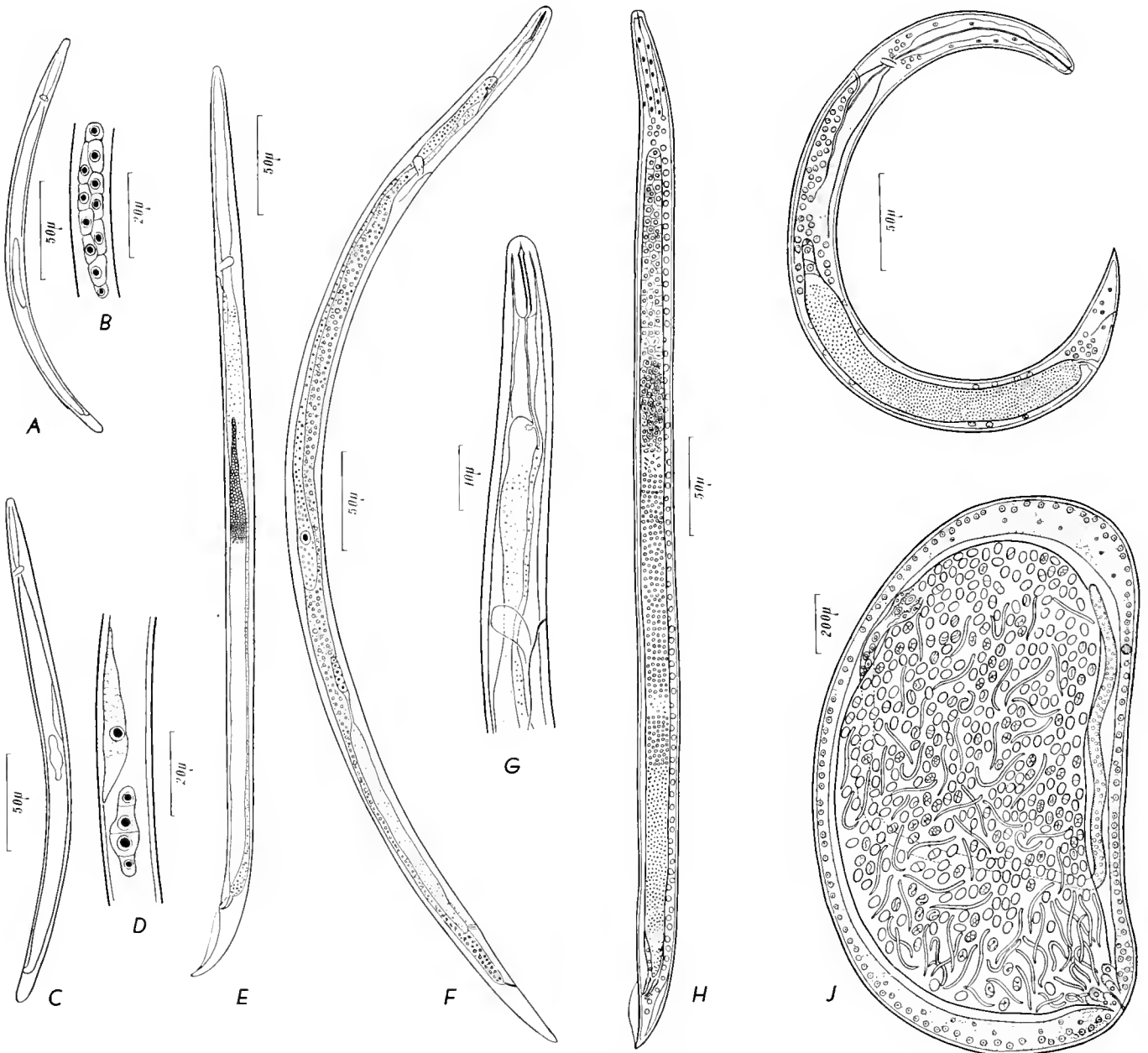


Fig. 171. ALLANTONEMATIDAE

A-G—*Tylenchinema oscinellae* (A—Very young male larva and, B, genital primordium of same; C—Very young female and, D, genital primordium and esophageal gland of same; E—Adult male; F—Adult, preparasitic female and, G, anterior end of same. For fully grown,

adult, parasitic female, see Fig. 115 J, p. 136). H-J—*Allantonema mirabile* (H—Adult male; I—Adult, preparasitic female after copulation; J—Fully grown, adult, parasitic female. For stage intermediate between I and J, see Fig. 115 I, p. 136). A-G, after Goodey, 1930; H-J, after Wülker, 1923.

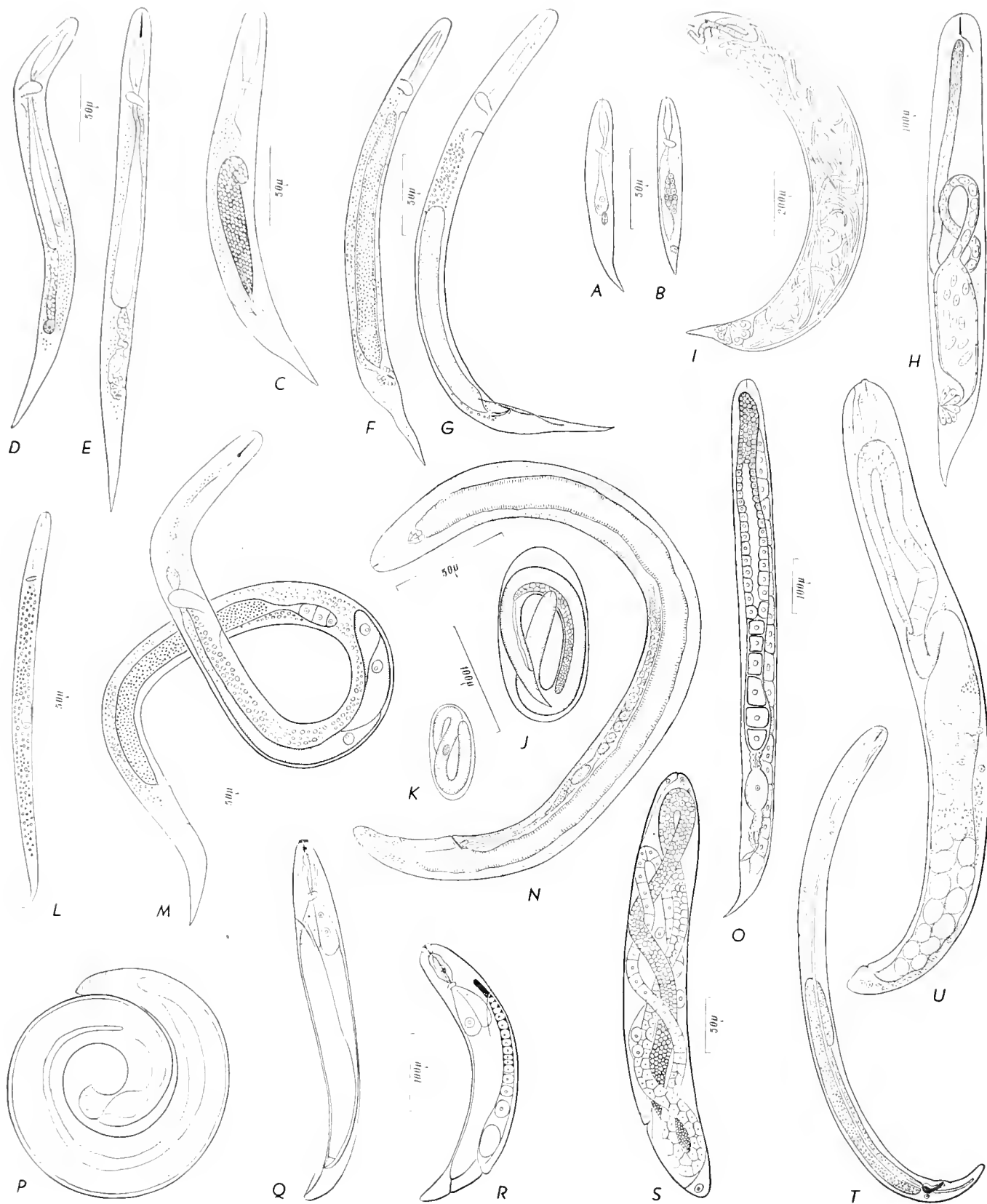


FIG. 172. ALLANTONEMATIDAE

A-I—*Scantonema wülkeri*. (A—Newly hatched female larva; B—Newly hatched male larva; C—Partly grown male larva; D—Female just before final molt; E—Adult preparasitic female after copulation; F—Male just before final molt; G—Adult male; H—Adult parasitic female a few days after entering host; I—Fully grown gravid female). J-O—*Heterotylenchus oberans* (J—Egg laid by female of gamogenetic generation; K—Egg laid by female of parthenogenetic generation; L—Newly hatched larva of gamogenetic generation; M—Adult preparasitic

female of gamogenetic generation; O—Fully grown female of parthenogenetic generation). P—*Aphelenchulus diplogaster*, adult parasitic female. Q-S—*Eceganobin curriei* (Q & R—Adult male and adult female, respectively, of “free-living” generation, i.e., from *Eucalyptus* galls; S—Gravid female of “parasitic” generation, i.e., from body cavity of gall fly). T & U—*Parasitylenchus dispar typographi*, adult parasitic male and adult parasitic female, respectively. A-I, after Bovien, 1932; J-O, after Bovien, 1937; P, T & U, after Fuchs, 1915; Q & S, after Currie, 1937.

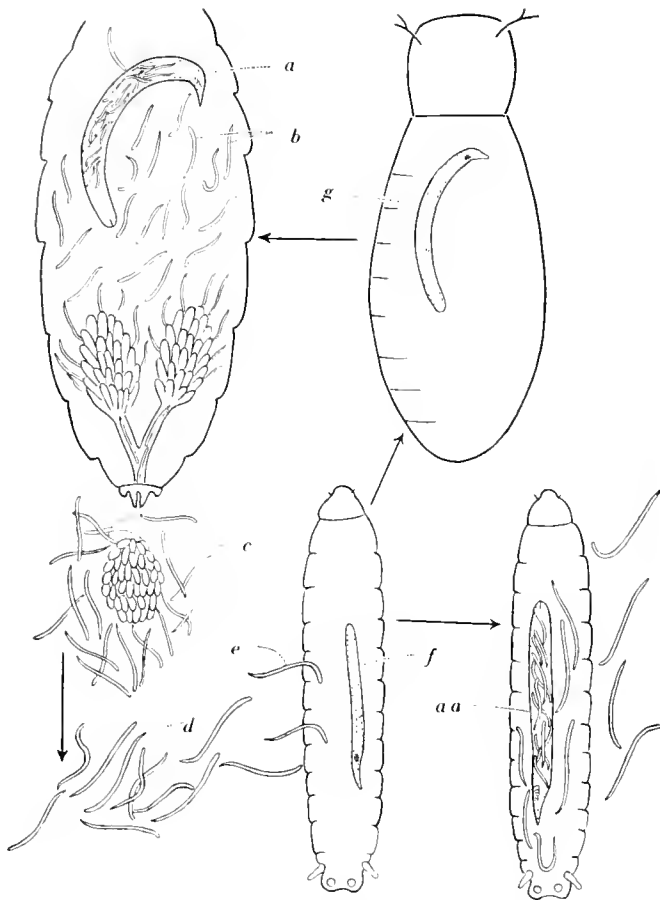


Fig. 173

Diagram illustrating life cycle of *Scatonema wülkeri*. The adult parasitic female (a) produces offspring (b) that eventually enter the female fly's reproductive organs and are extruded with the eggs (c). Outside the host these larvae develop into adults (d) and copulate whereupon males die and impregnated females (e) enter fly larvae. These females then undergo a period of growth (f) and may begin producing offspring (aa) while the fly is still in the larval stage or females may be only partly grown (though adult) (g) when the fly pupates and begin laying eggs (a) when the fly becomes adult. After Bovien, 1937.

nal [esophageal] gland, which prevents the normal growth of the host's sex-cells. At the same time it is quite likely that the same may be true of the host; if once its reproductive organs become sufficiently developed, then it is able to pour out some substance which definitely inhibits the growth of the worm.'

ALLANTONEMA MIRABLE Leuckart, 1884, is a body cavity parasite of the pine weevil, *Hylobius abietis* (L.) and occurs in Europe but has not been found elsewhere. This nematode differs from *Tylenchinema osecinellae*, not so much in its life cycle, which is essentially the same, as in the form and degeneration of the gravid female. Unlike most allantonematids, the fully grown female (Fig. 171 J) is oval, some 1.5 to 2 mm. in length and about half as wide as long. Its body is virtually a sac largely filled by the uterus as it becomes distended with eggs and larvae. The other internal organs degenerate to such an extent that if vestiges persist their identity has not been recognized.

Eggs hatch in the uterus where they begin to accumulate during late summer and where they remain during the winter undergoing little development. In the spring larvae begin to pass through the vulva into the body cavity of the weevil where they undergo two molts. Larvae finally leave the host by penetrating its alimentary tract and passing out through the anus.

The adult female of *Hylobius abietis* eats small holes in the bark on the trunk and roots of fir and certain other coniferous trees. In this cavity eggs are laid and hatch, the young weevils tunneling into adjacent tissues. In order to pass their free-living stages in the immediate vicinity of newly hatched weevils, the larval nematodes must escape when and where female weevils are laying eggs, albeit not through the genital aperture of the insect. Wülker (1923) observed only one molt during free-living development which took place after 8 to 10 days. Bovien (1937) found that larvae, taken from the rectum of adult weevils and placed in hanging drops of water, became

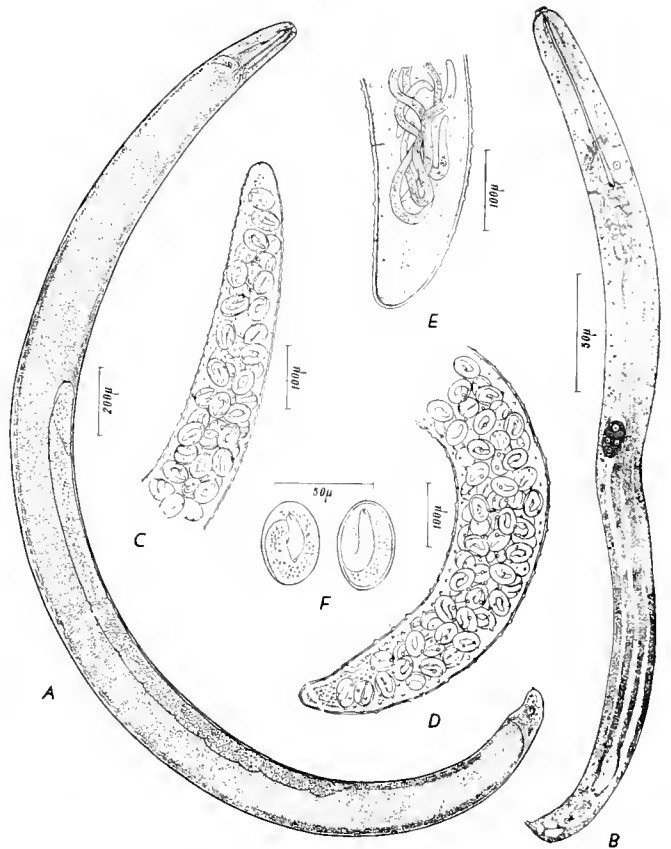


Fig. 174. *CHONDRONEMA PASSALI*

A—Oldest larva found within body cavity of host; B—Youngest larva found within body cavity of host; C-E—Portions of body of adult female filled with eggs or larvae and serving as brood sac; F—Eggs. All figures after Christie and Chitwood, 1931.

adult in 5 to 6 days. The final molt is followed very soon by copulation after which males die and impregnated females enter the body cavity of weevil larvae that, in the meantime, have hatched.

The adult preparasitic female of *A. mirabile* (Fig. 171 I) has a well developed stylet and Bovien (1937) figures two esophageal glands, one opening into the esophagus on the dorsal side near the base of the stylet and the other on the ventral side farther back. In the adult male (Fig. 171 II) a stylet is present though somewhat more weakly developed than in the female but the esophageal glands are inconspicuous or lacking.

By about July, when the weevils are pupating, the parasitic female nematode is producing ova. Fuchs (1915) states that *Hylobius abietis* lives for at least 31 months and, finding females of *A. mirabile* in 2-year-old weevils, he concludes that the nematode lives for at least 2 years.

SCATONEMA WÜLKERI Bovien, 1932, is a body cavity parasite of the dipterous insect, *Scatopse fuscipes* Meig., the immature stages of which develop in manure and other putrescent material. Eggs of this nematode hatch in the uterus where larvae (Fig. 172 A & B) undergo early development, the extent of this development varying considerably. In some cases, which Bovien (1932) regards as exceptional, an individual, while still within the uterus, may reach maturity and, in turn, develop larvae within its uterus, thus creating three generations, one within another. Most of the progeny, however, pass through the vulva into the body cavity of the host as partly grown larvae. These larvae enter the reproductive system of the insect and pass out with the eggs. When an infected fly dies not all the harbored parasites necessarily perish but some larvae may complete development, molt, and copulate after which impregnated females escape from the dead body. As male flies die soon after copulation Bovien concludes that, in moist surroundings, part of the nematodes may be able to



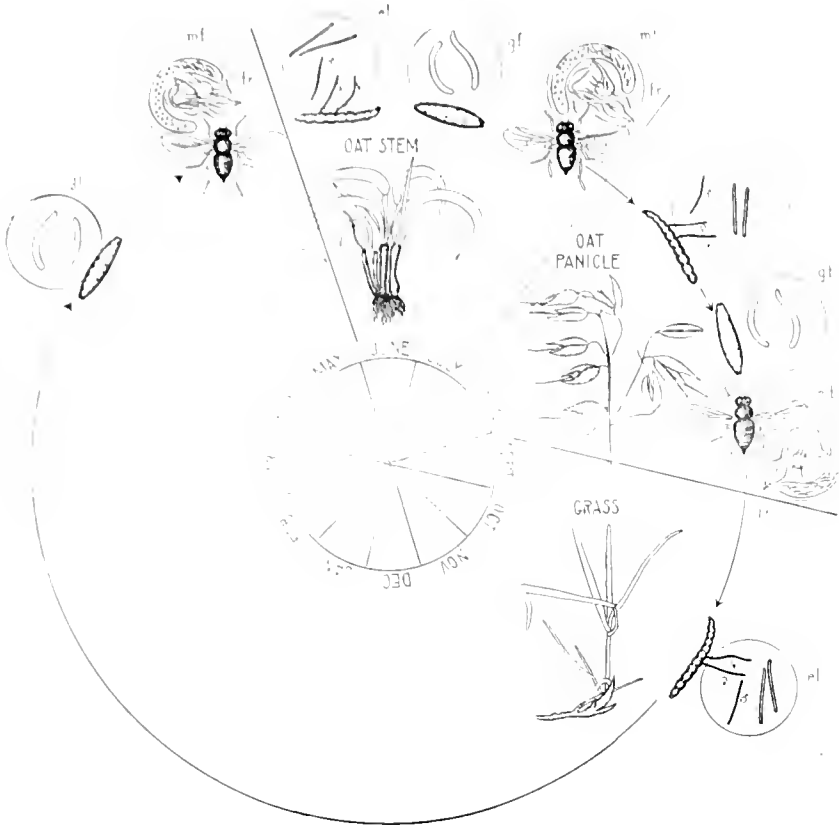


Fig. 175. *TYLENCHINEMA OSCINELLAE*

Schematic drawing illustrating life history of frit-fly in its three seasonal generations, and the approximate time occupied by each, linked with that of its parasite, *Tylenchinema oscinellae*. The various stages of the fly and worm are greatly enlarged whilst the oats and grass are smaller than natural size. Although the female fly only is shown it is to be understood that the male also carries the parasite. The dotted circles contain stages of the parasite related to the corresponding stage of the host. The circles cut into the pupa and imago but not into the fly larva in each case, thus showing that the parasite is within the pupa and fly but not within the larvae. *e l*, ensheathed larvae; *f r*, food reservoir of fly with larvae passing in; *g f*, growing female worms; *m f*, mature female worms. After Goodey, 1931, explanation quod verbatim.

escape from the decaying insects. Otherwise the fate of larvae harbored by male flies is unknown.

Bovien observed only one molt, the last, which may occur before, but usually not until after, emergence from the host. As this molt takes place not later than 24 hours after emergence and is followed immediately by copulation, the free-living stage is of short duration. In the adult, preparasitic female (Fig. 172 F) the stylet and esophageal glands (Bovien figures two) are well developed while in the adult male (Fig. 172 G) these structures are inconspicuous or absent. By the less well-developed genital primordium and the presence of esophageal glands one can distinguish female larvae while they are still within the egg.

Regarding penetration of preparasitic females into the body cavity of a fly larva Bovien (1932) writes as follows: "In many cases I found the nematodes in the act of entering the body of the larva. In a few cases I saw dead nematodes, which had not succeeded in penetrating the body-wall, held fast by it. The penetration may take place through all parts of the surface of the larva and no preference seems to be given to any particular region. The very beginning of this act, however, was not observed. I placed female worms in hanging drops together with *Scatopse*-larvae, the presence of which had an unmistakably attractive influence on the nemas. The nematodes slung themselves around the body of the larva, pressing their mouths against the skin without being able to puncture it. I suppose this failure may be ascribed to the lack of supporting surfaces. On the third day the worms were dead. An oblong, somewhat spiral-wound, coagulated mass of secretion had been ejected from the aperture of the buccal stylet, and the salivary [esophageal] glands appeared to be empty." The presence of the parasite does not result in sterility of the host.

*CHONDRONEMA PASSALI* (Leidy, 1852) Christie and Chitwood, 1931, is a body cavity parasite of the beetle *Popilius interrump-*

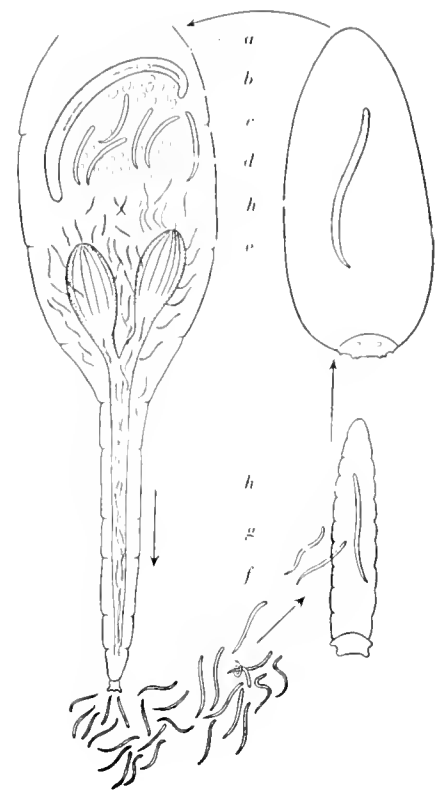


Fig. 176. *HETEROTYLENCHUS ABERRANS*

Diagram illustrating life cycle of *Heterotylenchus aberrans*. The adult parasitic female of the gamogenetic generation (*a*) lays eggs (*b*) that develop into females of the parthenogenetic generation (*c*). These females lay eggs (*d*) and the resulting larvae (*e*) enter the reproductive organs of the female fly and pass out through the genital aperture. Outside the host these larvae develop into adults of the gamogenetic generation (*f*) and copulate whereupon males die and impregnated females (*g*) enter fly larvae. While the fly matures and pupates the female grows (*h*) to reach full stature (*a*) and lay eggs (*b*). After Bovien, 1937.

*tus* (L.) (Syn. *Passalus cornutus* Fab.). This beetle occupies galleries in decaying stumps and logs where eggs are laid and where larvae develop and pupate. Leidy (1852) found 90 percent of the adult beetles infected and Christie and Chitwood (1931) estimated that each beetle usually harbors from 500 to 1,000 parasites. In the body cavity of the insect one finds larvae in all stages of development from young, newly hatched individuals (Fig. 174 A) to those that are fully grown (Fig. 174 B) but never adults.

Larval nematodes of both sexes taken from the body cavity of a beetle have a minute stylet, a moderately large esophageal gland (presumably the dorsal), and exceptionally large and conspicuous phasmids. Sex can be distinguished at a rather early stage partly through differences in the genital primordia but more especially through differences in the general appearance of the body, females being more opaque than males. Movement is sluggish.

The mode of exit from the host has not been determined. Once the nematodes have escaped neither males nor females again become parasitic but remain in the beetle galleries throughout the remainder of their lives. The mouth, anus, and vulva of the female become vestigial. If the vulva functions it is only during copulation. Eggs (Fig. 174 F) are retained within the body where they accumulate and hatch pushing aside the internal organs and converting the female into a brood sac (Fig. 174 C-E).

*C. passali* enters its host as a very young larva but it is not known how this is accomplished. Larvae of all sizes may be found in old beetles at any time of the year when the insects can be collected. The incidence of infection seems to be very much lower in larval beetles and pupae than in adults. These circumstances, together with the exceedingly large number of parasites usually harbored by a beetle, caused Christie and Chitwood (1931) to suggest that the larval nematodes enter *per os*, possibly the gravid female and her entire progeny being swallowed.

HETEROTYLENCHUS ABERKANS Bovien, 1937, is a body cavity parasite of the onion fly, *Hylemya antiqua* (Meig.), and its life history was studied by Bovien (1937) at Lyngby, Denmark. The onion fly hibernates in the pupal stage and emerges in May to lay eggs on onion plants or in nearby cracks in the soil. The young fly larvae move down the plant usually inside the sheath and finally burrow into the bulb. Pupation takes place in the soil or occasionally in the bulb. There are two or perhaps, occasionally, three broods a year with considerable overlapping.

In the body cavity of infected flies one finds from one to four large, adult females of *H. aberrans* (Fig. 172 N) and a greater number of smaller, adult females (Fig. 172 O). The larger individuals are females of the gamogenetic generation and the smaller ones are females of the parthenogenetic generation. The reproductive organs of a gamogenetic female, as compared with these structures in most allantonematids, are

exceptionally small. Much of the space within the body is occupied by the intestine which, according to Bovien, is without a lumen. A small stylet is present and the three esophageal glands, empty and reduced in size, are grouped around the base of the esophagus. Eggs (Fig. 172 J) are deposited in the body cavity of the host where they hatch and where the larvae develop into parthenogenetic females.

The outstretched reproductive organs of a parthenogenetic female are relatively much larger than those of a gamogenetic female. The esophagus and esophageal glands have almost completely degenerated but a small stylet is present and, according to Bovien, the intestine is represented by a single row of large, binuclear cells. Eggs (Fig. 172 K), which are smaller than those of the preceding generation, are deposited in the body cavity of the host and from them develop larvae of both sexes. These larvae remain in the host until they are ready to undergo their final molt when they penetrate the fly's ovaries,

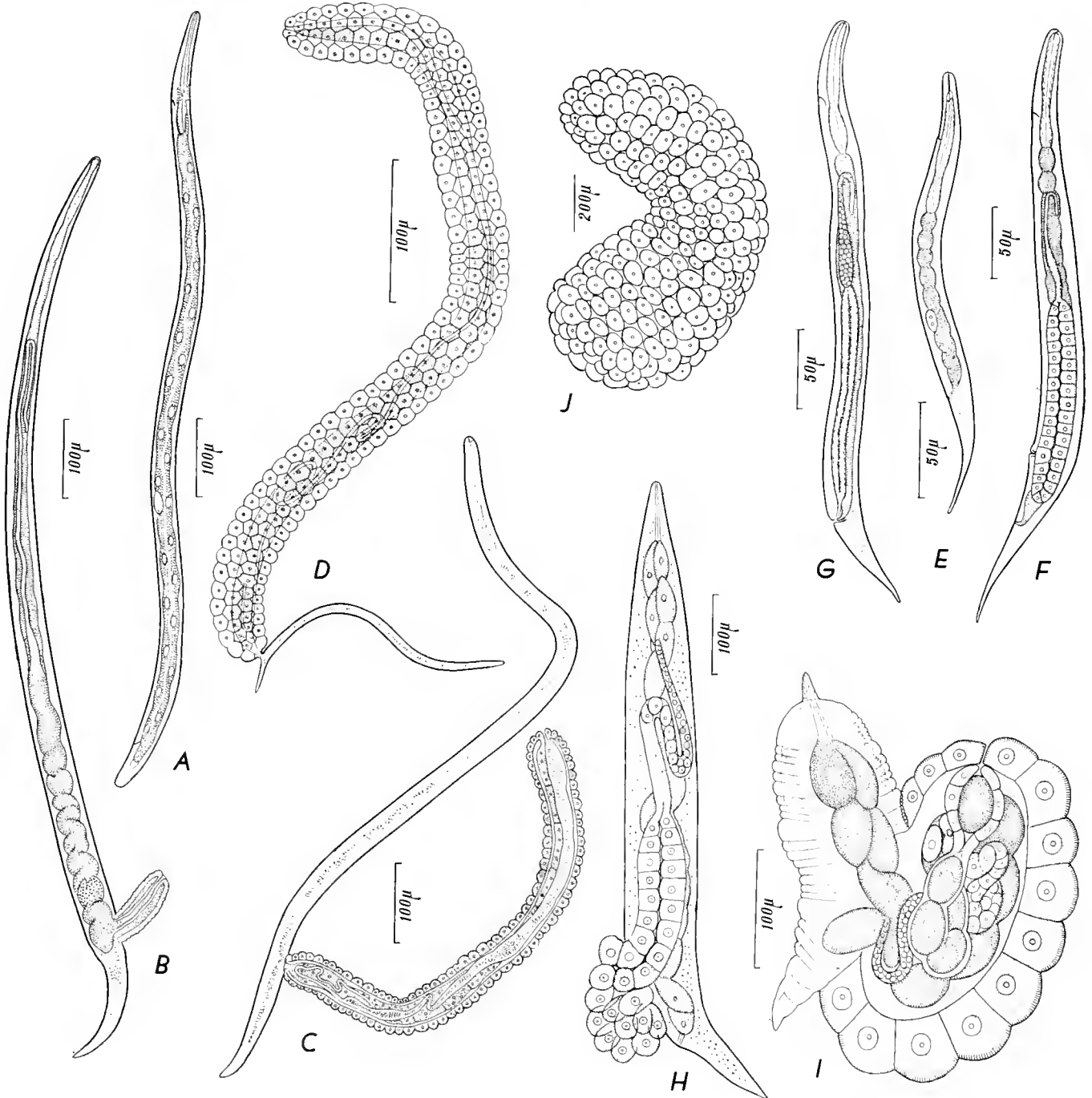


Fig. 177. SPHAERULARIINAE

A-D—*Sphaerularia bombi* (A—Fully grown larva; B-D—Adult parasitic females showing progressive stages in prolapsus of uterus. For fully grown adult parasitic female, see Fig. 115 A, p. 136). E-I—*Tripius gibbosus* (E—Newly born larva; F—Young but sexually mature female; G—Sexually mature male; H—Adult parasitic female showing

early stage in prolapsus of uterus; I—Adult parasitic female showing late stage in prolapsus of uterus. For stage intermediate between H and I, see Fig. 115 K, p. 136). J—"*Tylenchus sulphureus picar*," adult parasitic female. A-I, after Leuckart, 1887; J, after Fuchs, 1929

migrate to and assemble in the oviducts and escape through the genital aperture.

Both male and female flies are parasitized. Boyien found no evidence that male flies are rendered sterile but the ovaries of infected females fail to develop and because of this the nematode larvae can scarcely be transferred to onion plants with the eggs of their host. However, Boyien noted that infected female flies "stretched out the ovipositor, as if they wanted to lay eggs." As in the life cycle of *Tylochinema oxincillae*, infected female flies probably accompany normal females to the place where eggs are laid but deposit there, not eggs, but larval nematodes. Boyien found no evidence that the genitalia of male flies are invaded and he concluded that larvae have no way of escaping from male flies.

The free living stages of the nematode are passed in close association with the larval insects, either in the onion plant or in nearby soil. The nematodes reach maturity, copulate and the males die. Impregnated females (Fig. 172 M) enter larval flies presumably by penetrating the body wall though the act of penetration was not observed. The adult male has a stylet with basal swellings comparable to that of the female but somewhat more slender. All three esophageal glands are well developed in the preparasitic female but inconspicuous or lacking in the male. After entering the host a female develops into the large, parasitic individual of the gamogenetic generation.

The life cycle of *H. aberrans* (Fig. 176), therefore, consists of a parthenogenetic generation, passed entirely within the host, alternating with a gamogenetic generation that has both parasitic and free-living stages. If the parthenogenetic generation was omitted the life cycle would be essentially the same as that of most other members of this family.

*FERGUSOBIA CURRIEI* (Currie, 1937) n. comb. [Synonyms: *Anguillulina* (*Fergusonia*) *tumifaciens* Currie, 1937; *Anguillulina* (*Fergusonia*) *currii* (Currie, 1937) Johnston, 1938; not *Anguillulina tumifaciens* (Cobb, 1932)].

*Fergusonia currii* occurs in Australia where, in association with flies of the genus *Fergusonina*, it produces galls on *Eucalyptus* trees. This association was discovered by Morgan (1933) and later investigated by Currie (1937), the following account being based on the latter's observations. There are many species of *Fergusonina* that attack *Eucalyptus* trees in Australia and, according to Currie, all are probably associated with a nematode. Several species of *Eucalyptus* are attacked and galls may be formed on leaf buds, axil buds, stem tips, and flower buds, depending on the species involved. The following account of the life history of *Fergusonia currii* is based on a study of flower galls on *Eucalyptus macrorrhynchia* and of the associated fly, *Fergusonina nicholsonia*. Currie regards the stages of the nematode found in the galls as true plant parasites but is inclined to regard the relationship with the gall flies as symbiosis rather than parasitism.

Each female fly harbors two gravid female nematodes (Fig. 172 S). These nematodes deposit eggs and the resulting larvae, on reaching the proper stage, leave the body cavity and enter the reproductive system of the "host." Adult flies emerge during summer and females, after mating, lay eggs in young flower buds, depositing with each egg from 1 to 50 nematode larvae. The same fly or different flies may lay numerous eggs in a single bud as many as 74 eggs and 227 nematode larvae having been found. The larval nematodes immediately start to feed on the anther primordial cells that form a ring around the inner wall of the bud cavity and under this stimulus the tissue proliferates rapidly forming masses of large, thin-walled parenchymatous cells full of mucilaginous cell sap. The fly eggs hatch in about 6 weeks and by this time masses of gall tissue are already present in the bud. On hatching a fly larva moves in between two of these cell masses and tears out a small crypt in which to lie. The larval nematodes migrate into this crypt and quickly develop into adults all of which are parthenogenetic females (Fig. 172 R). Apparently the nematode passes through several parthenogenetic generations feeding on surrounding plant cells and in no way injuring the insect. During its first two instars the fly larva feeds on the viscous cell sap which oozes from surrounding cells that have been punctured by nematode stylets. During its last larval instar the fly larva tears down the walls of the cavity and feeds on the ruptured cells.

In autumn both male (Fig. 172 Q) and female nematodes appear that become the adults of the gamogenetic generation. This "preparasitic" female does not differ materially from the female of the preceding parthenogenetic generations (Fig. 172 R). Both the adult male and the adult "preparasitic" female have a stylet and three well developed esophageal glands. As in most other allantonematids, the male does not become parasitic. Just before pupating, if the fly larva is a female two adult, fertilized female nematodes enter its body cavity, presumably by penetrating the body wall. Male flies are never infected, female flies invariably so. Once in the body cavity of the "host" the

female nematodes proceed in their development and, by the time the fly has emerged as an adult, they are depositing eggs.

The life cycle of *Fergusonia currii*, therefore, consists of several parthenogenetic generations passed entirely outside the "host" alternating with a gamogenetic generation that has both a "free living" and a "parasitic" stage. If the parthenogenetic generations were omitted the life cycle would be essentially the same as that of most allantonematids.

In the case of *Fergusonia currii* associated with *Fergusonina nicholsonia* in galls of *Eucalyptus macrorrhynchia*, only two gravid "parasites" are normally found in each female fly but in some other species of *Fergusonina*, usually those of larger size, a female fly may harbor a greater number. As Currie suggests, further work may demonstrate that the nematodes associated with different species of flies are themselves specifically distinct.

*TRIPUS GIBBOSUS* (Leuckart, 1886) Chitwood, 1935 [Synonym: *Atractonema gibbosum* (Leuckart, 1886)] is a parasite of the dipterous insect *Cecidomyia pini* (DeGeer). Since the investigations by Leuckart (1887), following its original discovery in Germany, this nematode has not been reported elsewhere or received further study. Each infected larva of *C. pini* usually harbors a dozen or more, sometimes as many as 50, adult female parasites showing different degrees of development.

Eggs are laid in the body cavity of the host where they hatch and where larvae (Fig. 177 E) accumulate in great numbers. Leuckart could never find larvae in the alimentary tract or secure other evidence that they pass out through the anus and he was inclined to believe that they are liberated by the death and decomposition of the insect. The extrusion of larvae along with the eggs of the host when adult flies are ovipositing seems to be an uninvestigated possibility.

The free living period, passed in the soil, is of short duration and in a few days after leaving the host the larval nematodes have developed to adult males and females (Fig. 177 F & G). Leuckart mentions one molt, apparently the last, but noted that sometimes the cuticle shed by the male is double. After copulation males die and females enter new hosts. Leuckart did not determine how the young females reach the body cavity of larval flies but suggests entrance through the mouth or anus as a possibility. In the light of our present knowledge of this group, penetration directly through the body wall seems more probable. Fly larvae are susceptible to infection from the time they hatch until they go into the pupal stage.

During parasitic development of the female the uterus is gradually everted through the vulva (Fig. 177 H) and develops on the outside eventually forming an oval structure, somewhat exceeding in size, but always firmly attached to, the body proper that, in the meantime, has become greatly foreshortened. The remainder of the reproductive system and part of the modified intestine occupy this prolapsed uterus (Fig. 177 I).

The effect on the host is not pronounced and when the nematodes are present in moderate numbers fly larvae are able to pupate and become adults. However, Leuckart concluded that this parasite is not harmless and that heavily infected flies frequently die in the pupal stage.

*SPHAERULARIA BOMBI* Dufour, 1837. This remarkable nematode is a parasite of queen bumble bees. It has been reported from several species of *Bombus*, each host usually harboring one or, at the most, only a few adult female parasites though Leuckart (1887) found 32 in one bee. *Vespa rufa* and *V. vulgaris* have also been reported as hosts. This parasite has been found in several localities in Europe and North America and is apparently widespread.

*S. bombi*, in so far as information is available, has the typical allantonematid life cycle. Eggs are laid and hatch in the body cavity of the host and larvae, after a period of parasitic development, pass out by way of the anus and enter the soil. Here the nematodes reach maturity and copulate whereupon the males die and the impregnated females enter their new hosts. The free-living period, according to Leuckart, is of several months' duration.

Queen bees hibernate in the soil and Leuckart found that under coniferous trees where the soil is moist and covered with humus and moss is a favored place. Leuckart concluded that the bees become infected in autumn when they are penetrating the soil preparatory to hibernation and that this explains why only queens are parasitized. Infected queens, due to retarded development of the ovaries, are either unable to produce eggs or produce only a few and both Schneider (1885) and Leuckart were convinced that such queens never found colonies.

The interesting and unusual feature about this nematode is not its life cycle but the morphological development of the parasitic female. After entrance into the new host the body of the young female undergoes little or no increase in size. Instead the uterus is everted through the vulva, carries within

it the other reproductive organs as well as the modified intestine or "fat body," and develops outside the body proper (Fig. 177 B-D). This prolapsed uterus increases enormously in size while the body proper remains a relatively minute, functionless structure that may sometimes become detached (Fig. 115 A, p. 136).

**OTHER SPECIES.** In addition to the species already discussed, the number of allantonematids that have as yet been named and described is not great and for most of these information about life cycles, especially regarding free-living stages, is meager or lacking. A few exceptions, however, may be noted briefly.

*Bradynema rigidum* (v. Siebold, 1836) zur Strassen, 1892, is a parasite of the dung beetle, *Aphodius fimetarius* (L.) and *Bradynema strasseni* Wülker, 1921, is a parasite of the wood-boring beetle, *Spondylis buprestoides* (L.). These two nematodes have been rather extensively investigated in Europe (zur Strassen, 1892; Wülker, 1923) though the free-living stages of *B. rigidum* are still imperfectly known. Both have the typical allantonematid life cycle, larvae passing out of the hosts by way of the anus.

*Howardula benigna* Cobb, 1921, is a parasite of the cucumber beetle, *Diabrotica vittata* (Fab.) and, less commonly, of the related beetles, *D. trivittata* (Mann) and *D. duodecimpunctata* (Fab.). This nematode has the typical allantonematid life cycle, larvae passing out with the eggs of the host (Fig. 178). Beetles of both sexes are infected and the fate of larvae that find themselves in male beetles is not known. Cobb (1928) was of the opinion that these larvae may be transferred to female beetles during copulation. He found considerable numbers of larvae in the proximal end of the male genitalia but was unable to demonstrate experimentally that such larvae are actually transferred to female beetles.

In the genus *Aphelenchus* the adult, parasitic female is usually characterized by being curved dorsad with the vulva on the outside of the curve. *A. diplogaster* (Linstow, 1890), Filipjev, 1934 (Fig. 172 P) is a parasite of the bark beetle, *Ips typographus* (L.) and *A. tomiçi* Bovien, 1937, is a parasite of the bark beetle, *Pityogenes bidentatus* (Hbst.) (Syn. *Tomicus bidens* (F.)). These two nematodes are very closely related and both have the typical allantonematid life cycle, larvae passing out of the host by way of the anus (Fuchs, 1915; Bovien, 1937) to undergo free-living development in the frass of the beetle galleries.

"*Tylenchus*" *aptini* Sharga, 1932, was found in Scotland, where it is a parasite of the thrips, *Aptinothrips rufus* (Gmelin). Eggs of this parasite are deposited in the body cavity of the host and larvae leave by way of the anus. Males remain in the host until bursa, spicules and gubernaculum are formed and Sharga (1932), finding no evidence that males enter the gut or pass out through the anus, suggests that copulation takes place before the parasites leave the host. Furthermore, Sharga states that "after several ecdyses the mature female stage is reached" and his discussion and drawings seem to indicate that one or more of these ecdyses take place after the female has passed through the free-living stage and entered a new host. If copulation takes place before this parasite leaves the first host and the female molts after entering the second host, the life cycle is, indeed, a departure from that known for any other allantonematid.

*Parasitylenchus dispar* (Fuchs, 1915) Nicoletzky, 1922, subspecies, *typographi* (Fuchs, 1915) is a parasite of the bark beetle, *Ips typographus* (L.). In general this nematode has the typical allantonematid life cycle. The adult, parasitic female gives birth to larvae, large numbers of which accumulate in the body cavity of the host to eventually enter the gut and pass out through the anus. In one respect, however, the life cycle differs from that of most allantonematids. After completing free-living development young adults of both sexes enter the new host. One finds in the body cavity of infected beetles 200 to 300 adult parasitic females (Fig. 172 U) and an even greater number of adult parasitic males (Fig. 172 T).

Fuchs (1915) did not observe copulation or determine whether it takes place before or after entering the new host. However, he was able to rear to maturity larvae taken from the rectum of a beetle, the adult stage being reached in 7 to 10 days. The experimentally reared females did not lay eggs and it seems probable that eggs are not laid until after entrance into a new host. If this is true we have, not a free-living generation, as Fuchs called it, but a free living stage.

Ostensibly, *Tripius gibbosus* and *Sphaerularia bombi* are the only members of the Sphaerulariinae that have as yet been reported. It may be noted, however, that Fuchs (1929) has described two very unusual nematodes from bark beetles, viz., "*Tylenchus sulphureus piccae*" and "*Tylenchus sulphureus pini*." Fuchs maintained that in the case of these two nematodes the gravid female is not a prolapsed uterus basing his

contention, in part, on a failure to find any vestige of the body proper or transitional stages showing the uterus in the process of prolapsus. But in the case of *Sphaerularia bombi*, as Leuckart points out, the body proper is sometimes detached and one wonders if Fuchs' material included a sufficiently complete series of developmental stages. If the gravid female of "*Tylenchus sulphureus piccae*" (Fig. 177 J) is not a prolapsed uterus its resemblance to the gravid female of *Sphaerularia bombi* is, to say the least, very remarkable.

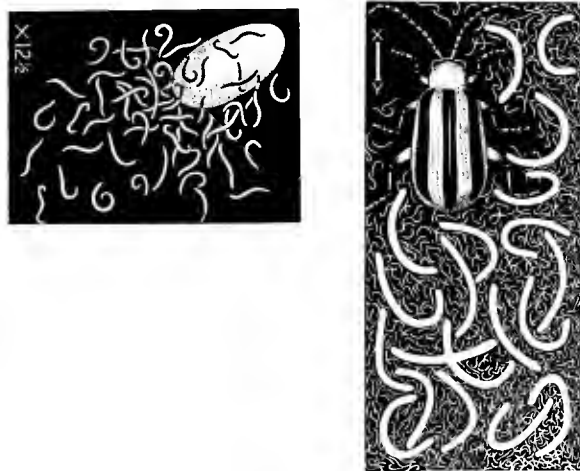


Fig. 178. HOWARDULA BENIGNA

Showing relative size of beetle, *Diabrotica vittata*, and of its parasites (line XY indicates actual length of beetle); also egg of beetle and larval nematodes deposited with it. After Cobb, 1921.

## Bibliography

- ACKERT, J. E. and WADLEY, F. M. 1921.—Observations on the distribution and life history of *Cephalobium microbivorum* Cobb and of its host, *Gryllus assimilis* Fabricius. Tr. Am. Micr. Soc., v. 40(3):97-115, 15 figs.
- ALICATA, J. E. 1934.—Observations on the period required for *Ascaris* eggs to reach infectivity. Proc. Helm. Soc., Wash., v. 1(1):12.
- BARTHÉLEMY, A. 1858.—Études sur le développement et les migrations d'un nématode parasite de l'oeuf de la limace grise. Ann. Se. Nat., Zool., 4. s., v. 10(1):41-48, pl. 5, figs. 8-15.
- BOVIEN, PROSPER. 1932.—On a new nematode, *Scatonema wülkeri* gen. et sp. n. parasitic in the body-cavity of *Scatopse fuscipes* Meig. (Diptera nematoceera). Vidensk. Medd. Dansk. Naturh. Foren, Kjøbenhavn, v. 94:13-32, figs. 1-7.
- 1937.—Some types of association between nematodes and insects. Ibid., v. 101:1-114, figs. 1-31.
- CLAUS, C. 1868.—Beobachtungen über die Organisation und Fortpflanzung von *Leptodera appendiculata*. Schrift. Gesellsch. Beförd. Ges. Naturw. zu Marburg, Suppl. Heft 3, 24 pp., 3 pls., 31 figs.
- CAULLERY, M. and COMAS, M. 1928.—Le déterminisme du sexe chez un nématode (*Paramermis contorta*), parasite des larves de chironomes. Compt. Rend. Acad. Se., Paris, v. 186:646-648.
- CHITWOOD, B. G. and CHITWOOD, M. B. 1937.—Snails as hosts and carriers of nematodes and Nematomorpha. Nautilus, Quart. J. Devoted to Interests of Conchologists, v. 50(4): 130-135.
- CHRISTIE, J. R. 1929.—Some observations on sex in the mermithidae. J. Exper. Zool., v. 53(1):59-76, figs. 1-5.
- 1936.—Life history of *Agamermis decaudata*, a nematode parasite of grasshoppers and other insects. J. Agric. Res., v. 52(3):161-198, figs. 1-20.
- 1937.—*Mermis subnitigrescens*, a nematode parasite of grasshoppers. Ibid., v. 55(5):353-364, figs. 1-6.
- CHRISTIE, J. R. and CHITWOOD, B. G. 1931.—*Chondronema pasali* (Leidy, 1852) n. g. (Nematoda), with notes on its life history. J. Wash. Acad. Se., v. 21(15):356-364, figs. 1-17.

- COBB, N. A. 1915. [Note dealing with a new species of free living nematode.] J. Parasit., v. 1(3):154.
1921. *Howardula benigna*; a nema parasite of the cucumber beetle. Science, n. s., v. 54(1409):667-670, figs. 1-4.
1921. Idem [Reprinted with only minor changes]. Contrib. Sc. Nemat., (10):1-4, figs. 1-4.
1928. Idem [Reprinted with additions] Ibid., (10):345-352, figs. 1-8.
- COMAS, M. 1927. Sur le mode de pénétration de *Paramermis contorta* v. Linst. dans la larve de *Chironomus rhummi* Kief. Compt. Rend. Soc. Biol., Paris, v. 96(10):673-675.
- CONTE, A. and BONNETT, A. 1903. Sur un nématode nouveau (*Angiostoma helicis* n. sp.) parasite de l'appareil génital d'*Helix aspersa* (Müll.). Compt. Rend. Soc. Biol., Paris, v. 55(5):198-199.
- CRAWLEY, W. C., and BAYLIS, H. A. 1921.—*Mermis* parasitic on ants of the genus *Lasius*. J. Roy. Micr. Soc., 1921:353-372, figs. 1-12.
- CUKRIE, G. A. 1937. Galls on *Eucalyptus* trees. A new type of association between flies and nematodes. Proc. Linn. Soc. N. S. Wales, v. 62(3-4):147-174, figs. 1-31, pls. 6-7.
- DOBROVOLNY, C. G. and ACKERT, J. E. 1934.—The life history of *Leidyema appendiculata* (Leidy), a nematode of coek-roches. Parasit., v. 26(4):468-480, figs. 1-10, pl. 23, figs. 1-3.
- DUJARDIN, FÉLIX. 1845.—Histoire naturelle des helminthes ou vers intestinaux. xvi + 654 + 15 pp. 12 pls. Paris.
- FUCHS, GILBERT. 1915.—Die Naturgeschichte der Nematoden und einiger anderer Parasiten. 1. des *Ips typographus* L. 2. des *Hyllobius abietis* L. Zool. Jahrb., Abt. System., v. 38(3-4):109-222, figs. a-b, pls. 17-21, figs. 1-82.
- 1929.—Die Parasiten einiger Rüssel und Borkenkäfer. Ztschr. Parasitenk., Abt. F, Ztschr. Wiss. Biol., v. 2(2):248-285, figs. 1-36.
- GALER, OSMAN. 1878.—Recherches sur les entozoaires des insectes. Organisation et développement des oxyuridés. Arch. Zool. Expér. & Gén., v. 7(2):283-390, pls. 17-26.
- GLASER, R. W. 1932.—Studies on *Xoanplectana glaseri*, a nematode parasite of the Japanese beetle (*Popillia japonica*). N. J. Dept. Agric., Circ. No. 211, 34 pp., 3 pls., 17 figs.
- GLASER, R. W., MCCOY, E. E. and GIRTH, H. B. 1940.—The biology and economic importance of a nematode parasitic in insects. J. Parasit., v. 26(6):479-495, figs. 1-8.
- GOODEY, T. 1930.—On a remarkable new nematode, *Tylenchinema oscinellae* gen. et sp. n., parasitic in the frit-fly, *Oscinella frit* L., attacking oats. Phil. Tr. Roy. Soc. London, s. B, v. 218:315-343, fig. 1, pls. 22-26, figs. 1-54.
- 1931.—Further observations on *Tylenchinema oscinellae* Goodey, 1930, a nematode parasite of the frit fly. J. Helm., v. 9(3):157-174, figs. 1-2.
- GÖSSWALD, KARL. 1929.—Mermithogynen von *Lasius alienus*, gefunden in der Umgebung von Würzburg. Zool. Anz., v. 84(7-8):202-204.
- 1930.—Weitere Beiträge zur Verbreitung der Mermithiden bei Ameisen. Ibid., v. 90(1-2):13-27, fig. 1.
- HAGMEIER, ARTHUR. 1912.—Beiträge zur Kenntnis der Mermithiden. I. Biologische Notizen und systematische Beschreibung einiger alter und neuer Arten. Zool. Jahrb., Abt. System., v. 32(6):521-612, figs. a-g, pls. 17-21, figs. 1-55.
- HUNGERFORD, H. B. 1919.—Biological notes on *Tetradonema plicans* Cobb, a nematode parasite of *Sciara coprophila* Lütner. J. Parasit., v. 5:186-192, 2 text figs., 1 pl., figs. 1-6.
- JOHNSON, G. E. 1913. On the nematodes of the common earthworm. Quart. J. Micr. Sc., v. 58(4):605-652, figs. 1-2, pl. 35, figs. 1-10.
- KABURAKI, TOKIO and IMAMURA, SHIGEMOTO. 1932. Mermithid worm parasitic in leaf hoppers, with notes on its life history and habits. Proc. Imp. Acad., v. 8(4):139-141, figs. 1-6.
- KABURAKI, TOKIO and IYATOMI, KISABU. 1933. Notes on sex in *Amphimermis zumashi* Kab. et Im. Proc. Imp. Acad., v. 9(7):333-336.
- KEHLIN, D. 1925. Parasitic autotomy of the host as a mode of liberation of coelomic parasites from the body of the earthworm. Parasit., v. 17(2):170-172.
- KEHLIN, D. and ROBINSON, V. C. 1933. On the morphology and life history of *Apraotonema entomophagum* Keilin, a nematode parasite in the larvae of *Sciara pullula* Winn. (Diptera Nematocera). Parasit., v. 25(3):285-294, figs. 1-2, pls. 19-20, figs. 1-19.
- KOHN, F. G. 1905.—Einiges über *Paramermis contorta* (v. Linstow) (*Mermis contorta* v. Linstow). Arb. Zool. Inst. Univ. Wien, v. 15(3):213-256, pl. 1, figs. 1-21.
- KREIS, H. A. 1932. *Trionchonema rusticum* n. g. n. sp., a parasitic nematode from the land snail, *Polygyra espicola* Bland (Helicidae). Tr. Am. Micr. Soc., v. 51(1):48-56, pls. 8-9, figs. 1-13.
- LEIDY, JOSEPH. 1852.—Some observations on *Nematoidea imperfecta*, and descriptions of three parasitic infusoriae. Tr. Am. Phil. Soc., Phila., n. s., v. 10(2):241-244, pl. 11, figs. 42-51.
- LEUCKART, R. 1887.—Neue Beiträge zur Kenntnis des Baues und der Lebensgeschichte der Nematoden. Abhandl. Math.-Phys. Cl. K. Sächs. Gessellsch. Wiss., v. 13(8):565-704, pls. 1-3.
- LINSTOW, O. v. 1890.—Ueber *Allantonema* und *Diplogaster*. Centralbl. Bakt. u. Parasitenk., v. 8(16):487-497, figs. a-f.
- MAUPAS, E. 1899.—La mue et l'enkystement chez les nématodes. Arch. Zool. Expér. & Gén., 3. s., v. 7:563-628, pls. 16-18, figs. 1-29.
- MCCOY, E. E., GIRTH, H. B. and GLASER, R. W. 1938.—Notes on a giant form of the nematode *Xoanplectana glaseri*. J. Parasit., v. 24(5):471-472.
- MERRILL, J. H. and FORD, A. L. 1916.—Life history and habits of two new nematodes parasitic on insects. J. Agric. Res., v. 6(3):115-127, figs. 1-3.
- MORGAN, W. L. 1933.—Flies and nematodes associated in flower and galls of spotted gum. Agric. Gaz. N. S. Wales, v. 44(2):125-127.
- OTTER, G. W. 1933.—On the biology and life history of *Rhabditis pellio* (Nematoda). Parasit., v. 25(3):296-307.
- PEREIRA, CLEMENTE. 1931.—*Myenchus botelhoi* n. sp., curioso nematoide parasito de *Limnodynella brasiliensis* Pinto (Hirudinea). Tese, Fac. Med., 29 pp., 5 figs., 2 pls., Sao Paulo, Brasil.
- SCHNEIDER, ANTON. 1859.—Über eine Nematodenlarve und gewisse Verschiedenheiten in den Geschlechtsorganen der Nematoden. Ztschr. Wiss. Zool., v. 10(1):176-178.
- 1885a.—Über die Entwicklung der *Sphärulearia bombi*. Zool. Beitr., Bresl., v. 1(1):1-10, pl. 1, figs. 1-5.
- 1885b.—Fortgesetzte Untersuchungen über *Sphärulearia bombi*. Ibid., v. 1(3):247-251, 1 fig.
- SCHUBERG, A. and SCHÜRÖDER, O. 1904.—*Myenchus bothryophorus*, ein in den Muskelzellen von *Niphelis* schmarotzender neuer Nematode. Ztschr. Wiss. Zool., v. 76(4):509-521, pl. 30, figs. 1-11.
- SEURAT, L. G. 1920.—Histoire naturelle des nématodes de la Berbérie. 1st part. Morphologie, développement éthologie et affinités des nématodes. vi + 220 pp., 34 figs., Algiers.
- SHARGA, I. S. 1932. A new nematode, *Tylenchus aptini* n. sp., parasite of Thysanoptera (Insecta: *Aplinothrips rufus* Gamelin). Parasit., v. 24:268-279, figs. 1-26.
- ZUR STRASSEN, O. 1892.—*Bradynema rigidum* v. Sieb. Ztschr. Wiss. Zool., v. 54(4):655-747, pls. 29-33, figs. 1-98.

- STRICKLAND, E. H. 1911.—Some parasites of *Simulium* larvae and their effects on the development of the host. Biol. Bull. Mar. Biol. Lab., Woods Hole, v. 21(5):302-338, pls. 1-5.
- VANDEL, A. 1930.—La production d'intercastes chez la fourmi *Pheidole pallidula* sous l'action de parasites du genre *Mermis*. Bull. Biol. France & Belg., v. 64(4):458-494, figs. 1-14, pl. 17.  
1934.—Le cycle évolutif d'*Hexameris* sp., parasite de la fourmi (*Pheidole pallidula*). Ann. Se. Nat., Zool., 10. s., v. 17:47-58, 1 fig.
- VAN ZWALUWENBURG, R. H. 1928.—The interrelationships of insects and roundworms. Hawaiian Sugar Planters' Assoc. Exper. Sta. Bull. (Ent. Ser.) No. 20, 68 pp.
- WHEELER, W. M. 1928.—*Mermis* parasitism and intercastes among ants. J. Exper. Zool., v. 50(2):165-237, figs. 1-17.
- WÜLKER, G. 1923.—Über Fortpflanzung und Entwicklung von *Allantonema* und verwandten Nematoden. Ergeb. u. Fortschr. Zool., v. 5(4):389-507, figs. 1-53.  
1926.—Über geschlechtsreife Nematoden im Regenwurm. Arch. Schiffs- u. Tropen-Hyg., v. 30:610-623, figs. 1-6.

## CHAPTER VI

### LIFE HISTORY (ZOO PARASITICA)

#### II. PARASITES OF VERTEBRATES

ASA C. CHANDLER, Rice Institute, Houston, Texas; J. E. ALICATA, University of Hawaii, Honolulu, T. H.; and M. B. CHITWOOD, Babylon, N. Y.

##### Introduction

The life cycles of the nematodes parasitic in vertebrates differ in no essential from those of free-living nematodes, but are subject to a number of modifications which enable the parasites to gain access to new hosts with more facility and greater certainty. With a few exceptions these nematodes have five stages of development separated by four moults as do most free-living nematodes, but in a few forms (e.g., *Contraecicum* and *Trichinella*) the number of moults is said to be increased, and in some forms one or more of them is suppressed to the extent of being passed through rapidly in the egg, or in hatched larvae with no intervening period of growth.

The outstanding feature in the life cycle of parasitic nematodes is a cessation of development of the young worms after reaching an infective stage, while they await an opportunity to gain access to a new definitive host. In most cases the organisms pass through this period of waiting outside the body of the original host, either (1) as embryos inside the egg shells (oxyurids, ascaridids, trichurids); (2) as free-living but nonfeeding third-stage larvae, often enclosed in the shed cuticle of stage two (*Strongyloides*, many strongylins); or (3) as third-stage larvae, usually encysted, in the body of an intermediate host, which in some cases is obligatory (e.g., spiruroids, camallanins, some metastrongylids) but in other cases is optional (e.g., *Capillaria annulata*, *Syngamus trachea*). In many cases such larvae are capable of re-encystment, sometimes over and over again, in other hosts—transport hosts—in which development to maturity does not occur. In a few cases such secondary intermediate hosts have become necessary parts of the life cycle (e.g., *Gnathostoma spinigerum*). In the filariae and a few other nematodes (e.g., *Habronema*) the infective larvae do not become encysted, and habitually emerge through a break in the labium of the vector as a result of their own activities. A striking exception to the usual waiting period outside the body of the host occurs in the case of *Trichinella spiralis*, which passes its waiting period encysted in the flesh of the parental host.

In considering the life cycles of parasitic nematodes from an evolutionary standpoint it is necessary to consider possible ways in which the nematodes may have developed into parasites of vertebrates. One method was presumably the result of ingestion by the host, followed by adaptation to the environment encountered inside the alimentary canal. It seems probable that the Oxyuridae, for instance, became parasitic in this manner. Such nematodes might be expected to have the simplest possible type of life cycle, reproducing generation after generation in the lumen of some part of the alimentary canal, with enough eggs or larvae escaping with the feces to allow for spread to other hosts through the medium of contaminated food or water. It seems remarkable that only a single instance (*Probstmayria vivipara*) is known of a parasite which has unequivocally adapted itself to this type of life. The nearest approach, with the exception of *Probstmayria*, is the facultative parasitism of a number of species whose congeners are saprozoic free-living forms, e.g., a species of *Longibucca* in the stomach of a snake (Chitwood, 1933), and another species of *Longibucca* in the stomach and intestines of a bat (McIntosh and Chitwood, 1934); *Diploscapter coronata* in ahydrochloric human stomachs (Chandler, 1938); and *Cephalobus parasiticus* in the stomachs and intestines of monkeys (Sandground, 1939). In addition to these cases, it is claimed by a number of writers (Koeh, 1925; Penso, 1932) that *Enterobius vermicularis*, *Passalurus ambiguus* and other oxyurids are capable of reproducing, generation after generation, in the lumen and walls of the intestine. This is denied by others (Zowadowsky and Schalimov, 1929; Lentze, 1935) because of the demonstrated need of oxygen by the embryos before they can complete their development. Even if the larvae can occasionally develop to maturity in the gut walls, such an occurrence can certainly be considered the exception rather than the rule. One other instance of repeated generations in a single host has been claimed for *Strongyloides stercoralis* (Nishigori, 1928; Faust, 1931) but this is a case of short-circuited rather than continuous development, and occurs only under exceptional conditions. The offspring of worms in the intestine do not grow to maturity directly in the intestinal lumen, but migrate

through the body as they would if they had infected from outside.

With the few exceptions mentioned above, the simplest type of life cycle in the case of obligatory parasites is that exhibited by most species of oxyurids, in which the eggs fail to develop beyond a certain point (morula stage in some, "tadpole" in others) until exposure to oxygen outside the body of the host, followed by reentrance of the embryonated eggs or hatched larvae into the same or another host with food or water contaminated by them. In many cases, possibly in all, this simple cycle is modified further by a stage in which the larvae attach themselves to the mucous membrane, bury their heads in it, or actually burrow into the walls of the gut before they take up their residence in the lumen as adults.

Few parasites other than the Oxyuroidea have as simple a life cycle as that described in the last paragraph. Most of them have an instinct for burrowing at some time during the course of their development and exercise it either (1) by burrowing through the skin and going on a tour of the body via the circulatory system, lungs and throat before reaching the intestine; (2) by burrowing into the mucous membranes of the alimentary canal, either being content to live buried in the gut wall for a few days, or entering the circulatory system and going on a tour of the body similar to that of the skin penetrators or, in some cases, burrowing directly through into the body cavity or through mesenteries, parenteral tissues, etc.; or (3) by burrowing into the body cavity or tissues of an intermediate host, either through the surface or through the walls of the gut after being ingested.

Two possible origins of this burrowing habit suggest themselves. One possible origin is as a useful instinct on the part of gut parasites to serve either one or both of two purposes, (1) to protect the young worms from being swept out of the intestine with the feces, and (2) to provide a better type of nourishment for the period of rapid growth and development. There can be little doubt but that the burying of the head of fourth-stage larvae of *Dermatophys veligera* (Wetzel, 1931) and the use of the "corpus" of the esophagus of *Oxyuris equi* as a mouth capsule (Wetzel, 1930) are steps in this direction. One could then visualize as further developments complete burrowing into the gut wall, penetration into the circulatory system, and the circuit through the body that would necessarily be entailed.

The alternative explanation is that the worms which migrate through the body originally became vertebrate parasites by burrowing through the skin. An initial step in this direction can be observed today in the occasional invasion of the skin of dogs and sometimes of other animals by *Rhabditis strongyloides*, the adults of which live in soiled straw bedding. Successful development of adult parasitism by this method would necessitate an ultimate location in the body whence the eggs or embryos could escape in order to reach new hosts. This condition would be fulfilled in the case of those parasites which, after penetration of the skin, reach the circulatory system and eventually arrive in the lungs, where, still imbued with an instinct for burrowing, they would escape into the air spaces. Here they could successfully reach maturity and reproduce (e.g., Metastrongylidae) or they could be carried passively, via trachea and throat, to the alimentary canal. Successful parasitism would, of course, be dependent upon loss of the burrowing instinct after the third moult, which actually occurs.

The temporary burrowing into the intestinal mucosa of the larvae of such worms as *Ascaridia*, *Haemonchus* and *Oesophagostomum* might, then, be construed either as a step in the direction of a more extended migration, as practiced by related worms, or as a step in the direction of the simple oxyurid type of life cycle, with abandonment of a primitive but no longer necessary migration from skin to intestine. In those species which usually perform the entire migration, the failure of some individuals to do so (e.g., *Ascaris*, hookworms) could be either atavistic or progressive. The fact that the curtailed migration is more likely to occur in the normal than in abnormal hosts is of little help in the matter, for it could be argued either that the reason for the failure of migration in normal hosts is due to less restlessness in such hosts and a consequent slipping back to ancestral ways, or that it is

due to more perfect adaptation and therefore more advanced evolution.

It is, as a matter of fact, probable that the migration is primitive for some worms and secondarily acquired for others. Worms which may be assumed normally to develop directly in the intestine are at least occasionally able to reach the intestine even if injected under the skin. This was demonstrated by Harwood (1930) in the case *Cosmocercoides dukae*, for although he found the larvae of this worm to be incapable of skin penetration, he succeeded in recovering a few worms from lungs and intestine after subcutaneous injection. There is some reason to believe that the Strongyloidea and Rhabdiasidae, the latter of which never establish themselves in the intestine at all, may be primitively skin penetrators, whereas it is very unlikely that the ascarids are. Whether parenteral migration is primitive or secondary among the Strongylina is not so easy to guess.

An interesting derivative of the migratory type of life cycle is the course of development of *Trichinella*. The unique life cycle of this worm has apparently resulted from a precocious development and hatching of the eggs in the uterus of the mother, accompanied by early acquisition of the burrowing instinct, the result being the invasion of the parental host instead of a new host. The *Strongyloides* life cycle is another derivative in which the parasitic worms have become parthenogenetic and a free-living sexually-reproducing generation may be interpolated in the course of a cycle of development which is otherwise similar to that of hookworms.

In the case of nematodes whose larvae hatch outside the body and have an instinct for burrowing it is easy to conceive of the accidental or, in time, routine invasion of intermediate or transport hosts. This might come about by invasion from the outside (e.g., Protostrongylinae), or by penetration through the gut wall after being swallowed (e.g., Anisakinae). Such penetration of hosts other than the definitive one, and subsequent encapsulation in parenteral tissues, is an extremely common phenomenon, and occurs in all the major groups of parasitic nematodes. In some instances it is a more or less exceptional phenomenon, e.g., the encystment of *Toxocara* larvae in mice (Fülleborn, 1921); in others it constitutes an important but not absolutely essential factor in the epidemiology, e.g., *Syngamus trachea*; and in still others it has become obligatory, the invaded hosts then becoming true intermediate hosts rather than transport hosts, e.g., spiruroids.

The frequent encapsulation of some nematodes in transport hosts and its non-occurrence in others is probably dependent upon the behavior of the larvae in the hosts concerned. Larvae that keep on the move do not become encapsulated. It is for this reason that most spiruroids are encapsulated, whereas *Habronema* and filariae are not. No encapsulation of hookworm or *Ascaris lumbricoides* larvae occurs when these enter rodents since the larvae complete the migration to the intestine, and are then evacuated because the environment is unsuitable for growth to maturity. On the other hand, since *Toxocara* is encapsulated in mice, it must be assumed that this worm loses its burrowing instinct before it has regained the alimentary canal, and then becomes quiet enough to be encapsulated by the host.

In the Metastrongylidae alone all gradations can be found from more or less accidental and unnecessary penetration of an intermediate host (e.g., *Dictyocaulus filaria*) to obligatory development in specific invertebrates (e.g., *Metastrongylus*, *Protostrongylus* and *Muellerius*). Similar obligatory dependence upon specific intermediate hosts has become the lot of the entire group of spiruroids, the Camallanina, the Dioctophymatina, and apparently at least one ascaridoid, *Subulura brumpti* (Aliata, 1939).

A clue to the origin of the filarioid type of life cycle, in which the microfilariae are withdrawn from blood or skin by blood sucking arthropods, and are eventually given an opportunity for reinvasion of the skin by these same arthropods, after development within them, is afforded by the habronemas (see p. 286). In these the larvae show a definite step towards the filarial type in that they fail to become encapsulated in the intermediate host, there to await passive transfer to the definite host, but instead remain free and active, and leave the intermediate host, under suitable conditions, of their own volition. The further steps to a filarial life cycle are merely (1) substitution of a parenteral for a gastro-intestinal habitat for the adult worms, and consequent liberation of the embryos into the blood or tissues whence blood-sucking insects can withdraw them; and (2) successful penetration of the skin by the infective larvae to reach their definitive location.

It will be seen that in no case is there reason to believe that intermediate hosts of nematodes are ancestral hosts, as is the case with intermediate hosts of flukes.

The same modifications in life cycle have a tendency to reappear over and over again in the various groups of para-

sitic nematodes, and sometimes several of the principal types may occur within a group of closely related genera. In the genus *Habronema*, for instance, the species parasitic in the stomachs of horses are deposited by the intermediate hosts on the lips or skin and they reach their destination by way of the mouth, either by direct migration into it or by being licked from the skin. In the habronemas parasitic in insectivorous birds, on the other hand, there can be little doubt but that they reach their destination in the orthodox spiruroid fashion, by the intermediate hosts harboring them being swallowed. In the species found in raptorial birds, however, a secondary transport host is usually if not always involved. Because of this lack of uniformity within even nearly related forms, and because of the endless number of minor variations by which one type of life cycle grades into another, we believe that a clearer picture of the life cycles of parasitic nematodes can be given by discussing the outstanding types and principal variations in each natural group, than by discussing types of life cycles irrespective of the natural groups in which they occur. By way of summary, however, we suggest the following classification of the principal life cycle types:

- A. Monoxenous or Direct (no intermediate host required).
  1. Continuous reproduction within host, generation after generation; various stages of worms occasionally carried out of body and infect other hosts through contaminated food. Ex., *Probstmayria*; facultative rhabditoid parasites.
  2. Discontinuous, eggs or embryos escaping habitat of adults, and usually leaving parental host.
    - (1) Without free-living phase.
      - a. Simple. Eggs leave body of host, usually becoming embryonated outside, reënter via the mouth usually before hatching, and grow to maturity in the alimentary canal. Ex., *Euterobius*, *Trichuris*.
      - b. With temporary burrowing into mucosa. Ex., *Ascaridia*.
      - c. With parenteral migration via blood system to heart and lungs, returning to intestine via throat. Ex., *Ascaris lumbricoides*.
      - d. With parenteral migration via blood system to definitive locations elsewhere in body. Ex., *Capillaria hepatica*.
    - (2) With free-living phase. Eggs usually hatch outside body of host into first-stage larvae which grow to third (infective) stage while free, but in some forms may develop to third stage before hatching.
      - a. With skin penetration and migration to intestinal tract via heart and lungs.
        - (a) Free-living forms larvae only. Ex., *Necator*.
        - (b) With possible development of an alternative generation of free-living adult males and females. Ex., *Strongyloides*.
      - b. Without skin penetration; infection by mouth.
        - (a) With temporary burrowing of larvae into mucosa. Ex., *Haemonchus*.
        - (b) With more extended burrowing and formation of nodules in intestinal wall. Ex., *Oesophagostomum*.
        - (c) Migration through intestinal wall and formation of nodules in parenteral locations. Ex., *Strongylus*.
      - c. With optional use of transport host. Infective larvae when ingested by various invertebrates become encysted and reach final host when transport host is eaten. Ex., *Syngamus*; *Dictyocaulus*.
- B. Heteroxenous or Indirect (development occurs only in an intermediate host)
  1. Passive Indirect. Embryonated eggs or larvae enter an intermediate host and become infective upon reaching third stage. Final host reached when intermediate host is eaten. Migration in definitive host, if any, via tissues or natural passages, not via blood system.
    - (1) Eggs or larvae leave host with feces.
      - a. Embryonated eggs or larvae are swallowed by intermediate host. Ex., *Metastrongylus*, spiruroids.
      - b. Larvae superficially penetrate foot of molluscs. Ex., Protostrongylinae.
    - (2) Larvae leave host through skin or by other parenteral routes. Develop after being swallowed by intermediate host. Ex., Draconuloidea.
  2. Active Indirect. Larvae actively leave intermediate host to reach skin of definitive host.
    - (1) Larvae reach intermediate host by eggs being eaten. Ex., *Habronema*.
    - (2) Larvae reach intermediate host by being sucked from blood or skin. Ex., Filariae.
  3. Double Indirect. Larvae utilize two or more successive intermediate hosts.



- (1) Second and subsequent intermediate hosts optional. Possible variation in many subdivisions above.
- (2) Two successive intermediate hosts obligatory; definitive host reached by eating of second intermediate host. Ex., *Gnathostoma*.

## RHABDITOIDEA

As noted on p. 267, there are a considerable number of nematodes belonging to this group which are facultative parasites of vertebrates, but only the Rhabdiasidae and Strongyloididae have become *obligatory* parasites of vertebrates. In both of these families there is a tendency for an alternation between free living and parasitic generations, and in both, except in one species, *Parastrongyloides winchesi*, there is a suppression of males in the parasitic generation. The larvae of most species penetrate the skin or mucous membranes and migrate to the lungs before growing to maturity. The Rhabdiasidae mature in the lungs, and this is probably the more primitive condition; the Strongyloididae only exceptionally mature in the lungs, ordinarily returning to the intestine before maturing.

### STRONGYLOIDIDAE

The life cycle of *Strongyloides stercoralis* of man, the main features of which were first elucidated by Grassi (1878) and Leuckart (1882), has been studied by a large number of prominent parasitologists, yet even today there is no unanimity of opinion about some phases of it. Grassi observed direct development into filariform\* larvae; Leuckart discovered that an alternation of generations might occur; van Durme (1902) first demonstrated that infection resulted from skin penetration; and Looss (1905) showed that the migration of the larvae after penetration paralleled that of *Ancylostoma*. Important additional details or interpretations with respect to this or related species have been added by Leichtenstern (1899, 1905), Fülleborn (1914), Sandground (1926), Nishigori (1928), Kreis (1932), Faust (1933), Lueker (1934), Graham (1936-1939), Beach, T. D., (1935-1936) and Chitwood and Graham (1940).

The parasitic females live more or less deeply imbedded in the mucous membrane of the small intestine where they produce embryonated eggs which in this species hatch promptly within the host. The embryos are rhabditiform, and resemble those of hookworms except for the very short stoma. They normally pass out of the body with the feces of the host, and then begin to feed and grow. From this point on they may follow either one of two courses of development, known respectively as the direct or homogonic type, and the indirect or heterogonic type.

In the homogonic type of development the rhabditiform larvae grow and transform into filariform larvae, sometimes in 24 hours or less. Looss reported two molts in the course of the development of rhabditiform to filariform larvae in the human and other species, and Lueker (1934) observed two molts in the larvae of *S. ransomi* of pigs, but other observers have not mentioned more than one molt. The second stage larva, according to Lueker, does not at first differ in any morphological characters from the first stage larva, but transition to the filariform type of larva begins soon afterwards. The first molt occurs 12 to 18 hours after hatching, the second within 48 hours, at room temperatures. The filariform larvae are unshathed and constitute the infective stage. They creep up on points of vantage in the soil or culture, often clustering together in brush-like groups to await an opportunity to burrow through the skin of a host.

In the heterogonic type of development the rhabditiform larvae, instead of developing into filariform larvae, change into adult free-living males and females. With the exception of Lueker (1934), no observer has mentioned or suggested more than a single molt in the course of this development, but Lueker has been able to trace the usual four molts and five stages. The only morphological changes are in size and in growth of the genital primordium until the fourth stage is reached, when the structure of the head simulates that of the adult, and the male and female characters are gradually assumed. At the time of the final molt the ovaries of the females and spicules of the males are fully formed. Adults may be found after 36 to 48 hours at room temperature.

The impregnated females produce eggs soon after they reach maturity, and these hatch soon after being deposited. These first-stage rhabditiform larvae are morphologically similar to those hatching from eggs deposited by the parasitic females, although a few small differences have been mentioned

by Kreis (1932). Although Kreis, like all others before him, fails to mention more than a single molt, Lueker was able to trace the orthodox two molts before the infective filariform larva was produced, just as in the case of filariform larvae of direct development. No difference between the two types of infective larvae has been noted.

Beach (1935-1936) showed that under particularly favorable conditions there may be several generations of free-living bisexual forms. Kouiri, Basuneyo and Arenas (1936) reported that *S. stercoralis*, after numerous free-living generations, becomes entirely free living; the females become parthenogenetic and there are no males, but the fecundity of the females gradually decreases until the cultures become sterile.

Nishigori (1928) first demonstrated the opposite extreme in the life cycle of *S. stercoralis*—internal auto-infection (called hyper-infection by Faust), with complete elimination of a free-living stage. Nishigori also suggested circumstances under which this might occur. Faust (1931) and Faust and Kagy (1933) confirmed Nishigori's observations, but the evidence was inconclusive for many until Faust and deGroat (1940) made observations at autopsy of a case which left no room for doubt but that under exceptional conditions in human beings auto-infection by filariform larvae of *S. stercoralis* through the walls of the colon can occur. There is no certainty, however, that it occurs in other species or hosts.

The filariform larvae of *S. stercoralis* and most other species normally penetrate the skin. If swallowed they burrow through the mucous membranes of mouth, esophagus or stomach. Mönig (1930), however, states that sheep are usually infected with *S. papillosus* by mouth, this species being a poor skin-penetrator, and that larvae administered by mouth do not migrate to the lungs. Lueker's (1934) experiments with *S. ransomi* suggest that in this species also migration to the lungs may not take place after oral infection.

The larvae of *S. stercoralis*, after penetrating skin or mucous membranes, enter the circulatory system and are carried to the lungs. Faust (1933) states that they reach the lungs unchanged; they are sometimes recovered as early as the third day, and sometimes as late as the thirtieth day. Although no molts are mentioned, Faust distinguishes post-filariform, pre-adolescent, adolescent, and mature female and male forms. The post-filariform type of larvae is found most commonly in the lungs about the fifth day; if carried to the digestive tract they seem unable to establish themselves. These larvae are slenderer than infective larvae, with longer esophagus, and are more plastic. The pre-adolescent forms also occur principally in the lung tissue and bronchioles, and are believed to be too immature to establish themselves in the intestine. At this stage, according to Faust, sexual differences are observable for the first time, the female being still more slender than the post-filariform type and with a longer esophagus, whereas the male shows decided resemblances to the rhabditiform larva. The adolescent forms are migratory, and are commonly found not only in the lungs but also in the upper parts of the respiratory tree, esophagus and intestines. Both mature females and males were reported from lung tissue and bronchioles, but only mature females from the intestine, where they burrow into the walls. Lueker (1934), studying *S. ransomi*, observed only a single molt after entering the body of an animal, this occurring in the intestine about 6 days after infection; Looss (1911) also reported only a single molt, but Fülleborn (1914) apparently considers that two molts occur. By analogy with other nematodes, and with the development of the free-living adults of *Strongyloides* itself, it would seem more probable that two molts do occur in the course of the development in the host.

There has been much difference of opinion on several points in connection with the life cycle of *Strongyloides*, particularly (1) the factors determining whether the development is homogonic or heterogonic; (2) the reproduction status of the parasitic females, and (3), since the work of Kreis (1932) and Faust (1933), the occurrence and function of parasitic males.

Sandground (1926) gave a brief but valuable summary of views up to the time of his writing on the factors determining direct or indirect development. Environmental factors were first thought to be the cause, but Brann (1899) and others showed that such was not the case; Sandground felt that there remained no substantial reason for questioning the generally accepted idea that the direction of development was fixed before the larvae entered their period of free life. Leichtenstern (1905) advanced the view that there were two genetically different varieties of the human species, differing in their life cycles, the indirectly developing variety being confined to the tropics, the directly developing one being especially characteristic of the temperate zone. Leichtenstern considered the heterogonic type to be the more primitive and gave a very plausible explanation for the evolution of the homogonic type. Darling (1911) suggested as a cause environmental effects on the rhabditiform larvae prior to leaving the host, and Brumpt

\*The term "filariform" is used here to denote the third stage larva of *Strongyloides* to distinguish it from the third stage larva of strongylids which is called "strongyliform" larva. Its use in no way signifies a similarity to any stage of filarids. Actually, the esophagus is very similar to that of an infective strongyl larva. B. G. C.

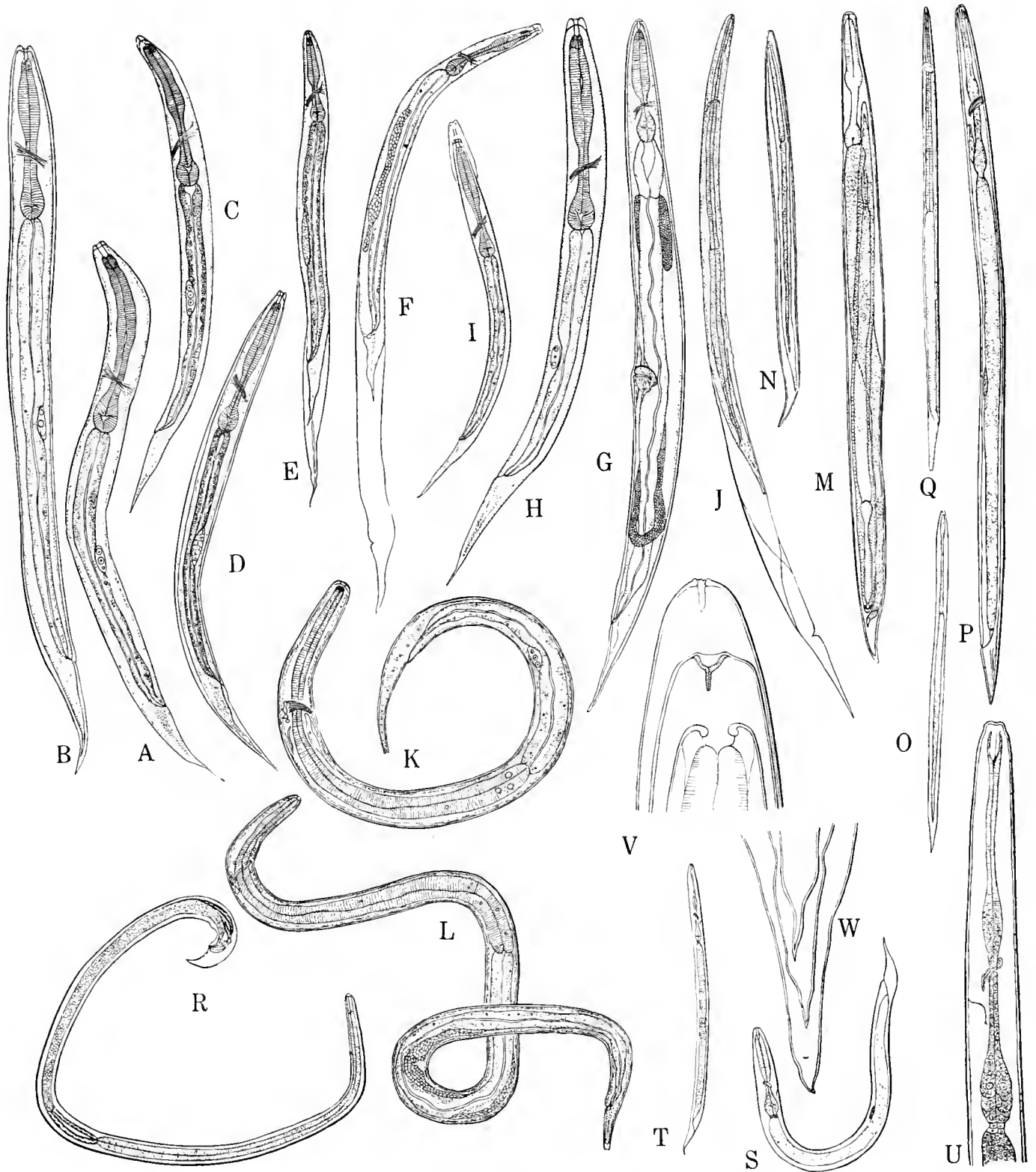


Fig. 179. DEVELOPMENT OF STRONGYLOIDES AND RHABDIAS

A-L—*Strongyloides ransomi* (A-B—Direct cycle, A—First stage larva, newly hatched; B—Larva in first molt; C-J—Indirect cycle; C—First stage larva, newly hatched; D—Larva in first molt; E—Larva in second molt; F—Larva in third molt; G—Larval female in fourth molt, H—First stage larva from free-living female, newly hatched; I—Second stage larva, immediately after first molt; J—“Filariform” larva undergoing second molt; K-L—Parasitic generation, K—Larva from small intestine of pig about 4 days after percutaneous infection; L—Larva from pig, showing early final molt). M-O—*Rhabdias fulleborni* (M—Free-living female with larva, the genital organs already destroyed; N—Cuticular hull of female with only one filariform larva, 115 hr. old culture; O—Filariform larva (Same as N) from which cuticle of fe-

male has been carefully removed). P-Q—*Rhabdias fuscovenosa* (P—Infective rhabditiform larva 72 hours after hatching from egg of parasitic generation; Q—Filariform larva (infective larva) from free-living generation). R—*Parastongyloides winchisi* male. S-W—*Rhabdias fuscovenosa*, direct development (S—Rhabditiform larva; T—Ensheathed infective larva; U—Anterior end; V—Anterior end during third and fourth molts; W—Posterior end of same). A.L., after Lueker, J. T., 1934, U. S. D. A. Bull. no. 437. M-O, after Travassos, L., 1926, Arch. Schiffs. u. Tropen-Hyg. v. 30. P-Q, after Chu, T. C., 1936, J. Parasit. v. 22 (2). R, after Morgan, D. O., 1928, J. Helm. (6). S-W, after Goodey, T., 1924, J. Helm. 2.

(1921) pushed the determination back still further, to the developing eggs. Sandground (1926), who reported the finding of sperms in female worms and concluded that the worms were hermaphroditic, suggested that the direction of development is determined by the chromosomal constitution of the eggs subsequent to fertilization. Faust (1933), having found what he interpreted as parasitic males, suggested that fertilized eggs give rise to heterogonic and unfertilized eggs to homogonic progeny. Subsequently Beach (1935-1936), working in Faust's laboratory, showed conclusively that the course of development can be influenced by nutritional conditions; as these become less favorable more and more of the rhabditiform larvae undergo direct development to filariform larvae instead of becoming males and females. The evidence indicated that the potential females are influenced in this way more readily than the potential males.

Meanwhile Graham (1936-1939) started two pure lines of *S. ratti* of rats from original single larva infections of the homogonic and heterogonic type, respectively, and found marked inherent differences between them. In each line over 85 per cent of the total progeny were of its own type, with an extreme difference in the number of males produced. Graham also observed that there was a falling off in heterogonic larvae in winter as compared with summer, brought about by climatic effects on the host, not on the developing larvae. The conclusion seems warranted, therefore, that the course of development is dependent upon nutrition or other environmental conditions and not upon genetic constitution, but that there are genetic differences in the extent to which different strains are influenced towards homogeneity by a given degree of unfavorableness in the environment.

The reproductive status of the parasitic females was brought into question by Sandground (1926); prior to that time it had been generally accepted that they were parthenogenetic, although Leuckart apparently suspected that they were hermaphroditic, by analogy with the condition in the parasitic generation of *Rhabdias*. Sandground believed them to be protandrous hermaphrodites; he described what he interpreted as sperms and observed what seemed to be fertilization in specimens of *S. ratti*. Faust (1933), having found male worms in the lungs, concluded that the sperms observed by Sandground probably were the result of copulation. He considered the worms to be bisexual early in life, later becoming parthenogenetic. Chitwood and Graham (1940) concluded that *S. ratti* was parthenogenetic since they were unable to find sperms and also unable to find fertilization membranes. The weight of evidence is therefore in favor of parthenogenesis.

The occurrence of parasitic males described by Kreis (1932) and Faust (1933) has not been confirmed by others. In an unpublished observation, one of us (J. E. A.) has noted adult rhabditiform males in the fresh feces from a case of human strongyloidiasis but it was unknown whether these were parasitic males or males developing from eggs of parasitic females. The fact that the supposed parasitic males of Kreis and of Faust were rhabditiform and practically identical with free-living males is sufficient cause for doubt that they are really males of the parasitic generation, for in the one other member of the Strongyloidea in which males have been found—*Parastrongyloides winchessi*, Morgan 1928—the parasitic males are filariform like the females. We suggest that, since Faust not only observed eggs and rhabditiform larvae, but also filariform larvae which he interpreted as the progeny of the parasitic worms, in the lungs and bronchioles of infected hosts, the males observed were free living males produced precociously in the lungs. The observations of Beach (l. c.) that males will develop more readily than females under suboptimal conditions would account for the failure to find free-living females. Graham's work with single-larva infections has shown clearly that males are at least unnecessary in *S. ratti*, though no conclusions can be drawn from this, for it is, of course, possible that there might be differences between species in this respect. For the present the occurrence of rhabditiform parasitic males in members of the genus *Strongyloides* must certainly be considered *sub judice*.

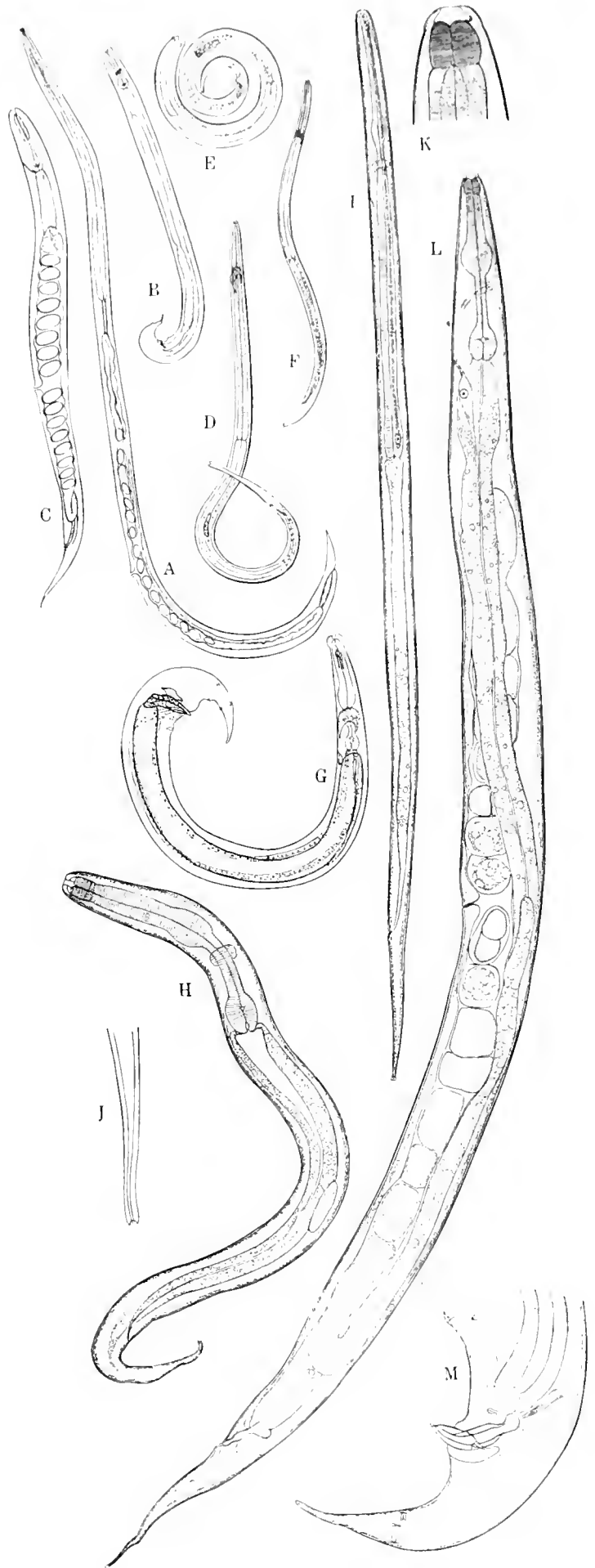


FIG. 1-80. DEVELOPMENT OF *STRONGYLOIDES*

A-G—*Strongyloides stercoralis* (A—Parasitic female; B—Free living female; C—Free living female; D—Filariform larva (human strain A), from lung tissue of experimental dog three days after skin inoculation; E—Post-filariform larva from same; F—Pre-adolescent female from lung of experimental dog 11 days after skin inoculation, note developing genital primordium; G—Adult male from lung tissue of experimental dog 57 days after skin inoculation). H-M—*Strongyloides* sp. from dog, free living generation (H—First stage larva; I—Infective larva; J—Tail of infective larva; K—Head, adult female; L—Adult female; M—Tail, male). A-C, after Faust, E. C., Human Helminthology, 1939. D-G, after Faust, E. C., 1933, Am. J. Hyg., v. 18 (1). Remainder original drawings by M. B. C.

The members of the genera *Rhabdias* and *Entomelas*, now separated into a separate family from *Strongyloides*, resemble Strongyloidea in having an alternation of generations, at least in some species. This double life cycle was first demonstrated by Meczniakov (1865) in the case of *R. bufonis*. Unlike *Strongyloides*, the parasitic generation, at least of some species of *Rhabdias*, consists of hermaphroditic females, possessing a well-developed seminal receptacle. Seurat (1920a), however, thinks that the parasitic forms of *Entomelas dujardini* and *E. entomelas* from *Anguis fragilis* are parthenogenetic rather than protandrous hermaphrodites, since he was unable to find seminal receptacles or to detect sperms.

As in *Strongyloides*, both homogonic and heterogonic types of development may occur in the free-living phase of the life cycle of *Rhabdias*. In most of the species one type or the other strongly predominates or may even occur exclusively, though in some of the forms in which one type of development was long thought to occur exclusively, the alternative type has more recently been observed. Travassos (1926) called attention to the fact that the species found in Amphibia and Lacertilia have indirect development, while those found in snakes have direct development. Chu (1936), however, reported some unpublished observations of Chitwood's, and also some of his own, in which both types of development were found in several amphibian and reptilian species, (*Rana castriceps*, *Fülleborni*, and *fuscovenosa* var. *catanensis*). In the last named species Chu observed only homogonic development except when an especially favorable culture medium was used, whereupon a small percentage of free-living adults, predominantly males, were usually found. The offspring of these adults failed, however, to infect snakes. It seems evident from this data that the course of development of *Rhabdias* is determined by factors similar to those operating in the case of *Strongyloides*.

Whereas in *Strongyloides* both direct and indirect infective larvae are filariform, in the Rhabdiasidae the direct larvae are rhabditiform while the indirect ones are filariform (cf. Figs. 179, P-Q). The free-living adults of different species vary considerably in their mode of reproduction. Travassos describes the free-living female of *R. fülleborni* of frogs as producing only one or two larvae, which may become fully developed within the mother, destroying her tissues, whereas Chu (1936) describes *R. fuscovenosa* var. *catanensis* as having a few eggs in each horn of the uterus, which are usually laid when little or no development has occurred.

According to Goodey (1924) the homogonic larvae of *R. fuscovenosa* undergo two ecdyses outside the body of the host, the second shed cuticle being retained as a tight fitting sheath for the infective larvae. The sheath is shed upon gaining entry to the host. The larvae molt twice more during development in the host's parenteral tissues, but both shed cuticles are retained as sheaths. Although the infective larvae of *R. bufonis* were reported by Fülleborn (1920) to penetrate the skin, and by the same writer (1928) to migrate to the lungs via the circulatory system, Goodey (1924) failed to get the infective larvae of *R. fuscovenosa* to penetrate skin, although their behavior outside the body was like that of skin-penetrating larvae, and he also thought it probable, from their distribution in the body, that they migrated to the lungs, after penetrating the gut wall, by direct migration through the mesentery and not via the blood stream. Fülleborn (1928) called attention to the fact that larvae of *R. bufonis* would also penetrate snails and possibly other invertebrates, where they remain unchanged for weeks, capable of infecting a frog when the snail is eaten. Similarly the larvae may sometimes become encapsulated parenterally in frogs which may then act as "transport hosts" for infection of larger frogs which eat them. Fülleborn suggests that since the skin of snakes is hard to penetrate transport hosts may constitute the principal method of infection for these hosts.

## STRONGYLINA

### I. STRONGYLOIDEA AND TRICHOSTRONGYLOIDEA

Three general types of life cycles, which more or less merge into each other, occur in the superfamilies Strongyloidea and Trichostrongyloidea of the suborder Strongylina. One of these, characteristic of the Ancylostomatidae and a few other forms in the Strongyloidea and Trichostrongyloidea, is essentially the same as the homogonic cycle of *Rhabdias*, except that the parasitic worms are bisexual. It involves development to the third (infective) stage outside the body of the host, skin penetration, and parenteral migration via the circulatory system after infection. The second, characteristic of most of the

Trichostrongyloidea and many of the Strongyloidea, differs in that there is no skin penetration, and no migration in the host beyond the walls of the alimentary canal. The third, characteristic of the Syngamidae in the Strongyloidea, involves development and molting within the egg, without feeding or growth, with at least optional establishment in an invertebrate transport host, and a parenteral migration which leads to the respiratory system but not beyond. We think it probable that both types 2 and 3 were derived from type 1, although it is also possible that type 2 is the most primitive, and that types 1 and 3 were both derived from this.

#### 1. ANCYLOSTOMA SPP.

The genus *Ancylostoma* will serve as a typical example of the first type, involving skin penetration and parenteral migration. The eggs of these worms are deposited by the adult females in the lumen of the small intestine, whence they make their exit with the feces; at the time of leaving the body of the host they are nearly always in the four-celled stage of development, normally being unable to progress beyond this point without free oxygen. Under optimum conditions of oxygen, moisture and warmth (75° to 85° F.) the eggs proceed with

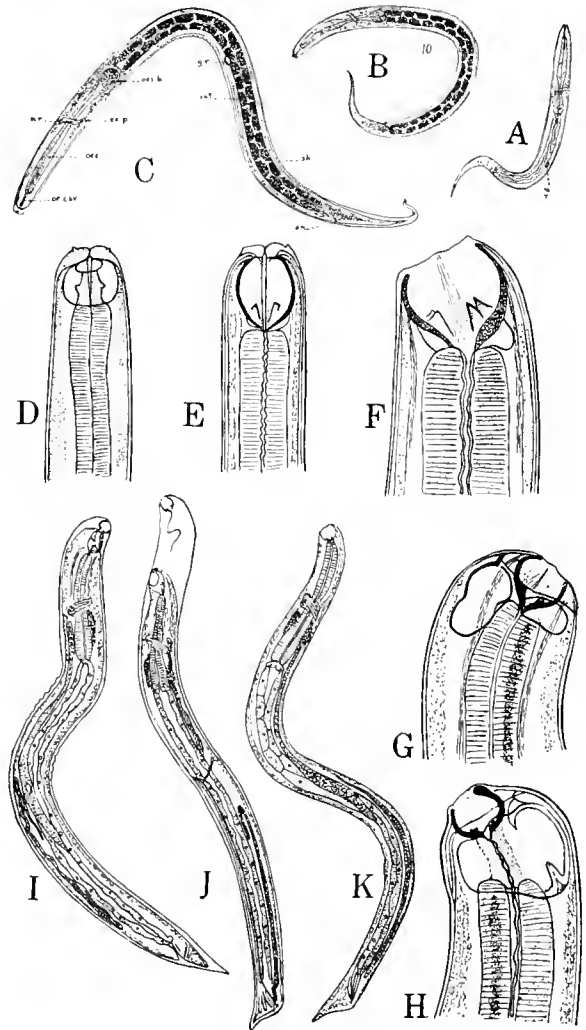


Fig. 181. DEVELOPMENT OF HOOKWORMS

*Ancylostoma duodenale*. A—First stage larva; B—Second stage; C—Third stage. D-H—Development of primitive and definitive capsules; (D)—Bladder-like structures forming around the larval oral cavity, with beginning of formation of the primitive larval teeth; E—Nearly completed primitive capsule, with triangular teeth at base; F—Fully developed primitive capsule with beginning of formation of bladders at its base; G—Later stage in development of dorsal and ventral bladders which will eventually form the definitive capsule; H—Later stage in development of definitive capsule, with primitive capsule still connected with esophagus by a strand of tissue; I—Female larva with definitive capsule formed but primitive capsule still attached; J—Male after final molt but last cuticle still enclosing it; K—Male larva with primitive capsule. After Looss, Chandler, A. C., 1929, Hookworm Disease.

then development rapidly, and a rhabditiform larva may hatch within 24 hours. This larva is about 250  $\mu$  long, with an elongated buccal cavity and a typical rhabditiform esophagus possessing esophageal valves. These larvae were shown by McCoy (1926) to develop normally with only pure cultures of certain species of living bacteria as food. Under favorable conditions the larvae undergo the first ecdysis or molt within 48 hours after hatching, but second stage larvae show very slight morphological differences from first stage larvae, although they are about 400 to 430  $\mu$  long. After a minimum of about 2 more days the larvae cease feeding, undergo a second ecdysis, and enter the third or infective stage. The cuticle shed at this molt is normally retained as a protective sheath, though it may occasionally be lost. The most important morphological changes in the infective larva are noted in the shape of the tail and the structure of the esophagus. The tail is shorter and more stumpy than that of the preceding stages. The esophagus is "filiform," or preferably "strongyloform," i.e., it is more uniform in width with tapering anterior portion, and the esophageal valves are lacking. The anterior portion of the lumen of the stoma is closed and the remaining posterior portion remains open in a characteristic shape. According to Alicata (1935) the various third stage larvae of strongyloid nematodes parasitic in swine can be differentiated, among other ways, by the form of the stoma; there is a possibility that this characteristic may hold true for other members of the Strongylini.

The infective larvae climb up on objects as high as a film of moisture extends, and show positive thermotropism and thigmotropism. They retire from excessive warmth in direct sunlight. They migrate vertically if buried in soil, but migrate laterally to a very slight extent (Chandler, 1925).

Although Leuckart (1866) showed that *A. caninum* of dogs could be transmitted *per os*, and Leichtenstern (1886) proved the same thing for *A. duodenale* of man, the usual mode of infection is by penetration of the skin; this method of infection was first demonstrated by Looss (1898). In subsequent work Looss (1905) established the course which the larvae follow in the body to reach the intestine. Skin penetration is accomplished in a few minutes when the larvae are able to obtain leverage, as in mud, to help them in their burrowing, but they are unable to penetrate when submerged in water. Within 35 to 40 minutes the larvae, having left their sheaths behind them, have reached the dermis and within a few hours are in the subcutaneous tissue. Many find their way into superficial lymphatic capillaries, and a few directly enter blood vessels. Some larvae are slow in entering the circulation, and may be encapsulated in the skin, especially in hosts sensitized by previous exposure. Certain "foreign" species of hookworms, e.g. *Ancylostoma braziliense* and *Uncinaria stenocephala* in man, commonly fail to enter the circulation at all but wander aimlessly in the skin, causing "creeping eruption." Although the larvae may remain in the skin for considerable periods no development takes place there (Fülleborn, 1927).

When larvae enter the lymphatics they are carried first to the regional lymph glands, and then to the main lymph channels leading to the thoracic duct, through which they enter the circulation. Such larvae, as well as those which entered the blood system directly, eventually reach the right heart, whence they are pumped out to the lungs. Here the majority burrow into the air spaces (Fülleborn, 1925), and are then mechanically carried in mucus, helped by epithelial cilia, to the trachea and throat. If swallowed they now pass to the alimentary canal, and grow to maturity in the intestine.

Although skin penetration is undoubtedly the usual mode of infection, infection by mouth can also occur. There has been considerable controversy as to whether swallowed larvae had of necessity to penetrate the mucosa and migrate to the lungs before growing to maturity, or whether they could develop to maturity without such migration. Yokogawa (1926) investigated the matter and found that when *A. caninum* larvae are fed to puppies a few penetrate the walls of the alimentary canal and enter the circulation, but the great majority of those which develop at all do so directly, without migration. In abnormal hosts, however, such as rodents, most of them perform the usual migration via the circulatory system, and a few migrate through the tissues to the body cavity whence they enter the liver, or go through the diaphragm to the pleural cavity, whence they enter the lungs. This work was confirmed by Scott (1928). Fülleborn (1926-1927) showed that the larvae of *Uncinaria stenocephala* of dogs also develop directly after oral infection, few migrating even in abnormal hosts. Several Japanese workers, however, (Myingawa, 1916; Myingawa and Okada, 1930, 1931; Okada 1931) have persisted in the belief that lung migration is a biological necessity for hookworms. Foster and Cross (1934) carried through some further experiments which conclusively confirm the earlier work, showing that the lung journey is not a biological necessity for these worms (though it apparently is for *Strongyloides stercoralis*.) Swallowed larvae rarely migrate in susceptible normal hosts, but

commonly do so in abnormal hosts and in resistant normal ones. Looss (1911) and Yokogawa (1926) observed that swallowed hookworm larvae remain in the stomach at least 2 days, and Fülleborn (1927) found they could remain there at least 5 days, partly in the lumen, partly deep in the mucous glands. He demonstrated that the larvae have an initial tendency to burrow into the glands, later to return to the lumen, as is the case with *Ascaridia*. He thinks that something in the secretion of the mucous glands causes the larvae to lose their mobility; possibly the same mechanism is responsible for the loss of the burrowing instinct in the larvae reaching the intestine from the lungs after skin penetration (see below).

The minimum time required for the larvae to reach the trachea after skin penetration is usually about 3 days, but the majority require 4 or 5 days, and some still longer. By the time the larvae appear in the bronchioles and trachea they have grown slightly in length, have developed a provisional mouth capsule, and are ready for the third molt, although there is no evidence that they ever complete it before reaching the digestive tract. The formation of the provisional, and subsequently of the definitive, mouth capsules is accomplished by the development of dorsal and ventral bladder-like structures posterior to the already existing mouth. These spread around the sides and finally unite (Looss, 1905) (Figs. 181).

Up to the time of the third molt the larvae grow very little in length, but increase from about 20  $\mu$  to 30  $\mu$  in diameter. The molt usually occurs very soon after the larvae reach the intestine, and the larvae at this time lose their tendency to burrow, so remain in the intestine. There is no evidence that they temporarily burrow into the glands of the stomach as do larvae that are directly swallowed. The young worms now grow very rapidly. They may reach a length of 2.5 to 3 mm within a few days. Sexual differentiation now begins, and in from 4 to 6 days after the third molt the definitive mouth capsule is developed. By the time the worms have reached a length of from 3 to 5 mm, the fourth molt takes place. Thereafter the worms grow to maturity, copulate, and begin egg production. In the case of *Ancylostoma duodenale* in man the eggs first appear in the feces 5 to 6 weeks after infection, whereas in *A. caninum* of dogs, eggs may appear as early as 15 days (Herrick, 1928).

## 2. HAEMONCHUS CONTORTUS

The life cycle of this worm as worked out by Ransom (1906), Veglia (1916) and others is essentially the same as that of the ancylostomas in its free-living phase. The infective, ensheathed third-stage larvae, however, are not skin-penetrators, but have a tendency to climb up on vegetation or other objects where they are in a favorable position to be ingested by their herbivorous definitive hosts. Here they curl up, and are remarkably resistant to cold and to moderate desiccation. Upon being ingested by the final host the larvae bury themselves in the mucous glands and crypts of the abomasum, where they undergo the third and fourth molts; the adult stage is reached after about the 9th to 11th days, and the worms emerge to live in the lumen of the organ, beginning egg production about 3 weeks after infection. Although there is no evidence that the worms perform a parenteral migration in sheep, Ransom (1920) showed that they do migrate to the lungs in guinea pigs.

## 3. SYNGAMUS TRACHEA

The life cycle of this worm was first experimentally worked out by Ortlepp (1923). The eggs of the worm are laid in the bronchi or trachea of the host in an advanced stage of segmentation. Under favorable conditions the first-stage larva is developed in about 3 days, but the egg does not become infective until after 1 to 2 weeks, whereupon they may or may not hatch. Ortlepp observed only a single molt during the course of development and interpreted the infective larva as a second-stage larva but Wehr (1937) demonstrated that the developing larva undergoes two molts within the egg. Buckley (1934), studying *S. irvi* of cats, also observed the usual two molts. Yokogawa (1922) also missed the first molt in the case of *Nippostrongylus muris*, and in spite of the large amount of experimental work done with that worm the missed molt was not discovered until 1936, when Lueker demonstrated it. The first cuticle in both these worms is extremely thin, and the second ecdysis may be in progress before it is completely shed.

Infective larvae, whether hatched or still in the eggs, are infective when directly swallowed by susceptible hosts, but very often they are swallowed by various invertebrates; when this happens they penetrate the gut wall and become encapsulated in the body cavity. Walker (1886) and Waite (1920) both called attention, on epidemiological grounds, to the importance of earthworms in the dissemination of this parasite, but Clapham (1934) first experimentally worked out the rôle played by these annelids. Subsequently Taylor (1935) showed that

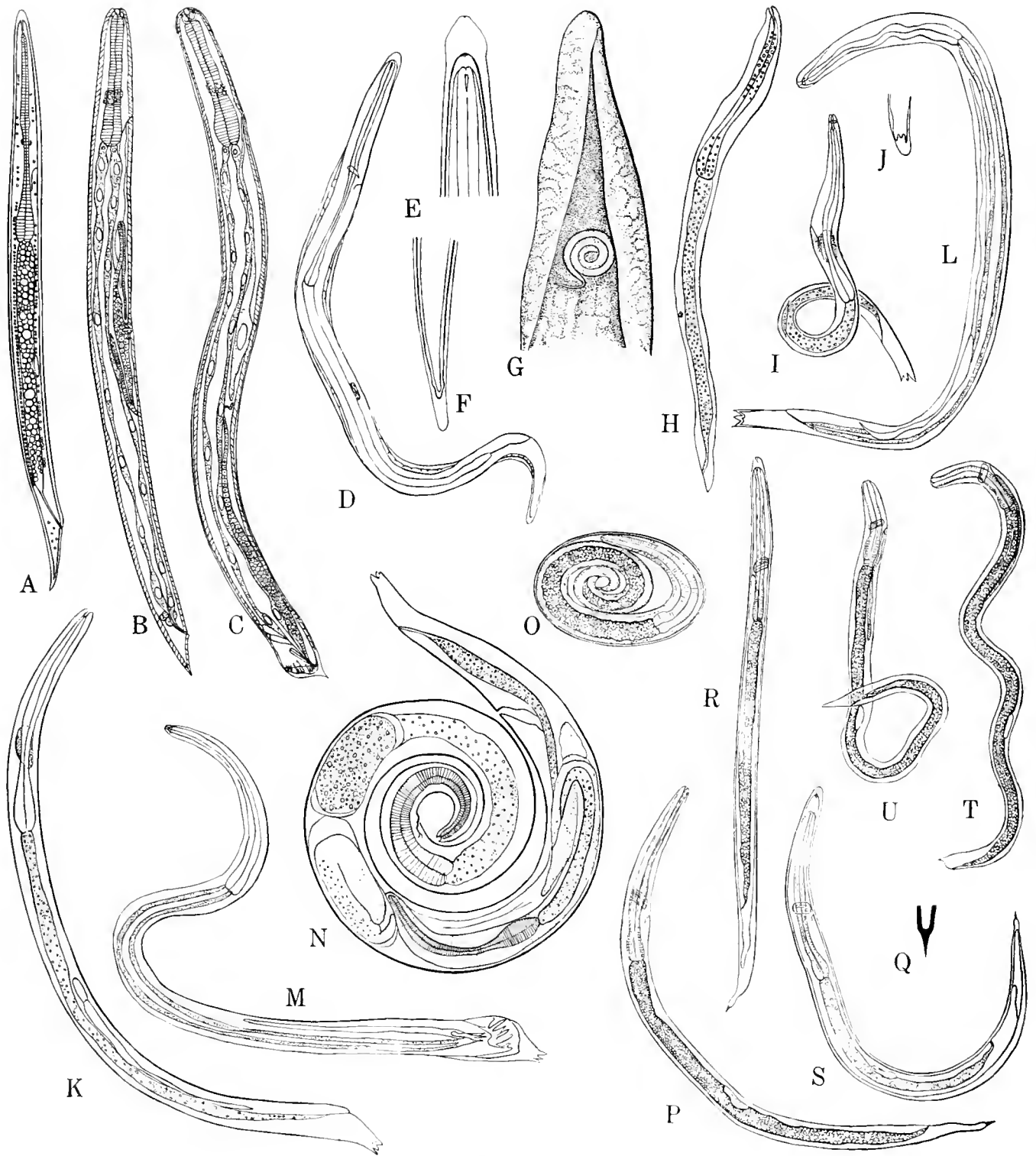


Fig. 182. DEVELOPMENT OF THE STRONGYLINA

A-C—*Syngamus trachealis*. (A—Ensheathed second stage larva; B—Third stage larval female; C—Ensheathed young fourth stage larval male). D-F—*Syngamus yerey* (D—Third stage larva; E—Anterior end of third stage larva; F—Tail of third stage larva). G—*Haemonchus contortus* on blade of grass. H-N—*Ollulanus tricuspis* (H—First stage larva; I—Second stage larva; J—Tail between first and second stage; K—Third stage (infective) larva; L—Fourth stage female; M—Fourth

stage male; N—Gravid female). O-U—*Dictyocaulus arnfieldi* (O—Egg from the feces; P-Q—First stage; R—Second stage; S—Third stage; T—Fourth stage male; U—Fourth stage female). A-C, after Ortlepp, 1923, J. Helm., v. 11. D-F, after Buckley, 1934, J. Helm., v. 72. G, after Ransom, 1906, U. S. Bur. An. Ind. Circ. 93. H-N, after Cameron, 1927, J. Helm., v. 5. O-U, after Wetzel and Enigk, 1938, Arch. Wiss. u. prakt. Tierheilk., 73(2).

snails and slugs would also serve as transport hosts, and later found that the encysted larvae would remain viable in these molluscs for several years. More recently Clapham (1939a, 1939b) showed that maggots, crane fly larvae, spring tails and centipedes would serve in a similar capacity, and that the worms were able to survive metamorphosis in the tissues of flies.

When ingested by these hosts the infective larvae hatch from the eggs if they have not already done so, penetrate the gut wall, and enter the body cavity, where they are eventually encapsulated by the host tissues. Clapham has shown that the life cycle is completed somewhat more readily with the aid of a vector than without, and was able to infect chickens readily with a starling strain when an earthworm vector was used, whereas Taylor (1928) had had difficulty in doing so by direct infection. Clapham calls attention to the fact that *Syngamus trachea* is evidently undergoing evolution in its life cycle; at present it can still develop without an intermediate host, and has not as yet adapted its requirements to any particular intermediate host, but can use almost any that happens to swallow it. She makes the reasonable suggestion, however, that in time different strains may adapt themselves to different intermediate hosts, as determined by the food habits of the final hosts, and thus perhaps give rise to new species. At present, however, the effect of living in a transport host seems to be to eliminate physiological differences; for example, in the case of starling strains developing in chickens. It is possible that some species of *Syngamus* may already have reached the stage of requiring an intermediate host, since Buckley (1934) was unable to infect cats with eggs containing third stage larvae of *S. iveri*.

After infection by swallowing eggs, free larvae, or larvae contained in invertebrate transport hosts, *S. trachea* apparently reaches the lungs via the circulatory system. Ortlepp (1923) found the larvae in the lung tissues within 24 hours and Wehr as early as 17 hours after infection. Wehr found fourth stage larvae after 3 days and immature adults after 7 days; some of the latter were already *in copula* even before entering the trachea.

#### VARIATIONS IN THE LIFE CYCLE IN OTHER STRONGYLOIDEA AND TRICHOSTRONGYLOIDEA

The parasitic stages of nearly all the members of the Strongyloidea and Trichostrongyloidea, except the Syngamidae, are remarkably similar, involving two free-living rhabditiform stages separated by a molt, and a strongyliform third stage, in which the shed cuticle is usually retained as a sheath. The time intervals between the molts and the total time required to reach maturity vary considerably; in some species, e.g. *Ornithostrongylus quadricolatus*, the infective third stage may be reached within 3 days. The infective larvae are distinguishable by characters of the mouth, buccal cavity, esophagus, shape of tail, length of sheath, etc., and also, as Lueker has shown in a series of papers (e.g., Lueker, 1938) by the number and arrangement of cells in the intestine.

The only important variation from this formula is the molting of some species within the egg, thus eliminating a period of feeding and growth outside the host; this, as already noted, occurs in *Syngamus* and it also occurs in *Nematodirus* spp. (Ransom, 1911; Manpas and Seurat, 1913) and in *Oswaldocruzia filiformis* (*Strongylus auricularis*, Zeder) (Manpas and Seurat, 1913). According to the latter authors, *Ostertagia marshalli* hatches as a second-stage larva and undergoes its second molt 2 or 3 days later without feeding. This is not true, however, of *O. circumcincta*. When both molts occur inside the egg the infective embryos may or may not hatch prior to being swallowed by a host, eggs containing infective third stage larvae being infective as well as the free larvae.

*Strongylacantha glycyrrhiza*, according to Seurat (1920b), hatches at the end of 48 hours but the larvae fail to feed, and at the end of a month have molted twice and are encysted in both shed cuticles, just as in the case of *Dictyoaulus* (see below).

A striking exception to the usual course of events occurs in the case of *Ollulanus tricuspis*, according to Cameron (1927). This parasite of the stomach of cats is viviparous. The eggs hatch in the uterus of the mother, and the larva undergoes its first molt before it is born, acquiring the typical tri-cuspid tail. Third stage larvae are found free in the stomach of the cat, but it is not certain whether the second molt occurs before or after birth. This form is believed by Cameron to leave the stomach with the vomitus of the cat. When eaten by another cat with the vomitus the larvae change to fourth stage larvae and finally adults. Some part of this development is believed to take place in the depths of the mucous membrane. No other method of exit from the cat has yet been found; no larvae were ever seen in the intestine, nor were mice infected when fed on cat stomach or infected vomitus. Continuous auto-infection is believed possible but improbable; Cameron suggests the pos-

sible production of a substance inhibiting complete larval development, as postulated by Filleborn in the case of *Rhabdias bufonis* in the lungs of frogs.

The mode of access of the infective larvae to the final host varies in different species, even, sometimes, within the same genus. There are three possibilities: (1) penetration of the skin; (2) ingestion with food or water; (3) ingestion with a transport host. Skin penetration is characteristic of most of the hookworms (Family Ancylostomatidae) *Ancylostoma*, *Uncinaria* and *Gaigeria* but *Bunostomum* seems to be an exception in that, although the larvae, at least of *B. trigonocephalum*, seem to be capable of penetrating under certain conditions (Ortlepp, 1937, p. 207), they do not do so as readily as other hookworm larvae (Cameron, 1923; Schwartz, 1925), and normally infect by mouth. Although most of the hookworms are able to infect the host by mouth as well as through the skin, and may even be able to dispense with the parenteral migration (see above), Ortlepp (1937) was unable to cause infection in sheep by the oral route with larvae of *Gaigeria pachyseclus*. Most other members of the Strongyloidea and Trichostrongyloidea fail to penetrate the skin although a few (*Stephanurus dentatus*, *Nippostrongylus muris*, *Longistriata musculi*, *Trichostrongylus calcaratus*) are able to do so. Other species of *Trichostrongylus* apparently do not penetrate the skin. *Nippostrongylus muris* is almost wholly dependent upon skin penetration (Yokogawa, 1922), whereas for *Longistriata musculi* oral infection is probably more important in nature (Schwartz and Alicata, 1936).

The great majority of the worms belonging to the groups we are considering normally enter the host by mouth, with contaminated water or food. In most cases the larvae climb up on living vegetation and are more or less resistant to desiccation. This is true of all the Strongyloidea so far as known (except *Stephanurus*), and all of the Trichostrongyloidea with the exception of the few mentioned in the preceding paragraph, and *Ollulanus*.

The development within the host involves varying degrees and types of migration. Skin-penetrating larvae usually follow the route described above for ancylostomes, but Schwartz and Alicata (1936) showed that the larvae of *Longistriata musculi* do not normally do so; they appear in the stomach within a few hours after skin penetration, and in the intestine soon after that, but they were not found in the liver, lungs or stomach walls. Their actual route was not determined. In the case of this worm, whether infection is by skin or mouth, the entire development takes place in the intestine, contrary to what happens in other skin-penetrating forms, even in the nearly related *Nippostrongylus*.

Nematodes infecting by mouth may or may not migrate via the blood stream. Most of the Trichostrongyloidea (e.g. *Cooperia*, *Ornithostrongylus*, *Ostertagia*, *Obliscoides*, *Graphidium*, *Haemonchus*, *Hyostrongylus*, most species of *Trichostrongylus*, *Nematodirus*) perform no migration at all beyond a more or less temporary invasion of the glands or crypts of the stomach or duodenum. Some forms, e.g., *Ornithostrongylus quadricolatus*, may reach the adult stage of development as early as the third or fourth day after infection (Cuvillier, 1937).

The Strongyloidea show various gradations from invasion of the circulatory system and transportation with the blood, to mere temporary invasion of the glands. Of the three common species of *Strongylus* in horses each shows characteristic features in its migration, the larvae of *S. vulgaris* being found in aneurysms in the anterior mesenteric vein, those of *S. edentatus* under the peritoneal walls of the abdominal cavity, and those of *S. equinus* in liver and pancreas. According to the usually accepted view (see, for example, Neveu-Lemaire, 1936) *S. vulgaris* penetrates the walls of the intestine and migrates through the body via the circulatory system, passing through the capillaries of both liver and lungs to be distributed all over the body by the systemic arterial circulation. Ninety percent stop in the anterior mesenteric artery, to the walls of which they adhere by using the mouth as a sucker. The resulting irritation leads to the formation of an aneurysm and thromboses. Here they remain for 5 months, meanwhile growing and passing through two molts; one at a length of 3 to 4 mm, the other at a length of 7 to 10 mm. Having passed the final molt they release their holds and are carried by the blood stream to the walls of the cecum or colon. They remain imbedded in the walls in little nodules under the mucosa for about a month, and finally make their exit into the lumen. Ott (1932) thinks that the normal migration is via the lungs and trachea as in the case of hookworms, but that some larvae burrow through the intestinal walls and between the laminae of the mesenteries until they reach a large blood vessel. If this is a large, heavy-walled vessel the slow passage through it leads to inflammation and the characteristic aneurysms. Wetzel and Enigk (1938a), on the other hand, believe they have convincing evidence that no



Fig. 183. *STRONGYLUS VULGARIS*

Verminous aneurysms affecting the anterior mesenteric artery. After Foster & Clark, 1937, *Am. J. Trop. Med.* v. 17 (1).

*Strongylus* larvae migrate via the lungs and trachea, but undergo their whole development within the abdominal cavity.

*S. edentatus* larvae penetrate the walls of the intestine and the majority come to rest under the peritoneum, though the route followed in reaching this location has not been traced. Some, probably carried by the blood stream, reach the liver and lungs. After about 3 months, during which they grow much larger, the larvae migrate to the roots of the mesenteries and travel between the laminae to the walls of the cecum and colon. Here they become lodged for about a month in large subserous hemorrhagic nodules which eventually open into the lumen of the intestine.

*S. equinus* larvae penetrate the walls of the intestine and make their way to the liver and pancreas. It has generally been assumed that they arrive in these places via the blood stream, but Wetzel's observations (i.e.) throw doubt on this. After development to the fourth larval stage they return to the walls of colon and cecum, again by an undetermined route, and continue their growth in nodules in the walls of these organs. After reaching the final stage of development by a fourth molt they pass into the lumen.

The Trichoneminae of horses are believed not to migrate out of the intestine at all. Many of them, perhaps all, penetrate into the walls of the mucosa where they develop in nodules. They undergo the third molt when about 1 mm long, becoming what Ihle and Oordt (1923) call "Trichonema" larvae, provided with a provisional mouth capsule. The final molt occurs in the lumen of the intestine.

*Triodontophorus tenuicollis* is believed by Ortlepp (1925) to develop directly in the lumen of the cecum and colon, without even temporarily burying itself in the mucosa. He was never able to find larvae of this species in nodules. However, only fourth stage larvae were found, and there is nothing in Ortlepp's observations to preclude a hookworm-like migration via lungs and trachea on the part of the third-stage larvae.

The Oesophagostominae have a life cycle in the host essentially the same as that of the Trichoneminae, the young worms tending to bury themselves in the mucosa, where they cause the formation of cysts or nodules. Here they undergo their development to the final stage, emerging into the lumen of the intestine at about the time of the final molt, or in some cases even later, when they have grown to a length of 4 or 5 mm.

According to Spindler (1933), *Oesophagostomum quadrispinulatum* (= *longicaudum*) of pigs produces inflamed liquefying cysts within 48 hours after infection, and the larvae begin escaping into the lumen after about 17 days. Similar inflamed cysts are produced by most other species of oesophagostomes, but Goodey (1924) failed to observe them in experimental infections with *O. dentatum* and Schwartz (1931) saw only small noninflamed nodules at the site of attachment of adult worms of this species in contrast to the inflamed lesions caused by *quadrispinulatum*. *Chabertia orinus*, though nearly related to *Oesophagostomum*, also fails to develop in submucous nodules.

*Stephanurus dentatus*, (see Schwartz and Price, 1932; Ross and Kauzal, 1932) whether entering by skin or mouth, migrates

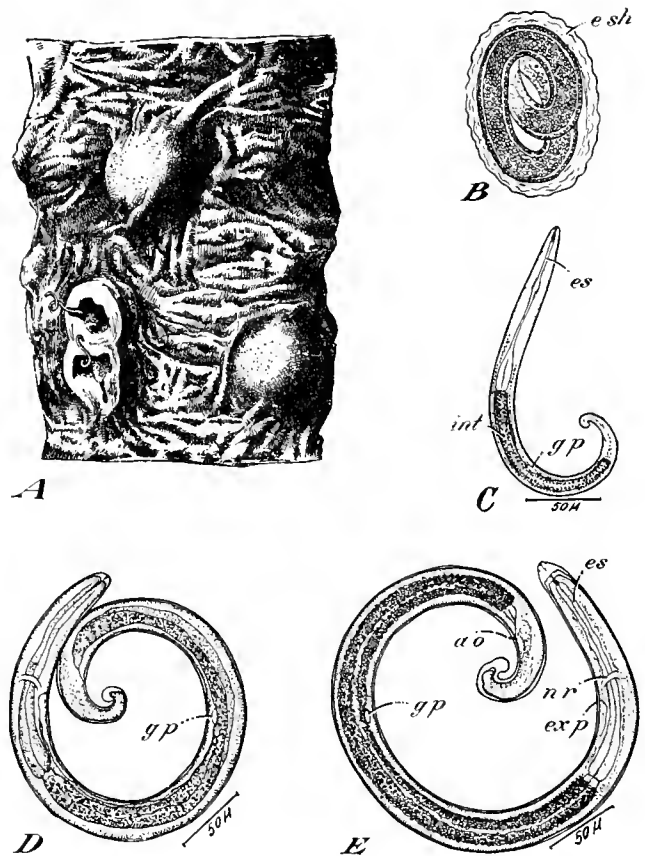


Fig. 184. *OESOPHAGOSTOMUM BIFURCUM* AND *METASTRONGYLUS SALMI*

A—Nodules of *Oesophagostomum bifurcum* in the large intestine of an African (after Brunpt). B-E—*Metastrongylus salmi* (B—Egg with fully developed embryo; C—Newly hatched first stage larva; D—First stage larva undergoing first molt; E—Second stage larva undergoing second molt while still enclosed within the cuticle of first molt). A. after Chandler, 1940 (fig. 146) *Int. to Parasit.* B-E, after Alicata, 1935, U.S.D.A. Tech. Bull. 489.

to the liver via the blood stream. The third molt occurs about 70 hours after infection, and the larvae have a provisional mouth capsule. Normally such larvae escape from the capillaries in the liver and wander in the hepatic parenchyma until they reach the surface capsule. They wander under this for a time but eventually, 3 months or more after infection, break free into the body cavity and make their way to the perirenal fat tissue, perforating the walls of the ureters to establish connection with the outside world. They themselves become enclosed in capsules of host tissue.

## II. METASTRONGYLOIDEA

In this superfamily of the Strongylina the early development follows somewhat different patterns from that of the other members of the suborder, except in a few instances (e.g., *Strongylacantha* resembles *Dictyoaculus* in hatching and then reaching the infective stage without feeding or growing, and the Syngamidae also resemble *Dictyoaculus* in having optional transport hosts).

Three principal types of development occur among the Metastrongyloidea: (1) the *Dictyoaculus* type, in which the larvae go through two molts and reach the infective stage, surrounded by one or both shed cuticles, without feeding or growing; (2) the *Metastrongylus* type, in which the first-stage larvae continue their development after ingestion by earthworms, and (3) the *Protostrongylus* type, in which the first-stage larvae, attracted by the mucus of snails or slugs, continue their development after entering the slime glands in the foot of these molluscs, and becoming encapsulated in the muscular connective tissue under the epithelium.



## 1. DIETYOCAULUS spp.

The eggs of *D. filaria* and *D. viviparus* hatch in the bronchi, or at least in the intestine, as they are leaving the body of the definitive host, but those of *D. arnfieldi*, according to Wetzel and Enigk (1938) fail to hatch in the lungs, and usually do not hatch until a few hours after leaving the body. The first molt usually takes place at room temperature in from 1 to 2 days, and the second in from 3 days (in *D. arnfieldi*) to about 12 days (*D. filaria*) later. Usually both sheaths are present in early third stage larvae, but the first cuticle is eventually lost. These infective larvae live a long time in moist soil or water, and are able to survive in earthworms if eaten by them, although they do not depend upon the earthworm as an intermediate host. The use of earthworms as transport hosts seems to be of less importance in the case of *Dietyocaulus* than in the case of *Syngamus* (see above). However, there is no evidence as yet that *Dietyocaulus* can use as large a variety of transport hosts as can *Syngamus*.

## 2. METASTRONGYLUS

*Metastrongylus longatus* (= *apri*), *M. submi*, and *Choerostongylus pudendolictus*. The eggs of these worms contain fully developed embryos when deposited. Although usually stated to hatch in the bronchi or intestinal tract during passage out of the definitive host, Alicata (1935) found that they are usually passed in the feces unhatched, and remain unhatched until taken into the body of a susceptible intermediate host. The eggs or embryos may, however, remain viable for 3 months in moist soil.

When ingested by earthworms (species of *Helodrilus* and *Lumbricus*) the larvae burrow into the walls of the esophagus and proventriculus of these hosts. Alicata has found them there 16 hours after exposure to infection. They also enter the circulatory system and may be found in the hearts, but Schwartz and Alicata (1929) showed that migration via the blood stream was not an essential part of the life cycle of this worm in its intermediate host. In the earthworm the first molt occurs about 8 to 10 days or more after infection, and the second one a few days later, this molt beginning before the first cuticle has been shed. The second cuticle is retained by the third stage larvae, which are now infective. The larvae do not spontaneously leave the host, and an earthworm may remain infective over winter, and probably at times for several years. Upon death of the earthworms the larvae are able to survive for 2 weeks in moist soil. Pigs become infected by eating infected earthworms or liberated infective larvae. After ingestion, according to Hobmaier and Hobmaier (1929), they migrate via the lymphatics or blood stream, and going the third molt in mesenteric lymph glands, and then proceed via the lymphatic and blood systems to the lungs, where they become mature after a fourth and final molt.

## 3. PROTOSTRONGYLINAE

All the members of the family Protostrongylinae resemble one another in requiring molluscs as intermediate hosts. In all cases the embryonated eggs hatch before leaving the body, or soon after, and the first-stage larvae may live in soil or water for several weeks, but without further development. The larvae are attracted by the slime of molluscs, and upon coming in contact with a mollusc they creep into furrows in the foot, whence they penetrate into mucous glands, burying themselves in the muscular connective tissue under the epithelium. Here they coil up and soon become enclosed in a tubercle resulting from encapsulation by the host. The first molt usually takes place after a week to 10 days at room temperature, the larvae having grown comparatively little in length, but having become thicker. The second molt usually takes place in from 10 or 12 days (*Aclurostrongylus*, *Muellerius*, *Crenosoma*) to 4 or 5 weeks (*Elaphostrongylus*), after which the larvae are infective when molluscs containing them are eaten. In most cases little specificity is shown with respect to the species of molluscs utilized as intermediate hosts, although, possibly because of the habits of the snails, certain species seem to be of prime importance. *Protostrongylus rufescens* develops primarily in *Helicella* (Hobmaier and Hobmaier, 1930); *Muellerius capillaris* can utilize a great variety of snails and slugs, although Pavlov (1937) found only *Helicella obesa* to be important in

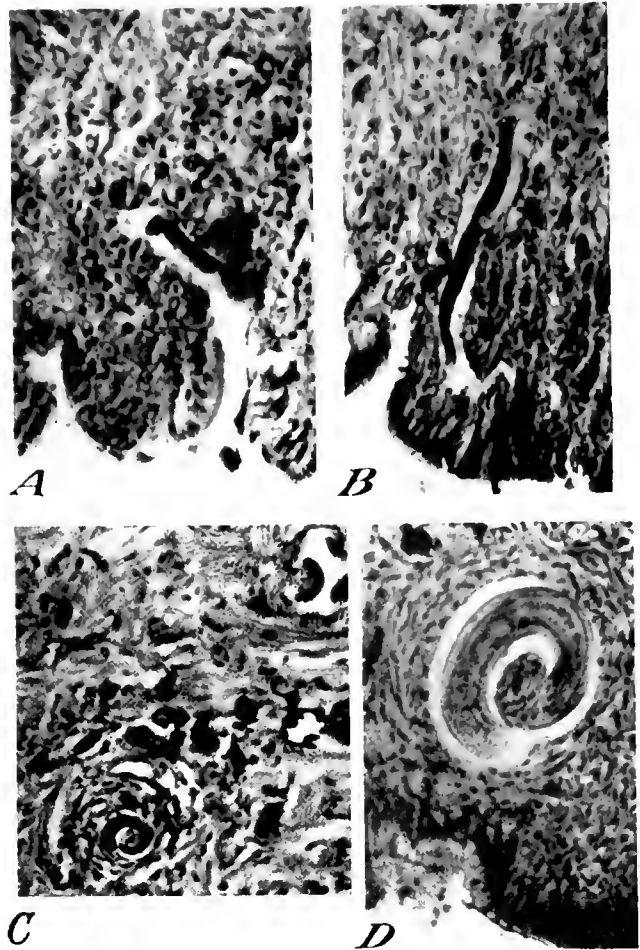


Fig. 185 DEVELOPMENT OF PROTOSTRONGYLINAE IN MOLLUSCS

Larvae of *Muellerius capillaris* in *Agriolimax agrestis*. A—Larvae in furrow of foot of mollusk a few hours after infection; B—On first day of infection (sagittal section); C—Coiled larva in foot on second day of infection (horizontal section); D—Larvae in sole of foot on 16th day of infection. After Hobmaier, 1934, *Ztschr. f. Parasitenk.*, v. 6 (5).

Jugoslavia; *Aclurostrongylus abstrusus*, reported by Cameron (1927) to utilize mice as intermediate hosts, apparently erroneously, according to Hobmaier and Hobmaier (1935) develops in a variety of snails and slugs, but *Epiphragmophora* proved most suitable. Other forms in which a variety of molluscs have been shown to serve as hosts are *Aclurostrongylus falciformis* (Wetzel, 1938), *Crenosoma vulpis* (Wetzel and Müller, 1935), and *Elaphostrongylus odocoi* (Hobmaier and Hobmaier, 1934).

Hobmaier (1934) believes that the utilization of molluscs as intermediate hosts by the Protostrongylinae grew out of the habit of the larvae of seeking protection from desiccation in the slime of the molluscs. This predilection for slime extends to the period of passage through the colon of the definitive host, for the larvae are commonly found burying themselves in the intestinal mucus and thus becoming located on the surface of fecal pellets instead of inside. In this position those larvae which were not protected from desiccation by the mucus, and subsequently the tissues, of snails would fail to survive. The larvae, as Hobmaier points out, differ widely in their habitat in the snail from the parthenitae of flukes, which probably develop in snails because these were ancestral hosts. Whereas fluke parthenitae are true internal parasites of molluscs, lungworm larvae are scarcely more than external parasites. Larvae ingested by snails usually pass all the way through the alimentary canal and fail to develop.

## OXYUROIDEA

## 1. ENTEROBIUS VERMICULARIS

In spite of the fact that the oxyurid type of life cycle is the simplest and probably the most primitive of any found among nematodes parasitic in vertebrates, a search of the literature has failed to reveal a single instance in which a detailed molt by molt account of the life cycle has been described. The life cycle of *Enterobius vermicularis*, so far as it is known, will serve as an example of its type.

The adult female worms, with the uteri filled with developing eggs, live in the lower part of the large intestine and particularly in the rectum. They do not ordinarily deposit their eggs in the lumen of the intestine, but crawl out of the anus and deposit them in the perianal region, leaving trails of eggs as they creep about. Contact with air is apparently a stimulus to oviposition (Philpot, 1924). Although they frequently remain outside the anus and release the eggs in showers when the body ruptures, MacArthur (1930) and others state that they commonly retreat into the rectum, to repeat their egg-laying expeditions out of the anus over and over again, particularly at night.

The eggs when deposited by the females, or contained in the uterus of females which have voluntarily migrated out of the intestine, are fairly uniformly in the "tadpole" stage of development, apparently being unable to progress beyond this point without free oxygen. Within 6 hours after leaving the body they develop a coiled larva (ring and a-half embryo) which is infective. According to Brumpt (1922) the larva undergoes no molt before hatching nor, according to Philpot (1924), as a free larva in water. However, Alicata (1934) suggested that a molt within the egg shell might be general for the Ascaridina, and *Enterobius* might well be reexamined. Chitwood (personal communication) believes he has seen a molt in the egg, and thinks there may be two.

Development of the larva in the egg will occur in oxygenated water, and in this medium the larvae commonly emerge in from 9 to 24 hours at 37° C., but they only live for a few days, so it is evident that water cannot be an important vehicle of infection. Exposed to air a considerable proportion of the eggs survive for at least 6 days at humidities above 62 percent (Jones and Jacobs, 1939).

When ingested the eggs hatch in the stomach or intestine, and the worms live during the early part of their development in the lower part of the small intestine, cecum and upper portions of the colon, not infrequently invading the appendix. Heller (1903) states that there definitely are two molts in the small intestine, and probably three. Chitwood, (personal communication) reports having seen a molt in the epithelium of the appendix. By analogy with other nematodes there is probably a total of four molts.

Although the worms have repeatedly been reported as burrowing into the mucous membranes, especially of the appendix (Penso, 1932), it seems probable that this is a habit only of the fourth-stage larvae. Chitwood (personal communication) reports having found the fourth-stage larvae in sections of the appendix. He has observed a definite period 6 to 9 days after infection when symptoms of invasion appeared, followed 4 to 7 days later by migration of the worms from the anus. Exposure to air after operation would account for the deposits of eggs which Penso reports and figures deep in the walls of the appendix.

There has been a large amount of discussion as to whether internal auto-infection by the worms can occur. The fact that infections persist even for many years in spite of the most careful efforts to prevent reinfection from the anus via the hands has lent support to this idea. However, the demonstration by Lentze (1935), and Nolan and Reardon (1939) of the ease with which airborne infections can occur seems sufficient to account for the persistence of infections. On the other hand, Zawadowsky and Schalimov (1929), Lentze (1935) and others have called attention to the failure of development and infection of eggs or embryos left under conditions such as exist in the lumen of the large intestine. It would be difficult to say that internal auto-infection could never occur, but the evidence is all in favor of the view that if it does occur it is an abnormal and exceptional condition.

Copulation of the young adult worms usually takes place in the upper parts of the colon or in the cecum, where the males live for some time. The females do not migrate to the rectum until they contain developing eggs. Ripe females begin to appear about 15 days after infection.

The life cycles of other Oxyuridae are the same in essential features, but differ in details. *Oxyuris equi* differs in that the fourth-stage larva has a special structural development of the anterior portion or "corpus" of the esophagus which enables the larva to use it as a highly developed buccal capsule for adhering to the mucosa (Wetzel, 1931). The ripe females of this species creep out of the anus as do those of *Enterobius*, but this is probably not true of forms parasitic in rodents. The fourth-stage larva of *Dermatoxys veligera* is also provided with a special structure for adhering to the mucosa, but in this case the end is accomplished by the development of four conspicuous hooks on the head (Fig. 156, X, Y) (Dikmans, 1931), which is buried in the mucosa (Wetzel, 1932). These specializations for maintaining a position in the colon are of interest as indicative of a need for some sort of protection against expulsion from the body before maturity is reached, a need which may perhaps, as has already been suggested, have led to a deeper burrowing into the mucosa and ultimately to a parenteral migration.

According to Philpot (1924), *Aspicularis tetraptera* has a life cycle strikingly like that of *Enterobius*, differing only in the earlier stage at which the eggs cease development before expulsion, and their failure to hatch outside the body. *Syphacia obvelata* differs in that the eggs have developed embryos when they leave the host. All stages of development from the youngest larva to adult can be found in the cecum of naturally infected mice, and are strikingly similar to those described and figured for *Aspicularis*. *Tachygonetria longicollis* and *T. dentata* definitely undergo a molt before hatching from the egg. *Passalurus ambiguus*, according to Penso (1932), is capable of infernal auto-infection; the gravid females burrow into the mucosa to deposit their eggs, and the larvae subsequently emerge to continue their development. Penso, however, postulates a similar behavior on the part of *Enterobius vermicularis*, and thinks that Wetzel's observations on *Dermatoxys veligera* were in error, the larvae with buried heads being emerging from, not entering, the mucosa. Although *Passalurus ambiguus* may sometimes deposit its eggs in the mucosa, Penso's observations need to be extended before this can be accepted as a normal or usual procedure.

*Probstmayria vivipara* (Atractidae) is, so far as known at present, unique among nematodes that are known to be obligatory parasites of vertebrates in reproducing continuously generation after generation in a single host. It is among the nematodes what the *Pupipara* are among the Diptera, or *Tunga* among fleas. Its larvae hatch in the uterus and grow almost to the size of the parents before being born (*vide* Ransom, 1907). They resemble the parents except for lack of development of the genital organs. No stage of development is known outside the body of the host. Transfer to new hosts is believed by Jerke (1902) to be accomplished by contamination of food or water by worms passed in the feces; such worms, he says, remain alive in feces for several days.

## ASCARIDOIDEA

In the Ascaridoidea there are always one or more molts before the embryos leave the eggs and, with few if any exceptions, there is a phase of burrowing into the mucosa, and in many cases more extensive migration from the lumen of the intestine to the body cavity, liver, lungs or other tissues of the definitive or of an alternating host.

## HETERAKIDAE

The members of this family bridge the gap between the typical oxyurid life cycle and that of the ascaridids. At least one species, *Sabalura brumpti* (*see* below), has become dependent upon an intermediate host.

The life cycle of *Heterakis gallinae*, according to Clapham (1933), is of the typical oxyurid type except that the females do not migrate out of the anus to deposit eggs, but oviposit in the ceca. Earlier writers have reported burrowing and encystment in the cecal walls, or penetration into cecal glands, but Clapham was unable to find any evidence of migration or burrowing, the larvae passing directly to the ceca within 48 hours and maturing in the lumen. The first molt occurs in the egg (Alicata, 1934), the third not until 10 days after infection.

Other species of *Heterakis* (*isolonehe*, *beramporia*) burrow into the intestinal mucosa at some time during development and reach maturity in tumors which form around them. This possibly is the first step in the direction of the *Ascaris* type of life cycle.

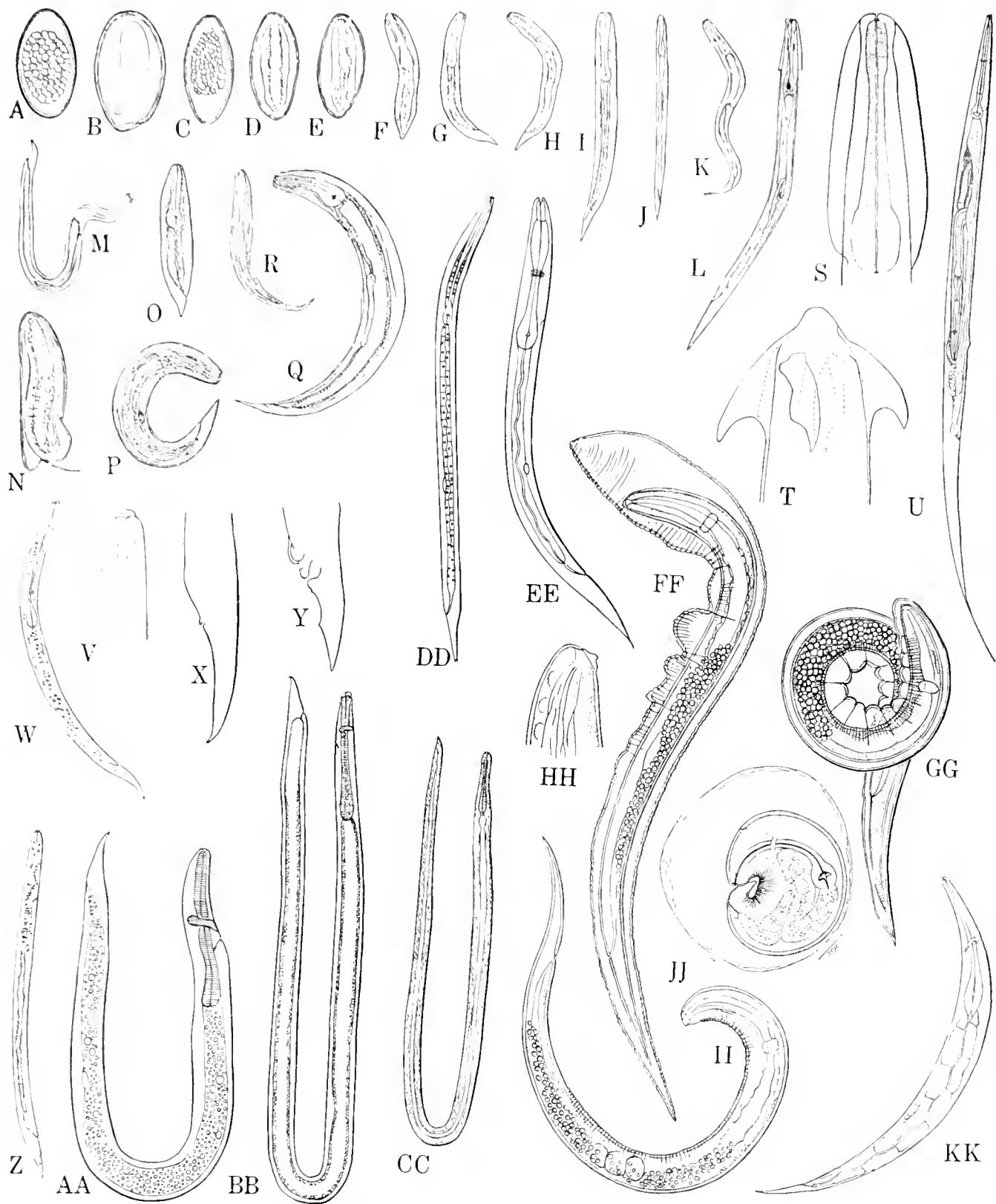


Fig. 186. DEVELOPMENT OF THE ASCARIDINA

A—*Passolobus ambiguus*. B—*Oxyuris brevicauda* showing emergence area and embryo in outline after incubation for 64 hours at 22° C. C—C M—*Aspicularis tetraptera* C—Egg incubated in water 24 hours; D—44 hours; E—68 hours; F—Larva from intestine 4 hours after feeding; G—Larva from caecum 4 hours after feeding; H—After 18 hours; I—After 44 hours; J, K—10 days after feeding; J, male; K, female; L, M—18 days after feeding, L, female; M, male; N, Q—*Siphacium obrolata* (N—Uterine egg containing mature embryo; Q—Hatched embryo; P—Youngest larva found in caecum; R—Male measuring .81 mm.). R—*Enterobius vermicularis*, larva three days after hatching in Ringer's solution. S—T—*Derodonturus setigerus* (S—Head, T—Head, fourth stage). V—*Probsthaueria rufipura*, lateral view of female containing a well developed embryo, a second less developed and two eggs. V—Y—*Ascaridia galli* (V—cephalic extremity of second stage larva showing oral prominence; W—Second stage, newly hatched; X—Tail of third stage female showing preanal swelling; Y—Tail of fourth stage

male). Z, CC—*Ascaris lumbricoides* (Z—Second stage (newly hatched); AA—Third stage; BB—Fourth stage (21 days); CC—Fifth stage (29 days old). DD—EE—*Casmocercoides dukae* (DD—Newly hatched larva; EE—Infertile larva). FF—II—*Contracaecum aduncum* (FF—GG—Hatched larvae, HH—Anterior end of larva armed with boring tooth; II—Larva from body cavity of *Ascartia bifilosa*). JJ—*Subulura beunpti*, encysted infertile larva recovered from body cavity of the beetle *Alphitobius diaperinus*. KK—*Heterakis gallinae*, infertile larva found newly hatched in the small intestine. A, R, after Philpot, J. Helminth., v. 2 (5), pp. 239-252. S, T, after Dikmans, 1931, Trans. Amer. Mic. Soc., v. 59 (4). U, after Ransom, 1907, Trans. Amer. Mic. Soc. v. 27. V, Y, after Roberts, 1937, Bull. 2 An. H. Sta. Queensland. Z, CC, after Roberts, 1934, Bull. 1, An. H. Sta. Queensland. DD—EE, after Harwood, 1930, J. Parasit., 17. FF—II, after Markowski, 1937, Bull. Acad. Polon. Ser. B, JJ, after Alicata, 1939, J. Parasit., 25. KK, after Clapham, 1933, J. Helminth., v. 11 (2).



Fig. 187.

*Dermatoxys veligera*. Photomicrograph of fourth stage larva penetrating mucous membrane. After Wetzel, 1931, J. Parasit., v. 18.



Fig. 188.

*Ascaridia galli*. Section of small intestine showing larva. After Roberts, 1937, Bull. No. 2, Annual Health Sta., Yeerongpilly, Queensland.

The life cycle of *Ascaridia galli* may well be a second step towards that of *Ascaris*. As elucidated by Aekert (1931), Alicata (1934) and Roberts (1937) this worm undergoes one molt in the egg and then normally remains enclosed in the egg until infection. There are three molts in the host, the first of these (second molt) occurring about 6 days after infection and the others at about 6 day intervals thereafter. After reaching the third stage, on about the ninth or tenth days, the larvae burrow down between the villi and penetrate into the glands of Lieberkuhn, the posterior ends of the bodies remaining free in the lumen. Itagaki (1927) observed that at certain seasons in Japan (midsummer and midwinter) the larvae habitually penetrated into the mucosa, about as described by Aekert and by Roberts, causing fibrous nodules, but that in spring and autumn they remain in the lumen. Roberts reported less tendency for the larvae to burrow into the mucosa in April and May than in November. Although on rare occasions the larvae penetrate too deeply and enter the peritoneal cavity, mesenteries, liver, or even the lungs (Aekert, 1923; Guberlet, 1924), it is clear that this is purely accidental.

*Subulura brumpti*, according to Alicata (1939), has departed from the usual heterakid life cycle pattern in requiring an intermediate host. This is the only member of the subfamily Subulurinae in which the life cycle has been investigated, and it is possible that the use of an intermediate host has become general in this group as it has in the Anisakinae.

Alicata was unable to infect chickens by feeding embryonated eggs, either just recovered from the uteri of gravid females, or incubated in water at about 24° C. for 1 week, but succeeded in producing infection by feeding naturally infected arthropods harboring the cysts in the body cavity. The cysts contain coiled nematodes having bulbous esophagi and conspicuous esophageal valves as in the adults (Fig. 186, J.L.). High incidences of natural infection were found in the following arthropods collected on poultry farms in Hawaii: (Coleoptera) *Dermestes vulpinus*, *Gonocephalus scitatum*, *Ammophorus insularis*, *Alphitobius diaperinus*; and (Dermoptera) *Euborrha annu-*

*lipex*. Encysted larvae were also found in grasshoppers (*Conocephalus saltator*) 15 days after experimental infection.

#### COSMOCERCIDAE

At least some of the members of this family resemble the typical Ascarididae in that the larvae, burrowing into the mucosa, enter the circulatory system and reach the lungs, where they escape into the air spaces and eventually make their way back to the intestine via trachea and esophagus. They differ, however, in having a free living phase outside the body. *Cosmocecea trispinosa* (= *Nematorxys longicauda*) has long been known to occur in the lungs of salamanders in an immature form, and in the intestine as an adult. Von Linstow considers its growth in the lungs as analogous to the growth of Anisakinae in an intermediate host. Harwood (1930) found that *Cosmoceceoides dukae* (his *Orysomatium variabilis*) undergoes a molt after 5 days of free life outside the body and, although his observations on development after infection are inconclusive, that the larvae are found in the lungs not only after subcutaneous inoculation but also after infection by mouth. They do not, however, penetrate the skin.

#### ASCARIDIDAE

The majority of the Ascarididae have a migratory phase before becoming adult in the intestine. The larvae, burrowing into the mucosa, enter the circulatory system and are carried via liver or lymphatic system to the heart, thence to the lungs where they become free in the air spaces, and thence via trachea and throat back to the alimentary canal. *Toxascaris leonina*, according to Wright (1935), does not perform this migration; the life cycle is similar to that of *Ascaridia* except that the second-stage larvae burrow into the mucous membranes almost immediately after hatching, and return to the lumen of the intestine after the third molt, on the 9th or 10th day. As shown by Fülleborn (1922) and others, some larvae penetrate all the way through into the body cavity and enter viscera by

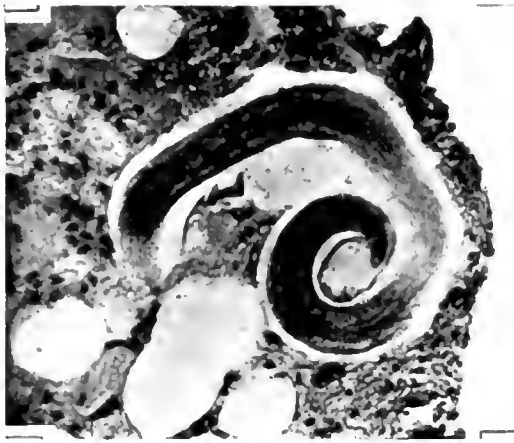


Fig. 189

*Ascaris suum*. Larva in section of mouse lung 1 week after infection. After Ransom, 1920, U.S.D.A. Yearbook.

this route, and some are probably picked up and carried by the circulatory system.

*Ascaris lumbricoides*. Long thought to have a direct development in the intestine, *Ascaris lumbricoides* was first shown to undergo a preliminary migration through the body by Stewart (1914); Stewart found that eggs fed to rats migrated to the lungs, and erroneously concluded that rats served as intermediate hosts. Shortly thereafter Ransom and Foster (1917) and Chandler (1918) called attention to the probability that the migration through the body was a part of the normal development in the definitive host; experimental proof, with details of the development, was supplied by Ransom and Foster in 1920. Details of the course of the migration were worked out and reported by Ransom and Cram in 1921, and further details were supplied by Roberts (1934).

The first-stage larva appears in the egg on about the eighth day at the optimum temperature of 30 to 33° C., and the first molt occurs in the egg on about the 18th day. Ransom and Foster (1920) first observed that the embryo underwent a molt in the egg. Later Alicata (1934) reported that the egg is not infective until after this molt; he also pointed out that the embryos of *Ascaridia lineata*, *Parascaris equorum*, *Toxocara canis*, *Toxascaris leonina*, *Heterakis gallinae* and the roach oxyurid *Blatticola blattae* also underwent a molt, a feature which may be common in the Ascaridina and which determines when the egg has reached the infective stage.

Normally the eggs of *Ascaris lumbricoides* hatch in the small intestine after being swallowed, but they will sometimes hatch when implanted subcutaneously or intraperitoneally (Ransom and Foster, 1920; Yoshida and Toyoda, 1938) or in artificial media containing glucose or various nitrogenous substances (Yoshida and Toyoda, *loc. cit.*).

The second stage larva has a small, sclerotized, knob-like structure at the anterior end, called the "boring tooth." The larvae bore into the intestinal wall, mainly in the duodenum and upper part of the jejunum, after hatching; the majority have disappeared within 2 hours. The majority enter the blood stream after some hours and are found in the liver in from 18 hours to several days after infection. A few apparently enter lymphatics since they are sometimes found in mesenteric lymph glands, but from here they seem to go via mesenteric venules to the liver rather than directly to the lungs. Within 5 or 6 days all have left the liver and have gone to the lungs via the blood stream; some appear in the lungs within 18 hours, and they may continue to be found there for 10 or 12 days, although most numerous on about the third to fifth days. During the first 2 days of this migration the larvae grow considerably. About the fifth or sixth day the larvae in the lungs, measuring about 0.8 to 1 mm in length, undergo the second molt. The third stage larva has three lips with papillae, lacks the boring tooth, has a highly developed muscular esophagus, has the intestinal cells packed with granules, has a distinct nerve ring and oval genital primordium, and a conical tail turned dorsad at the tip.

On the tenth to twelfth days the third molt occurs, also in the lungs. In the opinion of Roberts, although second and third stage larvae may be found in the intestine prior to the tenth day (Ransom recovered larvae from the trachea as early as the third day), these larvae have not completed their development in the lungs and probably fail to establish themselves in the intestine. The suggestion is made that the occurrence of such larvae in the intestine may indicate unfavorable conditions in the lungs resulting from excessive infections. Roberts found some hundreds of fourth stage larvae in the intestine on the 14th and 21st days, but no molting third stage larvae were found between the 14th and 14th days. Fourth stage larvae are 1.4 mm or more in length. The cuticle begins to show striations, fin-like lateral alae are present, the lips resemble those of the adult, the esophagus is less bulbous, and the sexes can be differentiated by a difference in length of tail. Rudimentary genital tubules are present in the body cavity.

After arrival in the intestine the larva grows enormously, reaching a length of 16 to 25 mm 29 days after infection. Larvae undergoing the fourth molt measure 17.5 to 22.5 mm (Roberts). The lateral alae have become inconspicuous, the genital tubules and body wall muscles are comparatively well developed, and the characteristic features of the tail of the two sexes are present. Growth to maturity and beginning of reproduction takes several weeks.

It is obvious that the only striking difference between this life cycle and that of the heterakids is the entrance into the circulatory system when burrowing into the intestinal wall, the consequence of which is the migration through the body via liver, heart and lungs. The determining factor seems to be the age at which the larvae do their burrowing. *Enterobius* and *Dermatophys*, as we saw, burrow as fourth-stage larvae, and some species of *Heterakis* do likewise and live as adults in the burrows; *Ascaridia* burrows while in the third stage; but *Ascaris* burrows immediately after hatching as a second-stage larva. The burrowing heterakid larvae are too large to enter or be sucked into blood vessels, whereas the *Ascaris* larvae can easily do so. The failure of *Toxascaris* larvae to enter the circulatory system except rarely may be found to be due to a difference in size, particularly in the diameter of the larvae.

*Toxocara canis* has essentially the same life cycle as *Ascaris lumbricoides*, and the same is true of *Neoascaris vitulorum* (*vide* Schwartz, 1922), of *Parascaris equorum* (*vide* Baylis, 1923), of *Ascaris columnaris* (*vide* Goodey and Cameron, 1923), and probably of all other Ascaridinae. According to Fülleborn (1921), *Toxocara canis* is frequently encapsulated in the tissues of mice or other abnormal hosts, which thereby become transport hosts.

#### ANISAKINAE

It has long been known that various members of this subfamily occur as immature worms in the body cavity, mesenteries and other organs of various vertebrates, and sometimes invertebrates, whereas the adults occur in vertebrates which prey upon these hosts. Although morphological characters often suggested affinities between larvae and adults there was little experimental evidence in support of them. Moreover the various larval forms were not clearly differentiated from each other. Baylis (1916) for instance, showed that a number of larval forms were confused under the name "*Ascaris capsularia*," which he believed on morphological and distributional evidence to be the larval form of "*Ascaris decipiens*" (now *Parroccacum decipiens*). The same confusion probably holds for other species.

Thomas (1937a, 1937b) experimentally worked out the life cycle of *Contracaecum spiculigerum*. Eggs obtained from the proventriculus of a cormorant contained active molted larvae with a boring tooth after being incubated in water for 5 days, and on the sixth day they molted a second time and then hatched. Many attached themselves by the anterior end of the sheaths, which seemed adhesive, but they swam freely when detached. On the thirteenth day a third molt was in progress, with a cuticular tooth still present. When swallowed by tadpoles or guppies (*Labistes reticulatus*) the larvae shed their sheaths and were found free in the intestine or in the body cavity. About 3 months later larvae were found encysted in the mesenteries; they had grown to 1.3 mm in length (from less than 400  $\mu$ ). In cysts developed by the host tissues they continue to grow until nearly adult size is reached. Unlike most nematodes the number of molts is not limited to four; as many as eight molted cuticles have been removed from encysted worms from a natural infection.

There is evidence that when an infected fish is eaten by another fish the larvae penetrate the intestinal wall and re

## SPIRURINA

### SPIRUROIDEA

Encyst in the mesentery. This was observed to occur when a parasitized guppy was fed to a black bass. In all cases the worms retain the cuticular "boring tooth" until the definitive host is reached, although three lips can be seen under the cuticles in older larvae. Natural infections with similar worms were found in several species of fish in Illinois. Sexual maturity is reached only in birds. Fledgling cormorants become infected when fed on infected guppies. The larvae at first penetrate into the glands of Lieberkuhn, and when fish are present in the ventriculus they leave the glands and penetrate into the food during its digestion.

Kahl, 1936, investigated the life cycle of *Contracaecum clavatum* and concluded that it can undergo partial development in a great variety of intermediate hosts, including *Sagitta*, Calanidae, amphipods and medusae among invertebrates, and in *Ammodytes* and *Merlangus* among fishes. Wülker, 1929, thought there was a succession of three hosts,—plankton, plankton-eating fish, and piscivorous fish, but Kahl thinks that all three hosts are not necessary; development to the stage infective for the definitive hosts can take place directly in such fish as *Merlangus merlangus*. *Merlangus* can also serve as a definitive host, if infective larvae are swallowed with the flesh of smaller intermediate hosts.

Markowski (1937), influenced by Wülker's work, found that certain species of copepods served as first intermediate hosts for *C. aduncum*, and presented evidence for the view that a variety of plankton-eating or carnivorous fish might serve as second intermediate hosts, although he expressed doubt that the larvae developing in the parenteral organs of a fish would develop to maturity in the intestine of the same fish, even if it were a suitable host. Markowski did not consider the possibility of a plankton host being unnecessary. According to Kahl the larvae undergo their early development in the intestine of the intermediate hosts, and then, when about 5 mm long, acquire a boring tooth and penetrate into the body cavity where they molt again, but retain the sheath with tooth and posterior spine until eaten by the final host. Essentially then, the life cycle of this species is similar to that of *C. spiculigerum*, although according to Kahl the eggs develop embryos only after being swallowed by a host. For a species living in marine hosts this might be necessary. It is probable that all the species of *Contracaecum* conform very closely to the same pattern.

Thomas (1937c) worked out the life cycle of *Rhaphidascaris canadensis*. The eggs of the species may become embryonated after 8 hours outside the host and are infective within 24 hours, after one molt within the egg. When eaten by nymphs of dragonflies, these eggs hatch, the first cuticle is shed, and the larvae penetrate into the body cavity. Infected nymphs caused infection in guppies, which in turn caused infection in fingerling muskellunge. In Douglas Lake the livers of all yearling *Perca flavescens* are full of *Rhaphidascaris* cysts, whereas the plankton-feeding fingerlings are free of infection. Guppies can be infected directly by the embryonated eggs, the intervention of an invertebrate host apparently being unnecessary, as in the case of *Contracaecum aduncum*. In small bottom-feeding or nymph-eating fish, then, they become encapsulated in the mesenteries and liver and continue growth until eaten by species of *Esox*, in which the cycle is completed. *R. acus* of Europe presumably has a similar cycle, since the larvae are found in the inner organs of various cyprinoid, salmonid and percid fishes, whereas the adults are found in *Esox*, *Perca*, *Alosa* and *Anguilla*.

The observation of Baylis on the probable relation between *Porrocaecum decipiens* of seals and walrus and encapsulated larvae in various fishes have already been mentioned. A number of European writers have reported encysted larvae of *Porrocaecum* in insectivores (moles, shrews, desman) and Schwartz (1925) has reported them from under the skin of moles and shrews in the United States; he, and also Solonitzine, who has found the larvae of a *Porrocaecum* on the serous surface of the stomach of a desman (*Desmana moschata*), think the adult stage is probably reached in a bird of prey.

Walton (1936a) found evidence for a similar life cycle for *Multicaecum tennicolle*. Encysted larvae were found in species of *Rana* and in *Siren*; 3 weeks after being fed to a young alligator, presumably parasite-free, several immature males and females were found. A similar cycle was found by Walton (1936b) for *Ophidascaris labiatopapillosa*; the larvae were encysted in mesenteries and muscles of *Rana* spp., the adults developing in *Natrix* spp. Similar larvae encysted in muscles of *Amphiuma*, however, failed to develop in *Natrix*. Ortlepp (1922) failed to get larvae of *O. filaria* to penetrate the mucous membranes when the ripe eggs were fed to a mouse, although those of *Polydelphus anoura* migrated to liver and lungs like typical Ascaridinae.

The members of this superfamily, with a few exceptions, show a striking degree of uniformity in the general features of their life cycles. Although many species tend to live in the walls of the alimentary canal or in more distant locations in the body, the eggs, usually embryonated, escape with the feces, and usually hatch only after being eaten by an intermediate host. The embryos of *Habronema*, however, hatch before escaping from the body. In most cases there is some degree of specificity with respect to the intermediate host, but usually it is not very close. After ingestion by the intermediate host the first-stage larvae emerge from the egg, penetrate into the body cavity or tissues, undergo two molts, and become encapsulated as third-stage larvae. These larvae are usually not sheathed, as are the larvae of metastrongyles; the second cuticle is not needed as a protection, since this is provided by a capsule produced by the host, so is completely shed.

Infection of the definitive host is nearly always by ingestion of the infected intermediate host, although an alternative method occurs in the case of *Habronema* (see below). Not infrequently transport hosts may intervene between the true intermediate host and the definitive host, and it is possible that this can occur in all spirurids. When the larvae are eaten by a host in which the worm is unable to reach maturity they burrow through the walls of the alimentary canal and become reencysted. In most cases this seems to be an optional course of development which is frequently favorable to ultimate access to a definitive host (e.g., *Spirocerca*, *Habronema mansioni*) but in the case of at least one species, *Gnathostoma spinigerum*, a second intermediate host has apparently become indispensable in the life cycle. After reaching the final host the worms undergo two more molts before reaching maturity. Being too large to enter blood vessels in the intestinal wall, they usually reach their destination, if this is outside the alimentary canal, by direct migration through tissues or along natural passageways.

*Gongylonema pulchrum* will serve as an example of a typical spirurid life cycle. *Gnathostoma spinigerum* and *Draschia megastoma* will serve to exemplify two important variations.

### GONGYLONEMA PULCHRUM

The adult worms live imbedded in the mucous membranes of the esophagus, tongue and oral cavity. The eggs escape into the lumen and leave the body with the feces in a fully embryonated condition. No further development takes place until the eggs are ingested by a suitable intermediate host. This may be any of a large number of beetles, particularly scarabaeids, or cockroaches. Twenty-four hours after ingestion by *Blattella germanica*, according to Alicata (1935), empty egg shells are found in the crop and intestine. The absence of larvae in the lumen or wall of the intestine and the presence of a few still adhering to the wall of the crop, apparently ready to invade the body cavity, suggests that hatching takes place in the crop, and that the larvae find their way into the body cavity by piercing the wall of the crop. Forty-eight hours after ingestion of eggs, first-stage larvae are found in the body cavity, especially in the thoracic region.

The newly hatched first-stage larva is cylindrical with a spine and a small hook near the anterior end on the ventral side, behind which about 20 rings of minute spines encircle the anterior end of the body (Fig 190B); the tip of the blunt tail is encircled by 8 to 10 small refringent points, a character which is diagnostic of the first-stage larva. The filariform esophagus and intestine are about equal in length, both transparent. The larvae wander about in the body cavity and grow to double their original length in about 2 weeks, and at this time are preparing for the first molt (Ransom and Hall, 1916; Alicata, 1935). The actual molt, according to Alicata, does not occur until about the 19th day.

The second-stage larvae lose the cuticular armature at the anterior and posterior ends, which are bluntly rounded. The slender esophagus occupies about one-half the body length, and in older larvae becomes differentiated into an anterior muscular portion and a posterior glandular portion. These larvae increase in size to a length of 1.5 to 2 mm by the end of the fourth week, when they begin the second molt. At about this time they usually penetrate the muscles of the body wall, and sometimes, in heavy infections, other muscles, and they may become partially encysted prior to the second molt.

Third-stage larvae are found encysted at the end of about a month. This stage is distinguished by a raised lateral bor-

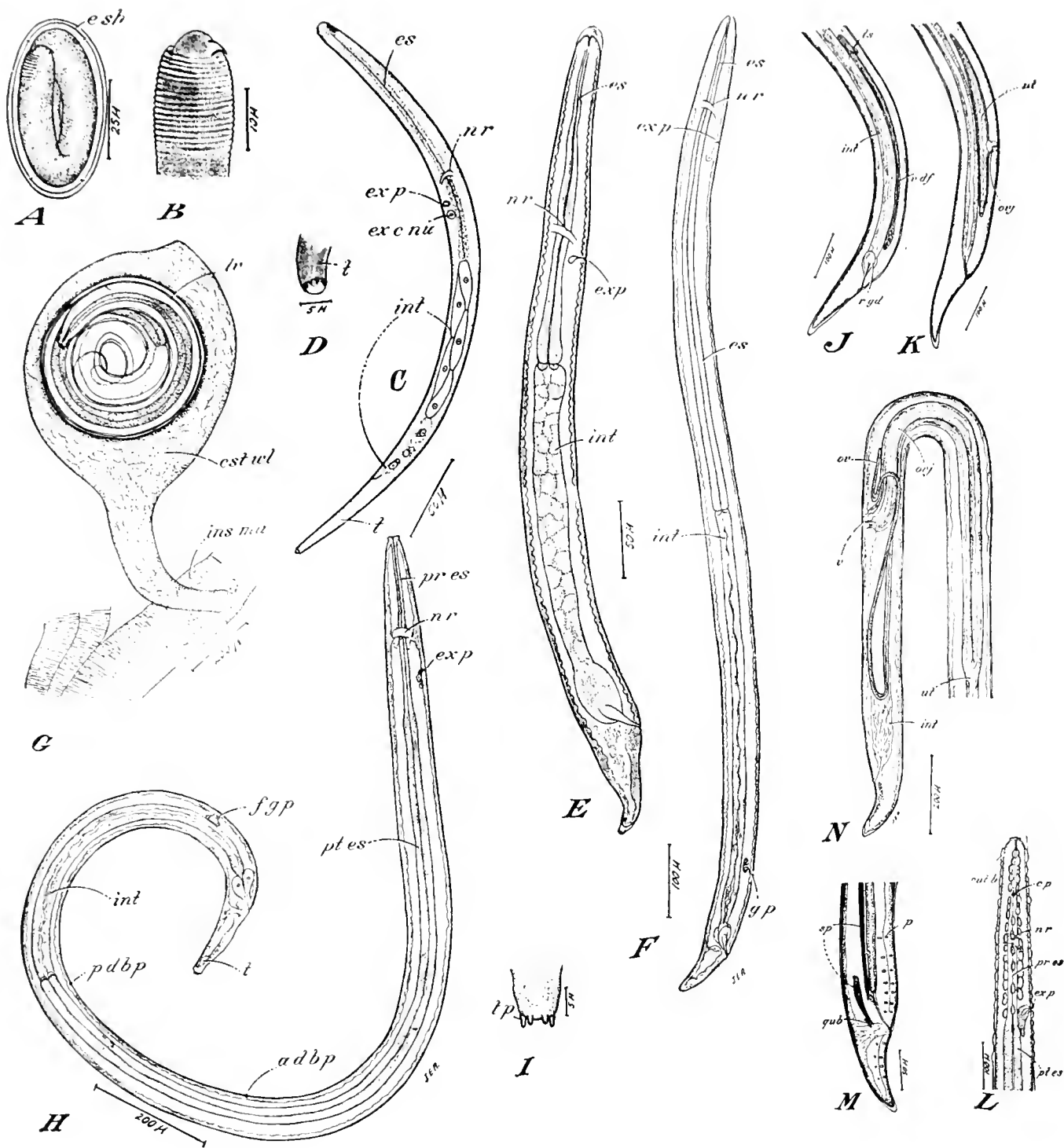


Fig. 190.

Development of *Gongyalanema pulcherrum*. A—Egg with fully developed embryo; B—First stage larva, anterior end; C—First stage larva from intermediate host, four days after experimental infection; D—Tail, lateral view; E—First stage larva undergoing first molt; F—Second stage larva; G—Third stage larva encysted in musculature of roach (*Blattella germanica*); H—Third stage, lateral view; I—Posterior end

showing four digitiform processes; J—Posterior end of male undergoing third molt; K—Posterior end of female undergoing third molt; L—Fourth stage larva, anterior end; M—Posterior part of male in fourth molt; N—Region of vulva of larva undergoing fourth molt. After Abcorta, 1935, U.S.D.A. Tech. Bull. 489.

der of the mouth and by four, occasionally only two, small digitiform processes on the tail. The larvae are found imbedded within the sarcoplasm surrounding a muscle fiber. As the cysts become well formed they are sometimes pushed out into the body cavity, remaining attached to the muscle by a thin strand, or eventually falling free. Baylis (1926) found that the larvae would escape from disintegrating cockroaches into water, and could be kept alive for a number of days, but since the larvae settle to the bottom he concluded that drinking water was not an important means of infection. Freed larvae were found to be incapable of skin penetration. The possibility exists, of course, that larvae, either in or out of their intermediate hosts, might reëncyst in some transport host; Alicata (l.c.) cites the finding of third stage larvae in the stomach wall of a mole.

Upon ingestion by a definitive host (Alicata used guinea pigs for experimental infections) the larvae are liberated in the stomach and may invade the esophagus within one-half hour after feeding, entering through the tissue at the gastro-esophageal junction. They migrate upward through the epithelium of the esophagus and may reach the tongue as early as the third day. Larvae begin the third molt on the ninth day after ingestion, and many fourth-stage larvae are present by the twelfth day. These larvae are characterized by development of the reproductive organs, gradual development of the characteristic cuticular bosses at the anterior end, and loss of the caudal appendages. The final molt occurs about a month after infection; the minimum time required for growth to maturity seems not to have been determined definitely, but Ransom and Hall (l.c.) report the finding of egg-bearing females in a sheep about 3 months after infection, and Alicata (1935) obtained an adult male 70 days after infection.

#### GNATHOSTOMA SPINIGERUM

The adult worms live in tumors in the wall of the stomach of Felidae, or of the esophagus of mink, the eggs escaping into the alimentary canal through openings which eventually develop from the tumors into the lumen. The eggs escape from the body in an early stage of development (one to two-celled stage according to Prommas and Daengsvang, 1933; one to many-celled according to Refuerzo and García (1938)). In aerated water they become embryonated in a minimum of about 4 or 5 days, and in 2 days or more thereafter the embryos emerge from the egg in an ensheathed condition, being, therefore, in the second stage. These larvae have smooth cuticles devoid of spines or striations, and are armed with a spine at the anterior end.

The larvae usually live for only a few days in tapwater (Prommas and Daengsvang, 1933) although sometimes they may live for a month or more (Yoshida, 1934). Further development is known to occur only when the larvae are ingested by *Cyclops*. Attempts to infect mammals, fish, frogs, fleas and Cladocera have all been negative. The development of the larvae in *Cyclops* was independently discovered by Prommas and Daengsvang (l.c.) in Siam and by Yoshida (1934) in Japan. These workers showed that sheathless motile larvae were found in the stomachs of *Cyclops* soon after experimental exposure, and that by the following day they could be found in the body cavity. According to Refuerzo and García (1938), the larvae in the body cavity 1 day after infection lose the sclerotized oral spine, and a fleshy enlargement representing the future lips develops at the anterior end. Three days later the cuticle becomes striated, its armature of spines develops, and a head bulb armed with four rows of spines, and connected with cervical sacs, is also present. The larvae seem to have completed their development to the infective stage by the sixth day.

Attempts to infect cats by feeding them infected *Cyclops* have been uniformly negative (Yoshida, 1934; Prommas and Daengsvang, 1936) but Prommas and Daengsvang succeeded in infecting a catfish, *Clarias batrachus*. The larvae were found in the muscles of the stomach or intestine of the fish 2 to 6 days after infection and after 6 days or more they were found, some free and some encysted, in body muscles. Chandler (1925a) had reported the presence of numerous gnathostome cysts in the mesenteries of Indian snakes, which he found to undergo further development in cats (1925a) until the adult morphology of *Gnathostoma spinigerum* was reached (1925b); Chandler also called attention to reports of probably identical larvae in pelicans and eagles. Subsequent to the work of Prommas and Daengsvang many other intermediate hosts, natural and experimental, have been added, including a considerable variety of fresh-water fishes, frogs, and snakes. In all of these the larva undergoes considerable growth, but does not develop more than 4 rows of spines on the head bulbs, in contrast to the 8 to 11 found in the adults

of *Gnathostoma spinigerum*. It is probable that the larvae always become encysted ultimately.

Chandler (1925a) showed that when gnathostome cysts in snakes are fed to cats they penetrate through the alimentary canal and can be found parenterally within 2 days after infection. Some are found free in the abdominal cavity, under the parietal peritoneum, or in the capsules of the kidneys, but the majority, and nearly all later in the infection, are found burrowing in the liver. A single larva was also found in the liver of an experimentally infected guinea pig. The larvae in the livers of cats grow somewhat, and a vulva and rudimentary genital tubes develop within 6 days. No further development was observed in cats infected for as long as 4 weeks, although in the meantime there was extensive damage done to the liver. Subsequently (1925b) Chandler found, in naturally infected cats, all stages of development from (presumably) fourth-stage larvae burrowing in the liver, exactly like those obtained from experimental infections, to forms, still sexually immature, which had undergone the final transformation to the adult morphology, and had 8 to 11 rows of hooks on the head bulb, and complex spines on the body. Some of the worms which had undergone the final molt were found still in the liver, but others were evidently migrating out of the liver; a few were found in the mesentery or in the diaphragm, and several were in the stomach wall; one was free in the stomach. The worms in the stomach wall were not yet enclosed in hard-walled tumors, but occurred in submucous purulent cavities. It was evident from these observations that the worms, upon gaining access to a definitive host, migrate through the walls of the stomach or intestine to the abdominal cavity and enter the liver, where they burrow and feed actively for several weeks. They finally enter the wall of the stomach from the peritoneal side, and grow to maturity.

Africa et al (1936a) fed rats with encysted larvae and found the larvae in the liver and body muscles 8 to 25 days later. Infection of cats fed on gnathostome cysts from cold-blooded hosts has been confirmed by Prommas and Daengsvang (1937), the prepatent period being 28 to 32 weeks, and by Africa et al (1936b), who found semi mature worms in the diaphragm and in nodules in the stomach wall nearly 4 months after infection. It is clear that the formation of a tumor about the worms in the wall of stomach or esophagus, which finally opens into the lumen, is a late stage of development. It also seems evident from observations made by the writer (1925b) that these tumors, when in the stomach of cats, frequently become perforated into the peritoneum and are then fatal. Yoshida's observation that in mink the tumors form on the esophagus in the lower part of the thoracic cavity suggests that this may be the normal host and habitat, and that in these circumstances there is less danger of fatal parenteral perforation.

#### DRASCHIA MEGASTOMA

The life cycle of this worm is of particular interest since it represents an intermediate evolutionary step from that of the typical spirurids to the filariae. It was first worked out in detail by Roubaud and Descazeaux (1921).

The female deposits embryonated eggs in the alimentary canal which, according to Roubaud and Descazeaux, hatch before leaving the body of the host. The first-stage larvae possess a hooklike structure similar to the hook of *Gongylonema*, but the larvae are in a very immature state, the contents of the body being granular in appearance, with no differentiation. These larvae are ingested by young maggots of flies, and there seems to be a fairly high degree of specificity. *Draschia megastoma* and *Habronema muscae* have been found to be capable of development in a number of species of *Musca* and also in *Muscina stabulans* and in *Fannia*, but actual transmission has been observed only in *Musca domestica*, and was definitely found by Roubaud and Descazeaux to fail in the case of *Muscina stabulans* because of inability of the larvae to escape from the proboscis of that species. *H. microstoma*, on the other hand, develops primarily in *Stomoxys*, but has been reported as developing in *Sarcophaga*, *Lyperosia* and *Musca* as well, though Roubaud and Descazeaux (1922b) state that it does not reach the infective stage in *Musca domestica*. Development of *Habronema* larvae has also been reported from *Drosophila*.

The ingested larvae bore through the walls of the alimentary canal of the maggots and enter the body cavity. They live free in the body cavity for only a brief time, and about the third day they penetrate into the Malpighian tubules. Here they become quiet, and undergo the first molt on the third or fourth day after ingestion. They lose the oral hook, become immobile, and grow very thick and sausage-



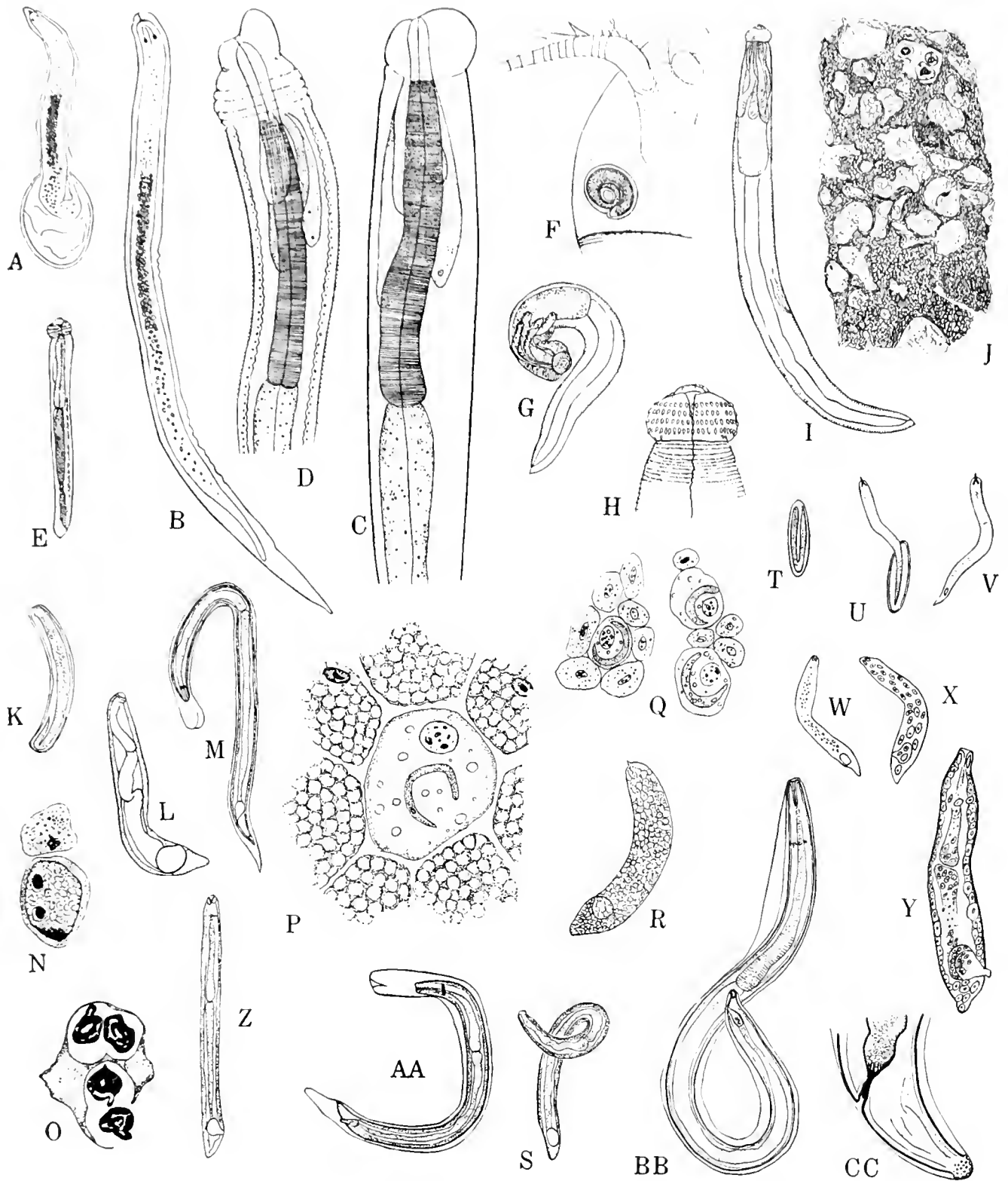


FIG. 191. DEVELOPMENT OF SPIRUROIDEA

A-D—*Gnathostoma spintgerum* (A—Larva emerging from egg through opercular end; B—Newly hatched larva with loose enveloping sheath, anterior spine; C—Anterior end of larva dissected from cyclops on first day of infection, no anterior spine but large fleshy lip, two pairs of contractile cervical sacs; D—Larva from Cyclops on fourth day of infection, cephalic bulb with four rows of minute spines, lip smaller; E—Larva in body cavity of cyclops; F—Same larva; G—Larva from cyst in mesentery of cobra; H—Head, bulb and lips of larva from liver of artificially infected cat; I—Gnathostome from liver of artificially infected cat; J—Section of liver of cat showing riddling of tissue by burrowing gnathostomes). K-M—Stages in the development of *Habronema muscae*. (K—Egg with embryo; L—Second stage larva, M—Third stage larva before the molt). N-O—Sections pointing out the histological reaction of the fat cells parasitized by *Habronema muscae* (N—Fat cells at the beginning of the infection showing peripheral thickening and hypertrophy of parasitized cell in relation to normal; O—Section of a fat sac enclosing many parasites). P—Fragment of

fat tissue of larva of *Musca domestica* showing aciculate larva of *H. muscae* in hypertrophied and transformed fat cell. Q-S—Development of *H. microstomum* in *Stomoxys*. (Q—Group of adipose cells of the larva of *Stomoxys* of which three are infested with a young larva of *H. microstomum*; R—Sausage shaped larva; S—Older second stage larva). T-CC—*Draschia megastoma* (T—Embryonated egg; U—Aciculate larva emerging from egg shell; V—Aciculate larva in intestine of fly; W—Second stage larva immediately after molt in malpighian tubules of fly larva; X-Y—Second stage larvae recovered from larva (X) and pupa (Y) of fly; Z—Full grown second stage larva; AA—Second stage larva about to molt; BB—Second stage larva, full grown and about to molt, removed from malpighian cyst; CC—Posterior end of same). A-D, after Refuero & Garcia, 1938, Philip. J. An. Ind. (5 (4)). E, F, after Promas and Daengsvang, 1933, J. Parasit. G-J, after Chandler, 1925, Parasit. v. 17. K-CC, after Roubaud and Descazeaux, Bull. Soc. Path. Exot., v. 14, 15.

like. An outline of the stoma appears at the head end, and a conspicuous caudal vesicle and outline of the pyriform rectum at the posterior end, but throughout the rest of the body the nuclei are still scattered without definite order. Gradually during the next few days the worm elongates, and the alimentary canal, nerve ring, and rectum become well developed. Meanwhile the tissue of the wall of the Malpighian tubule surrounding the larva degenerates and is finally reduced to a mere membrane, which serves as a sheath. On the eighth day, at about the time of emergence of the adult fly, the larvae begin to break loose into the abdominal cavity, still enclosed in the membrane, but they now molt a second time and their movements become very active, resulting in their soon freeing themselves.

These third-stage larvae, the infective forms, may appear as early as the ninth day. They migrate forward to the head of the fly, and collect in the interior of the labium. Attracted by warmth and moisture they move down into the labellum, and escape through the delicate membrane between the lobes of this structure when the fly is resting on a warm wet surface, e.g., the lips, nostrils or wounds of an animal. If on the lips, the larvae have an opportunity to reach the stomach via the mouth, and grow to maturity in a normal manner, but from the nostrils they reach the lungs, and from the skin the subcutaneous tissues, and in either case fail to grow to maturity. There is no doubt but that animals could also be infected by swallowing flies harboring infective larvae, but in the case of habronemiasis of horses this would probably not be a common method in nature. On the other hand it would probably be the principal if not the exclusive method in the case of habronemiasis of insectivorous birds. Still another possibility—ingestion by a transport host—is suggested in the case of habronemiasis in birds of prey; this is supported by the finding of abundant larvae of *H. mansonii* encysted in the stomach walls of toads by Hsü and Chow (1938). This species had previously been recorded from the bearded vulture, *Gypaetus barbatus*, but several species of falcons were experimentally infected by feeding them larvae from toads.

*Habronema muscae* and *H. microstomum* have similar life cycles (vide Roubaud and Descazeaux, 1922a), but different in details. These two species, instead of undergoing development in the Malpighian tubes, develop in cells of the fat body, the thickened walls of the cells serving as temporary "cyst" walls. *H. microstomum*, which develops in the blood-sucking *Stomoxys*, might be expected to be introduced into the tissues when the insect pierces the skin, and be forced to find its way to the stomach by some roundabout parenteral route, but Roubaud and Descazeaux (1922b) point out an interesting biological adjustment which makes this unnecessary. They point out that, as a result of interference by the worms in its proboscis, the fly is unable to rasp a hole in the skin to suck blood, and is forced to revert to the habits of its ancestors and non-blood-sucking relatives, and obtain moisture and nourishment from the lips or other exposed moist surfaces.

The failure of the larvae of *Habronema* to become encysted in the intermediate host, there to remain until eaten by a definitive host, and the substitution of a voluntary exit from this host in response to warmth and moisture, are definite steps in the direction of a filarial life cycle. As remarked by Roubaud and Descazeaux (1922b), however, the habronemas are imperfectly adapted for parenteral parasitic life. Their larvae, in spite of the fact that they leave the body of the intermediate host on the surface of the body of the definitive host, are unable to penetrate the tissues, and are unable to reach maturity outside the alimentary canal. With (1) development of a parenteral adult habitat (already attempted by many spiruroids but always hampered by the necessity for the eggs to reach the alimentary canal), and (2) development of ability to enter the skin on the part of the infecting larvae, the only important change necessary to bring about a filarial life cycle is the substitution of the blood or skin for the alimentary canal as a means of exit for the larvae. Such a development could hardly fail to occur in the case of a parenteral parasite with a blood-sucking intermediate host.

#### OTHER SPIRUROIDEA

The life cycle of the majority of the Spiruroidea in which it has been determined conforms in general pattern to that of *Gongylonema*, except for the intermediate hosts involved. In some cases there seems to be far less specificity with respect to intermediate hosts than in others, but some instances of apparent specificity are probably due to incomplete data. Thus *Chiclospirura hamulosa* was not known to develop in anything but grasshoppers until Alicata (1937) showed that an amphipod and 10 species of beetles belonging to 7 different

families, as well as several grasshoppers, could be utilized as intermediate hosts by this worm. On the other hand, Cram (1931) got negative results from feeding eggs of *C. spinosa* to cockroaches, ground beetles, sowbugs and crickets, but obtained development in two species of grasshoppers. Again, whereas *Tetrameres fissispina* is reported as capable of development in grasshoppers, roaches, *Daphnia*, *Gammarus* and earthworms, Swales (1936) found that the eggs of *T. crami* failed to hatch in various species of Cladocera, but developed readily in two species of amphipods. Members of the genera *Ascarops*, *Physocephalus* and *Spirocerca* seem to develop primarily in dung beetles; *Spirura*, *Protospirura* and *Gongylonema* in beetles or roaches; *Oxyspirura* in roaches; *Seurocyanea* in roaches and grasshopper nymphs; *Acuaria* in grasshoppers; *Tetrameres* in various Orthoptera and Entomotrachea; *Hartertia* in termites (workers); *Echinuria* in Cladocera, *Dispharynx* and *Hedruris* in isopods; *Cystidicola* in amphipods, and *Spiroxyis* in copepods. Spiruroid larvae, possibly *Protospirura*, have been found in fleas also. Under experimental conditions *Physaloptera turgida*, according to Alicata (1938), is able to develop in cockroaches, but there is a possibility that other arthropods are utilized under natural conditions.

*Spiroxyis contorta*, as reported by Hedrick (1935), differs from the majority of the Spiruroidea but resembles *Gnathostoma* in that the eggs become embryonated in water after leaving the body of the host. It differs from *Gnathostoma*, however, in that the definitive host can be infected directly by the third-stage larvae in *Cyclops*, without requiring a second intermediate host. In nature, however, transport hosts—fish, tadpoles, frogs, newts and dragonfly nymphs, and frequently turtles as well—are commonly made use of. The larvae of this worm are further peculiar in that they continue to grow after they reach the infective stage, both in *Cyclops* and in the various transport hosts. The development of a "sausage" form by the late first-stage larva of *Oxyspirura mansonii*, as figured by Kobayashi, is highly suggestive of *Habronema* or the filariae.

As far as known at present *Gnathostoma spinigerum* is the only spiruroid which requires a second intermediate host, but it is quite possible that this will be found to be true of other Gnathostomatidae as well, and perhaps of still other spiruroids. The larvae of *Echinoccephalus* (family Gnathostomatidae) have been found encysted in the tissues of a bivalve, *Margaritifera vulgaris*, which is presumably the first intermediate host. Similar larvae have been found in a sea urchin. Since the adults occur in oyster-eating fishes no second intermediate host may be necessary.

The course of migration in the definitive host is usually, as noted above, by burrowing directly through tissues or natural cavities, or by migration along natural passageways. The path of *Oxyspirura mansonii* to the eye, according to Fielding (1926), is by way of esophagus, mouth and lacrimal duct, the larvae sometimes arriving in the eye 20 minutes after infected roaches are fed to chicks.

The migration route of *Spirocerca lupi* (= *sanguinolenta*) is not so clearly known. Faust (1927) thought that the larvae, after ingestion with the flesh of a transport host (hedgehog), reach the aorta via the portal system and lungs, but does not

Fig. 192.

Development of Ascaropsinae larvae. A-E—*Ascarops strongylina* (A—First stage larva, anterior end, lateral view; B—Larva recovered from an intermediate host three days after experimental infection; C—Larva undergoing first molt; D—Third stage larva, lateral view; E—Encysted larva, third stage). F-K—*Physocephalus scalatus* (F—Anterior end, lateral view; G—Larva from intermediate host 2 days after experimental infection; H—Larva from intermediate host 12 days after experimental infection; I—Larva undergoing first molt; J—Encysted third stage larva (from Hobmaier, 1925); K—Third stage, tail). After Alicata, 1935, U.S.D.A., Tech. Bull. 489.

Fig. 193.

A-C & G—*Spiroxyis contorta*; (A—Free-living larva with sheath; B—Five-day old larva from cyclops; C—*Cyclops leuckarti* with three larval nematodes; G—Fully developed larva from body cavity of cyclops, showing genital primordium). D-E—*Dispharynx spiralis* (D—Head; E—Tail). F—*Tetrameres americana*, tail of third stage larva. H-I—*Tetrameres crami* (H—Third stage larva from *Gammarus fasciatus* 32 days after infection; I—Diagrammatic illustration of papillae on tail of third stage larva). J—Larval spiruroid larva from cat flea. K-M—*Protospirura muricola* (K—Lateral view of anterior extremity of infective larva; L—Lateral view of tail of 3.5 mm. specimen; M—Free-hand sketch of rosette of papillae on tail of same). N-P—*Oxyspirura mansonii* (N—Larva just after hatching; O—Larva at end of first larval stage; P—Mature larva). Q—*Habronema mansonii*, larva. A-C & G, after Hedrick, L. A., 1935, Tr. Am. Mic. Soc. v. 54(4). D-F, after Cram, E. B., 1931, U.S.D.A. Tech. Bull. 227. H, I, after Swales, 1936, Canad. J. Res. D. 14. J, after Alicata, J. E., 1935, J. Parasit. v. 21 (3). K-M, after Foster, A. O., and Johnson, C. M., 1939, Am. J. Trop. Med. v. 19 (3). N-P, after Kobayashi, H., 1928, Taiwan Igakk. Zasshi Formosa, No. 280. Q, after Hsü, H. F., and Chow, C. Y., 1938, China Med. J. Suppl. II.

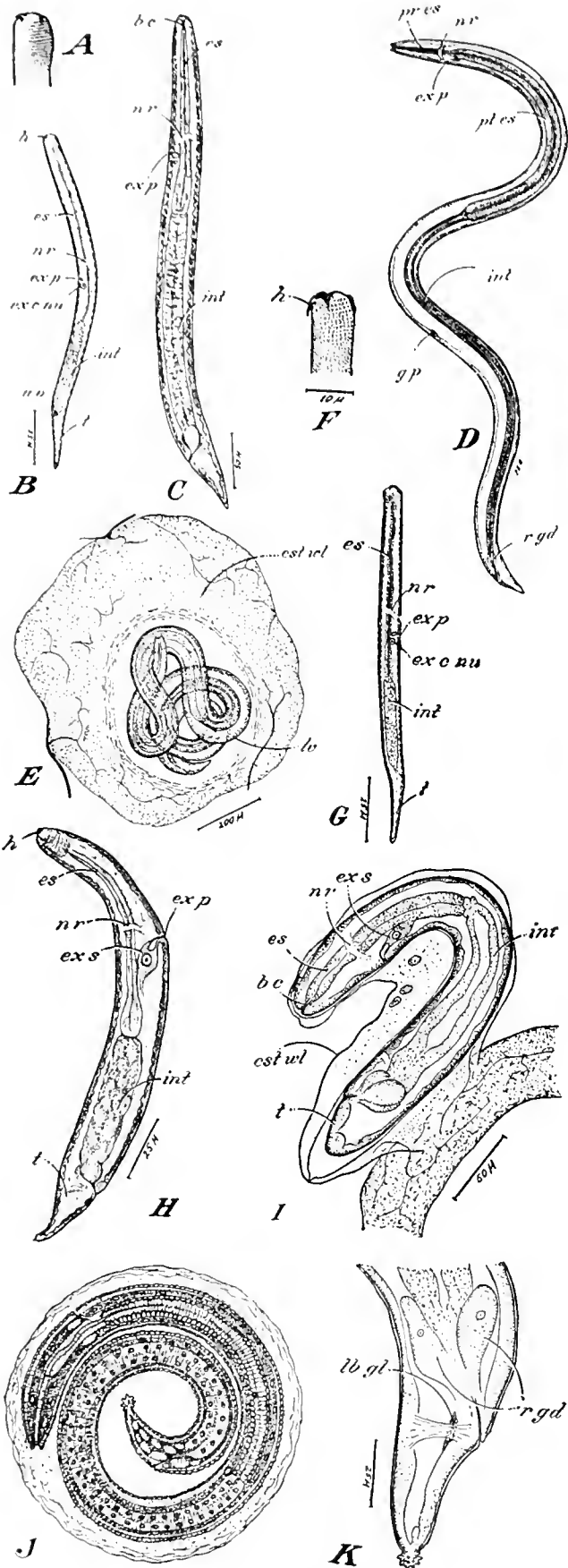


Fig. 192. DEVELOPMENT OF ASCAROPSINAE LARVAE

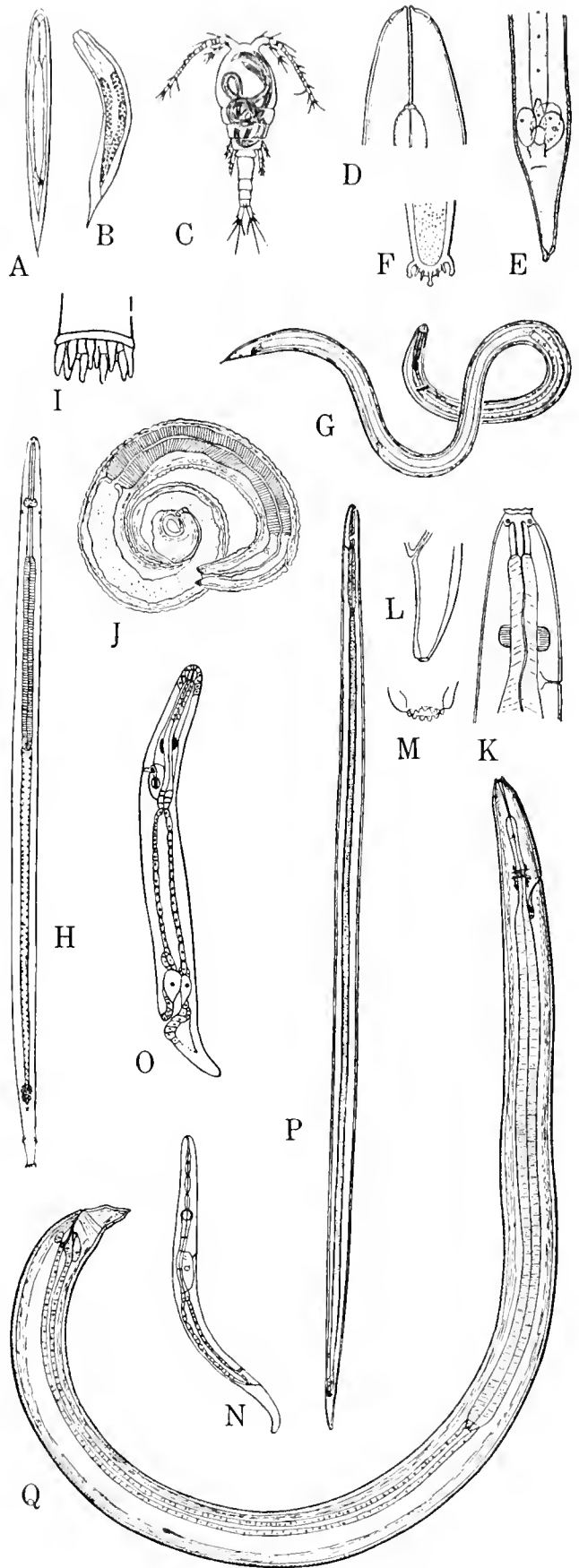


Fig. 193. DEVELOPMENT OF SPIRUROIDEA

make it clear how a worm 150  $\mu$  in diameter is able to pass through capillaries, or why the worms appear in the abdominal aorta before the thoracic, and never cause lesions in vessels anterior to the aortic arch. It seems far more likely that the larvae follow the route indicated by Hu and Hocppli (1936); after penetrating the gastric wall they proceed to the coronary, gastroepiploic and coeliac arteries, and via these to the upper abdominal and lower thoracic portions of the aorta, eventually reaching the upper thoracic aorta from below.

In the aorta the worms attach themselves to the wall and cause the formation of characteristic nodules. Some worms remain in this position but many migrate outward through the aortic wall and through the intervening tissue until they reach the esophageal wall, in which they find a favorable habitat in which to reach maturity and reproduce. The eggs reach the lumen of the esophagus through a secondary opening from the tumor in its wall.

## FILARIOIDEA

The Filarioidea are unique among nematodes, so far as is known at present, in having perfected a mechanism by which both exit from and entrance to a host takes place through the skin. The larvae of *Dracunculoidea* escape through the skin, though by a different mechanism, and the *habronemas* succeed in infecting a host when deposited on certain areas of skin (the lips) but in neither case is both exit and entrance accomplished by way of the skin. As noted under the discussion of Spiruroidea, the evolutionary process by which the life cycle of filariae developed is clearly foreshadowed by the course of events in the case of *Habronema*.

### WUCHERERIA BANCROFTI

Manson's (1878) discovery of the ingestion of filarial embryos by mosquitoes and their development in these insects set a landmark in the history of medical entomology, since it was the first instance of a human blood infection being transmitted by an insect. Low (1900) first demonstrated the mechanism by which the larvae were returned from mosquitoes to man, and Annett, Dutton and Elliott (1901), Lebrede (1905), and Bahr (1912) added further details.

The adult worms live in the lymphatic system and liberate their larvae, known as microfilariae, into this system, whence they eventually, unless blocked, make their way into the blood stream. Their presence in the peripheral blood is periodic in most parts of the world, being present at night, but not in the daytime. Similar periodicity, though often less complete, is observed in many other filarial infections; in some species, however, e.g., *Loa loa*, there is a diurnal periodicity, and in others, e.g., *Dipetalonema perstans*, no periodicity has been observed. Two principal theories have been proposed to account for the periodicity: one, originally advanced by Manson, is that the larvae retire to internal organs during the day and enter the peripheral circulation only at night; the other, advanced by Lane (1929), is that the worms have cyclical parturition, producing their entire day's output of larvae at the same time each day, and that these worms are all destroyed in the host within 12 hours after they appear in the blood stream. Some support is given to this theory by O'Connor's (1931) observation at autopsies that at certain hours all the adult female filariae have their uteri crowded with embryos, while at other hours they are uniformly spent. On the other hand, the persistence for a year or more of microfilariae transferred to an uninfected host (Underwood and Harwood, 1939) is against this theory, though the fate of microfilariae in infected and non-infected hosts may not be at all comparable. As yet there is no unanimity of opinion as to the reason for microfilarial periodicity.

The microfilariae of *Wuchereria bancrofti* as seen in blood smears are covered by a sheath which has very generally been thought to be not a shed cuticle but a delicate, stretched vitelline membrane. Augustine (1937) questioned this, since he observed that developing microfilariae in the uterus of *Vagrilaria columbigallinae* clearly show the vitelline membrane surrounding eggs containing coiled larvae, but none of the microfilariae from the vaginal region show any evidence of a sheath, and accumulations of crumpled hyaline objects interpreted as the remains of discarded vitelline membranes were found at a higher level in the uterus. Augustine was able to see no evidence of a sheath on the microfilariae of this species while they were in capillaries but was able to follow its formation on drying slides. He concludes, therefore, that the sheath is, as in other sheathed nematode larvae, the loosened but unshed cuticle from an incomplete ecdysis. This conclusion seems to us, however, to be very doubtful, since no other nematode larvae are known to molt at such an early stage in development, and

since two other molts have been observed during the course of development of the larvae in their mosquito hosts; this would bring them to the third stage, which is usual for infective larvae in intermediate hosts (see p. 237). Some species of filariae are not provided with sheaths.

The larvae are in a very immature state of development. They are covered by a layer of sub-cuticular cells, and within the body have a column of nuclei which subsequently develop into the esophagus and intestine.

This column of cells is broken at certain definite spots representing the future position of the nerve ring, the excretory pore and cell, and the anus. There are also a few large cells: an excretory cell just posterior to the excretory pore, a genital cell well behind the middle of the body, and a group of three cells previously reported as genital cells 2 to 4, but which Feng (1936) says give rise to the anus and rectum, and which Abe (1937) says belong to the sphincter between intestine and rectum, and are ultimately lost. There is a difference of opinion as to the existence of a stylet at the anterior end of the worm. The structure so called appears to be a rudimentary mouth cavity.

Upon ingestion by suitable species of mosquitoes the larvae become unsheathed in the stomach and penetrate into the body cavity, whence the majority migrate at once to the thoracic muscles, where development to the infective stage takes place. The factors which determine the suitability of particular mosquitoes have not been elucidated. Development takes place readily in mosquitoes of a variety of genera, including *Anopheles*, *Culex* and *Aedes*, but sometimes nearly related species within these genera differ widely in their ability to serve as nurses. For example, *Culex quinquefasciatus* and *C. pipiens* are good hosts, whereas *C. vexans* is not; and *Aedes variegatus* is a very good host whereas *A. aegypti* and *A. albopictus* are not. As yet nobody has succeeded in obtaining development in any arthropods other than mosquitoes.

Upon arrival in the thoracic muscles the larvae become quiescent, lying parallel with the muscle cells. Here in the course of 2 or 3 days they become considerably foreshortened, often to approximately half their original length, and at the same time grow considerably in girth, assuming what is known as the "sausage" stage. Only the caudal tip of the body fails to thicken, and is retained as an attenuated tail-like structure. Meanwhile a large excretory bladder develops and subsequently a large rectal cavity, and the outlines of the esophagus and intestine become defined. On the fifth day, according to Abe (1937), the larva undergoes its first molt, the cuticle developing an annular break near the anterior end. After this molt the larva reaches its maximum shortness and thickness and then, as the alimentary canal becomes well developed, begins to lengthen. As it approaches its maximum length it becomes active again and, according to Abe (l.c.), undergoes a second molt about the time it is ready to leave the thoracic muscles (in his experiments on the 13th day). The loosened cuticle breaks near the middle of the body and is shed. The larvae now become active and migrate out of the thorax. The majority go through the neck and head and move down into the interior of the labium, but a few get lost and can be found in the abdomen, legs, palpi, etc. Infective larvae commonly reach the labium about 2 weeks after infection in warm weather, but have been known to complete their development in 9½ days. In the labium they are stimulated by warmth, and when the mosquito is biting, escape through the delicate membrane where the labella join the shaft of the labium. The larvae do not, of course, interfere with skin-piercing as do the larvae of *Habronema* in the labium of *Stomoxys*, since in mosquitoes the labium itself is not a piercing or sucking organ. After leaving the proboscis and becoming free on the skin the larvae were believed by Fülleborn (1908), on the basis of experiments with *Dirofilaria immitis*, to penetrate into pores and enter through unbroken skin, but Yokogawa (1938) carried out a series of experiments which indicate that they can only enter broken skin, and presumably in nature use the wound made by the mosquito.

Nothing is known about the development of the larvae after they enter a human host until they reach maturity in the lymphatic system. *Dirofilaria immitis* requires about 9 months to reach maturity, and it is improbable that *Wuchereria bancrofti* takes any longer, if as long.

### OTHER FILARIAE

The life cycles of comparatively few species of filariae are known, but among those that are known there is comparatively little variation. As already noted, some microfilariae are sheathed and some are not, but there is no evidence that the presence of a sheath has a "muzzling" effect in keeping the microfilariae from passing in or out of the capillaries, as Manson had thought. This was shown by O'Connor (1931) in the

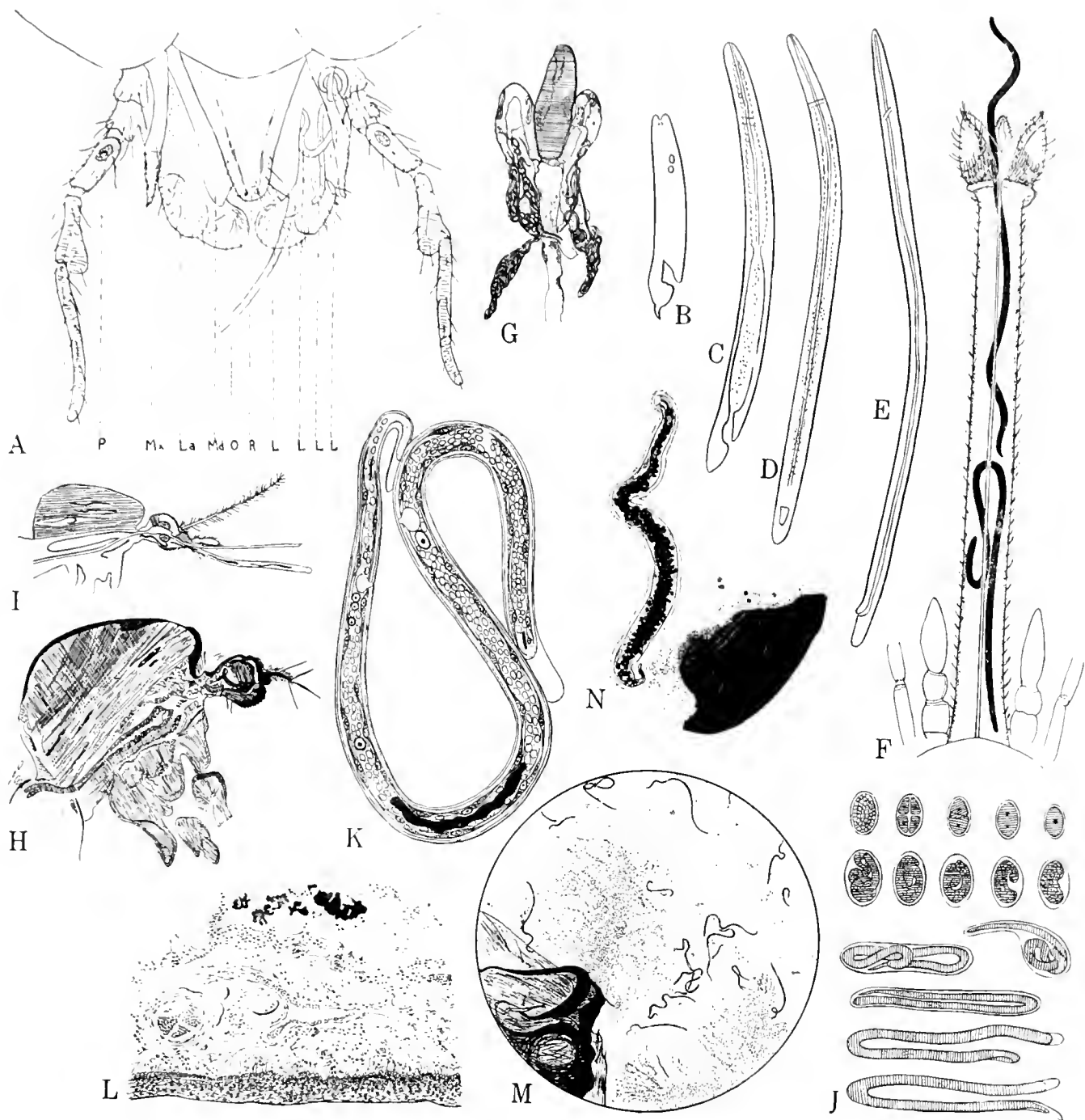


Fig. 194 DEVELOPMENT OF FILARIOIDEA

A—Mouth parts of *Simulium damnosum*, fixed in alcohol, cleared in warm clove oil, showing position of larvae of *Onchocerca volvulus* emerging and in situ. B-E—*O. volvulus* (B—Early thoracic form, second day; C—Thoracic form, seventh day; D—Slightly later thoracic form; E—Proboscis form, ninth day). F—Mature larva of *Wuchereria bancrofti* escaping from proboscis of *Culex fatigans*. G—Larvae of *Dirofilaria repens* in *Anopheles* (11 days). H—*Wuchereria bancrofti* larvae two days after entering *Aedes variegatus*. I—Mature larvae of *W. bancrofti* in thoracic muscles and proboscis of mosquito. J—Embryonic development of *Loa loa*. K—Detailed drawing of *Wuchereria bancrofti* larva. L—*Microfilaria* in deeper layers of conjunctiva in

case with disturbance of vision, keratitis, and iritis. M—Second larval stage of *Onchocerca* in the thoracic muscles of *Simulium metallicum*, approximately 48 hours after feeding upon infested patient. N—Third larval stage or so-called "sausage form" of *Onchocerca* on edge of thoracic muscles of *S. ochraceum* several days after feeding upon infested case. A-E, after Blacklock, D. B., 1926, *Ann. Trop. Med.*, v. 20 (2). F, after Francis, E., 1919, *Hyg. Lab. Bull.*, 117. G, H, & J, after Fuelleborn, F., *Handb. path. Mikr. Jena* v. 6. I & K, after Chandler, A. C., 1940 (Figs. 163 and 160). L, N, after Strong, et al., 1934, *Contrib. Dept. Trop. Med., Harvard*, VI

case of *Wuchereria bancrofti* and by Harwood (1932) in the case of *Litomosoides carinii*.

The microfilariae of *Onchocerca*, which are unshathed, differ from those of other filariae in that they live in the skin, and do not enter either the lymphatic or blood systems. The adult worms, living in subcutaneous tissues, are encapsulated by the host in hard nodules, through which the larvae are able to burrow and escape. The salivary secretion of the intermediate hosts (*Simulium*) seems to exert a definite chemotactic effect on the microfilariae, since they may be many times more numerous in the stomach of a fed fly than in a comparable quantity of tissue.

The intermediate hosts are usually Diptera. Fleas were stated by Breinl (1921) to serve as intermediate hosts for *Dirofilaria immitis* and Summers (1940) corroborated this, showing that development would occur in several species of fleas, and in a shorter time than in mosquitoes. Noë (1908) followed the development of *Dipetalonema grassii* in a tick, *Rhipicephalus sanguineus*; the microfilariae of this species are said to be too large to enter the blood circulation and are found in the lymph, which the ticks suck more than they do blood at the beginning of a meal. This work has not been confirmed and is open to suspicion in view of the fact that the embryos of related species (*Dirofilaria reconditum*, *Dipetalonema perstans*) live in the blood and develop in mosquitoes. Savani (1933) has also reported filariae in dog ticks in areas where *Dirofilaria immitis* is common. The intermediate hosts of *Wuchereria* and *Foleyella* are various mosquitoes; of *Dipetalonema perstans* and *Mansonella ozzardi*, *Culicoides*; of *Loa loa*, *Chrysops*; of *Onchocerca* spp., *Simulium* or *Culicoides*; and of *Dirofilaria*, fleas and mosquitoes.

There is some variation with respect to the site of development in the intermediate hosts. The majority of the species studied—*Wuchereria bancrofti*, *Microfilaria malayi* (adult perhaps unknown), *Dipetalonema*, *Mansonella*, *Dirofilaria reconditum*, and *Onchocerca* spp.—develop in the thoracic muscles of their dipteran hosts, but *Loa loa* develops principally in the muscles or fatty connective tissue of the abdomen of *Chrysops* (Connal and Connal, 1922), and *Dirofilaria immitis* develops in the haemocoel of fleas and in the Malpighian tubules of mosquitoes. These sites of development are of great interest in view of the similar sites utilized by *Draschia* and *Habronema* in muscoid flies.

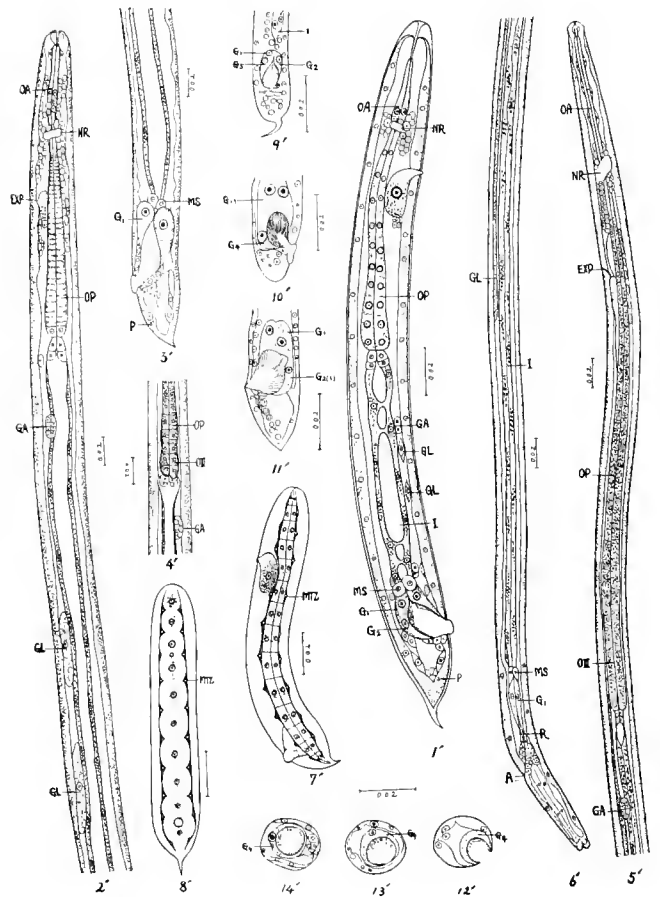
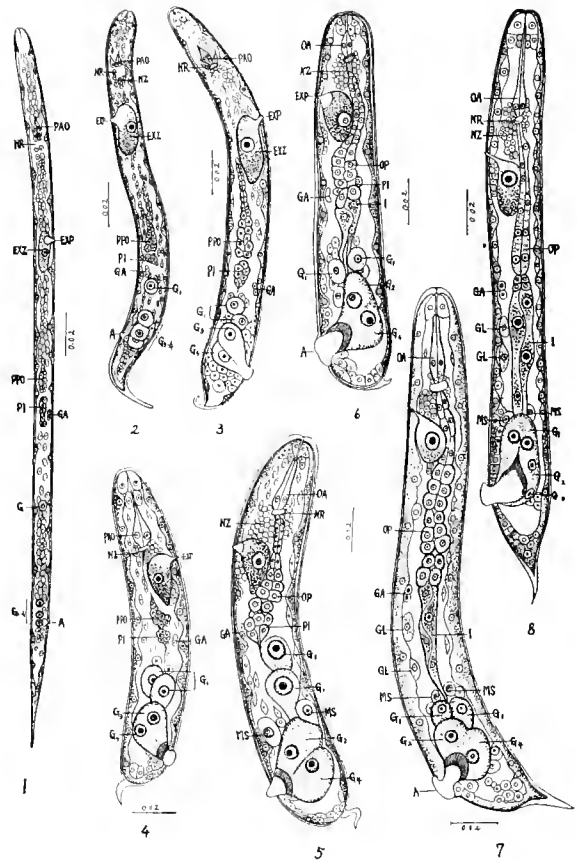


Fig. 195.

Development of *Wuchereria bancrofti*. 1—Larva 10 hours after infection. 2-4—Larva 2-3 days after infection. 5-6—Larva four days and three hours after infection. 7-8—Larva 5 1/2 days after infection. 1' Larva 5 1/2 days after infection, just before first molt. 2'-3'—Larva 7 1/2 days after infection. 4'—Larva 9 1/2 days after infection of posterior end of esophagus. 5'-6'—Larva 11 days and 10 hours after infection.

## CAMALLANINA

The members of both superfamilies of this suborder, Camallanoidea and Dracunculioidea, so far as known utilize copepods as intermediate hosts. There can be no doubt, from a consideration of the habitat and life cycle, that the Camallanoidea, dwelling as adults in the alimentary canal of aquatic hosts, are the more primitive, and that the tissue dwelling Dracunculioidea, sometimes occurring in land animals, are a later evolutionary development. The relation of these two groups is comparable, in a broad way, with the relation of the Spiruroidea and the Filarioidea. In the case of the Filarioidea a habitat in the tissues is accompanied by evolution of a new method of exit and entrance of embryos via the skin, whereas in the Dracunculioidea it is accompanied by a new—but different—method of exit via the skin, suitable for an aquatic animal, but with retention of the primitive oral path of entry.

### CAMALLANUS SWEETI

The life cycle of this worm was worked out by Moorthy (1938). The adult worms live in the intestine of a freshwater fish (*Ophicephalus gachua*) and produce free larvae which escape with the feces of the host. The embryos have a finely striated cuticle, a single dorsal denticle or boring cuticular tooth, and fairly well differentiated internal organs. On reaching water the larvae are swallowed by suitable species of *Cyclops* and reach the body cavity 2 or 3 hours after infection. These larvae undergo the first molt 24 to 36 hours later, and the second one after 5 to 7 days, in hot weather. The third-stage larvae are provided with ridged jaws suggestive of those of the adult, and have three unequal mucrones at the tip of the tail. No mention is made of these larvae becoming encysted in *Cyclops*. When infected *Cyclops* are eaten by small fish the larvae are activated by fish bile, escape from their copepod hosts and undergo further development, including possibly the third molt, in the intestines of these fish. The infection of the final host is thought to result from feeding on the second intermediate host, and the larvae undergo their fourth and final molt in the intestines of this host, acquiring the adult type of mouth. Whether the intervention of a second intermediate host is optional or obligatory was not determined, but in nature it would probably be the usual thing, since the final host does not ordinarily feed on *Cyclops* directly.

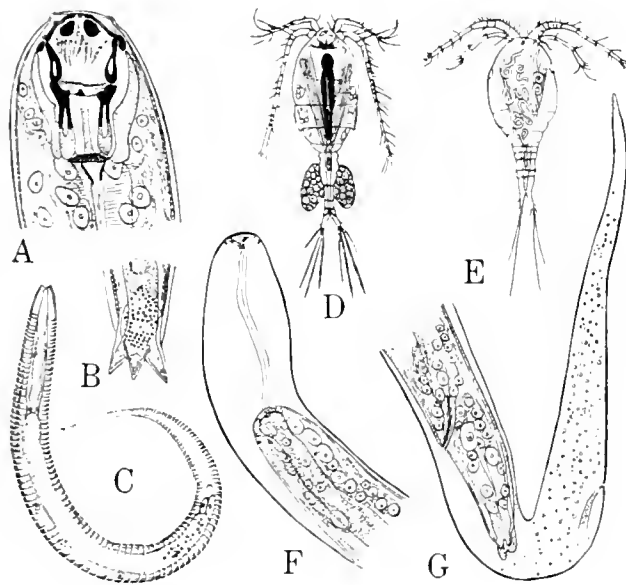


Fig. 196.

A-B—*Camallanus sweeti* (A—Head, fourth stage; B—Tail, same). C—*Procamallanus fulvidraconis*, mature embryo. D—Uninfected *Cyclops*. E—*Cyclops* infected with *Dracunculus medinensis*. F-G—*Dracunculus medinensis* (F—Cephalic region undergoing second molt; G—Tail, same). A-B, after Moorthy, 1938, *J. Parasit.*, v. 24 (4). C, after Li, 1935, *J. Parasit.*, v. 21 (2). D, E, after Fuelleborn, 1913, *Filariose des Mensch.* F, G, after Moorthy, 1938, *Am. J. Hyg.*, v. 27 (2).

No encysted forms of *C. sweeti* were found in fish hosts, nor was any evidence found of their penetrating the walls of the intestine, but camallanid larvae of another type were found encysted in the body cavity, loosely attached to the intestines. These were observed to exoest when eaten by *Ophicephalus gachua*, but failed to undergo further development in that host.

An essentially similar development in *Cyclops* has been demonstrated for *Procamallanus fulvidraconis* by Li (1935), except that only one molt was observed. It seems probable that the first one was overlooked, since Li's figure of a 6 day old larva corresponds with Moorthy's second stage larva of *Camallanus*, and his second stage larva with Moorthy's third stage. However, Pereira *et al.* (1936) state that *P. vearensis* develops only to the second stage in *Diaptomus*, the third and fourth stages being passed in the intestines of the fry of a fish other than the definitive host. Although they speak of this host as a "waiting host" (i.e., transport host) it would appear to be a true second intermediate host if their observation is correct that development to the third stage does not occur in *Cyclops*.

It will be seen that the camallanid life cycle is essentially the same as that of *Spiroxya* or of *Gnathostoma* except for the production of free embryos instead of eggs by the parent worms.

### DRACUNCULUS MEDINENSIS

The adult female guinea worm, *Dracunculus medinensis*, when preparing for parturition, appears in the subcutaneous tissues of her host and produces a small ulcer on the surface of the skin. Upon stimulation by chilling of the skin, which happens in nature when the skin is plunged into water, she contracts violently in such a manner that a portion of the larva-filled uterus is prolapsed through a rupture in the cuticle, and the prolapsed portion of the uterus, bursting, liberates a small cloud of larvae. These larvae are unusually large (about 600  $\mu$  long), have a striated cuticle, a cuticular boring tooth or denticle, well developed esophagus and intestine with dilated lumen, and a long filiform tail.

These larvae swim about in water and undergo further development only after being swallowed by certain species of copepods. The details of their development was worked out by Moorthy (1938). They reach the body cavity a few hours after being swallowed. They undergo two molts in the body cavity, the first one on the 5th to 7th day after infection, the second on the 8th to 12th day in hot weather. They start undergoing the second molt before casting off the exuviae of the first. The larvae grow very little in size, and actually decrease in length due to the loss of most of the filamentous tail. The third stage larvae increase slightly in size for about a week after the second molt, but after that undergo no further development; they are infective for the definitive host 4 to 8 days after the exuviae of the second molt are shed. They have a long esophagus of the adult type, and four mucrones at the tip of the tail. They remain active in the body cavity of the *Cyclops* for 4 or 5 weeks, but subsequently coil up and become quiet, but are not encysted. In addition to the usual type of larvae Moorthy also found a small proportion (1:900) of "abnormal" larvae in which the tail is malformed. Moorthy suggested that these may have been males, but it is more likely that they should be regarded as abnormal individuals.

The early development of the larvae in the definitive host has not been followed. Sexually mature females 12 to 24 mm in length were found by Moorthy and Sweet (1938) in deep connective tissues of experimentally infected dogs 67 days after infection, and Moorthy believed that at this time fertilization had already taken place. Migration of the worms to the subcutaneous tissue and the formation of an ulcer for the egress of larvae occurs about a year after infection in man.

An essentially similar life cycle occurs in the case of *D. aphidensis* of garter snakes (Brackett, 1938). *Cyclops* infected with this species may be eaten by tadpoles and possibly other transport hosts; in tadpoles the larvae were found to remain free and viable in the body cavity for at least 2 weeks, but no further growth or development was observed.

The Philometridae, which have been found in a great variety of parenteral locations in aquatic hosts, have a life cycle essentially similar to that of *Dracunculus*. Thomas (1929) found that the first stage larvae of *Philometra nodulosa* are devoured by *Cyclops* and invade its body cavity. Attempts at infection

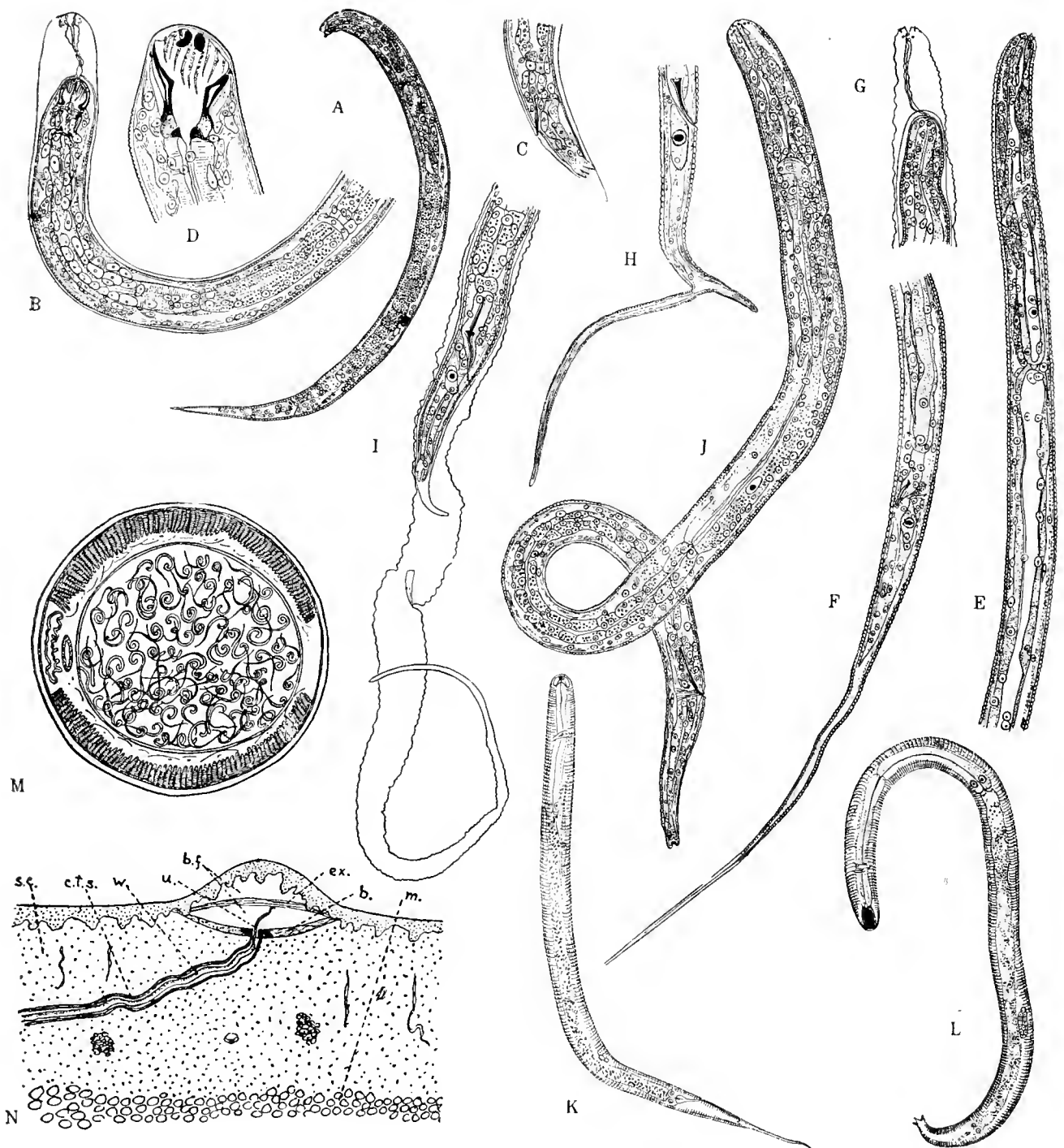


Fig. 197. DEVELOPMENT OF CAMALLANINA

A-D—*Camallonus sweeti* (A—First stage larva; B—Anterior end undergoing second molt; C—Posterior end undergoing second molt; D—Head, third stage). E-J—*Dracunculus oediniensis* (E—First stage, anterior end; F—Same, posterior end; G—Anterior end moulting larva; H—Posterior end; I—Posterior end of normal larva undergoing first molt; J—Normal third stage larva). K-L, *Procanallanus fulvidyaonis* (K—Larva, 6 days old; L—Larva, 14 days old). M—Cross-section of

guinea worm showing uterus filled with embryos, x about 30 (after Leuckart). N—Diagram of guinea worm in the skin at the time of blister formation. A-D, after Moorthy, 1938, *J. Parasit.*, v. 24 (4). E-J, after Moorthy, 1938, *Am. J. Hyg.*, v. 27 (2). K-L, after Li, 1935, *J. Parasit.*, v. 21 (2). M, N, after Chandler, 1940, *Introduction to Parasit.*



or fish from *Cyclops* a week after infection failed, presumably because of inadequate time for the larvae to reach the infective stage. Furnyama (1934) succeeded in completing the life cycle, in the case of *P. fujimotoi*, by feeding experimentally infected *Cyclops* to the definitive host. Young male and female worms were found in the body cavity, from whence the females subsequently migrated to their final habitat in the fins. In Philometridae the method of escape of the larvae is not as specialized as in the case of *Dracunculius*; the larvae of some species escape via the oviducts of the fish, while in the case of *P. fujimotoi* the ripe viviparous females leave the fins of their host, rupture, and liberate their larvae into the water. It is easy to see how the guinea worm life cycle could have evolved from the camallanoid type by the substitution of escape of embryos through the skin for escape via the anus, which would be very simple in the case of parasites which reproduced in peritrematid habitats in aquatic hosts.

## TRICHUROIDEA

The members of this superfamily, with the exception of *Trichinella* and *Cystoopsis* (see below), have a simple life cycle characterized by embryonation of eggs outside the body of the host; access to a new host by swallowing of eggs containing first stage larvae provided with an oral spear; and direct migration, via the blood stream if outside the alimentary canal, to the site of development, without preliminary development elsewhere in the body. The life cycle of *Capillaria columbae*, recently worked out in detail by Wehr (1939), will serve as an example of the typical Trichuroidea.

### CAPILLARIA COLUMBAE

The adults living in the small intestine are more or less imbedded in the mucosa, but the eggs make their way into the lumen and escape with the feces in an unsegmented state. Under favorable conditions of temperature, moisture, and oxygen segmentation occurs slowly, the first cleavage occurring in about 48 hours, the morula stage in about 3 days, and the infective first-stage larva in 6 to 8 days. No molting was observed to occur in the egg, and hatching does not normally take place before the egg is swallowed by a host. The entire development from newly hatched larvae to adult worms takes place in the small intestine of the definitive host.

The first-stage larva, like all other trichinoid larvae, has an oral spear. It has a long slender esophagus which posteriorly lies superficial to and only partly imbedded in the stichosome, which consist of two rows of opposing cells. The intestine is much shorter than the esophagus (ratio 1:3.5) and is terminated by a short rectum. The anus is subterminal.

The first molt occurs between 7 and 14 days after infection. The second-stage larvae are slenderer, and appear to have no oral spear; the stichosome consists of only a single row of cells, and the intestine is relatively longer. The second molt occurs about 14 days after infection. The third stage larvae are still slenderer, with relatively longer intestine, and the genital primordium is long. The third molt occurs between 14 and 19 days after infection. The fourth-stage larvae are very slender, and sexually differentiated. The time of the final molt was not determined, but some sexually mature adults with eggs were found by the 19th day.

### OTHER TRICHURIDAE

The available evidence indicates that the life cycle of *Trichouris* is essentially the same as that of *Capillaria columbae*, and it is probable that it is also the same for other species of *Capillaria* which inhabit the intestines of their hosts. The ability of *Capillaria* larvae to use transport hosts was shown by Wehr's (1936) demonstration that earthworms can serve as vectors for *C. anaalata*, the crop-worm of chickens. Fülleborn's (1923b) figures of *Trichouris trichiura* larvae are strikingly similar to Wehr's figures of the first stage larva of *Capillaria*. Although Neshi (1918, quoted by Yokogawa, 1920) reported the finding of four larvae of *Trichouris vulpis* in the lungs of a dog 21 hours after experimental infection, such migration on the part of *Trichouris* has not been observed by other workers either in normal or abnormal hosts (see Fülleborn, 1923a).

As Vogel (1930) pointed out, the entire group of Trichuroidea show a remarkable tendency to localization during their larval development in particular organs or tissues - what Vogel called "organotropism." In all cases except *Trichinella* this organotropism continues throughout the adult life of the worms. Different species of Trichuridae are known to develop and live as adults in the esophagus, stomach, small intestine, cecum, colon, respiratory tree, liver, spleen, urinary bladder, and epithelium. The available evidence indicates that the newly hatched larvae of those species which do not grow to maturity in the intestine itself reach their destination by burrowing into

the intestinal wall, entering the circulatory system, and escaping from the capillaries in the organ in which they are to develop. Good evidence for this has been obtained in the case of *Capillaria hepatica* of the liver of rats. Vogel (1930) showed that if young larvae of *C. hepatica*, recovered from the liver a few days after infection, were planted in the spleen, lungs, or under the skin, a few would succeed in reaching the liver. Normally this worm penetrates the cecum, sometimes as early as 6 hours after infection (Luttermoser, 1938b), and is carried directly to the liver via the hepatic portal system (Fülleborn, 1924; Nishigori, 1925), only an exceptional few penetrating into the abdominal cavity, or being carried beyond the liver to the lungs and systemic circulation. In the case of *Trichosomoides crassicauda* of the urinary bladder of rats, Yokogawa (1921) fed embryonated eggs to rats and 1 to 4 days later found a few larvae in the abdominal and pleural cavities and the lungs; these he thought were *Trichosomoides* larvae from his feeding, but their size makes it evident that they were not.

An unusual situation with respect to transfer of infection to new hosts exists in the case of *Capillaria hepatica*, which is suggestive of a possible step in the evolution of the *Trichinella* life cycle. The eggs of this worm are deposited in the liver tissues of rats or mice, and remain there in an early stage of development (one to four cells), viable for at least 7 or 8 months (Luttermoser, 1938a). Only exceptionally do any of the eggs escape from the liver to be voided with the feces, and eating of an infected liver by a susceptible animal cannot result in infection because the non-embryonated eggs are not infective. Momma (1930) suggested flies as a factor in disseminating the eggs from decaying carcasses, and also showed that eggs in the feces of cats that have fed on infected rats are viable. Troisier and Deschiens (1930) and Shorb (1931) independently suggested that the usual method of transmission in nature is by ingestion of eggs that have become embryonated after being freed from the liver of an infected animal, either by decomposition or by being eaten by another animal, usually the latter.

### TRICHINELLA SPIRALIS

The life cycle of this worm is unique among parasitic nematodes in that the period of waiting for a new host is passed in the parental host instead of in the open or in an intermediate host. The life cycle of *Capillaria hepatica*, described above, is a step in this direction, since in this case there are two periods of waiting, one in the liver tissue of the parental host, the other (the usual one) after embryonation in the open. In the case of *Trichinella* this double period is reduced to one by the complete elimination of the usual period of waiting outside the host, resulting from (1) precocious development to a burrowing larval stage in the uterus of the mother, and (2) consequent ability to infect the tissues of the parental host and to substitute development in this for the usual development in the open or in an alternate host.

The life cycle of this worm was one of the first to be worked out in its essential features, contributions having been made by Herbst, Küchenmeister, Leuckart, and Virchow from 1848 to 1860. The first entirely correct account of it was given by Leuckart (1860). The adult worms live in the small intestine. The females produce no egg shells, and the ova, unlike those of other Trichuroidea, develop precociously in the uterus, being born as active burrowing larvae, though in a very early stage of development, suggestive of microfilariae. There is an oral spear as in other members of the group, but the alimentary canal is rudimentary. This very immature larva enters the circulation, passing capillaries in both liver and lungs, and is distributed over the entire body. Presumably as the result of a special organotropism as suggested by Vogel (1930), the attraction in this particular case being the striated voluntary muscles, the larvae leave the capillaries and immediately penetrate through the sarcolemma into the interior of muscle cells, possibly by means of extra corporeal digestion. As to whether the larvae actually penetrated into the muscle cells has long been a matter of dispute, but seems finally to have been settled by Jensen and Roth (1938). Immediately after penetration, accomplished by a boring movement of the spear-bearing head end, the larva is seen lying lengthwise just under the sarcolemma, or between the sarcolemma and adjacent muscle cells. Jensen and Roth think it likely that a histolytic enzyme is also involved in the penetration of the muscle cells and in dissolving the fibrillae inside.

Once inside the cells the larvae come to rest and begin their growth and differentiation, the muscle substance meanwhile undergoing degenerative changes. By the 17th day, according to Jensen and Roth, the larva has grown from 100 to 400 or 500  $\mu$  in length and has its esophagus and intestine clearly differentiated. According to Stäubli, however, it may have increased its length 10 times, to 800 to 1,000  $\mu$ , in from 10 to 14 days. After 11 days it begins to roll up spirally in a spindle

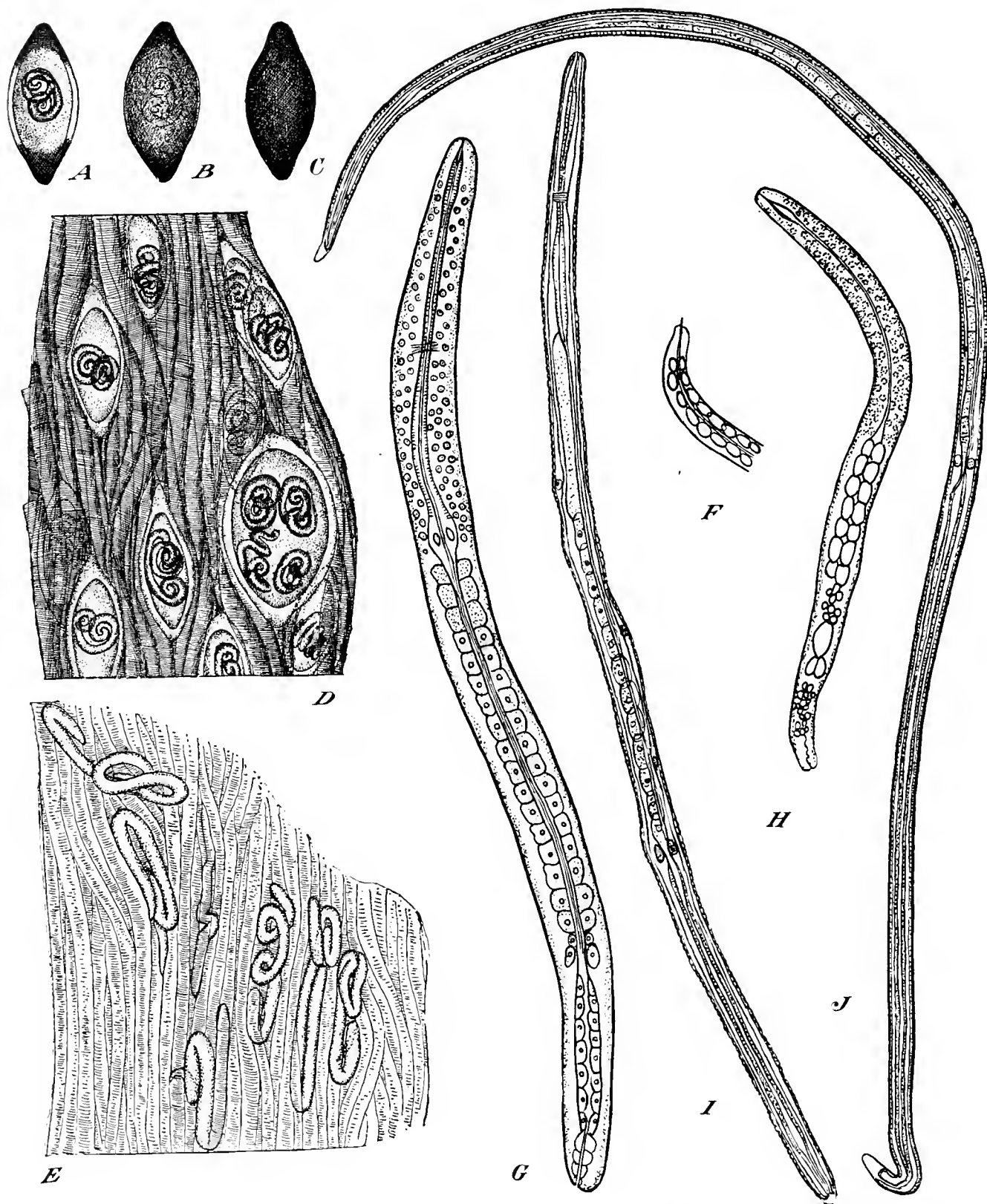


FIG. 198. DEVELOPMENT OF TRICHUROIDEA

A-C—Stages in calcification of *Trichinella* (A—Ends calcified; B—Thin layer of calcareous material over whole cyst; worm beginning to degenerate, C—Complete calcification). D—Larvae of *Trichinella spiralis*, encysted in striped muscle fibers in pork. Camera lucida drawing of cysts in infected sausage. E—Larvae of trichina worms burrowing in ham, flesh before encystment, from preparation from diaphragm

of victim of trichiniasis. F-J—*Capillaria columbae* (F—Anterior end of unhatched first stage larva; G—Late first stage larva from intestine of pigeon 7 days after infection; H—Embryo or unhatched first stage larva; I—Second stage larva, J—Third stage larva in molt. A-E, after Chandler, 1940. Introduction to Parasitology. F-J, after Wehr, 1939. U.S.D.A. Tech. Bull. 679

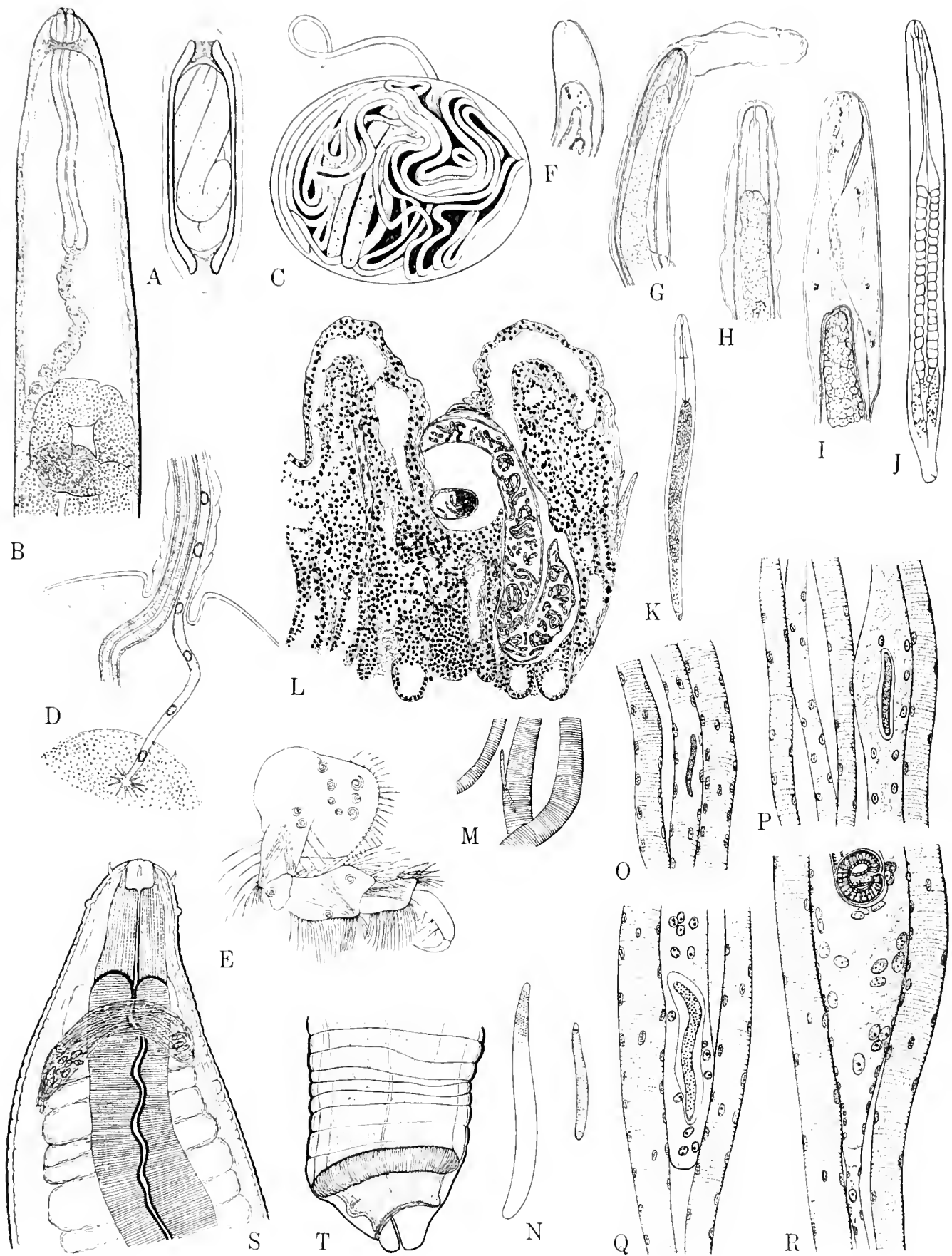


Fig. 199. TRICHUROIDEA AND DIOCTOPHYMATOIDEA

A-D—*Cystostephis acropensari* (A—Embryo; B—Head, male; C—Adult female; D—Connection of esophageal region and body proper of female.) E—*Cystostephis* larva encysted in appendage of *Gammareus platycheir*. F-I—*Trichinella spiralis* (F, Molt at fourth hour; G, Molt after 14 hours; H—Third molt of female after 48 hours; I—Molt after 70 hours). J-K—*Trichurus trichinra* (J—From the cocoon of a guinea pig; K—Larva pressed from egg). L-R—*Trichinella spiralis* (L—Section of

intestine showing female in tunica propria; M—Young larva entering muscle; N—Young larvae; O-R—Stages in encystment and calcification). S-T—*Eustrongylides* (S—Head; T—Tail). A-D, and E, after Janicki, C., and Rasini, F., 1930, Ztschr. Wiss. Zool., v. 136. F-I, after Kreis, H. A., 1937, Zentrabl. f. Bakt., v. 138. J-K, after Fuehlehorn, F., 1923, Arch. Schiff-u. Tropen, v. 27. L-R, after Staubli, in Handb. path. Mikroorg., v. 8. Remainder original.

shaped enlargement of the muscle fiber, and after 4 to 6 weeks becomes encapsulated. If not ingested by a host suitable for their future development, the larvae ultimately die and there is fatty degeneration and finally calcification of the cysts. Trichinae are said to remain alive and infective for as long as 11 years in the muscles of swine, and to have lived for 12 to 24 years in man, according to Baylis. Prenatal infection with trichinae has been demonstrated in guinea pigs by Roth (1936); Mauss got negative results in rats, rabbits and hamsters. In spite of the fact that the larva undergoes so much growth and differentiation Stäubli was unable to detect any evidences of molts, and the writer has seen no reports of any being seen by later observers. By analogy with other nematodes, however, it seems probable that the infective larvae have undergone at least two molts. Infection has not been obtained with larvae less than 19 days old and only after 21 days can one obtain a high percentage of infections. This seems to indicate that the larvae undergo a change, such as a molt, prior to that time.

When the larva has undergone its full development, whether encapsulated or not, it is infective when eaten by another animal. Development in the intestine is extremely rapid, sexual maturity being reached and copulation occurring on the third day, and embryo production beginning on the fifth day. According to Kreis (1937) there are four molts in this brief period, at about 4, 12, 48 and 70 hours after ingestion. However, his evidence is not very convincing. According to recent investigations one molt was obtained after ingestion and the cuticle of the resultant nema passed uninterrupted over the vulva, indicating that at least one more molt would be necessary before maturity.

It is evident from this account that *Trichinella spiralis* is not only unique among nematodes in utilizing the parental host as a sole resting place while awaiting an opportunity to gain access to another host by cannibalism (insofar as it passes from individual to individual of one species) but it is also unique among the Trichuroidea in having different "organotropisms" for the larval development and for the adult development, the former being the striated voluntary muscles, particularly the most active ones (pectoral and tongue), the latter the mucous membrane of the small intestine.

#### CYSTOÖPSIS ACIPENSERI

This is an aberrant worm with respect to both its morphology and its life cycle. The females with their large spherical bodies and the small cylindrical males live in pairs in cyst-like cavities just under the skin of young sturgeons. According to Janicki and Rasin (1930), a well-developed vulva and muscular vagina are present, but they seem to be used only for the entrance of sperms and not for the exit of eggs. The spherical body is filled with numerous coils of the uterus filled with embryonated eggs. These, according to the authors quoted, escape only by a bursting of the thin wall of the cyst and rupture of the parasite.

Experimentally the embryonated eggs are eaten by certain species of amphipods, and the larvae, liberated in the stomach, penetrate into the body cavity. The young larvae possess a mouth spear like other Trichuroidea, and are in a very early stage of development. At the end of about 2 weeks they have reached their full size, and then migrate into the appendages or into muscle layers. Here they coil up after the manner of *Trichinella* larvae and soon become encapsulated. The capsule thickens with time, and by the end of 3 months cannot be broken under a coverglass. No experiments have been performed to prove the infectiousness of the larvae encysted in *Gammarus*, but there seems to be no reasonable doubt but that young sturgeons are infected by eating amphipods, and that the young worms migrate through the tissues of the host to their location in the skin as do some species of Trichuridae.

#### DIOCTOPHYMATINA

The life cycles of members of this group are very imperfectly known. The available information concerning the genus *Eustrongylides* has been summarized by Cram (1927). Larvae described by Rudolphi as *Filaria cystica* from under the peritoneum and in the abdominal muscles of certain Brazilian fish were regarded by Jägerskiöld as belonging to this genus. Ciurea (1924) found similar larvae in other fish from the Danube, and he also regarded them as belonging to *Eustrongylides*. Larvae found in Brazilian fishes by Schneider and by Leuckart are stated by Jägerskiöld to resemble *E. ignotus* of water birds.

Chapin (1926) found the preadult stage of this species in *Fundulus diaphanus* at Washington, D. C. From one to three specimens were found in each fish, and adult characters could

be seen beneath the last cuticle, corresponding exactly to those of adult worms found by him in *Ardea herodias* from the same locality.

More recently Mueller (1934) reported similar larvae from *Fundulus*, in cysts attached to the mesenteries. They were 100 mm long by 0.685 mm in diameter, blood red in color, and the head was provided with 12 papillae, in 2 circles of 6 each, characteristic of the genus. Von Brand (1938) found a high percentage of *Fundulus* from Chesapeake Bay parasitized with this same larval form; individual fish harbored from 1 to 8 worms. Von Brand states that the encapsulated nematodes did not harm the host, but that after the host died they left their capsules and endeavored to escape from the dead host by burrowing through the tissues, eventually emerging through the gill region or body wall. He was able to keep the worms alive on sterile nutrient media for as long as 2 months, but there was no growth or development.

The larvae found by Ciurea are large, 28 to 70 mm long by 264 to 539  $\mu$  wide, and are rose-red or brown-red in color. On each side of the body near the anterior end is a row of small lateral papillae. The mouth aperture has the form of a cleft and has three small pointed papillae on each side of it, and three larger papillae just outside of these. The larvae have tails of two types, one enlarged near the end and regarded as that of the male, and the other rounded off without enlargement and regarded as that of the female. Whether the fish are first or second intermediate hosts is unknown. The thick-shelled eggs are undeveloped when they leave the body of the host.

Even less is known about the life cycle of *Dioctophyma renale*. The adults are usually found in the pelvis of the kidneys, particularly the right one, where they eventually destroy the entire parenchyma. Worms, often immature, are frequently found in other locations, particularly in the peritoneal cavity. The eggs, in an unsegmented condition, normally escape from the body with the urine. They develop slowly in water, requiring from 1 to 7 months for embryonation, according to the temperature, and then remain viable for at least 2 years, although they do not hatch. Beyond this point nothing is definitely known about the life cycle, but the frequency of infection in fish-eating animals makes its highly probable that fish serve as vectors for this worm as well as for *Eustrongylides*. Ciurea (1921) found a specimen 63 cm long in the peritoneal cavity of a dog fed, between 3 and 4 months previously, on 14 specimens of a cyprinid fish (*Idus idus*) from the Danube, but it is doubtful whether the worm was actually acquired from this feeding. Ciurea also found an active larva in the muscles of *Idus* which he thought might be that of *Dioctophyma*, but his figure and description are more suggestive of an ascarid larva. Swales (1933) reported *D. renale* as a very common and important parasite of mink in Canada, and stated that on mink farms the infection was definitely associated with the feeding of fish to these animals.

It has generally been assumed that *Dioctophyma*, after entering the alimentary canal of a definitive host, is carried via the blood stream to the kidneys as a young larva, there to undergo its growth to maturity. Its occurrence in the peritoneal cavity was thought to be accidental and rare, and due to rupture of the cyst-like remnant of the kidneys after the complete atrophy of its parenchyma. As a matter of fact, however, the worms are very frequently found in the abdominal cavity of dogs, in the majority of cases without evidence of damage to the kidneys. Wislocki (1919) found them in that location in every one of 12 dogs which he examined, and in only two cases could a portal of entry through a partially destroyed kidney be surmised. Brown, Sheldon and Taylor (1940) found 13 of 20 infected dogs in North Carolina with worms in the body cavity only, 6 had worms in the right kidney as well, and 1 had them only in the right kidney. Lukasiak (1930) called attention to the fact that in spite of numerous searches, especially in the kidneys, larvae have never been found, and relatively young forms have been found not in the kidneys but in the abdominal cavity, by preference between the lobes of the liver. Stefanski and Strankowski (1936) found a case in which a worm in the abdominal cavity was clearly in process of entering the right kidney; its anterior end was lodged in the tissue of the right kidney, the substance of which had not been destroyed. From this and similar cases which they quote from the literature, and from the other evidence cited above, these authors conclude that the larvae of the worm, travelling via the blood stream, stop in the liver, grow, and finally continue their development in the abdominal cavity, probably penetrating the kidney only after the final molt, and hollowing out a canal in the substance of this organ. Since the larvae are probably too large to enter capillaries, it seems to us more probable that the worms reach the body cavity by directly burrowing into it, as do *Gnathostoma* larvae; we know of no evidence that the liver is involved at all.



Fig. 200.

*Diotophyma renale* female anterior extremity of the parasite coiled in the pelvis of the right kidney. After Stefanski and Strankowski, 1936. *An. de Parasit. Humaine et Comp.*, v. 14 (1).

### Bibliography

- ABE, S. 1937. Development of the *Wuchereria bancrofti* in the body of the mosquito. [English summary.] *J. Med. Assoc., Formosa*, v. 36 (3): 516-519, 5 pls.
- AFRICA, C. M., REFUERZO, P. G. and GARCIA, E. Y. 1936a.—Observations on the life cycle of *Gnathostoma spinigerum*. *Philippine J. Sc.*, v. 59 (4): 513-521, 2 pls.
- 1936b.—Further observations on the life cycle of *Gnathostomum spinigerum*. *Philippine J. Sc.*, v. 61 (2): 221-225.
- ALICATA, J. C. 1934.—Observations on the period required for ascaris eggs to become infective. *Proc. Helmh. Soc. Wash.*, v. 1: 12.
- 1935.—Early developmental stages of nematodes occurring in swine. U. S. Dept. Agric., Tech. Bull., No. 489, 96 pp.
- 1937a.—The gizzard worm: and its transmission to chickens in Hawaii. *Hawaii Agric. Exper. Sta. Cir.*, No. 11, 7 pp.
- 1937b.—Larval development of the spirurid nematode, *Physaloptera turpida*, in the cockroach, *Blattella germanica*. *Papers on Helminthology, Jub. Skrjabin*, pp. 11-14.
- 1939.—Preliminary note on the life history of *Suhalaria brompti*, a common cecal nematode of poultry in Hawaii. *J. Parasit.*, v. 25: 179-180.
- ANNETT, H. E., DUTTON, J. E. and ELLIOTT, J. H. 1901. Report of the malaria expedition to Nigeria. Pt. II, Filariasis. *Liverpool Sch. Trop. Med., Mem.* 4.
- AUGUSTINE, D. L. 1923.—Investigations on the control of hookworm disease. XIX. Observations on the completion of the second oedysis of *Necator americanus*. *Am. J. Hyg.*, v. 3: 280-295.
- 1937.—Observations on living "sheathed" microfilariae in the capillary circulation. *Tr. Roy. Soc. Trop. Med. & Hyg.*, v. 31 (1): 55-60.
- BAHR, P. H. 1912. Filariasis and elephantiasis in Fiji. *Suppl. 1, J. Lond. Sch. Trop. Med.*, 192 pp., pls., maps.
- BAYLIS, H. A. 1916. Some ascarids in the British Museum (Natural History). *Parasit.*, v. 8 (3): 360-378, 4 pls.
- 1926.—Further experiments with the *Gongylonema* of cattle: II. *J. Trop. Med. & Hyg.*, v. 29 (20): 346-349.
- BEACH, T. D. 1935. The experimental propagation of *Strongyloides* in culture. *Proc. Soc. Exper. Biol. & Med.*, v. 32 (9): 1481-1486.
1936. Experimental studies on human and primate species of *Strongyloides*. V. The free living phase of the life cycle. *Am. J. Hyg.*, v. 23 (2): 243-277.
- BRACKETT, S. 1938. Description and life history of the nematode *Draconculus aphidensis* n. sp., with a re-description of the genus. *J. Parasit.*, v. 24 (4): 353-361.
- BRUNS, M. 1899. Bemerkungen über den "sporadischen Fall von *Anguillula intestinalis* in Ostpreussen." *Centralbl. Bakt. [etc.] Abt. 1, Orig.*, v. 26 (20-21): 612-615, 1 pl.
- BRUNI, A. 1921. Preliminary note on the development of the larvae of *Dirofilaria immitis* in dog fleas, *Ctenocephalus felis* and *canis*. *Ann. Trop. Med. & Parasit.*, v. 14 (3): 389-392.
- BROWN, H. W., SHELDON, A. J. and TAYLOR, W. W., JR. 1940. The occurrence of *Dioctophyme renale* in dogs of North Carolina [Abstract]. *J. Parasit.*, v. 26 (6) (Suppl.): 16.
- BRUMPT, E. 1921. Recherches sur la détermination des sexes et de l'évolution de anguillules parasites. *Compt. Rend. Soc. Biol. Paris*, v. 85 (23): 149-152.
- BUCKLEY, J. J. C. 1934.—On *Syngamus iveri* sp. nov. from domestic cats, with some observations on its life cycle. *J. Helmh.*, v. 12 (2): 89-98.
- CAMERON, T. W. M. 1923.—On the biology of the infective larva of *Monodontus trigonoccephalus* (Rud.) of sheep. *J. Helmh.*, v. 1: 205-214.
- 1927a.—Observations on the life history of *Aelurostrongylus abstrusus* (Railliet), the lungworm of the cat. *Ibid.*, v. 5: 55-66.
- 1927b.—Observations on the life history of *Ollulanus tricuspis* Leuck., the stomach worm of the cat. *Ibid.*, v. 5 (2): 67-80.
- CHANDLER, A. C. 1918.—Animal parasites and human disease, 1st ed., p. 275. New York.
- 1925.—The migration of hookworm larvae in soil. *Indian Med. Gaz.*, v. 60 (3): 105-108.
- 1925b.—A contribution to the life history of a gnathostome. *Parasit.*, v. 17 (3): 237-244.
- 1925c.—The helminthic parasites of cats in Calcutta and the relation of cats to human helminthic infections. *Indian J. Med. Res.*, v. 13 (2): 213-227, 2 pls.
- 1938.—*Diploscapter coronata* as a facultative parasite of man, with a general review of vertebrate parasitism by rhabditoid worms. *Parasit.*, v. 30 (1): 44-55.
- CHAPIN, E. A. 1926.—*Eustrongylides ignotus* in the United States. *J. Parasit.*, v. 13: 86-87.
- CHITWOOD, B. G. 1933.—On some nematodes of the superfamily Rhabditoidea and their status as parasites of reptiles and amphibians. *J. Wash. Acad. Sc.*, v. 23: 508-520.
- CHITWOOD, B. G. and GRAHAM, G. L. 1940.—Absence of vitelline membranes on developing eggs in parasitic females of *Strongyloides ratti*. *J. Parasit.*, v. 26 (3): 183-190.
- CHU, T. 1936a.—A review of the status of the reptilian nematodes of the genus *Rhabdias* with a re-description of *Rhabdias fuscorufosa* var. *catanensis* (Rizzo, 1902) new rank. *J. Parasit.*, v. 22: 130-139.
- 1936b.—Studies on the life history of *Rhabdias fuscorufosa* var. *catanensis* (Rizzo, 1902). *Ibid.*, v. 22: 140-160.
- CIUREA, J. 1921.—Sur la source d'infestation par l'eustrongyle géant (*Eustrongylus gigas* Rud.) *Comp. Rend. Soc. Biol. Paris*, v. 85 (27): 532-534.
- 1924.—Die *Eustrongylides* Larven bei Donaufischen. *Ztschr. Fleisch. u. Milchhyg.*, v. 34 (13) 134-137.
- CLAPHAM, P. A. 1934. Experimental studies on the transmission of gapeworm (*Syngamus trachea*) by earthworms. *Proc. Roy. Soc. Lond., Ser. B*, v. 115: 18-29.
- 1939a.—On flies as intermediate hosts of *S. trachea*. *J. Helmh.*, v. 17 (2): 61-64.
- 1939b.—Three new intermediary vectors for *Syngamus trachea*. *J. Helmh.*, v. 17 (4): 191-192.
- CONNAL, A. and CONNAL, S. L. M. 1922.—The development of *Loa loa* (Guyot) in *Chrysops silacea* (Austen) and in *Chrysops dimidiata* (van der Wulp.). *Tr. Roy. Soc. Trop. Med. & Hyg.*, v. 16: 64-89.

- CORT, W. W. 1925.—Investigations on the control of hookworm disease. XXXIV. General summary of results. *Am. J. Hyg.*, v. 5 (1): 49-89.
- CRAM, E. B. 1927.—Bird parasites of the nematode suborders Strongylata, Ascaridata, and Spirurata. *U. S. Natl. Mus., Bull. No. 140*, 465 pp.  
1931.—Developmental stages of some nematodes of the Spiruroidea parasitic in poultry and game birds. *U. S. Dept. Agric. Tech. Bull. No. 227*.
- CUVILLIER, E. 1937.—The nematode, *Ornithostrongylus quad-riradiatus*, a parasite of the domesticated pigeon. *U. S. Dept. Agric. Tech. Bull. No. 569*.
- DARLING, S. T. 1911.—Strongyloides infections in man and animals in the Isthmian Canal Zone. *J. Exper. Med.*, v. 14 (1): 1-24.
- DE BLIECK, L. and BAUDET, E. A. R. F. 1926.—Contribution a l'étude du développement des strongylidés (selérostomes) du gros intestin chez le cheval. *Ann. Parasit.*, v. 4 (1): 87-96.
- FAUST, E. C. 1927.—Migration route of *Spirocerca sanguinolenta* in its definitive host. *Proc. Soc. Exper. Biol. & Med.*, v. 25: 192-195.  
1931.—Human strongyloidiasis in Panama. *Am. J. Hyg.*, v. 14: 203-211.  
1933.—Experimental studies on human and primate species of *Strongyloides*. II. The development of *Strongyloides* in the experimental host. *Am. J. Hyg.*, v. 18 (1): 114-132.
- FAUST, E. C. and KAGY, E. S. 1933.—Experimental studies on human and primate species of *Strongyloides*. I. The variability and instability of types. *Am. J. Trop. Med.*, v. 13: 47-65.
- FENG, L. C. 1936.—The development of *Microfilaria malayi* in *A. hyrcanus* var. *sinensis* Wied. *Chinese Med. J., Suppl. I*, pp. 345-367.
- FIELDING, J. W. 1926.—Preliminary note on the transmission of the eyeworm of Australian poultry. *Austral. J. Exper. Biol. & Med.*, v. 3: 225-232.
- FOSTER, A. O. and CROSS, S. X. 1934.—The direct development of hookworms after oral infection. *Am. J. Trop. Med.*, v. 14 (6): 565-573.
- FREEBORN, S. B. 1923.—The control of the suckered roundworms of poultry. *Cornell Vet.*, v. 13: 223-231.
- FUELLEBORN, F. 1908.—Ueber Versuche an Hundefilarien und deren Uebertragung durch Mücken. *Arch. Schiffs- u. Tropen-Hyg., Beihefte 8*, 43 pp.  
1914.—Untersuchungen ueber den Infektionsweg bei *Strongyloides* und *Ankylostoma* und die Biologie dieser Parasiten. *Beihefte 5*, *Arch. Schiffs- u. Tropen Hyg.*, v. 18: 26-80 (182-236), figs. 1-14, pls. 1-7.  
1920a.—Perkutane Infektion bei *Angiostomum nigrovenosum*. *Ibid.*, v. 24: 176.  
1920b.—Ueber die Anpassung der Nematoden an den Parasitismus und den Infektionsweg bei Ascaris und anderen Fadenwürmern des Menschen. *Ibid.*, v. 24: 340-347.  
Fadenwürmern des Menschen. *Ibid.*, v. 24: 340-347.  
1921a.—Askarisinfektion durch Verzehren eingekapselter Larven und über gelungene intrauterine Askarisinfektion. *Ibid.*, v. 25: 367-375.  
1921b.—Ueber die Wanderung von Ascaris und anderen Nematoden-larven in Körper und intrauterine Askarisinfektion. *Ibid.*, v. 25: 146-149.  
1923a.—Ueber die Entwicklung von Trichocephalus im Wirt. *Ibid.*, v. 27: 413-420.  
1923b.—Ueber den "Mundstachel" der Trichotracheiden-larven und Bemerkungen über die jüngsten Stadien von *Trichocephalus trichinurus*. *Ibid.*, v. 27: 421-425, 1 pl.  
1924.—Ueber den Infektionsweg bei *Hepaticola hepatica*. *Ibid.*, v. 28: 48-61.  
1925.—Ueber die Durchlässigkeit der Blutcapillaren für Nematodenlarven. *Ibid.*, v. 29, Beiheft 3, 100 pp. 4 pls.  
1926.—Ueber das Verhalten der Hakenwurmlarven bei der Infektion per os. *Ibid.*, v. 30: 638-653.  
1927.—Ueber das Verhalten der Larven in Körper des Wirtes. *Ibid.*, Beihefte 2, v. 31, 56 pp., figs. 1-5.  
1928.—Ueber den Infektionsweg bei *Rhabdias bufonis* (*Rhabdonema nigrovenosum*) des Frosches nebst Versuchen ueber die Lymphzirkulation des letzteren. *Centralbl. Bakt. [etc.]*, Abt. I, Orig., v. 109: 444-462, 1 pl.
- 1929a.—On the larval migration of some parasitic nematodes in the body of the host and its biological significance. *J. Helm.*, v. 7: 15-26.  
1929b.—Filariosen des Menschen. *In Handb. Path. Mikroögg.*, 6, Lief. 28, 1043-1224, 3 pls.
- FURUYAMA, T. 1934.—On the morphology and life history of *Philometra fujimotoi* Furuyama, 1932. *Keijo J. Med.*, v. 5 (3): 165-177.
- GOODEY, T. 1922.—Observations on unsheathed larvae of nematodes. *Ann Applied Biol.*, v. 9: 33.  
1923.—Experiment on the feeding of embryonated eggs of *Ascaris megalocephala* to domesticated animals. *Ann. Appl. Biol.*, v. 10: 116-121.  
1924a.—The anatomy of *Oesophagostomum dentatum* (Rud.) a nematode parasite of the pig, with observations on the structure and biology of the free-living larvae. *J. Helm.*, v. 2: 1-14.  
1924b.—The anatomy and life history of the nematode *Rhabdias fuscovirvosa* (Railliet) from the grass snake *Tropidonotus natrix*. *Ibid.*, v. 2 (2): 51-64.
- GOODEY, T. and CAMERON, T. W. M. 1923.—Observations on the morphology and life cycle of *Ascaris columbiana* Leidy, a nematode parasite of the skunk. *J. Helm.*, v. 1: 1-8.
- GRAHAM, G. L. 1936.—Studies on *Strongyloides*. I. *S. ratti* in parasitic series, each generation in the rat established with a single homogonic larva. *Am. J. Hyg.*, v. 24 (1): 71-87.  
1938.—Idem. II. Homogonic and heterogonic progeny of the single homogonically derived *S. ratti* parasite. *Ibid.*, v. 27 (2): 221-234.  
1939a.—Idem. IV. Seasonal variation in the production of heterogonic progeny by singly established *S. ratti* from a homogonically derived line. *Ibid.*, v. 30 (Sect. D.), 15-27.  
1939b.—Idem. V. Constitutional differences between a homogonic and a heterogonic line of *S. ratti*. *J. Parasit.*, v. 25 (4): 365-375.
- GUBERLET, J. E. 1924.—Note on the life history of *Ascaridia perspicillum*. *Tr. Am. Micr. Soc.*, v. 43: 152-156.
- HARWOOD, P. D. 1930.—A new species of *Oryzomatium* (Nematoda) with some remarks on the genera *Oryzomatium* and *Aplectana* and observations on the life history. *J. Parasit.*, v. 17: 61-73.  
1932.—A note on the tissue-penetrating abilities of sheathed microfilariae. *Tr. Am. Micr. Soc.*, v. 51 (2): 153-154.
- HELLER, A. 1903.—Ueber *Oxyuris vermicularis*. *Deutsch Arch. Klin. Med.*, Berlin, v. 77, (1-2): 21-28, pls. 1-3.
- HERRICK, C. A. 1928.—A quantitative study of infections with *Ancylostoma caninum* in dogs. *Am. J. Hyg.*, v. 8: 125-157.
- HERRICK, L. R. 1935.—The life history and morphology of *Spiroxyis cantortus* (Rudolphi); Nematoda: Spiruridae. *Tr. Am. Micr. Soc.*, v. 54 (4): 307-335.
- HOEBAIER, M. 1930.—Life history of *Probostrongylus* (*Syn-thetocaulis rufescens*). *Proc. Soc. Exper. Biol. & Med.*, v. 28: 156-158.  
1934a.—Lungenwurmlarven in Mollusken. *Ztschr. Parasitenk.*, v. 6: 642-648.  
1934b.—*Elaphostrongylus odocoilei* n. sp., a new lungworm in black-tail deer (*Odocoileus columbianus*). Description and life history. *Proc. Soc. Exper. Biol. & Med.*, v. 31: 509-514.  
1935.—Intermediate hosts of *Aclurostrongylus abstrusus* of the cat. *Ibid.*, v. 32: 1641-1647.
- HOEBAIER, A. and HOEBAIER, M. 1929.—Die Entwicklung der Larve des Lungenwurmes *Metastrongylus elongatus* (*Strongylus paradoxus*) des Schweines und ihr Invasionsweg, sowie vorläufige Mitteilung über die Entwicklung von *Chorostrongylus brevicaudatus*. *München. Tierärztl. Wochenschr.*, v. 80: 365-369, illus.
- HU, C. H. and HOEPLI, R. J. C. 1936.—The migration route of *Spirocerca sanguinolenta* in experimentally infected dogs. *Chinese Med. J., Suppl. I*, pp. 293-311.
- HSÛ, H. F. and CHOW, C. Y.—On the intermediate host and larva of *Habronema mansoni* Scurat, 1914 (Nematoda). *Chinese Med. J., Suppl. II*, pp. 419-422.

- IBLE, J. E. W. and v. OORDT, G. J. 1924. On the development of the larva of the fourth stage of *Strongylus vulgaris* (Looss). Proc. K. Akad. Wetensch. Amsterdam, v. 27 (3-4): 194-200.
- IFAGAKI, S., 1927. On the life history of the chicken nematode, *Ascaridia perspicillum*. Rpt. Proc. 3rd World's Poultry Cong., Ottawa, pp. 339-344, Figs. 1-22.
- JANICKI, C. and RASIN, K. 1930. Bemerkungen über *Cysto-opsis acipenseris* des Wolga-Sterlets, sowie über die Entwicklung dieses Nematoden im Zwischenwirt. Ztschr. Wiss. Zool., v. 136: 1-37.
- JENSEN, V. and ROTH, H. 1938. Zur einwanderung der Trichinellarlarve in die Quergestreifte Muskelfaser. Acta Path. & Microbiol. Scand., Suppl., v. 37: 259-268, 6 abb.
- JERKE, H. W. M. 1902. Eine parasitische Anguillula des Pferdes. Arch. Wiss. u. Prakt. Tierheilk., v. 29 (1-2): 113-127, 1 pl.
- JONES, M. F. and JACOBS, I. 1939. Studies on the survival of eggs of *Enterobius vermicularis* under known conditions of humidity and temperature [Abstract]. J. Parasit., v. 25 (6), Suppl., p. 32.
- KAHL, W. 1936. Beitrag zur Kenntnis des Nematoden *Contracaecum claratum* Rud. Ztschr. Parasitenk., v. 8: 509-520.
- KOBAYASHI H. 1928. On the life history of *Oxyuris mansoni* and the pathological changes in the conjunctiva and the ductus lacrymalis caused by this worm, with further observations on the structure of the adult worm. [In Japanese]. 50 pp., 8 figs., pls. 1-2, figs. 1-24; English summary, pp. 1-6. Reprinted from J. Formosa Med. Soc., Taiwan Igakukai Tasshi, (280), July, 1928.
- KOCH, E. W., 1925. Oxyurenfortpflanzung im Darm ohne Reinfektion und Magenpassage. Centralbl. Bakt. [etc.], Abt. I, Orig., v. 94: 208-236.
- KOURI, P., BASNUEVO, J. G. and ARENAS, R. 1936. Contribución al conocimiento del ciclo evolutivo del *Strongyloides stercoralis*. Nota previa. Rev. Parasit., Clin. & Lab., v. 2: 1-6.
- KREIS, H. A. 1932. Studies on the genus *Strongyloides* (Nematodes) Am. J. Hyg., v. 11 (2): 450-491.  
1937. Die Entwicklung der Trichinellen zum reifen geschlechtstier im Darne des Wirtes. Zentralbl. Bakt. [etc.], Abt. I, Orig., v. 138: 290-302.
- LANE, C. 1929. The mechanism of filarial periodicity. Lancet, Lond., (5521), v. 216, v. 1 (25): 1291-1293, 1 fig.
- LEICHTENSTERN, O. 1886. Fütterungsversuche mit *Ankylostoma-Larven*. Eine neue *Rhabditis*-Art in den Fäces vom Ziegelerbeitern. Centralbl. Klin. Med., v. 8 (39): 673-675.  
1899. Zur Lebensgeschichte der *Anguillula intestinalis*. Centralbl. Bakt. [etc.], Abt. I, Orig., v. 25: 226-231.  
1905. Studien über *Strongyloides stercoralis* (Bayan). Arb. K. Gesundheitsamte., Berlin, v. 22 (2): 309-350.
- LENTZE, F. A., 1935. Zur Biologie des *Oxyuris vermicularis*. Centralbl. Bakt. [etc.], Abt. I, Orig., v. 135: 156-159.
- LEUCKART, R. 1860. Untersuchungen über *Trichina spiralis*. Zugleich ein Beitrag zur Kenntnis der Wurmkrankheiten. 57 pp., 2 pls., Leipz. & Heidelberg.  
1866. Zur Entwicklungsgeschichte der Nematoden [In his Helminthologische Mitteilungen]. Arch. Ver. Wiss. Heilkunde, Leipz., (n. F.), v. 2: 195-235.  
[1883]. Ueber die Lebensgeschichte der sog. *Anguillula stercoralis* und deren Beziehungen zu der sog. *Ang. intestinalis*. Ber. Verhandl. K. Sächs. Gesellsch. Wiss. Leipz., Math. Phys. Cl. (1882), v. 34: 85-107.
- LI, H. C.—The taxonomy and early development of *Procamallanus fulvicaecus* n. sp., J. Parasit., v. 21 (2): 103-113.
- LOOSS, A. 1898. Zur Lebensgeschichte des *Ankylostoma duodenale*. Centralbl. Bakt. [etc.], Abt. I, Orig., v. 24: 483-488.  
1905. Die Wanderung der *Ancylostomum*- und *Strongyloides*-Larven von der Haut nach dem Darm. Compt. Rend. 6. Cong. Internat. Zool., Geneva, pp. 225-233.  
1911. The anatomy and life history of *Ancylostoma duodenale* Dub. A monograph, Part 2. The development in the free state. Rec. Egypt. Govt. Sch. Med., v. 4: 159-613.
- LOW, G. C. 1900. A recent observation on *Filaria nocturna* in *Culex*, probable mode of infection in man. Brit. Med. J., v. 1: 1156-1157.
- LÜCKER, J. T. 1934a. Development of the swine nematode *Strongyloides ransumi* and the behavior of its infective larvae. U. S. Dept. Agric. Tech. Bull. No. 437, 30 pp.  
1934b. The morphology and development of the pre-parasitic larvae of *Posteriostrongylus ratzii*. J. Wash. Acad. Sc., v. 24: 302-310.  
1936. Preparasitic molts in *Xippostrongylus muris*, with remarks on the structure of the cuticula of *Trichostrongyles*. Parasit., v. 28 (2): 161-171.  
1938. Description and differentiation of infective larvae of three species of horse strongyles. Proc. Helmh. Soc. Wash., v. 5 (1): 1-5.
- LUKASIAK, J. 1930. Anatomische und entwicklungsgeschichtliche Untersuchungen an *Diocetophymi ronale* (Goeze, 1782). Arch. Nauk. Biol. Towarzyst. Nauk. Warszawsk., (1929), v. 3 (3): 100 pp., figs. 1-6, pls. 1-6.
- LUTERMOSER, G. W. 1938a. Factors influencing the development and viability of the eggs of *Capillaria hepatica*. Am. J. Hyg., v. 27 (2): 275-289.  
1938b. An experimental study of *Capillaria hepatica* in the rat and the mouse. Ibid., v. 27 (2): 321-340.
- MACARTHUR, W. P. 1930. Threadworms and pruritis ani. J. Roy. Army Med. Corps, v. 55: 214-216.
- MCCOY, O. R. 1929. The growth of hookworm larvae on pure cultures of bacteria. Science, v. 69 (1777): 74-75.
- MCINTOSH, A. and CHITWOOD, B. G. 1934. A new nematode *Longibucca lasiura*, n. sp. (Rhabditoidea, Cylihydrogastridae) from a bat. Parasit., v. 26: 138-140.
- MANSON, P. 1878. On the development of *Filaria sanguinis hominis*, and on the mosquito considered as a nurse. J. Linn. Soc. Lond., Zool., (75), v. 14: 304-311.
- MARKOWSKI, S. 1937. Ueber die Entwicklungsgeschichte und Biologie des Nematoden *Contracaecum aduncum* (Rudolphi, 1802). Bull. Internat. Acad. Polon. Sc. e Lett., Ser. B. Sc. Nat. (11), pp. 227-242, 2 pls.
- MAUPAS, E. and SEURAT, L. G. 1913. La Mue et l'enkystement chez les Strongles du tube digestif. Compt. Rend. Soc. Biol. Paris, v. 74: 34-48.
- MECZKOW, E. 1865. Ueber die Entwicklung von *Ascaris nigrovirens*. Arch. Anat., Physiol. & Wiss. Med., Leipz., pp. 409-420, pl. 10, figs. 1-11.
- MİYAGAWA, Y. 1916. Ueber den Wanderungsweg der *Ankylostoma duodenale* innerhalb des Wirtes bei Oralinfektion und über ihren Hauptinfektionsmodus. Mitt. Med. Fakult. K. Univ., Tokyo., v. 15: 411-452.
- MİYAGAWA, Y. and OKADA, R. 1930. Biological significance of the lung journey of *Ancylostoma* larvae in the normal host. First Report. Jap. J. Exper. Med., v. 8: 285-308, 1931.—Idem, Second Report. Ibid., v. 9: 151-207.
- MOMMA, K. 1930. Notes on modes of rat infestation with *Hepaticola hepatica*. Ann. Trop. Med. & Parasit., v. 24 (1): 109-113.
- MÖNNIG, H. O. 1930. Studies on the bionomics of the free-living stages of *Trichostrongylus* spp. and other parasitic nematodes. Union So. Africa Dept. Agric., 16th Rpt. Dir. Vet. Service, pp. 175-198.
- MOORTHY, V. N. 1938. Observations on the development of *Draconulus medincensis* larvae in Cyclops. Am. J. Hyg., v. 27 (2): 437-469.  
1938b. Observations on the life history of *Camallanus suctus*. J. Parasit., v. 24 (4): 323-342.
- MOORTHY, V. N. and SWEET, W. C. 1938. Further notes on the experimental infection of dogs with draconiasis. Am. J. Hyg., v. 27 (2): 301-310.
- MORGAN, D. C. 1928. *Parastrongyloides wuchesi* gen. et sp. nov. A remarkable new nematode parasite of the mole and the shrew. J. Helmh., v. 6: 79-86, 4 figs.
- MUELLER, J. F. 1934. Additional studies on parasites of Oneida Lake fishes including descriptions of new species. Bull. N. Y. State Coll. Forestry, v. 7: 335-373.

- NESHI, G. 1918.—Ueber die Entwicklung des *Trichocephalus* innerhalb des Wirtes. (Japanese). Tokyo. Med. Wochenschr., No. 2980.
- NÉVEU-LEMAIRE, M. 1936.—Traité d'helminthologie médicale et vétérinaire, xxiii + 1514 pp., Vigot Frères, Paris.
- NISHIGORI, M. 1925.—On the life history of *Hepaticola hepatica*. Second Report, J. Formosa Med. Soc., v. 247: 3-4.  
1928.—The factors which influence the external development of *Strongyloides stercoralis* and on auto-infection with this parasite. *Ibid.*, v. 277: 1-56.
- NOE, G. 1908.—Il ciclo evolutivo della *Filaria grassii*, mihi, 1907. *Atti R. Accad. Lincei, Roma, Rendic. Cl. Sc. Fis., Math. & Nat.*, an. 305, 5. s., v. 17 (5), 1. semester: 282-293, figs. 1-4.
- NOLAN, M. O. and REARDON, L. 1935.—Studies on oxyuriasis, XX. The distribution of the ova of *Enterobius vermicularis* in household dust. *J. Parasit.*, v. 25: 173-177.
- O'CONNOR, F. W. 1931.—Filarial periodicity with observations on the mechanism of the migration of the microfilariae from the parent worm to the blood stream. *Puerto Rico J. Pub. Health & Trop. Med.*, v. 6: 263-272.
- OKADA, R. 1931.—Experimental studies on the oral and percutaneous infection of *Anchylostoma caninum* (Four Reports), *Jap. J. Exper. Med.*, v. 9: 209-280.
- OLT, A. 1932.—Das Aneurysma verminosum des Pferdes und seine unbekannt Beziehungen zur Kolik. *Deutsch. Tierärztl. Wochenschr.*, v. 40 (21): 326-332, figs. 1-3.
- ORTLEPP, R. J. 1922.—On the hatching and migration in a mammalian host of larvae of ascarids normally parasitic in cold blooded vertebrates. *J. Trop. Med. & Hyg.*, v. 25: 97-100.  
1923.—The life history of *Syngamus trachea* (Montagu) v. Siebold, the gapeworm of chickens. *J. Helm.*, v. 1 (3): 119-140.  
1925.—Observations on the life history of *Triodontophorus tenuicollis*, a nematode parasite of the horse. *Ibid.*, v. 3: 1-14.  
1937.—Observations on the morphology and life history of *Gaigeria pachyseclis* Raill. and Henry, 1910: a hookworm parasite of sheep and goats. *Onderstepoort J. Vet. Sc. & Anim. Indus.*, v. 8 (1): 183-212.
- PAVLOV, P. 1937.—Recherches expérimentales sur le cycle évolutif de *Synthetocaulus capillaris*. *Ann. Parasit.*, v. 15: 500-503, pl. 14.
- PEREIRA, C. V., VIANNA DIAS, M. and DE AZEVEDO, P. 1936.—Biologia do nematoide *Procamallanus caracensis* n. sp. (English summary, p. 225). *Arch. Inst. Biol., Sao Paulo*, v. 7: 209-226.
- PHILPOT, F. 1924.—Notes on the eggs and early development of some species of Oxyurides. *J. Helm.*, v. 2: 239-252.
- PROMMAS, C. and DAENGSVANG, S. 1933.—Preliminary report of a study on the life cycle of *Gnathostoma spinigerum*. *J. Parasit.*, v. 19 (4): 287-292.  
1936.—Further report of a study on the life cycle of *Gnathostoma spinigerum*. *J. Parasit.*, v. 22 (2): 180-186.  
1937.—Feeding experiments on cats with *Gnathostoma spinigerum* larvae obtained from the second intermediate host. *Ibid.*, v. 23 (1): 115-116.
- RAILLIET, A. 1859.—Evolution sans hétérogonie d'un angiotome de la couleuvre à collier. *Compt. Rend. Acad. Sc., Paris*, v. 129 (26): 1271-1273.
- RANSOM, B. II. 1906.—The life history of the twisted wire worm (*Haemonchus contortus*) of sheep and other ruminants. *Bur. Anim. Indus., U. S. Dept. Agric., Cir. No. 93*.  
1907.—*Probstmayria vivipara* (Probstmayr, 1865), Ransom, 1907, a nematode of horses heretofore unreported from the United States. *Tr. Am. Mier. Soc.*, v. 27: 33-40.  
1911.—The nematode parasites in the alimentary tract of cattle, sheep and other ruminants. *Bur. Anim. Indus., U. S. Dept. Agric., Bull. No. 127*.
- RANSOM, B. II., and CRAM, E. B. 1921.—The course of migration of *Ascaris* larvae. *Am. J. Trop. Med.*, v. 1: 129-156, 2 pls.
- RANSOM, B. II. and FOSTER, W. D. 1917.—Life history of *Ascaris lumbricoides* and related forms. *J. Agric. Res.*, v. 11: 395-398.  
1920.—Observations on the life history of *Ascaris lumbricoides*. *U. S. Dept. Agric., Bull. No. 817*, 47 pp.
- RANSOM, B. II. and HALL, M. C. 1916.—The life history of *Gongylonema scutatum*. *J. Parasit.*, v. 2: 80-86.
- REFUERZO, P. G. and GARCIA, E. Y. 1938.—The crustacean intermediate hosts of *Gnathostomum spinigerum* in the Philippines and its pre- and inter-crustacean development. *Philippine J. Anim. Indus.*, v. 5 (4): 351-362, 5 pls.
- ROUBAUD, E. and DESCARZEUX, J. 1921.—Contributions à l'histoire de la mouche domestique comme agent vecteur des habronémoses des équidés. Cycle évolutif et parasitisme de l'*Habronema megastoma* (Rudolphi, 1819) chez la mouche. *Bull. Soc. Path. Exot.*, v. 14: 471-506.  
1922.—Evolution de l'*Habronema muscae* Carter chez la mouche domestique et de l'*H. microstomum* Schneider chez le stomoxe. Note préliminaire. *Ibid.*, v. 15: 572-574.  
1922.—Évolution de l'*Habronema muscae* Carter chez leurs rapports avec l'évolution des Habronèmes d'équidés. *Ibid.*, v. 15: 978-1001.
- ROBERTS, F. II. S. 1934.—The large roundworm of pigs, *Ascaris lumbricoides* L. 1758. *Anim. Health Sta., Queensland Dept. Agric. & Stock, Bull. No. 1*, 81 pp., 11 figs., 3 pls.  
1937.—Studies on the biology and control of the large roundworm of fowls, *Ascaridia galli* (Schrank, 1788). *Ibid.*, Bull. No. 2, 106 pp., 7 pls.
- ROSS, I. C. and KAUFAL, G. 1932.—The life cycle of *Stephanurus dentatus* Diesing, 1839; the kidney-worm of pigs; with observations on its economic importance in Australia and suggestions for its control. *Austral. Council Sc. & Indus. Res., Bull. No. 58*, 80 pp., illus.
- SANDGROUND, J. II. 1926.—Biological studies on the life cycle in the genus *Strongyloides*, Grassi, 1879. *Am. J. Hyg.*, v. 6 (3): 337-388.  
1939.—*Cephalobus parasiticus* n. sp. and pseudostrongyloidiasis in *Macaca irus mordax*. *Parasit.*, v. 31 (1): 132-137.
- SCHWARTZ, B. 1922.—Observations on the life cycle of *Ascaris vitulorum*, a parasite of bovines in the Philippine Islands. Preliminary Paper. *Philippine J. Sc.*, v. 20 (6): 661-669, 1 pl.  
1925a.—Two new larval nematodes belonging to the genus *Ferrocaecum* from mammals of the order Insectivora. *Proc. U. S. Natl. Mus.*, v. 67, Art. 17, 8 pp., 1 pl.  
1925b.—Preparasitic stages in the life history of the cattle hookworm (*Bunostomum phlebotomum*). *J. Agric. Res.*, v. 29: 451-458.  
1931.—Nodular worm infestation of domestic swine. *Vet. Med.*, v. 26: 411-415.
- SCHWARTZ, B. and ALICATA, J. E. 1929.—The development of *Metastrongylus elongatus* and *M. pudendotectus* in their intermediate hosts. [Abstract]. *J. Parasit.*, v. 16: 105.  
1936.—Life history of *Longistriata musculi*, a nematode parasite of mice. *J. Wash. Acad. Sc.*, v. 25 (3): 128-146.
- SCHWARTZ, B. and PRICE, E. W. 1929.—The life history of the swine kidney-worm. *Science*, v. 70 (1825): 613-614.  
1931.—Infection of pigs through the skin with the larvae of the swine kidney worm, *Stephanurus dentatus*. *J. Am. Vet. Med. Assoc.*, v. 79: 359-375.  
1932.—Infection of pigs and other animals with kidney worms, *Stephanurus dentatus*, following ingestion of larvae. *J. Am. Vet. Med. Assoc.*, v. 81, n. s., v. 32 (3): 325-347.
- SCOTT, J. A. 1928.—An experimental study of the development of *Ancylostoma caninum* in normal and abnormal hosts. *Am. J. Hyg.*, v. 8 (2): 158-204.
- SEURAT, L. G. 1920a.—Histoire naturelle des nématodes de la Berbérie, Algiers. 221 + vi pp., 34 figs.  
1920b.—Développement embryonnaire et l'évolution du *Strongylacantha glycirrhiza* Beneden (Trichostrongylidae). *Compt. Rend. Soc. Biol., Paris*, v. 83: 1472-1474.
- STORCK, D. A. 1931.—Experimental infestation of white rats with *Hepaticola hepatica*. *J. Parasit.*, v. 17: 151-154.
- SPINDLER, L. A. 1933.—Development of the nodular worm *Oesophagostomum longicaudum* in the pig. *J. Agric. Res.*, v. 46: 531-542.
- STAUBLI, C. 1913.—Trichinose. In *Handb. Path. Mikroörg., Kolle u. Wassermann, Aufl. 2, Bd. 8*, pp. 73-120, 3 pls.



- STEFFANSKI W. and STRANKOWSKI, M. 1936.—Sur un cas de pénétration du strongle géant dans la rein droit du chien. *Ann. Parasit.*, v. 14: 55-60, 1 pl.
- STELLS, C. W. and HASSALL, A. 1899. Internal parasites of the fur seal. In Jordan, D. S., et al. The fur seals and Fur Seal Islands of the North Pacific Ocean. Pt. 3, pp. 99-177, figs. 1-100. Washington.
- SUMMERS, W. A. 1940. Fleas as acceptable intermediate hosts of the dog heartworm, *Dirofilaria immitis*, v. 43: 448-450.
- SWALES, W. E. 1933.—A review of Canadian helminthology. II. Additions to Part I, as determined from a study of parasitic helminths collected in Canada. *Canad. J. Res., Sect. D*, v. 8 (5): 478-482.
- 1936.—*Tetrameres crami* Swales 1933, a nematode parasite of ducks in Canada. Morphological and biological studies. *Ibid.*, v. 14: 151-164.
- TAYLOR, E. L. 1928.—*Syngamus trachea* from the starling transferred to the chicken, and some physiological variations observed. *Ann. Trop. Med. & Parasit.*, v. 22: 307-318.
- 1935.—*Syngamus trachea*. The longevity of the infective larvae in the earthworm. Slugs and snails as intermediate hosts. *J. Comp. Path.*, v. 48: 149-156.
- THOMAS, L. J. 1929.—*Philometra nodulosa* nov. spec., with notes on the life history. *J. Parasit.*, v. 15: 193-197.
- 1937a.—On the life cycle of *Contracaecum spiculigerum* (Rud.), *Ibid.*, v. 23: 429-431.
- 1937b.—Further studies on the life cycle of *Contracaecum spiculigerum*. *Ibid.*, v. 23: 572.
- 1937c.—Life cycle of *Rhaphidascaris canadensis* Smedley, 1933, a nematode from the pike, *Esox lucius*, *Ibid.*, v. 23: 572.
- TRAVASSOS, L. 1926. Entwicklung d. s. *Rhabdias fülleborni*, n. sp. *Arch. Schiffs- u. Tropen-Hyg.*, v. 30: 594-602.
- TROISIÈRE, J. and DESCHENS, R. 1930.—L'Hépatocoliase. *Ann. Méd.*, v. 27: 414-425.
- UNDERWOOD, P. C. and HARWOOD, P. D. 1939. Survival and location of the microfilariae of *Dirofilaria immitis* in the dog. *J. Parasit.*, v. 25: 23-33.
- VAN DURME, P. 1902.—Quelques notes sur les embryons de *Strongyloides intestinalis* et leur pénétration par le peau. Thompson Yates Lab. Rpt., Liverpool, v. 4 (2): 471-474, pl. 7.
- VEGLIA, FRANK, 1916.—The anatomy and life history of the *Haemonchus contortus* (Rud.), Dept. Agr. Union S. Africa, 3d & 4th Rpts. Director Vet. Res., pp. 349-500, figs. 1-60.
- VOGEL, H. 1930.—Ueber die Organotropie von *Hepaticola hepatica*. *Ztschr. Parasitenk.*, v. 2 (4): 502-505.
- VON BRAND, T. 1938.—Physiological observations on a larval *Eustrongylides* (Nematoda). *J. Parasit.*, v. 24: 445-451.
- WHITE, R. H. 1929.—Earthworms—the important factor in the transmission of gapes of chickens. Md. State Coll. Agric., Bull. No. 234, pp. 103-118.
- WALKER, H. D. 1886.—The gapeworm of fowls (*Syngamus trachealis*), the earthworm (*Lumbricus terrestris*) its original host, etc. *Bull. Buffalo Soc. Nat. Sc.*, v. 5 (2): 47-71.
- WALTON, A. C. 1937.—The Nematoda as parasites of Amphibia. III. Studies on life histories. *J. Parasit.*, v. 23: 299-300.
- WEHR, E. E. 1936. Earthworms as transmitters of *Capillaria annulata*, the cropworm of chickens. *N. Am. Vet.*, v. 17 (8): 18-20.
1937. Observations on the development of the poultry gapeworm, *Syngamus trachea*. *Tr. Am. Micr. Soc.*, v. 56: 72-78.
1939. Studies on the development of the pigeon capillariid, *Capillaria columbae*. U. S. Dept. Agric., Tech. Bull. No. 679, 19 pp.
- WETZEL, R. 1930. On the biology of the fourth stage larva of *Oxyuris equi* (Schrank). *J. Parasit.*, v. 17: 95-97.
1931. On the biology of the fourth stage larva of *Dermatocys veligera* (Rudolphi, 1819) Schneider, 1866, an oxyurid parasite in the hare. *Ibid.*, v. 18: 40-43.
1938. Untersuchungen über die Entwicklung der Pferdstrongyliden. Sitzungsber. Gesellsch. Naturf. Freunde, Mar. 8, 1938, 18-19.
1938. Zur Biologie und systematischen Stellung des Dachslungenwurmes. Livro Jub. Travassos, pp. 531-535, 1 pl.
- WETZEL, R. and ENIGK, K. 1938a.—Wandern die Larvender Palisadenwürmer (*Strongylus spec.*) der Pferde durch die Lungen? *Arch. Wiss. & Prakt. Tierheilk.*, v. 73 (2): 84-93.
- 1938b.—Zur Biologie von *Dictyoacanthus arnfieldi*, den Lungewurm der Einhufer. *Ibid.*, v. 73 (2): 94-114.
- WETZEL, R. and MÜLLER, F. R. 1935.—Die Lebensgeschichte des schachtelhalmförmigen Fuchslungenwurmes *Crenosoma vulpis* und seine Bekämpfung. *Deutsch. Peltztierzüchter*, v. 19: 361-365.
- WRIGHT, W. H. 1935.—Observations on the life history of *Toxascaris lewina* (Nematoda: Ascaridae). *Proc. Helm. Soc. Wash.*, v. 2: 56.
- WÜLKER, G. 1929.—Der Wirtwechsel der parasitischen Nematoden. *Verhandl. Deutsch. Zool. Gesellsch.*, v. 33: 117-157. (*Zool. Anz.*, Suppl. 4).
- YOKOGAWA, S. 1920.—On the migratory course of *Trichosomoides crassicauda* (Bellingham) in the body of the final host. *J. Parasit.*, v. 7: 80-84.
- 1922.—The development of *Heligmosomum muris* Yokogawa, a nematode from the intestine of the wild rat. *Parasit.*, v. 14: 127-166.
- 1926.—On the oral infection by the hookworm. *Arch. Schiffs- u. Tropen-Hyg.*, v. 30: 663-679.
- 1938.—Investigation on the mode of transmission of *Wuchereria bancrofti* (Preliminary Report). *Tr. Soc. Path. Jap.*, v. 28: 619-624.
- YOKOGAWA, S. and OISO, T.—Studies on oral infection with *Ancylostoma*. *Am. J. Hyg.*, v. 6 (3): 484-497.
- YOSHIDA, S. 1934.—Contribution to the study on *Gnathostomum spinigerum* Owen, 1836. Cause of esophageal tumor in the Japanese mink, with especial reference to its life history. *Tr. 9th Cong. Far East. Assoc. Trop. Med.*, Nanking, v. 1: 625-630.
- YOSHIDA, S. and TOYODA, K. 1938.—Artificial hatching of *Ascaris* eggs. Livro Jub. Travassos, Rio de Janeiro, pp. 569-577.
- ZAWADOWSKY, M. M. and SCHALIMOV, L. G. 1929.—Die Eier von *Oxyuris vermicularis* und ihre Entwicklungsbedingungen sowie ueber die Bedingungen unter denen eine Autoinfektion von Oxyuriasis unmöglich ist. *Ztschr. Parasitenk.*, v. 2: 12-43.

## CHAPTER VII

### EPIDEMIOLOGY AND SANITARY MEASURES FOR THE CONTROL OF NEMIC PARASITES OF DOMESTICATED ANIMALS

T. W. M. CAMERON, Institute of Parasitology, Macdonald College, Quebec, Canada

The parasitic existence of a nematode is dependent on its finding a suitable environment in which it can mature and reproduce and this involves four sets of factors:

(a) **SUCCESSFUL ADMISSION TO THE HOST.**—The host must traverse the ground where the free stages of the parasite are found, it must eat suitable foodstuffs in or on which the larval stages occur; it must be exposed to the intermediate host, and so on. Even slight differences in habits; e.g., such as exist between a sheep and an ox in eating grass, may make all the difference in the parasite gaining admission. The anatomy of the host, including the thickness of the skin is a factor to be considered under this head.

(b) **SUITABLE ENVIRONMENTAL CONDITIONS IN THE HOST.**—Once inside the host, the parasite must find a suitable habitat—type of mucosa in the intestine, length of intestine, presence of suitable food, and so on.

(c) **POSSESSION OF A SUITABLE PROTECTIVE MECHANISM AGAINST THE NORMAL METABOLIC PROCESSES OF THE HOST.**—When the parasite lies in the alimentary system, it must possess some means of preventing itself being digested or being passed out by peristalsis, etc.

(d) **ABSENCE OF A HOST REACTION THAT WOULD INTERFERE WITH THE NORMAL METABOLISM OF THE PARASITE.**—This factor applies most obviously to parasites that leave the lumen of the alimentary tract at some period of their life, but it may apply to all. If there is any host reaction, the parasites must be able to resist its effects.

Under natural conditions, nematodes are more or less specific to a single species or a group of closely related species of animals. In general, it may be taken that nematodes of ruminants are not transmissible to horses, pigs, poultry, nor those of the latter to each other. However, many parasites of wild ruminants are transmissible to domesticated ruminants, of wild carnivores to dogs, cats and foxes, of wild birds to domestic ones, and so on. The important exceptions to this generalization include the *Trichina* and some members of the genus *Trichostrongylus*.

Very few parasitic nematodes can complete their entire life cycle within the same vertebrate animal, any more than they can live a free independent existence. At one stage or another they must leave the host to undergo some form of development outside of it—either free, or in an alternate or intermediate host. In effect, this means that a single young nematode develops into a single adult only; there is no multiplication as in the case of bacteria. In both groups, disease depends upon numbers, but whereas the entrance of a single bacterium into the body may cause disease, the entrance of a single larval nematode usually does not. Parasitic disease depends on actual numbers entering the body.

The stages which leave the body, are never immediately infective. Some essential development must take place before they are ready to re-enter and this development takes a definite period of time and requires a definite set of conditions—heat, moisture, oxygen, presence of correct intermediate host and so on—before they are able to infect. Once the infective stage is reached, they are often able to live for a long period before re-entry; while the minimum time necessary outside of the body can be fairly accurately ascertained, the maximum time is much more difficult to determine.

In many cases, after entrance to the body, complicated migrations through various organs are an essential part of the life-cycle and the greatest damage to the host is often caused at this period. We know of no means of preventing these migrations and we know of no therapeutic agents which can affect the nematodes during migration. Accordingly, prevention of ingress is of the greatest importance.

Scientific control consists in making development as difficult as possible and so depends essentially on a knowledge of the life history and bionomics of the parasite involved. This necessitates the correct identification of the nematode concerned. The need for correct identification is most important as not only are no two parasites quite alike in their biology, but treatment is often different.

In determining control methods it is important to remember that there are economic aspects of the problem to be considered. The cost of control may be excessive and it must be balanced against the loss to the stockowner—and loss should include not only actual, but potential future loss. It is often accordingly necessary to adopt several methods of control

simultaneously rather than to employ a single method. Control may be nation-wide or it may be individual. Individual control is at best a palliative, and campaigns directed over a wide area and infinitely more satisfactory. This not only involves cooperation between veterinarians, agriculturists, administrators and parasitologists but it involves careful co-ordination as well. A central authority and enabling legislation are almost essential, but the legislation to be successful must come as the result of a demand from the majority of the farmers involved. A central laboratory with a good information service is also desirable, with adequately staffed branch or associate laboratories throughout the country.

Control measures aim at breaking at some point the essential life-cycle of the parasite. If more than one point is attacked, the chances of successful results are increased. These measures will be discussed conveniently in several groups, although it must be understood that such a hard and fast division as is here adopted does not occur in nature and that methods described under one may be equally applicable under another. The parasite may be attacked at one or other of the following points in its life cycle:—

1. While in the ovum or as a young developing larva.
2. During the developed infective stage, which does not grow and “rests” until it enters the host.
3. Before entrance of this larva into the host.
4. Within the intermediate host or vector, within which 1 and 2 may be found and which may be the means of entry to the host.
5. During the parasitic stages in the host.

#### 1. Methods of Destroying the Eggs of Pre-Infective Larvae

These are those stages passed in the faeces as well as the subsequent stages which develop therein. (In a small minority of cases the larvae are not passed in the faeces, but may leave by the mouth or the urinary system or be abstracted from the blood by blood-sucking animals, or may come to rest in the host's muscles). As we know of no efficient method of destroying the eggs in the host, this section is accordingly mainly concerned with manure and its treatment. It is not a new subject, having been advocated for years in connection with human hookworm disease, but curiously enough, very little indeed has been done about it in connection with nematodes of animals in which it is infinitely more important.

There are two ways of treating manure. The first is to disregard its parasite content and concentrate on its dispersal in such a manner as to keep it out of harm's way. The second is to treat it in such a way that its parasite content is destroyed. Some twenty years ago, the late Dr. Maurice C. Hall stated that this subject offered a field for a large amount of investigation but this investigation has not been done.

“Broadly speaking,” he said, “one would have to determine how long the larvae and eggs of the various species of worms involved live in manure piles, in spread manure, in closely packed manure; the effect of sunlight, of moisture, of various chemicals, the chemicals in turn being of a nature not to injure the fertilizing value of the manure. There are practically no data on this and little could be surmised without such data.”

Since that was written a considerable amount of investigation has been carried out on this subject and it may be considered under the following headings:

1. **STORAGE.** The prompt daily collection of all manure in the stables is an essential routine in farm practice and if correctly carried out is a valuable preventive measure. If this manure were stored in a proper container for a sufficient length of time, without any other treatment all eggs and larvae would be destroyed. Unfortunately, the time factor is too long to be practical and resource to additional methods is necessary.

2. **HEATING.** In piles of horse manure, all eggs and larvae of the strongyle type are destroyed in 4 days by the natural heat generated, with the exception of those in the outer 6 inches. As a temperature as high as 107 degrees F. is generated in the central zone, all other parasites should be destroyed also, although we are without definite knowledge of this. If

the manure is confined within wooden boxes—either above or below ground—all except the outer layer of 3 inches becomes hot enough to destroy the parasites, while if the wooden boxes are double walled with sawdust between, all are killed in a week. Experiments with artificial heat, have shown that steam, at 15 lbs. pressure, destroyed in 30 minutes all eggs and larvae in a special manure box.

While these data indicate that manure can be effectively sterilized, the methods with the exception of the first, have the disadvantage of requiring special apparatus. This entails expense and limits their application to well-equipped stables. However, a compact manure pile, which has the outer 6 inches turned in every 5 days or so, will undoubtedly kill a very high percentage of the eggs and larvae.

3. DRYING. This is a method which is only occasionally possible and the drying of large quantities of manure is practically confined to countries with a hot, dry climate where dried dung is used for fuel, or where its value is secondary to military conditions and it may be spread out in the hot sun. In the latter case, however, immediate daily drying is essential as only the eggs and pre-infective stages are easily destroyed by this method. Drying is of some value also in destroying larvae on bare ground. Burning of manure is the logical extension of drying it, but this is seldom practicable.

In a rather different way this method has been used to combat the lung and intestinal nematodes of silver foxes in Canada. There the animals are raised on floor-boards which are easily kept dry by being roofed. This inhibits development of the eggs and larvae of the worms so effectively that it is now almost routine practice.

In the open, short grass assists drying and is frequently of value in reducing the infectivity of pastures, as of course, is drainage. Very wet pastures, however, are not suitable environments for the development of most nematodes (except *Dictylocaulus* of horses, sheep and oxen). Damp pastures are more generally favourable for parasitism and every effort should be made to render them unsuitable for the development of eggs into larvae.

4. CHEMICAL TREATMENT. Comparatively little has been done to find chemical methods of destroying the free-living stages of parasites. It is a problem which presents many difficulties, probably the greatest of which is the fact that the eggs and larvae are always in close contact with faeces, soil or grass, and many chemicals which might be used, are partially or completely counteracted by contact with organic matter. Nevertheless, chemical control of all the free-living stages does appear to have considerable practical possibilities.

It offers the opportunity of using the faecal material as manure and even of enriching the manurial value and it does not necessitate special equipment.

The method has been sporadically used with a certain limited success in some human hookworm areas, the chemicals employed being kainit, lime and some nitrogenous fertilizers such as nitrate of soda, sulphate of ammonia, and calcium cyanamide.

The addition of a chemical to faeces containing nematode eggs, may have varied effects on the eggs and larvae depending not only on the nature but also on the quantity of chemical.

1. It may increase the percentage of larvae which reach the infective stage and which continue to survive. Even in small cultures of fresh horse faeces in sterilized containers, fungi parasitic on nematodes have occasionally become established very rapidly and in a short time have destroyed all the larvae. The evidence suggests that some chemicals may destroy or retard the growth of these fungi without harming the larvae; flowers of sulphur is an example. Other evidence suggests that some chemicals, which are lethal to eggs or larvae when mixed with faeces in a certain proportion may, in a lesser proportion, be lethal to the fungi without harming the larvae; it follows that these chemicals may, if used too sparingly, actually increase rather than decrease the number of larvae which survive. Fungi are more likely to be common in manure pits and similar locations than in cultures and may be of practical importance as a method of natural biological control. It is probable that there are many chemicals of quite different types which possess this danger. A chemical may also decrease putrefaction which may be lethal to larvae.

2. It may have no effect at all on the eggs or larvae. The majority of the chemicals which can be added to faeces without affecting the eggs or larvae, are those which are most inert; examples are ferrous sulphide, ferric oxide, ground limestone, rock phosphate, basic slag, derris root, white hellebore, and pyrethrum powders.

3. It may allow larvae to reach the infective stage but cause many to exsheath. Some chemicals cause larvae to exsheath without necessarily causing their immediate death, although most of these chemicals are lethal in higher propor-

tions. Lapage has shown that the factors which are important in causing exsheathment when free of faeces, include age of the larvae and pH of their environment, and that chlorine and sulphides make the sheath more permeable. In the presence of faeces, some chlorides and sulphates, sodium and potassium hydroxide and potassium permanganate occasionally cause exsheathment. These chemicals, in slightly greater proportions, generally cause the death of larvae.

4. It may allow many larvae to reach the infective stage but subsequently cause their death. Many chemicals mixed with faeces in certain proportions, allow a considerable number of the eggs to hatch and the larvae to reach the infective stage and then kill them comparatively rapidly. The most outstanding examples so far noted with this property are quicklime, (in quantities too small to cause death through the heat of the chemical reaction), cupric, ferric and ferrous sulphates, zinc, cupric nitrate, sodium fluosilicate, and oxyquinoline sulphate. When applied to fresh faeces, in some cases only a third of the quantity of chemical may be required to cause delayed death compared with the quantity required to cause death before the infective stage is reached. Numerous other chemicals (but to a lesser extent) have the characteristic of causing delayed death under certain conditions; examples are nicotine sulphate, trisodium phosphate, sulphate and chloride of manganese, sodium and magnesium borates, strong cresol, phenol, and calcium hypochlorite.

5. It may allow a few larvae to survive. It is probable that the thickness of the sheath may be an important factor in preventing the action of some chemicals on the larvae. If this is so, it would account for the fact that, in spite of careful mixing, a few larvae survive in cultures treated with sufficient (or more than sufficient) chemical to kill most of them. In many cases when a few larvae have survived they have been mainly the larger sclerostome larvae. (In some cases the position of the eggs, e.g., in the centre of a lump of faeces, may have enabled them to survive.)

6. It may rapidly kill all the eggs or free-feeding larvae. When a chemical is highly lethal its method of causing death may be of considerable practical importance. Chemicals which are lethal but insoluble—they are not common—and which kill by contact would be difficult to use in practice, because many larvae would escape by remaining away from the chemical, e.g. inside a lump of faeces. These chemicals would, therefore, be especially useless in dry conditions. Applied dry, chemicals which are deliquescent have more prospect of being of practical value, for the moisture which they attract may also attract the larvae. With solutions it is much easier to obtain effective contact with eggs or larvae scattered throughout the manure, especially if the chemical is sufficiently lethal to be effective when applied as a very weak solution. With very lethal chemicals such as iodine salts, it has been found that very considerably less chemical is necessary to produce sterilization with a very weak rather than with a very strong solution. In extreme cases a chemical applied as a 1:2 solution may require from 20 to even 50 times the amount necessary when applied as a 1:500 or a 1:1,000 solution.

Some chemicals—including some which are very effective (such as chloropicrin, calcium cyanide, naphthalene, Ortho- and Para-dichlorobenzene)—can be used as gases. Application of this class of sterilizing agent should have many practical advantages, provided that a suitable container is available and that the faeces are not packed too tightly for the gases to permeate them, but results suggest it is possible for a small percentage of eggs or larvae to avoid gases, again probably when they are in the centre of a lump of faeces. However, whether this class of chemical is applied as a solid or fluid, it is probable that the chemical will distribute itself effectively through the faeces. Unfortunately, some of the most effective gases are dangerous or at best, most unpleasant, to man himself.

Apart from the effectiveness of a chemical and ease with which it can be applied, the practicability of its use depends largely on its cost. When sufficient urine is available it has practically all the possible advantages. The next most practical group of agents is the artificial fertilizers, since if care is taken to avoid loss of nitrogen, part or all of their cost may be recovered in increased manurial value.

Occasionally it may be possible to use a chemical which is not only lethal to nematode larvae, but also to other pests, such as fly larvae; if this can be done the advantages are obvious. Unfortunately, it by no means follows that because a chemical is lethal to fly larvae, it also kills nematode larvae. For example, hellebore, borax, aniline and pyridine have all been shown to be effective in fly control. Hellebore, however, is not lethal to sclerostome larvae, while borax has to be used in larger quantities than for fly control and in greater quantities than are practicable if the faeces are to be used as fertilizer (because of the toxicity of an excess of borax to plants). Ani-

line and pyridine, on the other hand, are both very lethal to the free-living stages of sclerostomes.

The cost of treating faeces can also be reduced if a lethal by-product is available or if a chemical is being bought in bulk for other purposes. In these cases the possibility of the presence of impurities must be considered, as they may alter the lethal value of the chemical or they may make it unsuitable by containing a plant poison.

A large number of chemicals has been tested at the Institute of Parasitology for their effect, under controlled laboratory conditions, on the pre-infective stages of horse sclerostomes. The more "practical" of these are given in the following table, with the percentage required to completely sterilize small quantities of horse manure:

| Percentage | Chemical             | Remarks                 |
|------------|----------------------|-------------------------|
| 0.043      | Chloropicrin         |                         |
| 0.19       | Calcium cyanide      |                         |
| 0.25       | Paradichlorbenzene   |                         |
| 0.33       | Formalin             | (in weak solution)      |
|            | Sodium fluoride      | (in very weak solution) |
| 0.37       | Phenol               | (in weak solution)      |
|            | Naphthalene          |                         |
| 0.62       | Cresol               | (in weak solution)      |
| 0.75       | Urea                 | (in strong solution)    |
| 1.0        | Sodium borate        | (in weak solution)      |
|            | Zinc chloride        | (in weak solution)      |
|            | Ammonium thiocyanate | (in medium solution)    |
| 1.25       | Calurea              |                         |
| 1.5        | Potassium cyanate    |                         |
| 1.9        | "Powdered" Cyanamide |                         |
| 2.5        | "Granular" Cyanamide |                         |
|            | Sodium chloride      | (in medium solution)    |
|            | Sodium hydroxide     |                         |
| 3.1        | Carbon disulphide    |                         |
|            | Calnitro             | (in medium solution)    |
|            | Ammonium nitrate     | (in medium solution)    |
|            | Kainit               | (in medium solution)    |
| 3.75       | Sodium nitrate       | (in medium solution)    |
| 4.4        | Potassium nitrate    | (in strong solution)    |
| 4.5        | Nitro chalk          | (in medium solution)    |
| 5.75       | Calcium hypochlorite | (in strong solution)    |
| 6.0        | Ammonium sulphate    | (in medium solution)    |
| 6.25       | Carbonate of potash  | (in medium solution)    |
| 8.0        | Carbon tetrachloride |                         |
| 14.0       | Sulphate of potash   | (in strong solution)    |
| 20.0       | 20% Superphosphate   |                         |
|            | Phenothiazine        |                         |
| 23.0       | Dog urine            |                         |
|            | Horse urine          |                         |
| 37.0       | Cow urine            |                         |
| 40.0       | 16% Superphosphate   | (in strong solution)    |
| 50.0       | Quicklime            |                         |
| 65.0       | Hydrated lime        |                         |

*Of No Value*

- Flowers of sulphur
- Ground limestone
- Raw rock phosphate
- Basic slag
- Pyrethrum powder
- Derris powder
- White hellebore powder

Among the numerous other chemicals tested, in attempts to find reasons for the lethal factors, compounds of iodine were found to be of a very high efficiency indeed; thus for example, 0.01% of methyl iodide (in a dilution of over 1:200) was completely effective. Iodine salts, however, are expensive.

On farms the most easily obtained (in efficiently drained stables) is urine; in addition there are many artificial fertilizers with lethal properties, part or all of the cost of which may be recovered in added manurial value.

The chemical and lethal composition of urine varies considerably, not only according to the species, but also according to the food and health of the animal from which it is taken. In a few cases its lethal value may be almost nil, but generally speaking, about 30 to 40 percent of the weight of urine to fresh faeces kills the free-living stages of sclerostomes. Of the artificial fertilizers, urea is the most potent, requiring about three-quarters of 1 percent by weight of the fresh faeces to sterilize them against sclerostomes. Calurea should be used at the rate of 1¼ percent, while about 2 percent of powdered cyanamid is needed and another half of 1 percent if used in the granular form. A high grade kainit is one of the next most lethal fertilizers and it should be used at the rate of 5 lbs. to 100 lbs. of manure. Closely following in potency are many other artificial fertilizers which should be used at the rate of about 6 percent or slightly over, compared with the weight of fresh faeces.

It must be remembered that the addition of some alkali fertilizers to faeces will cause the loss of ammonia. With urea and calurea, much ammonia escapes as gas.

The quantities mentioned above would be too great in many cases for common manurial practice if the whole manure heap had to be treated, but since the heat of fermentation, lack of oxygen and other factors, prevent the development of larvae in the centre of a well-built heap, it should only be necessary to treat the top and sides, provided that the faeces are put there as soon as they are passed, that the fertilizer is immediately well mixed in, and that the pile is sufficiently well-packed and protected to keep the fertilizer in contact for some time.

The use of a well-constructed manure pit is highly desirable and ideally should be divided into two portions—one to contain manure under treatment while the other is being filled; the first portion is then emptied and the procedure reversed. The size and design will depend on local circumstances. Manure stored in yards, no matter how stored, should be inaccessible to stock.

**SELECTIVE DISPOSAL.** As there is normally a marked specificity shown by the parasites of various species of animals, the manure, especially if untreated, should be used on ground which is inaccessible to the species of animal from which it came. Thus, horse manure should not be used for top-dressing pastures to be used by horses, but it is safe—or reasonably so—to use it for pastures used by cattle and sheep. It may also be used for growing crops—except hay crops which will subsequently be fed to horses.

**PLOWING UNDER.** Wherever possible, manure should be plowed under. However, this procedure cannot be guaranteed to keep eggs and larvae below ground. Earthworms bring some to the surface and strongyle larvae are capable of a certain degree of upward migration. The horse sclerostomes have practically no migrating ability in clay soil (provided that there are no cracks in the soil), but in sandy clay they can migrate 4 inches and in sandy loam 5 inches upwards; moreover, they can live for over 4 years under these circumstances. The sheep nematodes *Ostertagia* and *Nematodirus* can regain the surface after being plowed under and survive for 8 to 10 months; *Haemonchus* survives less well. Plowing-in may actually assist development by breaking up the soil and faeces.

**FLY DESTRUCTION.** Flies are important in connection with manure as mechanical carriers of worm eggs (e.g. *Ascaris*) and as actual essential intermediate hosts of worms (e.g. *Habronema*); the part they play as mechanical carriers is probably of secondary importance.

From the second point of view, flies must be prevented from feeding on horse manure; this, if perfect, would completely eradicate *Habronema* from horses. Cleanliness in stables is essential, even small quantities of manure being removed daily. Spraying manure with hellebore (½ lb. dissolved for 24 hours in 10 gallons of water, will treat 10 cubic feet) or powdered borax (at the rate of 1 lb. to 16 cubic feet) are recommended by the United States Department of Agriculture for the prevention of fly breeding. The hellebore has no injurious effect; the borax also is not injurious if the manure is not used in excess of 15 short tons to the acre. Creosote oil also has been recommended as a deterrent; it is mainly useful under war conditions and for dead horses.

The heat generated in the centre of a well-constructed manure pile or throughout the manure in a box will destroy many maggots. The outer layers of the pile, however, will not become sufficiently hot and will require treatment.

Comparatively few eggs or larvae leave the host other than in the faeces. Those that do include the pinworms, trichina worms, kidney worms and microfilaria.

**PINWORMS.** The female *Oxyuris equi*, the only known pinworm of importance in domestic mammals, leaves the host to deposit her eggs on the perianal skin or stable furnishings; sometimes she is evacuated in the faeces or, dying in the rectum, her eggs are so passed, but this is exceptional and the few cases in which it occurs are provided for by the usual procedures. Most eggs are actually laid on the skin and although very little is yet known of their bionomics, it would seem that excess of water is quickly fatal to them. Accordingly, washing of stable, stable furniture, grooming kit and perianal skin, would reduce the possibility of reinfection. The removal of eggs from the skin by washing will also reduce the local irritation and render reinfection less probable, while in heavy infections, warm water enemas will remove gravid females mechanically and so reduce the egg output. Infection is by swallowing the embryonated eggs either directly from the skin (where the irritation causes the horse to bite) or as a contamination in food. General cleanliness and the use of hot water (as for *Ascaris*) will undoubtedly reduce infections.

**TRICHINA.** While a few larvae pass in the faeces of car-

myores and pigs, they do not appear capable of becoming infective. Those which will become infective pass into the muscles. Control lies entirely in appropriate feeding. Pigs must not be permitted to eat uncooked meat foods.

**KIDNEYWORMS.** The eggs of the swine kidney worm are passed in the urine and exposure to drying and sunlight will destroy them. This can often be done by the provision of a bare lot around the hog lot, kept free from grass or shade and well drained. The eggs of the kidney worm of carnivores are also passed in the urine but nothing is yet known of their bionomics and so no control measures can be adopted.

**MICROFILARIA.** Few filaria worms are important in stock. All depend, however, on their removal from blood or skin by a blood-sucking insect. Control accordingly depends on insect control, screening of houses and related measures.

## 2. Methods of Destroying Infective Larvae (free or enclosed in egg shells)

### (a) Disinfection

(i) **Chemical**—There is no good chemical disinfectant for larvae enclosed in their egg shells (e.g. Ascarids) and disinfectants for this purpose are practically useless; in fact they may assist the larvae by destroying fungi and bacteria which are themselves harmful to the parasites. The use of chemicals against free larvae has been discussed above as it is not possible to separate the actions of chemicals on the preinfective larvae from the action on infective larvae, although in general the latter are more resistant.

(ii) **Heat** is lethal for all forms of parasites and, in the form of very hot water or live steam, is one of the most efficient disinfectants at our command. It is the only one of any practical value against Ascarids and its use is fundamental in the control of these very serious parasites in swine and carnivores. It can be used either as hot water (with lye or soap to loosen the dirt) or steam. Its use is recommended in all kinds of stables as it kills every kind of larva. While its value has been recognized for many years, it is only recently that accurate knowledge has been obtained on the amount of heat required and it was found that ascarid eggs could be killed in 1 second at 158° F., 2 seconds at 149° F. and 5 seconds at 140° F.

(iii) **Cleanliness** both of animals and of quarters, is of great value in reducing numbers of parasites. Washing of madders of sows with warm water and soap removed many infective ascarid eggs. Washing of stables (including window-sills and other places where dust lies) mechanically removes sclerostome larvae which have very great powers of resisting drying and which otherwise would be blown on to the animals' feed or water.

### (b) Pasture

Little work has yet been done on the control of parasites on pasture and arable land although the subject is of great importance in all parts of the world. Pastures cannot be treated daily as can a manure heap and so it is mainly infective larvae which have to be killed. They are then in position in which destruction is difficult. Chemical treatment has many disadvantages—cost being one of the most obvious. Nevertheless some chemicals have been tried; copper sulphate, bleaching powder and lime have proved unsatisfactory in practice. The most promising appear to be those which could be applied as a gas and retained on the ground by a mulch of paper, a tarpaulin trailed behind a tractor or some similar method. Such gases as chloropicrin (a tear gas), calcium cyanide and others mentioned have at least possibilities in that direction. Alternately it may be practical to use a deliquescent salt alone or mixed with a very lethal chemical so that the larvae may be attracted toward the moisture.

These are suggestions for the future; meanwhile, the only effective way is to collect the droppings daily before the larvae can migrate to soil or grass. Obviously this method has very considerable limitations, but it has been done on stud farms.

As infective larvae of bursate nematodes do not feed but live entirely on foodstuffs stored up in their bodies, their life can be shortened by causing them to use up this source of energy more quickly than usual. Moderate warmth and light are natural stimulants and, as continual spreading and harrowing exposes them to these physical agents, it is of assistance in reducing the numbers but will not completely eradicate them. It is especially effective in dry, warm climates.

The burning of grass—often a valuable agricultural practice—theoretically must destroy some larval worms on the pasture and in the ground beneath; it cannot be relied upon to destroy them all and it may also give the larvae access to the more succulent grass beneath. It does, however, help to raise the nutrition plane of the animals.

All worm larvae require a degree of moisture for development, although only a few (e.g. *Dioctyocephalus*) are capable of development in water alone. Drainage, an essential step in the control of these lungworms, is always of general value in damp pastures. It improves the quality of the grass and so improves the resistance of the host, but it is doubtful if it destroys many larvae.

Drying of larvae has very variable results. Ordinary drying is quickly lethal to many of the nematode larvae of sheep but only slightly so to other larvae and forms enclosed in egg shells, like Ascarids. Ten per cent of sclerostome larvae can survive 4 months air drying in an incubator at 75° to 80° F. and they have been found alive in window dust in stables out of use for several years. Ordinary drying destroys only some larvae but it may destroy sufficient to prevent disease.

Drought has not necessarily the same effect as draining of pastures. In dry seasons grass is short and scarce, more must be eaten, (especially by sheep) it is eaten "short" and a greater area of the pasture is grazed daily. Larvae of some worms tend to live near the roots of the grass and so under these conditions many more reach the host; the parasite intake varies directly with the time of grazing. The resistance of the host, through poorness of the feed, is lowered and so serious disease may result.

Moreover, embryonated but unhatched eggs of sheep gastro-intestinal nematodes are often very resistant to drought and so these tend to accumulate. When the drought breaks they hatch simultaneously, and if the moist weather continues for a week or so, they may cause an explosive outbreak of disease. However, short periods of drought interrupted by short showers have an opposite effect.

Heavy rain is inimical to the development of sclerostome larvae in their preinfective stages; whether this is due to the fact that subsequent drying is easier, is not yet known. Heavy rainfall in hill country often has the effect of mechanically washing larvae and faeces off the hillsides and so reduces the number of infective larvae; this is especially true in tropical islands and uplands where rainfalls are often very heavy. However, it may concentrate infections in the valleys. In flat country the larvae are washed off the grass but quickly crawl back again. (It should be noted that an excessive growth of grass may encourage sheep to feed between the tall grasses. It is in this position that most larvae are found and so heavy infections may result).

Continued exposure to extreme cold undoubtedly has a serious effect on the free stages of parasites but in many cases we cannot rely on the natural cold of winter to act as an important agent. Destruction depends at least to some extent on snowfall. Where the snow is adequate, the temperature of the ground beneath is almost independent of the air temperature, and even when the air temperature falls to 0° F., the ground temperature is still little below freezing. The type of winter most destructive is the *snowless* winter and a comparatively mild winter with little snow, is much more destructive than a severe one with a heavy snowfall.

Moreover, in countries with a normally cold winter, animals of all kinds are stabled during cold weather and the parasites can be carried over as adults in the host or as eggs in the manure in the barn.

In countries with a mild winter climate, frost may actually increase the life span of the parasites, although repeated freezing and thawing is much more lethal than continued freezing. There is little accurate knowledge yet available on the lower thermal death points of parasites. It is known, however, that some forms, such as horse sclerostome larvae, can survive very low temperatures (36° F.) for long periods; others such as sheep nodular worms, are easily killed by cold. Each species has its own critical temperature or range of temperature and this tends to control its distribution independently of man. However, man may often permit a parasite to survive in an otherwise unfavourable environment—by suitable methods of animal husbandry. Eastern Canada has a hot summer and a cold winter, but animals are housed for the coldest months of the year. This permits of the existence of such parasites as *Oesophagostomum columbianum* in eastern Canada, although it is absent from British Columbia and Great Britain, both of which are less extreme in temperature.

Sunlight is harmful to most nematodes but whether because of light or heat or both is not definitely known. Its value in destroying larvae of pig kidney worms is well recognized.

Dung-feeding insects such as beetles, are known to destroy many worm eggs but in some cases, the eggs appear to pass through them and they may act as distributors rather than destroyers. Such insects may act as true vectors of some worms.

Mixed grazing on pasture is usually of great value, as horses will eat and digest the larvae of worms which mature in cattle and so on. In this way, the number of infective larvae swal-

lowed by each kind of host is greatly reduced. This simple procedure often causes quite startling results and may, by itself, be sufficient to prevent actual disease. Thus, on heavily infested horse pastures, sheep may remove 100,000 eggs per head daily.

Hay often has the effect of mechanically removing a large number of infective larvae from a pasture.

Actual removal of the turf and re-sowing to permanent pasture may be and has been used in extreme cases. But this can only be recommended when heavily infested but very valuable land is concerned or where the turf may be sold for urban purposes.

### 3. Avoidance of Infection of the Host and Prevention of Ingress of the Parasite

(a) *General Hygiene*—This is of the utmost importance. Many horses are taken to stables throughout the year and when they are kept clean and permanent litter avoided, the worm infections are at a decidedly low level. Ordinary methods of cleanliness alone can be of great value. This indeed is one of the basic principles underlying the very successful Me-Lean County Sanitation System for controlling Ascarids in pigs; in its essentials, this system simply requires cleanliness of sow and breeding places and avoidance of exposure of the young animals to infection.

(b) *Disinfection*—Small quantities of faeces, overlooked in general cleaning of stables are important sources of infection. These may be sterilized by very hot water or steam. The general bactericidal disinfectants are not very good for this purpose, the least ineffective being 3 percent lye and 5 percent lysol. These are of value both for horse and sheep strongyles, but require an hour's contact to destroy them. They are practically useless against Ascarids and related worms, however. Lye in its usually applied strength (about 1 percent) is quite useless as a disinfectant for any kind of worm, although it is useful in freeing parasite eggs from dirt and making them more readily available to the destructive action of other agents.

(c) *Permanent Pastures*—There is no doubt that permanent pastures form the greatest single danger to stock and that Maurice Hall's dictum "Permanent pastures perpetuate parasites" is still of the utmost importance. The pastures concentrate eggs and larvae, and improved pasture culture, by increasing the stock-carrying capacity, still further increases the danger. At present there is no effective method of preventing infection on them.

It was once believed that temporary pastures, plowed in and re-sown would overcome this difficulty, but recent work has shown that heavy infections of sheep may result from such a practice. The eggs and larvae plowed in are protected from sun, heavy rain and drought and many emerge with the new grass.

So far attempts at altering the pasture flora to produce an environment less suitable for development, or a type of grass less suitable for migration of the worms and so less likely to cause an infection, have been comparatively ineffective but are still being tested. Taylor finds that larvae climb higher on clover than on grass and that such fertilizers as basic slag, by encouraging clover growth, increase parasitism. On the other hand, sainfoin carries only 550 larvae per pound, under conditions where grass carries 1,900, probably because of the relatively slight contact with the ground which the large sainfoin plant makes.

Manuring a pasture by nitrates encourages rapid growth of grass and may lead to a reduction in larvae per pound of grass and so produce a smaller intake. However, a dense growth of grass provides favourable cover for parasites and infection is proportional to density of cover; sparse growth permits natural agencies to reach them.

(d) *Rotations*. Rotation of permanent pasture when this is possible, is of value but it postulates a large amount of pasture land and much fencing as the animals have to be moved on before the eggs they have passed give rise to infective larvae (5 to 7 days) and kept away from the "used" land until all larvae are dead; this period varies with the parasite concerned, the soil and the climate and no general rules are yet possible. The cleanest land should always be reserved for growing stock which is more susceptible to worm disease than older stock.

However, a certain amount of rotation of stock may be practiced. Not only may horses follow ruminants, but cattle may, to some extent, follow sheep and old animals follow young ones. Under these circumstances, a shorter fallow period is possible as subsequent animals eat many of the larval parasites of their predecessors.

It is often practicable to graze lambs on clean pasture and follow them by old sheep which have some degree of resist-

ance to gastro-intestinal nematodes. In choosing a rotation such as this (or such as cattle following sheep) care must be taken to know which parasites are concerned as only some may thus be treated. The simultaneous grazing of several kinds of animals (such as horses and sheep) is only an extension and improvement of this method.

(e) *Bare Lots*—The use of bare lots for young stock has much to recommend it. There is no grass for them to eat and conditions for development of worms are highly unfavourable. Moreover, as the animals must be watered from troughs the danger of infection is further decreased.

This method has enabled lambs to be raised in districts where worm infections are so high as to kill a large percentage of animals raised on pasture.

Partial bare lots in pig pastures, in lands where the kidney worm is prevalent, are of assistance in controlling this parasite also. Faeces also are concentrated, making their removal or treatment easier.

(f) *Fencing*—Fencing is of value in dividing pastures for rotation, or to ensure uniform grazing and to avoid overgrazing of certain parts. Temporary fencing (as in folding) so arranged to allow lambs (but not ewes) to reach new pastures in a progressive system of feeding, is also of value, but entails a certain amount of labour, as the fences must be moved weekly. In this case the lambs, if they are not weaned, have the run of the pasture ahead as well as the one in which the ewes are kept, or, if they are weaned, the one ahead only. The very greatest care must be taken to prevent the lambs gaining access to old ground already grazed by the ewes and from which they have been moved on. If this is not done a heavy infection is extremely probable.

(g) *Limitation of Numbers*—Limitation of numbers is really an attempt to return to nature from artificial conditions of modern farming. It is the rational method with permanent pastures where over-stocking has such disastrous results. A reduction of 50 percent in numbers on a pasture means a much greater reduction than that in parasites. It also spreads out the rate of intake of parasites and allows resistance to develop. Moreover, overstocking decreases the food yield of the pasture and encourages closer grazing and a higher worm intake. The poorness of the food supply decreases body resistance and so encourages parasitic disease.

(h) *Night Housing*—Night housing is often valuable as most strongyle larvae are able to climb on to grass but do so only when it is damp, retreating towards the soil as it dries. Heavy dews are very suitable for this upward migration and the simple procedure of keeping stock—especially young stock—off the pasture until the dew has evaporated, has frequently made all the difference between health and disease. Husk in cattle is often caused by early grazing and night housing is particularly useful with that disease.

(i) *Raised Troughs*—Feed racks, raised troughs and clean water—especially in connection with bare lots and permanent pastures which are heavily stocked—is valuable in reducing intake of parasites. It is of value also in stables where the floors are often heavily contaminated with infective larvae and it is especially valuable for young stock.

(j) *Silage and Folding*—The use of silage helps to reduce numbers of larvae taken in by the host, and the temperature generated in its preparation may destroy some larvae and shorten the life of others; in general, silage does not carry the heavy infections that grass does.

Folding on green crops, with the aid of hurdles, is also of value, provided the same ground is not used too often for this purpose. The animals must be moved every 6 days or so and the young must have first choice—even going one fold ahead of the adults—and must not under any circumstances be permitted to enter old, used folds.

Rotational grazing (e.g., nitrogenous stimulation of grass which is grazed on successive fields) may cause a heavy infection but generally increases resistance to disease.

The penning of stock on arable ground is more dangerous than the free ranging of stock on permanent pastures as they tramp faeces into the ground and so improve the chances of the parasites developing; under these conditions sandy soil is probably more dangerous than clay.

Food cabinets, as recently developed, afford a method for the quick growing of green crops under circumstances which preclude any infection at all. The animals, if housed on concrete and kept clean, need never acquire any intestinal parasites at all with this system. The food cabinets permit of the growing of "grass" in a week from seed without the aid of soil. This is done by using perforated trays of certain grains (notably barley, wheat and maize) in a constant temperature cabinet and exposing them to the action of moisture and artificial fertilizer daily. By a suitable rotation a constant

output of grass is thus obtained, free from disease organisms and independent of climate.

(k) *Cooking*—Larvae carried by food animals are destroyed by heating and so all meat and fish foods fed to pigs or carnivores should be cooked unless free from suspicion; this is highly important in the control of the trichina.

(l) *Floorboards*—The use of floorboards, wire or concrete floors for pigs, carnivores and poultry, provided they are kept clean, not only permits of efficient egg destruction or elimination, but prevents infection, or at least reduces it very considerably.

(m) *Quarantine*—New stock should not be introduced to a worm controlled farm, until it has been carefully examined for parasites. A single boar, for example, may introduce ascariids to a worm free piggery. Known carriers of any kind of parasites already present should be treated, isolated or at least excluded from common grazing. Wild animals which harbour parasites communicable to domestic animals should be denied access or be destroyed.

## Vectors

Many roundworms of domesticated animals, require essential intermediate hosts. These may be arthropods, earth worms, snails, or vertebrates such as fish, amphibia, and even other mammals. A knowledge of the life history of both carrier and parasite is essential before control can be undertaken. This may involve not only destruction of the vector but avoidance of infection of the vector, in many cases a procedure of almost equal importance.

Destruction of infective larvae in vertebrates may be undertaken by meat inspection and physical or chemical destruction of condemned material. Cooking of all garbage fed to pigs is an invaluable means of controlling *Trichina* in pigs and so in man. In the case of pork for human consumption, thorough cooking until the flesh turns white, is a perfect safeguard; pork is often eaten undercooked. Where it is eaten raw, it should be subjected to chemical treatment, chilling or heating (as laid down in the regulations of the United States Bureau of Animal Industry).

Earthworms carry a considerable number of parasites to domestic animals and their control is extremely difficult. Chemicals and sand have been used but more successful results are obtained by avoidance of infection.

Insect destruction is almost as difficult. Most of the important carriers are dung feeders and are not easily attacked. Many of the usual contact or stomach poisons are available but their use has been extremely limited. A more rational means of control is an attack on the breeding places and this is feasible for house flies, mosquitoes and ecto-parasites.

Avoidance of infection of the vectors is almost as important as their destruction. This can be effected not only by proper manure disposal and treatment, but by means designed to keep animals and vectors apart. Thus, for example, the lungworms of swine are carried by manure-frequenting earthworms; if swine faeces are disposed of in situation where the swine themselves cannot reach the parasites can be controlled. Swine confined entirely to proper concrete pens, should never have lungworms.

In cases where the larvae are actually removed from the body by biting flies, protection from these will not only prevent vectors becoming infected but will prevent the hosts being infected in turn. In addition, of course, measures for the control of these insects (mosquitoes, midges, black flies and stable flies) should be undertaken.

As the spirurid nematodes are carried largely by dung frequenting insects, manure treatment and disposal will help to reduce infections.

## Destruction Within the Host

Antiparasitic drugs are used for two purposes—to treat clinical cases or to provide a clean herd. The first requires the removal of only sufficient parasites to relieve the symptoms; the second postulates a much more efficient drug, one which would destroy all parasites being the ideal. There are few such drugs available as yet and such as are, may be used with success against a comparatively small number of species; fortunately, however these include some of the important forms. Where these drugs are available, for either internal or external parasites, their use as a means of control is highly important. All members of the herd should be treated regularly until all parasites have disappeared and no residual infection left to act as a starting point for re-infection.

This mass treatment, where it has been employed correctly and under strict supervision, has given excellent results. It is

necessary to emphasize the necessity for strict supervision. All drugs used to destroy parasites are animal poisons, at least to some extent, and their indiscriminate use by laymen is apt not only to nullify their results but to be actually dangerous to the animals. Their use accordingly requires the aid of the practicing veterinarian. No other person knows the habits and location of the parasite, the physiology of the host, the correct drug to use, the technique of its administration and its contra-indications. If this principle is accepted, it follows that co-operative district schemes, involving panels of practitioners, are essential. It is useless, as a control measure, to eradicate any particular parasite on one farm if the next remains heavily infested. The first steps to be taken must be those of an educational nature to be followed by some enabling order from a higher authority; this order, however, should come only as the result of a demand from the district itself. Thereafter, by a suitably designed veterinary panel treating animals in groups in sub-districts, the entire population in the district can be treated quickly, cheaply and efficiently.

## Reservoir Hosts

Wild animals belonging to the same major groups as domesticated ones, often harbour parasites transmissible to them and there are innumerable cases on record of such animals or even animals more distantly related, acting as reservoirs of infection as well as transmitters of new species to domestic animals. It is of importance to know what parasites occur in the wild fauna of a country or district and to take such steps as may be possible to keep those parasites within control.

At present only one or both of two plans of action are available; either to destroy all the wild carriers or to prevent their intermingling with domestic ones by suitable segregation.

Indigenous wild mammals and birds not only possess parasites transmissible to man and to domesticated stock but may become infected from the introduced stock and act as uncontrolled reservoirs of the new parasites. An adequate knowledge of the parasitic fauna of the indigenous wild animals is an essential step in controlling parasites of domestic stock. It is surprising how little has been done. The Institute of Parasitology in Canada is conducting such a parasitological survey to ascertain the distribution and intensity of infections in all forms of animal life in the Dominion. A large amount of voluntary assistance in collecting has been readily given by all classes of persons—official, commercial and private—and although many years must pass before the survey is even approximately complete, it has already yielded invaluable results. This survey has also been extended to the West Indies and it is to be hoped that other countries will take similar steps and enable a world map to be prepared showing the distribution and importance of all parasites in all kinds of animal life.

## Avoidance of Parasitic Disease

Effective control of any species of parasites will eliminate the disease caused by it, but even when this cannot be done, steps should be taken to reduce or avoid the disease. With a very few obvious exceptions, disease depends on numbers of parasites present but we are unable to state the exact point at which clinical disease begins, even if we admit the theoretical concept that even a single parasite causes some disease. There is too little real knowledge about the action of parasites and too many factors involved, including nourishment, resistance and presence of other parasites.

In general, however, it may be stated that any attempt to reduce the number of parasites ingested, to increase the resistance of the host or to raise the standard of fitness of an animal offsets the effects of the parasite to some extent and helps to reduce parasitic disease even if it does not eliminate parasitism.

(a) *Preventive Licks*—The theory underlying the use of preventive licks is that a small daily dose of some drug taken in a mineral lick, will either kill the larvae taken in the food or else render their environment so abnormal that they will not develop; it is not suggested in this way to administer drugs against adult parasites. There is no conclusive proof yet that preventive licks are satisfactory. Good results have been claimed with tobacco and bluestone, but the subject must still be considered as in the experimental stage.

There is no doubt that the efficiency of some of the older worm medicines was due to their "tonic" action on the body and that this was particularly the case with such elements as iron, copper, cobalt, arsenic, phosphorus and calcium. These appear to be used by the body to repair or counteract damage done by the parasites, as well as in some cases to destroy the parasites themselves. A supply of such materials in mineral licks is often of great importance in preventing the develop-

ment of disease symptoms. It not infrequently happens that animals fed on a "natural" diet may be receiving insufficient phosphates or calcium or other elements, and the use of licks containing these substances immediately increases the general condition of the animal and so assists in control of parasitic diseases.

(b) *Diet*—It is extremely difficult to separate many parasitic diseases from those caused by deficiencies in diet. There is evidence to suggest that in many cases the two are so closely interwoven as to be inseparable and that their effects are mutually cumulative. There is no doubt that in very many cases, a sufficient, well-balanced diet, balanced in all its accessory factors as well as its main constituents, will prevent parasitic disease and will often actually reduce the numbers of parasites harboured. Pasture treatment, such as "top-dressing," is often a valuable way of doing this. This adequate diet is particularly important for immature animals and every effort should be made to secure this. This is, of course, true for all preventive measures to be taken but is especially important in connection with diet.

### Bibliography

- ACKERT, J. E. 1939.—Factors in the resistance of chickens to parasitic worms. Proc. 7th World's Poultry Cong. & Expo., Cleveland, pp. 265-276.
- CAMERON, T. W. M. 1933.—The bursate lungworms of domesticated animals. Imp. Bur. Agric. Parasit., 36 pp.  
1934.—The internal parasites of domestic animals. xii + 292 pp., London.
- HALL, M. C. 1917.—Manure disposal as a factor in the control of parasitic diseases of live stock. J. Am. Vet. Med. Assoc., n. s., v. 4: 675-678.  
1936.—The control of animal parasites. 162 pp., Evanston, Illinois.  
1938.—Three parasites which habitually surmount our sanitary barriers. Livro Jub. Travassos, Rio de Janeiro, pp. 195-211.
- MONNIG, H. O. 1938a.—Veterinary helminthology and entomology. 2nd ed. xv + 409 pp. London.  
1938b.—Pasture hygiene with respect to parasitic diseases. 13th Internatl. Vet. Cong., v. 1: 666-671.
- PARNELL, I. W. 1934.—Studies on the bionomics and control of the bursate nematodes of horses and sheep. Pt. I. Canad. J. Res., Sect. D, v. 10: 532-538. 1936a.—Idem. Pt. II. Ibid., v. 14: 71-81. 1936b.—Idem. Pt. III. Ibid., v. 14: 172-180. 1937.—Idem. Pt. IV. Ibid., v. 15: 127-145. 1938.—Idem. Pt. V. Ibid., v. 16: 73-88. 1939a.—Idem. Pt. VI. Ibid., v. 17: 68-86. 1939b.—Idem. Pt. VII. Ibid., v. 17: 187-204. 1940.—Idem. Pt. VIII. Ibid., v. 18: 371-394.  
1935.—On the control of the free-living larvae of bursate nematodes of domestic animals. Trop. Agric., v. 12: 109-113.  
1936.—Notes on the survival of the eggs and free-living larvae of sclerostomes on pasture. Sc. Agric., v. 16: 391-397.  
1939.—Some notes on the chemical control of the free-living stages of bursate nematodes. Canad. J. Comp. Med., v. 3: 84-87.
- RAFFENSPERGER, H. B. 1927.—The swine sanitation system as developed by the Bureau of Animal Industry in McLean County, Ill. U. S. Dept. Agric. Tech. Bull. No. 44, 20 pp.
- RAILLIET, A. 1918.—Oxyurosis in the horse. Vet. Rev., v. 2: 139-157.
- RANSOM, B. H. and HALL, M. C. 1920.—Parasitic diseases in their relation to the live-stock industry of the southern United States. J. Am. Vet. Med. Assoc., n. s., v. 10: 394-413.
- RANSOM, B. H. [1927].—The prevention of roundworms in pigs. U. S. Dept. Agric. Leaflet No. 5, 8 pp.
- ROSS, J. CLUNIES and GORDON, H. McL. 1936.—The internal parasites and parasitic disease of sheep. xx + 238 pp. Sydney, Australia.
- ROSS, I. CLUNIES, CHAMBERLIN, W. E. and TURNER, H. N. 1937.—The influence of improvement of pastures and rotational grazing on parasitism, body weight and fleece production in crossbred sheep in Tasmania. J. Council Sc. & Indus. Res., v. 10: 313-326.
- SCHWARTZ, B. 1928.—Gastro-intestinal parasites of equines and control measures. J. Am. Vet. Med. Assoc., n. s., v. 26: 855-870.  
1934.—Controlling kidney worms in swine in the southern states. U. S. Dept. Agric. Leaflet No. 108, 6 pp.
- SWALES, W. E. 1927.—The practitioner's role in parasitology. Canad. J. Comp. Med., v. 1: 17-21.  
1940a.—The helminth parasites and parasitic diseases of sheep. Pt. I. Canad. J. Res., Sect. D, v. 18: 29-48.  
1940b.—Idem. Pt. II. Canad. J. Comp. Med., v. 4: 155-161.  
1940c.—The use of phenothiazine in veterinary parasitology. Ibid., v. 4: 333-341.
- SWALES, W. E. and FROMAN, D. K. 1939.—An apparatus for measuring the "flash" thermal death point of microscopic animal organisms and its use with ova of *Ascaris lumbricoides*. Canad. J. Res., Sect. D, v. 17: 169-177.
- TAYLOR, E. L. 1931.—The faeces in worm diseases: their value for diagnosis, and their hygienic disposal on the farm. Vet. Rec., v. 18(24): 639-644.  
1933.—The control of red-worms in horses. Ibid., n. s., v. 13(18): 399-506.  
1935.—The veterinary aspect of parasitology. Ibid., v. 15(5): 103-114.  
1938a.—Grazing hygiene with respect to parasitic diseases. Proc. 13th Internatl. Vet. Cong., v. 1: 672-682.  
1938b.—Observations on the bionomics of strongyloid larvae in pastures. I. The duration of infection in pasture herbage. Vet. Rec., v. 50(40): 1265-1272.  
1939.—The role of pastures in the development of the strongyloid diseases of grazing animals. Ibid., v. 51(16): 495-504.



## CHAPTER VIII

### EPIDEMIOLOGY AND SANITARY MEASURES FOR THE CONTROL OF NEMIC PARASITES OF MAN

WILLIAM W. CORT, Department of Helminthology, School of Hygiene and Public Health, the Johns Hopkins University, Baltimore, Md.

ELOISE B. CRAM, Division of Zoology, National Institute of Health, U. S. Public Health Service, Washington, D. C.

DONALD L. AUGUSTINE, Department of Comparative Pathology and Tropical Medicine, School of Medicine and Public Health, Harvard University, Boston, Mass.

#### General Discussion

W. W. C.

After the discovery of a human parasite the next step in the sequence leading to effective control must be the determination of its life cycle and method of human infection. Such information suggests the broad lines along which control measures can be developed, but needs to be supplemented by epidemiologic studies to gain information on the various factors involved in dissemination in population groups. These factors differ greatly for the different nemie parasites of man, which vary in their host relations and life cycles.

In those species in which eggs or larval stages have a free life, viz., the hookworms, ascaris, trichuris, enterobius, etc., knowledge is essential on the effect of general environmental factors, such as temperature, moisture, and physical and chemical conditions of the soil; for only when the environment outside the host is favorable can these free stages persist and infect man. The relations of these factors depend on the amount of development outside the host necessary before the infective stage is reached and vary greatly between such species as enterobius and the hookworms. In those species with intermediate hosts the relations outside the definitive host are still more complicated, since they involve all the factors related to the infection of the intermediate host and the transmission by it of the parasite. Thus all the relations of *Trichinella spiralis* to the rat and pig become of vital significance in its transmission; with the guinea worm, *Dracunculus medinensis*, the cyclops is brought into the picture; and in the filariae the relations of mosquitoes and certain other blood sucking flies must be considered.

Equally significant in epidemiologic studies of nemie parasites is the consideration of human habits in relation to transmission. For those species in which the eggs pass out with the feces, habits of excreta disposal are of great significance. Equally important also are all the human habits that make possible the entrance of the free stages into the human body. With those nematodes which have intermediate hosts the human habits that are related to spread are entirely different and vary greatly with the species. In the filariae, habits that bring about exposure to the bites of the insect vector are important both in relation to the infection of the insect and in the transmission of the parasite back to man. In certain cases the human relations may be very peculiar. As for example with *Trichinella spiralis* where the methods of feeding pigs and habits in relation to pork eating have to be considered; or with the guinea worm where transmission depends on the drinking of water containing cyclops, in which infected individuals have waded or washed their feet.

Another phase that cannot be neglected is the host parasite relations. For example, the development of a specific immunity or the presence of an age resistance may be important in determining the distribution of the parasite in the population; or undernourished individuals may be more susceptible than are those on a good diet. We know least about these factors and in the present state of our knowledge their relation to epidemiology is difficult to evaluate.

It is evident that the more extensive is the understanding of the epidemiology of a parasite, the more effective the control program can be made; thus, weak links in the cycle of transmission can be more effectively discovered and mistakes avoided. Most effective in control are attempts to change human habits that make possible transmission. In fecal borne infections the improvement of sanitation to prevent soil pollution is most important. Where transmission is by insect vectors, control measures are chiefly concerned with the protection of the people from their bites and with their eradication from areas near human habitations. Where treatment is effective and easily applied to large groups, mass treatment may be an efficient control measure in breaking the cycle at the stage passed in the human body.

In the following discussion only the most important and best known of the nemie parasites of man will be considered, viz., the hookworms, *Ancylostoma duodenale* and *Necator americanus*; the large round worm, *Ascaris lumbricoides*; the whip worm, *Trichuris trichiura*; the pin worm, *Enterobius vermicularis*; the flesh worm, *Trichinella spiralis*; the guinea worm, *Dracunculus medinensis*; and the most important of the filariae, *Wuchereria bancrofti*, *Onchocerca volvulus*, and *Microfilaria malayi*. For most other human nematodes there is little information on epidemiology or control methods, and they are for the most part of minor significance as human parasites. Also, the knowledge presented on the more important forms gives a background for understanding similar relations for the other species.

#### The Hookworms

W. W. C.

In ancient Egyptian, Arabian, and Greek writings are found descriptions that may possibly have referred to hookworm disease. Also, accounts in medical treatises of the 17th and 18th centuries from Brazil, Guadeloupe, and Jamaica almost certainly referred to this disease. Modern knowledge dates from the description of *Ancylostoma duodenale* by Dubini (1843). In 1878 Grassi and the brothers Parona demonstrated that hookworm infection could be diagnosed by fecal examinations. Leichtenstern in 1887 demonstrated experimentally that infection could be brought about by the ingestion of larvae. Looss (1898) first discovered infection by skin penetration with migration through the lungs and over a period of years carried out extensive investigations on hookworm biology which culminated in his 1911 monograph.

By the beginning of the 20th century the scene had shifted to the Western Hemisphere, where Lutz in Brazil, Ashford in Puerto Rico, and Stiles in the United States had demonstrated the importance of hookworm disease. Of especial significance was the discovery by Stiles (1902) of the second species of human hookworms, *Necator americanus*. Very important also was the work of the Puerto Rico Anemia Commission from 1904 to 1908 (Ashford and Gutierrez, 1911) which carried out the first extensive investigation and control program in a tropical country. Later (1909 to 1914) came the campaign of the Rockefeller Sanitary Commission in the southern United States, which was followed in 1914 by the establishment of the International Health Board of the Rockefeller Foundation, which in the next few years extended hookworm control campaigns widely into other parts of the world.

The true human hookworms, *Ancylostoma duodenale* and *Necator americanus*, are widely distributed between the 36th parallel north latitude and the 30th parallel south. Within this belt there are extensive regions where the combination of favorable temperatures and rainfall make possible the development of widespread heavy infections and clinical disease in populations living close to the soil under primitive conditions of sanitation. Such populations are still found in very limited areas in the southern United States and more extensively in the West Indies, Central America, northern South America, Tropical Africa, and in certain parts of southern Asia and the East Indies. While *A. duodenale* and *N. americanus* are present together in a considerable part of the hookworm belt they show important differences in geographical distribution and appear to have originated in different parts of the world (Darling, 1920).

*A. duodenale* and *N. americanus* differ greatly in the morphology of the adults. The former is larger, appears to be more injurious to its host and is harder to eliminate with anthelmintics (Darling, Barber, and Haeker, 1920). The female of *A. duodenale* produces about 22,000 eggs every 24 hours while

\*For a detailed discussion of the geographical distribution of hookworm disease see Chandler, 1929, pp. 18-54.

that of *N. americanus* only about 8,000 to 10,000 (Soper, 1927). Also the infective larvae of the first species are slightly larger, of different structure, and more resistant to environmental conditions than those of the second (Svensson, 1925). In experimentally infected human volunteers, the adults of *A. duodenale* lived almost 7 years and those of *N. americanus* over 5 years (Kendrick, 1934). In these infections, however, the egg counts in the individuals infected with the first species fell to a very low level in less than 2 years, and in those harboring the second species they were greatly reduced in about a year. In spite of all these differences, both species are very similar in their host relations and life cycles; and the symptomatology, epidemiology, treatment, and control of the diseases they produce are alike in all essential particulars.

Although in a few cases the human hookworms have been reported incidentally in other hosts, and *N. americanus* appears to be a normal parasite of anthropoid apes, there is at present no good evidence that such animals serve as true reservoir hosts.

Besides the true human hookworms there are several others that have some relation to man. The dog and cat hookworms, *A. caninum*, *A. braziliense*, and *Uncinaria stenocephala* have been extensively used in studying host-parasite relation problems. The larvae of the last two have been shown to produce linear skin lesions in man, and *A. braziliense* is the causative agent of creeping eruption which is especially prevalent in certain parts of the southern United States (Fülleborn, 1928; Kirby-Smith, Dove, and White, 1929). *N. suillus*, described by Ackert and Payne (1923) and by some workers considered as a synonym of *N. americanus*, has received consideration in relation to the possibility that its host, the domestic pig, may serve as a reservoir host for human hookworm disease.

#### FACTORS AFFECTING THE FREE STAGES OF THE HOOKWORM LIFE CYCLE

Development of hookworm larvae can be completed at temperatures ranging from about 12° to 37° C., with the optimum from about 25° to 30° C. (Stiles, 1921; McCoy, 1930). Below 22° C. the development is greatly slowed up; and at temperatures approaching 37° C., although development is very rapid, a considerable proportion of the larvae either fail to develop or soon die. The eggs and larvae are quickly killed by temperatures above 40° C. and have little resistance to temperatures close to freezing (Looss, 1911; Svensson, 1925). The injurious effect of low temperatures on hookworm eggs and larvae is the determining factor in limiting the distribution of hookworm disease almost entirely to tropical and subtropical regions.

All the free stages of the hookworm life cycle are quickly killed by desiccation. Therefore, in regions of low rainfall infection is absent or kept at a low level (Chandler, 1926-1928; Sawyer, 1923; Docherty, 1926). On the other hand, while the eggs and infective larvae will live for a considerable period under water, they will not develop either under water or in cultures that are saturated with moisture. Therefore, in areas where the soil is flooded for a part of the year hookworm infection may be kept at a low level (Chandler, 1926; Barnes and O'Brien, 1924). Hookworm larvae require the presence of oxygen for development (McCoy, 1930) and it is probably its absence that prevents their development in a saturated medium. Also, they require a loose porous culture medium and do not develop well in clay soils (Stoll, 1923b). Soil relations are very important in the southern United States where infection is almost entirely absent in areas with clay soil and is particularly intense in those with a loose sandy soil (Augustine and Smillie, 1926; Rickard and Kerr, 1926).

The developing larvae can apparently feed normally only on living bacteria, which must be present in considerable numbers for development (McCoy, 1929). It seems probable that the growth of enough bacteria for the needs of the larvae depends chiefly on the mixture of feces with the soil and if the eggs become separated from the fecal material in which they are passed development will be checked.

Epidemiologic studies of recent years have given illustrations of the types of field conditions that are suitable or unsuitable for the development of the hookworm larvae in the soil. Loose porous humus, sandy, or loam soils that are well shaded give the best development. Places of intense soil infestations under such conditions have been reported in fields of sugar cane in Trinidad (Cort and Payne, 1922), in coffee groves in the hills of Puerto Rico (Cort, Riley, and Payne, 1923), and in fields of cultivated mulberry trees in the Yangtze Delta region of China (Cort, Grant, and Stoll, 1926). In clay soil not covered by a layer of humus or a growth of grass almost no larvae will develop even where the rainfall is considerable (Cort and Payne, 1922). Even on soils of loose texture in regions of abundant rainfall, development of soil infestation will be greatly inhibited if there is no shade, since exposure of the soil surface to the sun's rays produces alternate periods of wetting and

drying which quickly destroy a large proportion of the larvae (Augustine, 1923c). Unshaded areas covered with a thick growth of grass have in some cases been reported as very favorable for development (Korke, 1925). In hookworm infected population groups, therefore, significant sources of infection may be limited, even where there is extensive soil pollution, to the comparatively few places where the eggs are deposited on a loose soil that is well shaded.

When the larvae develop in the soil they migrate toward the surface and are found frequently singly or in clumps extending from the particles (Augustine, 1922b; 1923b). In only a few cases have they ever been reported at depths below the superficial surface layers (Baermann, 1917b). When covered with a loose soil they can migrate vertically from considerable depths (12 to 36 inches), while in a water soaked or stiff clay soil almost no upward migration occurs (Payne, 1922 and 1923). Lateral migration is very restricted and they will not spread out from the place of development unless carried by water or animals (Augustine, 1922a; Chandler, 1925). After the second molt they no longer feed and will continue to live only as long as their reserve of food material lasts. Therefore, the more active they are the shorter will be their life. Under artificial conditions in water, however, infective hookworm larvae have been kept alive for as long as 18 months (Ackert, 1924). In the soil in the tropics their life may be limited to only 6 to 9 weeks, with the great majority dying in 3 or 4 weeks (Augustine, 1922c and 1923c). Under conditions less favorable for activity they may persist in the soil for periods up to 4 to 6 months (Hirst, 1924; Baermann, 1917b). There is also evidence that the larvae of *A. duodenale* live somewhat longer than those of *N. americanus* (Svensson, 1925).

A consideration of the activities of the infective hookworm larvae in the soil lead to certain practical considerations in relation to the epidemiology and control of hookworm disease. The larvae tend to remain in "nests" where the stools are deposited; so only limited places are sources of infection. Further, the burying of feces except under a very stiff clay soil is dangerous because the larvae will soon reach the surface. There is no evidence, however, that they will migrate out of latrines (Payne, 1922). Finally, where soil pollution is stopped, sources of infection will be naturally sterilized in a comparatively short time by the death of the larvae.

#### HOST RELATIONS TO HOOKWORM INFECTION

The penetration of the infective hookworm larvae through the skin produces lesions which are commonly known as ground itch. Secondary bacterial infection frequently increases the severity of this condition. Also, the type of reaction suggests in many cases an allergic condition associated with the presence of immunity. Thus Sarles (1929) noted a much more severe skin reaction to hookworm larvae in old resistant dogs than in susceptible puppies.

Lung symptoms produced by the migrations of the larvae have frequently been noted. They are only occasionally at all severe except in extremely heavy infections, suggesting that the larvae usually enter a few at a time.

In the intestine, the hookworms bite deeply into the mucosa and appear to suck blood constantly throughout their adult life (Wells, 1931; Nishi, 1933). It seems evident that they feed chiefly on elements derived from the blood (Hsü, 1938). They move from place to place and, therefore, when numerous injure the intestinal wall over considerable areas. Blood continues to flow from the lesions even after they have moved away. Disturbances of the digestive system which are commonly present in moderate as well as heavy infections have been explained chiefly in relation to the injury of the intestinal mucosa produced by the worms.

Anemia is the most prominent symptom of hookworm disease. Indeed, most of the long train of symptoms found in chronic hookworm patients can be related to the presence of long standing anemia. The etiology of hookworm anemia has been the subject of considerable controversy. A review of the literature indicates that there is no convincing evidence that it is caused by toxic products of the worms. Recent investigations have emphasized the importance of blood loss in the production of the anemia. In experimentally infected dogs the blood picture follows exactly that produced by artificially induced hemorrhage (Foster and Landsberg, 1934; Landsberg and Cross, 1935; Landsberg, 1937). Apparently, blood loss produced by the worms is only one factor in the production of the anemia in hookworm infected populations. Disturbances produced by dietary deficiencies, particularly lack of iron, have been emphasized as important additional factors (Rhoads, Castle, Payne, and Lawson, 1934 a & b; Cruz, 1934). More recent work, however, stresses general dietary deficiency rather than lack of iron alone (Otto and Landsberg, 1940; Payne and Payne, 1940). Anemia produced by other diseases especially malaria

may also be a complicating factor. It seems evident also that the chronic blood loss produced by the worms might in certain cases be one of the factors that would finally lead to the development of one of the "idiopathic" anemias. In addition, it seems certain that anemias of a variety of etiologies are frequently referred to hookworm infection in cases where the few worms present have little if any part in the production of the anemic condition (Andrews, 1940).

Recently it has been shown that a specific immunity is acquired by dogs in response to repeated infections with *A. caninum* in which antibodies are formed chiefly in response to the secretions and excretions of the worms (Kerr, 1936; Otto and Kerr, 1939; Otto, 1940). It seems practically certain that a similar immunity develops in man in response to hookworm infection. In fact, several workers have recently expressed the view that host immunity must play an important role in the regulation of human hookworm infection (Fülleborn, Dios, and Zuccarini, 1928; Fülleborn, 1929; Cort, 1932; Pessoa and Pascale, 1937 a & b; Cort and Otto, 1940). Such a postulation makes it easy to explain the relatively moderate infections and slight evidence of hookworm disease found in many individuals and groups of people who appear to live under conditions giving constant opportunity for the invasion of the larvae. Severe cases, especially in children, might perhaps be explained in part by exposure to infections so extreme that the development of the immunity is prevented. Also, it seems probable that undernourishment or other debilitating factors prevent the development of the immune reactions. In experimental infections in young dogs either undernourishment or too rapid infection which weakens the host from extreme blood loss will prevent the immune response (Otto and Kerr, 1939); and the immunity already developed in highly resistant older animals is easily broken down by placing them on a deficient diet (Foster and Cort, 1932; 1935). If the same relations hold in human infection, individuals or groups that are badly debilitated by undernourishment or other factors may be expected to acquire heavy worm burdens and will also be less able to compensate by the regeneration of new blood for the losses caused by the worms. It seems probable also that malaria and other diseases are more important than is at present realized in weakening the defense mechanism against hookworm infection. In fact, the hypothesis has recently been suggested that widespread chronic hookworm disease of the type found specially in tropical countries seldom results from uncomplicated hookworm infection, but is produced by hookworm infection plus undernourishment or other debilitating factors that weaken the host defense (Cort and Otto, 1940; Cort, 1940).

#### HUMAN HABITS IN RELATION TO HOOKWORM DISSEMINATION

Insanitary methods of excreta disposal and activities bringing about contact with infested soil are the most important human habits in hookworm dissemination. Careless depositing of stools on the ground (soil pollution) is a widespread habit among most of the population of the world especially in tropical and subtropical regions. Recently, epidemiologic evidence has emphasized soil pollution in the general vicinity of dwellings as important in hookworm infection. Adults and older children are apt to go for defecation to protected places not far from their houses and often the most important contact with infested soil appears to come about during the act of defecation (Cort, 1925; Cort, Stoll, Sweet, Riley, and Schapiro, 1929; Chandler, 1928). Young children usually defecate in the dooryards close to the houses or even under or in the houses themselves, where the soil conditions are usually not suitable for the development of hookworm larvae.

It is usually difficult to determine the extent to which field work brings the laborer into contact with sources of infection. People living near cultivated areas such as vegetable gardens, banana groves, sugar cane fields, or coffee groves, may by their defecation habits produce concentrated places of soil infestation that will infect field laborers. Usually, however, stools passed by laborers at work are widely scattered and would be only occasionally sources of infection, as compared with the constant exposure in the defecation areas near the houses.

Some occupational relations especially important in hookworm dissemination have been noted. Coffee picking in the hills of Puerto Rico has been shown to be responsible for extremely heavy infection (Ashford and Gutierrez, 1911; Cort, Riley, and Payne, 1923). Here groups of people work in the groves for long hours and spread their stools widely when they pick the coffee at weekly intervals for 6 or 7 weeks. Toward the end of the picking season the soil of these groves becomes so impregnated with infective larvae that extensive infection of the workers occurs. In places in the Orient where human excrement is used as fertilizer, the practices in connection with the cultivation of particular crops determine the extent of hook-

worm dissemination. In regions in China where sericulture is important hookworm infection may be widespread because the methods of fertilizing the mulberry trees make possible the development of intense soil infestation (Cort, Grant, and Stoll, 1926); from such places the people who pick the mulberry leaves to feed the silkworms become intensely infected. Other occupational relationships that produce sources of infection might be cited, but as we consider the evidence it becomes more and more evident that, except where human excrement is used as fertilizer, soil pollution in the vicinity of the dwellings is by far the most important factor in hookworm dissemination.

#### DISTRIBUTION OF HOOKWORM INFECTION WITHIN POPULATIONS

The use of the Stoll dilution egg counting method in the extensive epidemiologic studies of hookworm disease of the last two decades has given a large amount of information from different parts of the world on the distribution of hookworm infection in population groups. Estimates of worm burdens by this method have made it possible to compare quantitatively the infection according to age, sex, occupation, race, and other categories, as well as to compare the distribution in populations living under different conditions. Thus data can be obtained for a scientific planning of control programs and the results of the campaigns in reducing the intensity of infection can be measured. Attention has, therefore, been turned from the percentages of positive cases and has been focused on the number of worms harbored (worm burden).

There has been an increasing emphasis on the importance of a proper evaluation of the lightly infected cases, especially those that might be considered as carriers or subclinical, as compared with the heavier cases. A high incidence of hookworm infection may occur in groups where the number of worms present is so small that they have little if any injurious effect. Such situations may be found, as in certain parts of North China (Cort, Grant, and Stoll, 1926) and Egypt (Scott, 1937) where human habits are favorable for hookworm dissemination but climatic conditions are unfavorable. Similar widespread, practically sub-clinical infections are also present where sanitation and treatment have reduced the intensity of infection to a low level, but where widely scattered light sources of infection still exist. It is not possible to indicate definitely the actual number of worms necessary to produce clinical symptoms since this would vary in relation to a variety of factors; also it is not easy to accurately evaluate the injury to a population produced by widespread light infections. It can be said, however, that light infections are of but little consequence as compared with heavy; and that hookworm disease becomes a real public health problem only in groups with fairly heavy worm burdens.

The individual family except when isolated is not nearly so much the unit of hookworm infection as is the case with ascariis. This appears to be due to the fact that sources of infection are fairly widespread and because defecation places near dwellings are commonly shared by more than one family. Frequently almost all the individuals in even large populations are infected.

The relative intensity of infection in the sexes and in different age groups varies greatly in different populations. Usually, however, infection is almost completely absent in children under 3 years of age, gradually increases up to 10 years and reaches the adult level somewhere in the early teens or even later (Smillie, 1922; Payne, Cort, and Riley, 1923). It may vary considerably in the different age groups of middle life and most often has a tendency to decline in older people. Females usually have a distinctly lower level of infection intensity than males (Carr, 1926; Hill, 1927a; Cort, Stoll, Sweet, Riley, and Schapiro, 1929). It has been suggested that this type of age and sex distribution is most typical of situations where infection comes from soil infestation in the general vicinity of the houses. It can be suggested that in most situations the children only have considerable exposure to infection when they begin to visit adult defecation places. Greater activity of boys than of girls brings greater contact with infection; and adult males usually have more contact with sources of infection away from home than do females. Unusually heavy infection in very young children has been noted in certain groups in Panama (Cort, Stoll, Sweet, Riley, and Schapiro, 1929), in the Argentine (Fülleborn, Dios, and Zuccarini, 1928), in Puerto Rico (Hill, 1927a), and in southeastern Georgia (Andrews, 1940). This seems to occur only where soil conditions in the dooryards are favorable for the development of hookworm larvae. Heavier infections in women than in men have been found in a few places like the areas of coffee cultivation in Puerto Rico (Cort, Riley, and Payne, 1923) and in certain groups engaged in sericulture in China (Cort, Grant, and Stoll, 1926) where the women are engaged to a greater extent than

the men in occupations that bring them into contact with unusually intense sources of infection. In parts of the southern United States the level of infection rises rapidly from 6 to 15 years and then declines rapidly until after 20 years the worm burden is almost negligible (Smillie and Augustine, 1925, Chart 4). Such a situation can probably be attributed to the wearing of shoes and the greater use of sanitary facilities by the adults.

Evidence on racial differences in hookworm infection and disease is rather conflicting. It does, however, seem clear that negroes in the southern United States have much lighter infections than whites (Knowlton, 1919; Smillie and Augustine, 1925; Keller, Leathers, and Densen, 1940). It seems possible that this difference is due to a true racial immunity in the negro race, although further investigations are needed before differences in environment and nutrition can be completely ruled out. There is also evidence that suggests that groups with negro and negro-indian blood are more resistant to the injurious effects of the worms than those of the white race (Gordon, 1925; Cort, Stoll, Sweet, Riley, and Schapiro, 1929). However, it is difficult to rule out other factors and here also the whole question needs much further investigation.

#### CONTROL OF HOOKWORM DISEASE

Four different methods of preventing the spread of hookworm infection have been generally recognized, viz., (1) disinfection of feces or infested soil, (2) the encouraging of wearing shoes, (3) anthelmintic treatment, and (4) improvement in sanitation. Extensive experimentation has shown that hookworm eggs in feces and the larvae in the soil can be killed by the application of salt, lime, or other chemicals. Such methods are useful in limited areas such as mines (Fischer, 1928), or in sterilizing human excrement which is to be used as fertilizer (Cort, Grant, and Stoll, 1926). The wearing of shoes has been shown to be a potent factor in keeping hookworm infection at a low level (Smillie, 1922; Davis, 1925; Chandler, 1929, pp. 208-211, 380-382). However, attempts to increase the wearing of shoes in hookworm infested populations by propaganda or legal requirements do not seem to have been very effective. Hookworm control campaigns, therefore, have been organized chiefly around treatment and sanitation. The use of anthelmintics improves the health of the people and reduces soil infestation. The sanitary phase of the program is a fight against soil pollution and involves education and the introduction of latrines. Much work has been done in developing sanitary conveniences suitable for people of different types. The pit latrine (privy) has been most widely used in the Western Hemisphere; and the recently developed bored hole latrine (Yeager, 1931 and 1934) seems to be best adapted for the peoples of certain countries of Asia and Africa.

In the early hookworm campaigns in Puerto Rico and the southern United States the so-called "dispensary method" was used. This consisted of the examination and treatment of large groups of people who flocked to the numerous dispensaries that were set up. Significant results were attained in the treatment of severe cases and in preliminary education, but only a beginning was made in the reduction of infection and in the improvement of sanitation.

As a reaction against the inadequacy of the "dispensary method," the "intensive method" was developed by certain members of the field staff of the International Health Board of the Rockefeller Foundation. Its objective was the complete eradication of hookworm infection by a systematic program of sanitation and treatment to "cure" of all infected individuals (Howard, 1919). First, every effort was made to get latrines installed in every house in a given area. Then, after systematic stool examinations, the positives were given treatment. They were later reexamined, and those still infected were given a second treatment. Reexamination and retreatment were supposed to be continued until the stool samples of all the people of the area were negative for hookworm eggs. Sometimes as many as 9 or 10 treatments were required for "cure." Efforts to improve the sanitation were continued during and after the treatments. Although hookworm infection was never completely eradicated from any area by this method, striking results were obtained in a number of places. Least defensible of the procedures of the intensive method was "treatment to cure" in which much effort and money were wasted in treating very light infections and in trying to remove the last few worms by retreatments. On the other hand, the emphasis on intensive sanitation especially before treatment and on a careful following up of the sanitation after treatment, was an important contribution to hookworm control procedures.

The "mass treatment" method came as a reaction against the complete ineffectiveness of the intensive method to cope with the situation in a large country such as Brazil. As advocated by Darling (1922) mass treatment required first the de-

termination of the index of infection (approximate worm burden) by the examination by worm counts of a representative sample of the population. Later, the development of the Stoll dilution egg counting method (Stoll, 1923a) made it possible with much less effort to obtain a better estimate of infection intensity. Then, wherever incidence was high, a whole group was simultaneously given anthelmintic treatment of known efficacy without a previous diagnostic examination and without reexamination. Thus large groups of people could be rapidly treated. An adequate sanitary program was sometimes combined with mass treatment. In many places, however, the great emphasis on treatment and the rapidity with which the campaign moved brought about a neglect of sanitation. Whenever this was true reinfection occurred at a rather rapid rate as suggested by the investigations of a number of workers (Baermann, 1917b; Sweet, 1925; Docherty, 1926; Hill, 1925, 1926, 1927b). Mass treatments, therefore, were particularly effective if repeated at intervals of 2 or 3 years (Rice, 1927; Lambert, 1928). Perhaps of greatest importance was the emphasis on the quantitative viewpoint; the object of the campaign was to reduce the worm burden of the population and not to cure cases. Also important was the idea that a preliminary survey was needed to estimate the "index of infection" before control work was started.

A campaign against hookworm disease at the present time can be planned on the basis of the wealth of experience of the last 25 years. Such a campaign under ideal conditions might include five steps which have actually been utilized in campaigns; and to these a sixth might be added. (1) a presurvey to evaluate the problem quantitatively; (2) presanitation to reduce soil pollution as much as possible before treatment; (3) mass treatment to reduce the worm burden of the group to a subclinical level in as short a time as possible; (4) follow-up sanitation to keep soil pollution at a low level; and (5) a post-survey to measure quantitatively the results of the campaign. Finally (6), every effort possible should be made to improve the general health by the correction of dietary deficiencies and the elimination of other diseases.

The central feature of the presurvey should be an examination by the dilution egg-counting method of a representative sample of the population to obtain information on the quantitative distribution of hookworm infection in the population and on the extent of true hookworm disease. Investigations of the amount of sanitation present and of soil pollution habits will aid in planning the program for sanitary improvement which in most situations is by far the most important part of the campaign. In regions where hookworm infection is found to be chiefly at or near the subclinical level, even if its incidence is high, control work may well be limited entirely to sanitation. When the preliminary survey shows heavy infection and widespread disease every effort should be made to reduce soil pollution to the greatest possible extent before treatment is started by the introduction of latrines and education in their use. This is done in order to reduce the amount of reinfection after treatment.

A course of treatments should be chosen which has been shown by quantitative study on a group of considerable size to reduce the worm burden by at least 90 percent. If the incidence of infection shown by the preliminary survey is over 90 percent, treatment without diagnostic examination according to the mass treatment method may seem desirable. Such a procedure, however, should not be applied to the youngest age group where infection is almost always least and danger from treatment greatest. Mass treatment should be given by population units so that all the people living in the same environment would be freed of their worms as nearly at the same time as possible. Individual examinations after treatment and retreatments cannot be justified where the object is to reduce the worm burden as much as possible with a given amount of treatment. In tropical regions treatments toward the end of the dry season, when the soil has been unsuitable for a considerable period for the development of the larvae, will have more lasting value than those given during the rainy season, when reinfection is very extensive (Chandler, 1929, pp. 405-408; McVail, 1922); and in colder regions treatments at the end of the winter will be most effective.

Even in the best organized campaigns a varying percentage of the worm burden will be left after treatment. Whether infection will soon return to a high level again depends on the extent to which the people are prevented from returning to their former habits of soil pollution. Real success in hookworm control, therefore, will be achieved only where efforts to improve sanitation have been permanently organized and effectively continued over long periods of time as part of the permanent public health program.

The final step in an ideal program for hookworm control would be a resurvey carried out about 2 or 3 years after the

completion of the treatments by the same methods used in the preliminary survey. Such an investigation will make it possible to check the sanitation and to determine whether the level of infection has returned to a point where further treatment is needed.

Most important in attacking the hookworm problem is the acceptance of the quantitative point of view and the using of quantitative methods to determine the "hookworm index" in the preliminary survey and the resurvey. Much effort and money have been wasted in trying by active treatment campaigns to reduce hookworm infection in populations where it was already close to the subclinical level. Most fundamental perhaps of all is the changed objective of the modern hookworm campaign, which is to reduce hookworm infection to a subclinical level by treatment and to keep it there by permanent improvement in sanitation.

Finally, certain new viewpoints need to be developed on account of the recent new information on the significance of acquired immunity in hookworm infection and its relation to undernourishment and other debilitating factors. If the immune response in man to his hookworms proves to be of the same grade as that of the dog to *A. caninum*, measures to remove factors that interfere with the normal host responses are just as important in hookworm control as those directed against the spread of infection. In fact, it seems altogether likely that if it were possible to eliminate dietary deficiencies from a population suffering from hookworm disease by furnishing an adequate food supply, the restoration of the normal host resistance would in itself strikingly reduce hookworm infection and disease (Otto and Landsberg, 1940; Cort and Otto, 1940). Emphasis in hookworm control, therefore, should be placed not on isolated spectacular treatment campaigns, but on the attempt to reduce hookworm infection by all the methods that will improve the sanitation and raise the general economic and health level of the infected populations.

### *Ascaris lumbricoides*

W. W. C.

References to human ascaris are found in the ancient medical literature of the Chinese, Egyptians, and Greeks. Edward Tyson in 1683 and Francesco Rodi in 1684 studied the anatomy of this parasite, distinguished the sexes, and expressed the view that it reproduced by eggs and not by spontaneous generation. From that time on, *Ascaris lumbricoides* became a favorite object for study, and investigations on its anatomy laid the foundation of our present knowledge of nematode structure. Although much information on the prevalence, pathology, and geographical distribution of ascaris in man has been long available, it is only recently that much attention has been paid to the epidemiology of ascariasis in relation to control.

#### PATHOLOGY AND SYMPTOMATOLOGY

In laboratory animals and in pigs the migrations of ascaris larvae are known to produce lesions in the intestinal wall, liver, lymph nodes, and especially in the lungs (Ransom and Foster, 1920; Yokogawa, 1923; Martin, 1926; Roberts, 1934). The lesions in the lungs consist of petechial hemorrhages and inflammatory processes. In heavy infections the lungs may be very extensively involved, being edematous, hemorrhagic, and even completely consolidated. The picture is that of a multiple lobar pneumonia, which frequently causes the death of experimental animals. A disease of young pigs known as "thumps" has been identified as ascaris pneumonia (Ransom, 1920). In man severe pulmonary symptoms may be produced by heavy infections (Koino, 1922) and in some tropical regions lung symptoms, especially in children, have been attributed to ascaris infection. In most infected populations, however, it is extremely difficult to assign a definite symptomatology to the lung migrations of ascaris (Keller, Hillstrom, and Gass, 1932).

It is not easy to define clearly the symptoms produced by the worms in the intestine. Perhaps the most common complaint is an intermittent intestinal colic. Normal digestion may be disturbed and there may be loss of appetite and insomnia. Nervous symptoms are particularly common among heavily infected young children. Individuals having a special sensitivity may develop a generalized toxemia or specific nervous symptoms. In young children very heavy infections may cause severe illness or even death. Large numbers of ascariids may produce intestinal blockage. Also, the migrations of adult worms sometimes produce penetration of the intestinal wall and severe injury to the appendix, liver, lungs, or other organs. However, only a small proportion of infected individuals show symptoms that can be definitely attributed to ascariasis.

#### DISTRIBUTION AND EPIDEMIOLOGY

During the last 15 years our knowledge of the factors influencing the dissemination of ascaris has been very greatly in-

creased by a number of specific epidemiologic studies in different parts of the world. The distribution of the worm burden has been studied by the Stoll dilution egg counting method, and attempts have been made to get at the sources of infection by the observation of soil pollution habits and by the isolation of eggs from the soil (Spindler, 1929a; Maplestone and Mukerji, 1936). Data from these investigations and information on the factors influencing the development and viability of ascaris eggs outside the body of the host have given a fairly good body of epidemiological knowledge on which to base control measures. In addition, recent studies indicate that host relations may be of importance in determining the distribution of ascaris in populations.

#### DISTRIBUTION

*A. lumbricoides* in man has a world wide distribution and appears to rival *Enterobius vermicularis* for the distinction of being the commonest of all human parasites. It has been found within the Arctic Circle and in regions where almost desert conditions prevail. It is most abundant in tropical countries with a heavy rainfall and is especially widespread in the Orient, although extensive endemic centers are also present in Europe and in the United States (Otto and Cort, 1934a; Denecke, 1937; Girges, 1934).

Recently the information on the distribution of ascaris within population groups has been greatly increased. The family is almost always the unit of infection (Cort, Stoll, Sweet, Riley, and Schapiro, 1929; Cort, Otto, and Spindler, 1939; Otto, Cort, and Keller, 1931). This is true in urban as well as rural areas (Headlee, 1936; Winfield and Chin, 1938). Only in Egypt (Scott, 1939) and in certain special institutional situations (Caldwell, Caldwell, and Davis, 1930) was a larger group indicated as the unit. In numerous situations negative or lightly infected families are found living close to those that are heavily infected. With few exceptions (Scott, 1939) about 50 percent of the total worm burden of any population group is concentrated in about 5 percent of the infected individuals. These heavy cases are largely found in a small number of families, the so called "ascaris families." Usually the peak of the infection curve comes early in life, sometimes even in the 5 to 9 age group, and the worm burden in adults is only a fraction of that in children. Also, women of child bearing age are frequently more heavily infected than men of the same age groups. However, heavy infections are sometimes found in adults, especially in certain places in the Orient (Cort and Stoll, 1931).

Ascaris is in general a parasite of people on a low economic and social level. "Ascaris families" are usually among the poorest and most degraded of the population. Not infrequently, however, infections in children, sometimes rather heavy, are found in families of a higher type living under favorable environmental conditions. In the Orient, also, ascaris is often widespread in people of the better classes (Mills, 1927). It is not primarily a parasite of rural districts since in many parts of the world it is present and sometimes very common in cities of various sizes. The reasons for most of the peculiarities in the distribution of ascaris which have just been summarized become clear when the knowledge available on the various factors that influence dissemination is considered. These factors may be grouped under (1) host relations, (2) general environmental factors, and (3) human habits.

#### HOST RELATIONS

In this connection one of the important problems is the relation of the ascaris of pig to human infection. Extensive investigations have shown no differences between the ascariids of these two hosts in morphology or in physiological and biochemical relations (Schwartz, 1920; Bakker, 1921; Barker, 1923). Almost all attempts to infect pigs with eggs from human sources have been unsuccessful (Payne, Ackert, and Hartman, 1925; Martin, 1926). Also, the attempts to infect man with the pig ascaris have given negative results (Koino, 1922; Payne, Ackert, and Hartman, 1925; Buckley, 1931). Several workers have expressed the view that the human and pig ascariids are physiological or host varieties which have each lost their infectivity for the other host. As Laue (1934) has suggested, however, the evidence from these experimental infections is not very conclusive because of the lack of adequate controls and because of the difficulty reported by a number of workers of infecting pigs with the pig ascaris (see also Roberts, 1934). Also, de Boer (1935a & b) reported that he succeeded in infecting suckling pigs with eggs from both pig and human sources and Hiraiishi (1928) and others in Japan have infected pigs deficient in vitamin A with human ascaris. It is difficult, however, to escape the conclusion that under field conditions infection of man with pig ascaris is at least very infrequent. In fact, no evidence has been found in the reports of epidemiological studies of undoubted human infection from pig sources. Attention has also been called to areas in which differences in the

incidence of the pig and human ascaris are very great under conditions that would seem to favor cross infection (Payne, Aekert, and Hartman, 1925; Caldwell and Caldwell, 1926; Martin, 1926; Roberts, 1934). Therefore, until some evidence can be presented of human infection with ascaris from the pig, it hardly seems reasonable to consider the domestic pig as a reservoir host of any significance in the dissemination of ascaris in human populations.

There is some evidence that a specific immunity is acquired to infection with *A. lumbricoides*. Some of the studies showing this have been made on abnormal hosts and, therefore, involve only the stages of the cycle through the lung migration (Kerr, 1938). Other workers have reported experiments that suggested the development of immunity in pigs (Morgan, 1931; de Boer, 1936; Roberts, 1934). In pigs also infection is very much greater in young than in old animals (Ransom and Foster, 1920; Roberts, 1934). Such differences might be explained as the result of an immunity produced by repeated infection. There is some suggestion also that in older animals poor nutrition may increase susceptibility (Morgan, 1931; Hiraishi, 1928). Possibly in man a part at least of the reduction of infection in adults as compared with children may be due to the development of an acquired immunity, although difference in habits cannot be excluded. Also, it seems not unlikely that undernourishment or other debilitating factors may influence susceptibility to this parasite.

There is some suggestion that *A. lumbricoides* is not well adapted to its host. This has been suggested by several workers because of the difficulty of producing experimental infections in pigs (Ransom and Foster, 1920; Martin, 1926; Hiraishi, 1928; Roberts, 1934). A similar relationship in man may explain the rarity of heavy infections. Another significant host relation is the rapid turnover of the infection and the constant loss of worms in infected populations (Otto, 1930). Individuals frequently pass worms; heavy worm burdens are only kept up by constant reinfection; and groups removed from exposure to reinfection soon lose their worms (Keller, 1931). It is not clear whether this instability of infection is due to lack of attachment of the worms or to immunity reactions of the host. Finally, it seems probable that host reactions have a part in keeping ascaris infections in human populations at a low level except under extreme conditions of exposure to infection, and in establishing the peculiar age distribution of this parasite.

#### GENERAL ENVIRONMENTAL FACTORS

The eggs of *A. lumbricoides* live for long periods of time and are remarkably resistant to most external conditions. They have been kept alive for 4 to 5 years (Davaine, 1858 and 1863; Martin, 1926) and under natural conditions will live for 1 to 2 years and survive the winter (Brown, 1928; Roberts, 1934). Under field conditions, where they would be exposed to a larger variety of factors, it seems probable that they remain viable for somewhat shorter periods, although it is evident that infested soil remains dangerous for a very much longer time than is the case with hookworm.

Ascaris eggs have been shown to have a remarkable resistance to a wide variety of chemical agents (Yoshida, 1920; Ransom and Foster, 1920). It seems evident, therefore, that in nature they would rarely if ever meet chemical conditions in the soil that would be unfavorable. The eggs require a constant supply of oxygen for their development. They can, however, live for several weeks under anaerobic conditions and can develop in cultures where oxygen tension in the surrounding water is only a fraction of saturation (Brown, 1928a). It seems evident, therefore, that under natural conditions they can readily find oxygen enough for development except in polluted water or saturated media where bacterial growth would use up the supply.

Ascaris eggs in all stages of development can withstand freezing temperatures for surprisingly long periods of time (Cram, 1924; Nolf, 1932) and will develop slowly at temperatures as low as 12° C. The optimum temperature for development seems to be about 30° to 33° C. and development is almost completely inhibited at temperatures of about 37° C. Higher temperatures are very injurious to the eggs and at temperatures above 50° C. they are killed in a short time (Ogata, 1925; Nolf, 1932). Desiccation is also an important factor in killing ascaris eggs, although they will remain viable for several days when dried on glass slides and kept at a relative humidity of about 50 percent (Otto, 1929; Roberts, 1934). On the dry surface of soil they survive much longer (Caldwell and Caldwell, 1928; Brown, 1928b). Their resistance to desiccation is also greatly increased by low temperatures (Martin, 1926). They will develop normally when air dried on glass slides and kept in an incubator with a relative humidity above 80 percent (Otto, 1929). A number of authors have reported that direct sunlight is lethal to ascaris eggs, although in many of the experiments the effect

of high temperature was not excluded. There is, however, definite evidence that sunlight *per se* does injure the eggs since Nolf (1932) demonstrated that they were quickly killed by ultra violet light. Under conditions in the field, a combination of high temperatures with desiccation is probably most important in killing the eggs as is shown by the rapidity with which they die when exposed to direct sunlight on certain types of soils (Brown, 1927b; Otto, 1929).

The resistance of the eggs of the human ascaris to external environmental conditions accounts for its wide geographical distribution. Studies in the United States (Otto, Cort, and Keller, 1931) have shown that they can develop and persist on the hard-packed clay soil of unshaded dooryards where the eggs of trichuris and hookworm are soon killed. Certainly, *A. lumbricoides* is less restricted in its spread by climatic and soil conditions than any other human parasite with free stages. Of course, tropical and semitropical countries with a high rainfall offer the most favorable conditions for its spread; but where human habits are particularly favorable a high incidence with heavy infections may occur in regions, such as in certain places in North China, where there is low rainfall and a long cold winter (Cort and Stoll, 1931; Winfield, 1937a).

#### HUMAN HABITS AND SOURCES OF INFECTION

Studies of the last few years in such widely separated regions as tropical America, the southern United States, North China, and the Philippine Islands, have shown that the chief sources of ascaris infection are from eggs deposited by young children in the yards, under the houses, and even within the houses themselves (Brown, 1927a; Cort, Stoll, Sweet, Riley, and Schapiro, 1929; Cort and Stoll, 1931; Cort, Otto, and Spindler, 1930; Nair, 1935; Otto and Cort, 1934a; Tubangui, Basaca, and Pasco, 1934; Winfield, 1937 a & b). This household pollution by young children results in the accumulation of large numbers of viable eggs in the dooryards which are frequently carried into the houses. Under these conditions eggs can easily contaminate food and water and also infect directly by hand to mouth transfer the youngest children who play in the dirt and are most careless in their habits. More general areas of concentrated soil infestation are frequently found such as those near unsanitized schools or in the yards of institutions (Caldwell, Caldwell, and Davis, 1930). In Egypt the sources of infection appear to be chiefly from eggs on the floors of the houses (Scott, 1939). The point has been repeatedly stressed that heavy infection of a family can only be brought about by the grossest type of soil pollution close to the house combined with very careless habits, especially in the children. Families without infection have frequently been found living next door to heavily infested "ascaris families." Also, in certain regions, as for example western Tennessee, where there is little or no sanitation in some of the rural areas, ascaris infection may be at a low level or absent where the stools are deposited at some distance from the dooryards (Otto, Cort, and Keller, 1931). Such relations, and the rarity of heavy infections, can only be explained by postulating that in man, as has been shown in the pig, infection is difficult. The ingestion of large numbers of eggs is evidently necessary to produce even moderate infections of adult worms. When the constant loss of worms is also considered, it is easy to understand why constant exposure to intense infection is necessary to produce heavy infections.

The contamination of drinking water has been frequently suggested as a method of infection with ascaris. In most of the epidemiological studies that have been made in the United States, Tropical America, and the Orient the possibility of infection from this source has been practically ruled out. In certain parts of India, however, evidence was found that the contamination of shallow pools of water was a factor in infection (Chandler, 1928). Recently the suggestion has been made (Lane, 1934) that the breathing in of dust containing viable ascaris eggs might be a source of infection of considerable significance. While infection in this way seems possible, it could hardly be a method of major importance except under very unusual circumstances.

It has been commonly considered that vegetables fertilized with human feces are an important source of ascaris infection (Mills, 1927; Yoshida, 1925; Walker, 1927; Khalil, 1931; Robertson, 1936). This would explain, as suggested by Mills (1927), the distribution of ascaris among all ages and classes of the population in Korea. Several workers in the Orient have found viable eggs of ascaris clinging to vegetables that are used for food uncooked (see summary by Winfield and Yao, 1937). Also, where human excrement is used as fertilizer, the storage, transportation, and distribution of night soil on the fields would serve to scatter the eggs of ascaris widely in the general environment of the villages. However, definite evidence has been presented in studies in China that pollution by children in the yards and streets of the villages is a very common and perhaps the most important method of ascaris dissemina-

tion (Cort and Stoll, 1931; Winfield, 1935b; Winfield and Chin, 1938). Also, in North China, Winfield and Yao (1937) could find no evidence of ascaris eggs on vegetables after they were prepared for food, and expressed the opinion that infection from this source was of little if any significance in this part of China. It seems clear, however, that the use of human excrement as fertilizer does spread ascaris probably in a number of different ways, since in China, Korea, and Japan infection with this parasite appears to be more common, especially in the adults, than anywhere else in the world.

#### CONTROL OF ASCARIASIS

Treatment of infected populations and improvement in household sanitation are the obvious suggestions for the control of ascariasis. On account of the enormous numbers of eggs produced and their great resistance to chemicals, sterilization of sources of infection would seem to have a very limited value.

In spite of the availability of effective anthelmintics (Brown, 1934), there is clear evidence that mass treatment of infected populations is not an effective control measure against ascaris because of rapid reinfection (Cort, Schapiro, and Stoll, 1929; Otto, 1930; Otto and Cort, 1934b). Perhaps if the treatments could be made almost 100 percent effective in removing the worms, and if they were administered at the end of a dry season or winter when the numbers of viable eggs in the soil would be reduced (Cort, Schapiro, Riley, and Stoll, 1929), they might have some real value as a control measure. At any rate, treatment in ascaris-infected populations is important for relieving heavily infected individuals, especially young children, of dangerous worm burdens.

There are also certain difficulties to be met in the attempt to control ascaris by improved sanitation (Cort, 1931, p. 137). It was found in Panama (Cort, Stoll, Sweet, Riley, and Schapiro, 1929) that in certain areas sanitary improvements that had definitely reduced the level of hookworm infection did not appear to limit the spread of ascaris. Also, more than half of the families with heavy ascaris infection that were studied in the mountains of Tennessee had privies which in almost all cases were in use (Otto, Cort, and Keller, 1931). Examples have also been reported from cities of families with flush toilets connected with the sewage system in which the children had considerable ascaris infection (Otto and Cort, 1934a; Headlee, 1936). Under all these conditions the infection is kept up because the young children fail to use the sanitary facilities and deposit their stools in the yards close to the houses.

In attempts to improve sanitation in rural districts certain practical points of special importance in the control of ascaris seem to have been entirely overlooked in a number of places. First, the latrines should be placed near enough to the houses so that they can be reached by the young children, and in the second place they should have special seats for the children. Usually seats are designed only for adults and are difficult or even dangerous for children to use. In addition, real progress in ascaris control will have to depend on the instruction of the children and their parents in the homes and in the schools in the dangers of soil pollution and in the minimum requirements of a proper household sanitation. In most places widespread ascaris infection is associated with general low standards of living, and any raising of standards will have a tendency to reduce infection.

#### *Trichuris trichiura*

W. W. C.

The whipworm, *Trichuris trichiura*, was first described by Roederer in 1761, although it was apparently observed much earlier. Davaine (1858 and 1863) studied the development of the eggs. Leuckart (1866) demonstrated experimentally the direct development of the trichuris of the sheep and pig, and Grassi (1887) produced experimental infection with *T. trichiura* in man. About the beginning of the 20th century the pathogenic role of trichuris was greatly emphasized and it was considered to be an important factor in infection with such diseases as typhoid fever, cholera, appendicitis, and dysentery (Guiart, 1911). More recently, however, these views have been discounted by most workers. The extensive surveys of the last three decades by fecal examination have greatly extended the knowledge of the distribution of trichuris. Also, considerable information on the factors influencing its dissemination has been obtained, chiefly in connection with field studies on ascaris and hookworm.

The adults of *T. trichiura* are most frequently found in the caecum, vermiform appendix, and colon with their long attenuated anterior ends sewed into the superficial mucosa. The great majority of infections with the human trichuris involve only a few worms, but in occasional cases hundreds may be present. The length of life of the adult worms is not definitely known, although it appears to be much greater than that of

ascaris. Also, there is no evidence of the constant loss of worms and rapid turnover of infections found in that species. There is some evidence that an acquired immunity is developed in trichuris infections (Suzuki, 1934; Miller, 1941).

#### PATHOGENECITY

The adult worms produce some injury to the intestinal mucosa and when present in large numbers may cause considerable inflammation. Therefore, in the heaviest cases they may produce rather severe intestinal disturbances. There is no real evidence that they serve as a "lancet of infection" for other diseases as suggested by many earlier workers (Guiart, 1911) and their relation to the production of anemia is rather doubtful (Otto, 1935; Swartzwelder, 1939). In most cases their presence would pass unnoticed except for the finding of the characteristic eggs in fecal examinations.

#### DISTRIBUTION AND EPIDEMIOLOGY

*Trichuris trichiura* is widely distributed in the world and is frequently found, especially in tropical and subtropical regions, associated with both ascaris and hookworm. Its range is not as extensive as that of ascaris, especially in the temperate zones and it is absent in the colder regions. In the majority of places where both these parasites are found together the incidence of trichuris is lower. In the mountain regions of the southeastern United States, where the incidence of ascaris is several times that of trichuris, families are common that harbor only ascaris, but almost always where trichuris is found ascaris will also be present (Otto, Cort, and Keller, 1931). There are, however, many situations where examinations have shown the incidence of trichuris to be equal to or even higher than that of ascaris. Such situations are usually in tropical or subtropical countries, but there are a number of places in Europe, especially in the U. S. S. R., where the incidence of trichuris is surprisingly high.

Examinations of the last few years by the dilution egg counting method have given us considerable information on the distribution of trichuris within population groups, especially in the United States (Cort, Stoll, Sweet, Riley, and Schapiro, 1929; Otto, Cort, and Keller, 1931; Cort and Otto, 1937). Its distribution resembles that of ascaris in having the family as the unit of dissemination, and in the concentration of a large proportion of the worm burden in a small percentage of heavy cases, usually grouped in families. Also, the distribution of trichuris according to age and sex is much like that of ascaris except that the peak of infection comes almost always at a somewhat later age. Usually adult females are more heavily infected than males of the same age groups.

The human habits involved in the spread of trichuris and ascaris appear to be exactly the same. Differences in egg production, susceptibility of hosts to infection, stability and persistence of infection in the hosts, or in immunity relations cannot be evaluated in the present state of our knowledge in relation to differences in the methods of dissemination or distribution of these two parasites. Therefore, in attempting to explain such differences we must concern ourselves chiefly with the differences that have been found in the resistance of their eggs to external environmental factors. The eggs of trichuris are much less resistant to low temperatures than are those of ascaris, and are somewhat less resistant to high temperatures (Nolf, 1932). They are also less resistant to desiccation, require slightly more moisture for development, and develop more slowly when the moisture is reduced (Caldwell and Caldwell, 1928; Spindler, 1929 a & b; Nolf, 1932; Onorato, 1932). They are very long lived and like those of ascaris are very resistant to chemicals, and they are considerably more resistant than the eggs of ascaris to ultra violet light (Nolf, 1932).

The differences just discussed in the resistance to external environmental conditions of the eggs of ascaris and trichuris appear to explain satisfactorily at least some of the differences in their distribution. Certainly the absence of trichuris in cold regions and its scarcity wherever there is a long cold winter can be explained on the basis of the lack of resistance of its eggs to low temperatures. It seems unlikely that the eggs of trichuris on the soil could live through even a short period of freezing temperatures.

Following suggestions that trichuris is limited more in its distribution by dry conditions than ascaris (Sweet, 1924; Chandler, 1928), this relation was first carefully studied by Spindler (1929b) in the United States. It was found that in the mountains of southwestern Virginia trichuris occurred in a much lower incidence than ascaris except in a few limited localities, where dense shade in the yards produced moist conditions where the eggs were deposited. This finding led to the suggestion supported by a careful review of the literature that the incidence of trichuris tends to be as great or greater than ascaris only where there is a considerable amount of moisture

in the soil due to heavy rainfall or protection by dense vegetation. Later epidemiologic investigations elsewhere in the United States supported this view by showing that in other areas where the incidence of ascaris was much higher than trichuris, infections with the latter were largely limited to households where dense vegetation or poor drainage produced moist areas around the dwellings where the eggs were deposited (Otto, Cort, and Keller, 1931; Otto, 1932); Cort and Otto, 1937).

Certain field studies in the United States (Cort and Otto, 1937; Otto, 1932; Caldwell, Caldwell, and Davis, 1930) were made of situations where the incidence of trichuris was higher than ascaris. In these places the soil where the eggs were deposited was moist and appeared very favorable for development. The soil pollution in the yards and close to the houses, however, seemed to be considerably less than that found associated with heavy ascaris infections. It was suggested, therefore, that given favorable conditions for the development of the eggs the advantage in dissemination would be in favor of trichuris on account of its longer life and greater stability in the host. Undoubtedly, other differences in the life cycle, host relations, and general environmental relations of these two parasites also produce differences in their distribution.

Finally, since the human habits responsible for the spread of trichuris and ascaris appear to be the same, control measures would be the same for both.

### *Trichinella spiralis*

#### E. B. C.

The old Mosaic law against eating pork is perhaps traceable to suspicions regarding the casual relationship between pork and the disease later called trichinosis. From very early days epidemics were recorded with symptoms strikingly similar to those of trichinosis; Glazier (1881) refers to such a disease among the Carthaginians sent (B. C. 427) to subjugate Sicily; descriptions of outbreaks from the 15th century on, in Germany, France, the British Isles, and America correspond so closely with those of trichinosis that there is now no doubt as to the etiology. However, it was not until the 19th century that evidence was produced as to the cause of the disease.

The principal hosts of *Trichinella spiralis* are swine, rat and man. In addition, however, the following other animals either have been found naturally infected or have been experimentally

infected. Naturally infected: mice, rabbits, beaver (coypu), domestic cat, palm civet, dog, wolf, coyote, fox, pole cat, martin, ferret, European and American badgers, raccoon, polar bear, common bear (*Ursus* sp.), and mongoose. Experimentally infected: guinea pig, monkeys, sheep, cattle, horse, young chickens, pigeons, magpies, and rooks. In young chickens the larvae in the muscles soon die (Augustine, 1933; Matoff, 1938). Larvae developed to the infective stage in very young pigeons (Matoff, 1936; 1938) and in adult pigeons affected by avitaminosis (Pavlov, 1940). In the latter case infectivity was demonstrated 32 days after the feeding of trichinous meat to the pigeons (personal communication). Cold blooded animals are apparently immune (Pavlov, 1937).

#### DIAGNOSIS

During life, diagnosis of trichinosis may be made from the clinical history, the differential blood picture, and immunological tests. Other tests, sometimes used but less reliable, are the following. *Stool examination*: the evidence indicates it is of little or no value. *Biopsy*: a bit of muscle excised usually from the deltoid, biceps or gastrocnemius is examined as a fresh press preparation and by digestion, as the trichinae are thus more easily detected than in sections. This method has the limitation of being not dependable until the end of the third week after infection and in addition a negative biopsy does not exclude trichinosis. *Examination of blood and cerebrospinal fluid for larvae*: For a period of about 3 weeks, beginning about 1 week after infection, the larvae may be present but are not always easy to find.

Immunological reactions, consisting of intradermal and precipitin tests, are more reliable when properly used and interpreted. Bachman (1928) initiated both tests; they have been somewhat modified by Augustine and Theiler (1932) and other workers. Bozicevich (1939) developed an improved antigen, superior in having larvae with a minimum of debris and in being extracted with neutral 0.85 percent solution of sodium chloride, without preservatives; the result is much greater specificity and improved maintenance of potency. With the improved antigen a positive skin test in rabbits may be obtained 8 or 9 days after infection; however, in man positive reactions are seldom obtained until after the second week of infection. Both skin and precipitin tests should be used for diagnosis, even though the skin test is negative. Positive precipitin re-

TABLE 9. Findings of trichinae in local surveys in the United States

| Author                  | Date | Place                         | Number of examinations | Number positive | Percent positive | Method  |
|-------------------------|------|-------------------------------|------------------------|-----------------|------------------|---|
| Whelpley                | 1891 | St. Louis, Mo.                | 20                     | 1               | 5.0              | Microscopic   |
| Thornbury               | 1897 | Buffalo, N. Y.                | 21                     | 3               | 14.3             | Microscopic   |
| Williams                | 1901 | Buffalo, N. Y.                | 362                    | 21              | 5.8              | Microscopic   |
|                         |      | Philadelphia, Pa.             | 7                      | 0               | 0.0              | Microscopic   |
|                         |      | Baltimore, Md.                | 126                    | 5               | 4.0              | Microscopic   |
|                         |      | Denver, Colo.                 | 10                     | 1               | 10.0             | Microscopic   |
| Queen                   | 1931 | Rochester, N. Y.              | 344                    | 59              | 17.2             | Digestion   |
|                         |      | Boston, Mass.                 | 58                     | 16              | 27.6             | Digestion   |
|                         |      | Denver, Colo.                 | 431                    | 70              | 16.2             | Digestion   |
| Riley and Scheifley     | 1934 | Minneapolis, Minn.            | 117                    | 20              | 17.1             | Microscopic   |
| Hinman                  | 1936 | New Orleans, La.              | 200                    | 7               | 3.5              | Digestion   |
| McNaught and Anderson   | 1936 | San Francisco, Cal.           | 200                    | 48              | 24.0             | Digestion   |
| Magath                  | 1937 | Rochester, Minn.              | 220                    | 17              | 7.7              | Microscopic   |
| Sawitz                  | 1937 | New Orleans, La.              | 200                    | 10              | 5.0              | Micos. & digestion                                      |
| Pote                    | 1937 | St. Louis, Mo.                | 1,060                  | 163             | 15.4             | Sections  |
| Scheifley               | 1938 | Minneapolis & St. Paul, Minn. | 118                    | 15              | 12.7             | Microscopic   |
| Walker and Breckenridge | 1938 | Birmingham & Tuscaloosa, Ala. | 100                    | 33              | 33.0             | Micos. & digestion                                      |
| Evans                   | 1938 | Cleveland, Ohio               | 100                    | 36              | 36.0             | Micos. & digestion                                      |
| Hood and Olson          | 1939 | Chicago, Ill.                 | 208                    | 12              | 5.8              | Digestion   |
|                         |      |                               | 220                    | 25              | 11.4             | Digestion & micos.                                      |
| Sawitz                  | 1939 | New Orleans, La.              | 200                    | 14              | 7.0              | Micos. & digestion                                      |
| Butt and Lapeyre        | 1939 | Los Angeles, Cal.             | 170                    | 31              | 18.2             | Digestion   |
| Gould                   | 1939 | Eloise, Mich.                 | 90                     | 11              | 12.2             | Digestion   |
| Gould                   | 1939 | Eloise, Mich.                 | 410                    | 82              | 20.0             | Micos. & digestion                                      |
| Harrell and Johnston    | 1939 | Durham, N. C.                 | 44                     | 0               | 0.0              | Digestion   |
|                         |      |                               | 6                      | 0               | 0.0              | Microscopic   |
|                         |      |                               | 55                     | 3               | 5.4              | Micos. & digestion                                      |
| Oosting                 | 1940 | Dayton, Ohio                  | 134                    | 27              | 20.1             | $\frac{2}{3}$ Digestion; $\frac{1}{3}$ Micos. & digest. |
| Catron                  | 1940 | Ann Arbor, Mich.              | 300                    | 44              | 14.7             | Digestion and 270 microscopic                           |
| Totals                  |      |                               | 5,531                  | 774             | 13.9             |   |

\*Reported by Scheifley, 1938.



actions appear usually at the end of the third week. Limitations of the use of these reactions for diagnosis of the disease should be kept in mind; after an attack of trichinosis, a positive skin test may be obtained for as long as 7 years and a positive precipitin reaction for as long as 2 years. In addition, persons with subclinical trichina infections may also give positive skin and precipitin reactions.

For postmortem diagnosis, the compressor method and the digestion Baermann method are used. The former consists of direct microscopic examination of a press preparation of muscle. The latter method consists of the digestion of muscle in artificial gastric juice, the digested material being put through the Baermann apparatus and examined microscopically for larvae. Either of the two methods has special value and certain limitations for certain types of infection, the two methods being supplementary (Hall and Collins, 1937). Both methods have therefore been used in recent surveys and on a quantitative basis of trichinae per gram of diaphragm muscle examined. The two methods have been described in detail by Nolan and Bozicevich.

Investigation (Sawitz, 1937; Schapiro et al, 1938) has shown a correlation between the skin test for diagnosis of trichinosis in living persons and postmortem findings.

#### SYMPTOMATOLOGY

Trichinosis is characterized by lack of regularity in its course (Ransom, 1915; Hall, 1937; Kaufman, 1940). A history of eating raw or undercooked pork containing trichina may or may not be followed by a gastrointestinal disturbance, including abdominal pains, nausea, vomiting, diarrhea or constipation or one succeeding the other, and intestinal hemorrhages. Eosinophilia of 10 to 45 percent and at times 68 to 78 percent may be present; on the other hand it may not be present at all. There may be edema (usually periorbital), high fever, myositis and pneumonia. The heart may be involved. There may be nervous derangement, including encephalitis, meningitis and delirium. The variegated clinical picture results from differences in intensity of infection, organs invaded and resistance of the patient. A clinical but nonfatal case may show at biopsy as few as 8 larvae per gram of gastrocnemius (Ferenbaugh et al). Hall (1937) tentatively designated as "heavy to critical" cases showing 101 to 1,000 larvae per gram. Conclusions concerning man can not be drawn from quantitative data from laboratory animals, as in man (Nevimny, cited by Roth, 1939) inflammatory and other injurious processes are more pronounced and extensive, than in those animals. Schwartz (1938) found that experimentally infected hogs showed no symptoms when there were less than 800 to 900 larvae per gram of diaphragm muscle tissue.

#### EPIDEMIOLOGY

In California outbreaks of trichinosis have resulted from the eating of jerked bear meat (Walker, 1932; Geiger and Hobmaier, 1939) and in Europe from the meat of the polar bear and the dog (cited by Kaufman, 1940) and the Coypu (Rubli, 1936). These cases are rare, however; swine are the principal source of infection to man and it is probable that most cases of infection of other animals could be traced back ultimately to swine.

The incidence of trichinae in swine varies according to the locality and manner of feeding, the principal source of the infection being uncooked pork scraps fed to swine in garbage or swill; the eating by swine of infected rats or of carcasses of infected pigs are very minor sources. Hall (1937a), Schwartz (1938) and Wright (1939) have analyzed the data from different parts of the United States; trichinae were found in only about 0.5 percent of swine fed on cooked garbage and southern swine which range the fields and woods and get little garbage; in 1 to 1.5 percent of swine in the Central West, where feeding of grain predominates over garbage feeding; in 4 to 6 percent of swine fed on uncooked garbage; and in 10 to 20 percent of swine fed on slaughter house offal, this last group now being small.

As regards the incidence in man, data have been inadequate; due to the variability of symptoms, cases are frequently unrecognized. To reports of outbreaks of acute trichinosis, which have often involved large numbers of persons, and those of sporadic cases must be added necropsy findings which detect subclinical infections as well as previously undiagnosed clinical cases. In the 94 year period, 1842-1936, according to Sawitz (1938), there were between 5,000 and 6,000 clinical cases of trichinosis diagnosed and recorded in the United States. Stiles (1901) found that in Germany between 1860 and 1898 there were reported 14,820 cases of trichinosis with 831 deaths, a mortality of 5.6 percent. Hall (1938a) points out that in the 1880's competent authorities maintained that the incidence of

trichinae was much greater in the United States than in Europe and that these opinions, long neglected, were borne out by later findings which indicate that the United States has the greatest trichinosis problem of any country in the world. The incidence here is about 5 times greater than in middle Europe (Magath, 1937), or even higher (Hall, 1938a). Comparative data are lacking from many parts of the world. A very low incidence has been found in England (Van Someren, 1937). Eleven clinical cases are known from the Hawaiian Islands (Alicata, 1938); trichinae have been found in the rat, mungoose and wild and domestic pigs there. Apparently only one human case has been reported from China although the dog, cat and swine have been found infected there (Ch'u, 1937).

Early examinations of necropsy material were confined to direct microscopic examination of muscle by the compressor method and for the most part did not represent real surveys. In more recent years a digestion method has also been used; there is evidence that either technique alone fails to detect one-third of the infections, so that a correction figure of 33 1/3 percent should be applied if only one method is used. Local surveys have been made in various parts of the United States (Table 9) and a nationwide survey of unselected cases from various population groups (Table 10) is in progress. To date the findings from necropsy examinations of over 9,000 persons show that 15 percent were infected with trichinae, and the actual incidence figure would be higher if corrections could be made to eliminate all variables. As regards severity of infection, the majority of 488 positive cases (Wright, 1939) showed less than 11 larvae per gram of diaphragm but 2.5 percent of the cases had between 101 and 1,000 larvae per gram.

An analysis of the findings according to sex, age, race, occupation and social-economic status of 2,000 individuals (Wright, 1939) failed to show any special correlation in most of the groups represented. However, a geographical correlation is indicated, especially as regards reported cases of clinical trichinosis; the heaviest incidence is found along the North Atlantic coast and along the Pacific coast, correlated with garbage feeding. In New York, Massachusetts and California from 501 to over 1,000 clinical cases per state were reported up to 1938 (Hall, 1938); these are areas where there is extensive feeding of uncooked garbage to swine.

#### CONTROL

With regard to trichinosis, the significance of chemotherapy is decidedly different than in the case of most other helminth infections, as in oxyuriasis (p. 324). In trichinosis it is entirely therapeutic, administered only for the patient's sake; in oxyuriasis it is both prophylactic and therapeutic, preventing reinfection of the patient and infection of others.

Since human trichinosis results from the operation of two factors (Hall, 1938), its prevention lies in control of those factors—namely, (1) food habits of the individual, including infection from accidents and failures of cookery, and (2) the frequency of occurrence of live trichinae in swine supplying the pork. It is evident from necropsy findings as well as from numerous sporadic cases and occasional outbreaks of trichinosis, that a very large number of persons have eaten unprocessed, uncooked or undercooked infected pork. The great majority of swine are free from trichinae; pork from a small minority of swine serves as the principal source of both human and porcine trichinosis. This source may be combatted (Hall, 1936; 1937a; 1938a; Schwartz, 1938; Wright, 1939) by (1) meat inspection; (2) avoidance of the use of raw or inadequately cooked pork or pork products; (3) the swine sanitation system; (4) cooking of garbage; and (5) rat destruction.

In Germany microscopical inspection of pork for trichinae was instituted in 1875. Exclusion of American pork from Germany caused a loss of millions of dollars to farmers and exporters in the United States and led to diplomatic complications. Stiles' (1901) study of the German system indicated that inspection can not detect all infected meat, that there was a false sense of security from inspected meat and that the system was very elaborate and expensive. In the United States there has never been federal meat inspection for trichinae in pork intended for domestic consumption. The principal measures relied upon have been education as to cooking pork thoroughly, and the preparation under meat inspection of pork products customarily eaten raw. Freedom from infective trichinae is assured by cooking at 137° F. (that this temperature requirement, originally set by Ransom and Schwartz (1919), is adequate has been verified by Otto and Abrams, (1939), by refrigeration at 5° F. for not less than 20 days (Ransom, 1916), or by special processing of the pork (Ransom, Schwartz and Raffensperger, 1920). That the intradermal test be applied to all hogs killed in all slaughter houses, for the detection of trichina infections, has been advocated (Nelson, 1939) but the evidence (Spindler and Cross, 1939; Lichterman

and Kleeman, 1939) indicates that the test does not detect all trichina-infected swine.

The evidence shows that the control measures of the past have been palliative and casual and have not controlled trichinosis; a basic program should aim at elimination of the principal source of infection of swine—namely, pork scraps in garbage or slaughter house waste. The swine sanitation system is of great value where used; pigs are raised on pasture, are not fed swill or garbage, and have little or no opportunity to eat rats or other pigs. As already noted, the incidence of porcine infection is correlated with the method of feeding pigs. Localities, as England (Van Someren, 1937), which require cooking of any garbage fed to pigs have a low incidence. A survey made by Wright (1940) shows that in the United States over 50 percent of reporting cities having a population of 10,000 or over dispose of municipal garbage in whole or in part by feeding it to swine. Very few cities cook it; it is evident therefore that municipalities are contributing substantially to the spread of trichinosis. The problem of control clearly lies in that field.

TABLE 10.—Findings of trichinae in National Institute of Health nationwide survey\*  
Direct microscopic and digestion-Bacmann methods

| Series  | Number of diaphragms examined | Number of diaphragms positive | Percent positive |
|---|-------------------------------|-------------------------------|------------------|
| Base (10 hospitals in Washington, D. C., 2 Marine and 4 Naval hospitals in eastern seaboard cities) | 3,000                         | 488                           | 16.3             |
| Random (diaphragms selected at random from hospitals selected at random throughout U. S. A.)        | 436                           | 80                            | 18.3             |
| Negative (from States in which clinical trichinosis has never been reported)                        | 140                           | 26                            | 18.6             |
| Traumatic (persons suffering traumatic death and not hospitalized)                                  | 212                           | 38                            | 17.9             |
| Jewish (orthodox and unorthodox Jews)   | 134                           | 1                             | 0.7              |
| Totals  | 3,922                         | 633                           | 16.1             |

\*As reported by Hall and Collins, 1937; Nolan and Bozicevich, 1938; Wright, 1939; Kerr, 1940; and Kerr, Jacobs and Cuvillier (in press).

### The Filariae D. L. A.

The superfamily Filarioidea contains a large number of species of which *Wuchereria bancrofti* (Cobbold, 1877) Seurat, 1921 and *Onchocerca volvulus* (Lenekart, 1893) Railliet and Henry, 1910, are important pathogens for man. Less important species that infect man include *Loa loa* (Cobbold, 1864) Castellani and Chalmers, 1913; *Dipetalonema perstans* (Manson, 1891) Yorke and Maplestone, 1926; and *Mansonella ozzardi* (Manson, 1897) Faust, 1929. Several other species have been reported from man which are known only in the immature stages. Of these only *Microfilaria malayi* (Brug, 1927) appears to be of clinical importance. The following discussion will be limited to the first two species named and *Microfilaria malayi*.

#### 1. BANCROFTIAN FILARIASIS

The enormous enlargements of parts of the body, particularly of the legs and external genitals, so frequently accompanying Bancroftian filariasis were noted and much studied long before the etiological agent, *Wuchereria bancrofti*, was discovered. According to Menon (1935), the first, and a very good description of these conditions was written about 600 B. C. by Sushruta in India. The disease was probably also well known in Persia, Arabia, Egypt, and parts of Africa at that time. Hillary (1766) gives a very good account of its occurrence in Barbados, describing the recurring attacks of fever, the lymphangitis, the lymphadenitis, and the slowly increasing swellings of the affected part up to the stage at which typical elephantoid appearances become definite and prominent. Hillary was certain that the disease had been brought to the West Indies from Africa by Negro slaves and, at his time, was observed to be "too frequent among them and among the white people also." Neumann (O'Connor and Beatty, 1938) estimated that 6 percent of the population of St. Croix, Virgin Islands, had elephantiasis in 1881.

Observations demonstrating the etiology of elephantiasis were initiated in 1863 by the French surgeon Demarquay, who found microfilariae in chylous urine of a person who had lived in Cuba, were continued by the investigations of Lewis (1879) in India and by others, and culminated in the research of Patrick Manson in China between 1876 and 1900. Early in his investigations Manson discovered filarial periodicity and experimentally demonstrated that a mosquito, *Culex fatigans*, was an essential intermediate host and the agent for dissemination of the parasite. More recent investigations have been largely epidemiological and pathological. Noteworthy among these are the studies of Bahr (1912), Low (1913), O'Connor (1923; 1931), Anderson (1924), Fülleborn (1929), Iyengar (1938), and Paynton and Hodgkin (1938).

The adult worms are parasites only of the lymphatic system of man. They may occur at any level of the system, but are found most frequently in the limbs, serotum and inguinal regions. The two sexes are frequently coiled together in the periglandular tissues, the lymphatic vessels of the capsule and in the cortical sinuses. In heavy infections they may also occur in the medulla.

The microfilariae (for life history see p. 288) occur in the lymph, the blood stream, and, under certain conditions (chyluria), may be found in the urine. These larvae characteristically exhibit a marked nocturnal periodicity. They are found in greatest numbers between 10 o'clock in the evening and 2 o'clock in the morning, but during the day they may be entirely absent from the blood. In the Philippine Islands and largely throughout Polynesia, however, the microfilariae show no periodicity; while in Australia and New Guinea, periodicity is the rule, but both types do occur. As far as is known both types represent one and the same species. In order to continue their life cycle microfilariae must be taken up by an appropriate mosquito.

#### GEOGRAPHICAL DISTRIBUTION

*Wuchereria bancrofti* is practically world-wide in distribution, but is largely limited to tropical and subtropical countries. Its spread necessarily depends on the extent of the migrations of individuals showing microfilariae in the blood, and on the presence or absence of appropriate mosquitoes in new areas to serve as intermediate hosts. The parasite is characteristically found in island populations or along more or less broad low-lying coastal areas of larger islands and continents. In Asia it is established along coastal areas from Arabia to the Shantung Province in Eastern China, and cases have been reported as far north as Manchuria. It is prevalent in the islands of the East China Sea, southern Japan, southern Chosen and throughout Oceania. In Australia its distribution is mainly along the Queensland coasts.

Bancroftian filariasis is common across tropical Africa, Madagascar, Mauritius and neighboring islands and along the Mediterranean shores. It has been reported to be indigenous in Spain, Hungary and Turkey. It is of very common occurrence throughout the West Indies, the Guianas, and Venezuela, and is less frequent in northern Colombia and eastern Brazil (Bahia). It appears not to have become established along the Pacific coast. A small endemic focus was reported in 1915 and again in 1919 in North America near Charleston, South Carolina (Francis, 1919). Sporadic cases of infection have been noted from time to time in various parts of the United States, but these, invariably, were of foreign origin or from the Charleston area. Thus, it is evident that Bancroftian filariasis has a world-wide geographic distribution. Its distribution within any given country is, however, characteristically and markedly spotted and discontinuous due to the local physical factors and the differences in social standards and sanitation.

#### PATHOLOGY

It is generally held that living microfilariae are not pathogenic. It has been observed that microfilariae readily pass through lymph nodes without phagocytic filtration (Drinker, Augustine and Leigh, 1935). They are exceedingly active in the blood stream. They are not only passively carried about with the blood stream but actively move against the blood stream in the arterioles, making slow progress by bracing themselves through the crests of the alternate undulations of their bodies against the walls of the vessel. They frequently occlude the capillaries and then make their way through the stagnated column of blood cells to reenter the active circulation. They apparently never make permanent plugs or form emboli (Augustine, Field and Drinker, 1936; Augustine, 1937).

The serious disorders are brought about by dead microfilariae and the living and dead adult worms. These disorders in every case can be traced to interference with the lymphatic system. Living worms, however, apparently cause little damage other than varying degrees of blockage of the afferent approach of the vessels in which they lie. However, when the adult

worms die they become foreign bodies. Inflammation follows their death and degeneration, giving rise to lymphangitis, lymphadenitis, inflammatory varicose groin glands, abscess and fever. The obstructed and dilated lymphatics sometimes rupture, with escape of chyle into the bladder, and less frequently into the intestine and the abdominal cavity, and thus give rise to chyluria, chylous diarrhea and chylous ascites. Without rupture of the vessel, superficial or deep lymph varices may develop, such as varicose glands of the groin or axilla, hydrocele and lymph serotum.

All active changes are associated with the degeneration and absorption of dead parasites. The end result is always fibrosis with complete occlusion of the parasitized vessel. Elephantiasis, one of the commonest lesions, is the result of long and widespread lymphatic obstruction.

Pyogenic bacteria, streptococci and staphylococci have been isolated fairly frequently from the region of the lymphedema. Their responsibility in the disease syndrome, however, is not clear. Drinker, Field and Homans (1934) and Drinker (1936) have shown experimentally that loss of lymph circulation predisposes to streptococcal infection, that these bacteria cause attacks of severe chill and high fever, and usually can be isolated from the tissue fluids only in the early stages of the seizures.

The diagnosis of infections with *W. bancrofti* is made by finding the characteristic microfilariae in the blood. Many cases, however, having clinical symptoms show no microfilariae in the blood nor in the contents of the dilated vessels. In such instances the infection is usually of long standing and either the adult worms have died or the lymphatics draining the affected area have become obstructed by the worms and their products to such an extent that the microfilariae cannot pass along the vessels to enter the circulating blood.

#### EPIDEMIOLOGY

Bancroftian filariasis characteristically occurs in low lying coastal areas and along the shores of lakes and rivers. Indigenous infections are seldom to be found in the foothills or beyond coastal ranges. The incidence of infection and clinical manifestations in endemic regions vary greatly in adjoining areas. The incidence of infection is always in direct relation to the prevalence of the mosquito concerned; and, in turn, the prevalence of these mosquitoes in an area is in direct relation to the favorableness of that area for mosquito breeding. The parasite is naturally limited to the range of its insect vectors, and local physical factors, such as temperature, humidity, porosity of the soil, prevailing winds, and character of the vegetation which may influence the development and presence of the vector, will also indirectly influence the incidence and intensity of filariasis in the human population.

O'Connor (1923) found a very low incidence of filariasis and but a very few mosquitoes on some atolls of the Ellice group having narrow, broken land strips and lacking depth of bush favorable to *Aedes variegatus*. On other atolls with larger land areas, the central lagoon reduced to a swamp, and covered with dense, dark bush, *Aedes variegatus* was observed in swarms throughout the day, and over 70 percent of the inhabitants over 16 years of age showed some sign of filarial infection. Again, a favorable high temperature with a suitable amount of moisture is absolutely necessary for the development of the parasite in the mosquito and for its transfer from the mosquito to the human skin. The low incidence of infection or absence of infection in many places, particularly in the interior of China where proved mosquito carriers are present, is attributed to cold or to dryness and high temperature (Feng, 1931). Thus, local conditions may have a marked influence on the distribution of the infection within an endemic area.

While the mosquito is the sole vector of *Wuchereria bancrofti*, as in malaria, the transmission is accomplished with much less certainty and promptness in the case of filariasis. There is no multiplication of filaria larvae in the intermediate host. There develops but one infective larva from each microfilaria sucked in by the mosquito, whereas the malaria parasite multiplies enormously and the chance of the infection being returned to man is by thousands of times more likely. The actual number of microfilariae sucked up by the mosquito is also relatively small in comparison with the number of malarial organisms in the blood which may be taken up in a similar manner. A high mortality occurs among the microfilariae which actually reach the stomach of the mosquito.

O'Connor and Beatty (1938) estimated that about 35 percent of the microfilariae ingested by mosquitoes die within 20 hours after an infective meal in the stomach blood clot, that a very heavy mortality of larvae may occur after their arrival in the thorax, and that many infected mosquitoes may die during the first few days after such feeding. Of 5,000 wild *Culex fatigans* collected over a period of 12 consecutive months in St.

Croix, V. I., from within and near dwellings occupied by persons with filariasis, only 2.3 percent were found to contain fully "infective" larvae. These authors believe that the percentage of *C. fatigans* which actually transmit the infection to man is much smaller, due to death of mosquitoes from various causes such as strong winds, torrential rains, spiders, bats, lizards and chickens. Chickens wandering into the laboratory were observed to search and eagerly devour mosquitoes resting in the darker corners of the room. It was also observed that *C. fatigans* readily feeds upon domestic fowl. It is probable that many parasites are deposited on the skin or feathers of birds and thus become lost.

Bancroftian filariasis characteristically occurs in small, densely populated and poorly sanitized villages. It is particularly common in the overcrowded dwellings of poor people, and the incidence and morbidity in a given family may be striking. Infection is usually commoner in males than in females, which difference in China is attributed to the custom among women of wearing more clothes while sleeping at night, and thus exposing less body surface to the attack of mosquitoes (Lee, 1926). The severity of the disease characteristically increases with advancing age, thus indicating absence of any development of immunity in filarial infection. All races of mankind appear to be equally susceptible. Differences noted, particularly absence of infection or lighter infection among North Americans and Europeans residing in the area are due to their better sanitary conditions, better protection against mosquitoes, and homes removed from the overcrowded dwellings of the native population. Better housing is always essential in control.

In view of the fact that the parasite is transmitted solely through the bites of mosquitoes, its prevention is primarily one of mosquito control, and measures taken against these insects in the control of malaria and yellow fever are equally effective against infections with *Wuchereria bancrofti*. *Culex fatigans* is a domestic mosquito which breeds near dwellings in cisterns, rain barrels, discarded tin cans, sillage drains, ditches, etc. Tight screens and gauze coverings will prevent mosquito breeding in cisterns, vats and rain barrels. Discarded pots, tins and other utensils should be buried or destroyed, and drains and ponds kept clear of vegetation in order to effect proper mosquito control. All breeding places should receive weekly treatment with larvicides. In the Oceanic Islands, where *Aedes variegatus* is the most important vector, attention must be centered on discarded coconut husks and shells, natural and artificial cavities in trees, tin cans, and other possible containers of clean water. O'Connor (1923) observed that the Pacific rat makes breeding places for *A. variegatus* in trees by gnawing and cutting young cocoa-pods. The pods then die, become dry, and form hanging breeding places for the mosquito.

With modifications to meet local conditions, the methods advocated by O'Connor and Beatty (1938) to reduce Bancroftian filariasis in Christiansted, St. Croix, might be effectively applied elsewhere. They include the following recommendations:

1. The general measures adopted should be as follows:
  1. The incidence of persons with microfilariae should be determined at the same time for the whole population.
  2. The percentage of infective mosquitoes should be determined in the same houses and outhouses, etc. The mosquito "infective" incidence may be more valuable than the microfilarial incidence, partly because while some natives do not readily submit to having blood taken from them, yet when the reasons are explained to them they rarely object to their mosquitoes being collected. Furthermore, a person with microfilariae having been infected in another locality may be in a place where there are few or no mosquitoes and so will not be a serious menace. On the other hand, the repeated finding of infected mosquitoes is proof positive that one or more persons with microfilariae is near by.
  3. These studies might well be repeated about every 3 years.
2. In houses of high human and mosquito infectivity incidence, the following local measures should be carried out:
  1. The nature of filariasis, its transmission and prevention should be completely and simply explained to the occupants of the house where control measures are instituted.
  2. Suitable containers for potable and other water supplies should be adequately screened with wire netting. Where containers are not suitable they should be replaced.
  3. The use of the mosquito net should be demonstrated. (If the occupants cannot afford them these should be provided from public funds.)
  4. The proper maintenance and use of all screening should be supervised at intervals by the existing sanitary officers.
  5. When possible occupants should be encouraged to keep fowls in their yards near the house.
  6. The number of mosquitoes in the houses and the percentage of these which are infective should be recorded from time to

time in order to evaluate the results of preventive measures.

7. Efforts to have adult mosquitoes killed daily by the inhabitants while highly desirable will usually be found impracticable. This measure would be too expensive for government maintenance, but where full cooperation is assured it should be adopted to supplement the foregoing.

The control of filariasis in the Orient is complicated by the presence of another filariid, quite recently discovered, which has long been confused with *Wuchereria bancrofti*. This species, *Microfilaria malayi*, was discovered in 1927 by Leichtenstein in the Dutch East Indies. Leichtenstein had failed, after numerous attempts, to infect *Culex fatigans* and other eulieine mosquitoes with microfilariae of the area, and noting the absence of acute forms of the disease, although elephantiasis of the leg was common, it occurred to him that he might be dealing with a new species of filaria. Brug (1927) examined Leichtenstein's material, found morphological characters distinct from Bancroftian microfilariae, and proposed the name *Filaria malayi* for the parasite. Brug's observations have since been confirmed by various workers, and the species now appears established on morphological characters of the microfilariae and extensive epidemiologic studies, although the adult worms are yet to be discovered.

Thus far, *Microfilaria malayi* appears to be strictly oriental in geographic distribution. It is known to occur in the Federated Malay States, Sumatra, Java, Ceylon, parts of India, Indo-China and in north-eastern Chekiang Province of China. It is often the dominant species of a given region and occurs typically in rural districts along river or forest settlements. Elephantiasis of the feet and legs is typically associated with *M. malayi* infection. The genitals and upper extremities are rarely involved as in Bancroftian filariasis. The microfilariae show nocturnal periodicity, but do not disappear entirely from the peripheral blood during daytime. Mosquitoes of the genus *Mansonia*, subgenus *Mansonioides*, are the principal vectors, particularly *M. (Mansonioides) annulifera*. These are nocturnal feeders and are most active during the evening from 7 p.m. to 9 p.m.

Recently, extensive studies have been made on the control of filariasis in India, particularly in Travancore, where *Microfilaria malayi* is chiefly concerned (Sweet and Pillai, 1937; Iyengar, 1938). It was demonstrated by these investigators that the presence of a floating plant, *Pistia stratiotes*, is essential for the breeding of *Mansonia*. The female mosquito does not ordinarily lay eggs except on the leaves of *Pistia*, and the larvae, being structurally adapted to obtain their supply of oxygen from the air cavities in the root, are not capable of living apart from this particular plant.

In experimental areas the clearance of ponds and tanks of *Pistia* markedly reduced the incidence of *Mansonia* mosquitoes and checked further spread of the infection. *Pistia* plants can be cheaply and effectively removed by hand. Here we have an excellent example of the suppression of a mosquito borne disease by a strictly limited species control of the carrier.

## 2. ONCHOCERCIASIS

Onchocerciasis in man is caused by *Onchocerca volvulus* (Leuckart, 1893), the adult forms of which are characteristically found in prominent, subcutaneous, fibromatous tumors. The microfilariae appear in large numbers in the skin, especially in the skin in the vicinity of the tumor, the eyes, the conjunctivae and the cornea, and in the central portion of the tumor with the adult worms. They do not appear in the circulation but may rarely occur in the deeper tissues and viscera (Rodhain, 1937). When seen in fresh sections of the epidermis or conjunctiva they are actively motile and possess no sheath. Two types are clearly distinguished. It has been suggested that the smaller forms with a more compact arrangement of the nuclei may represent male, and the longer ones female microfilariae.

It is believed that the tumor results from the irritation produced by the presence of the adult worms and the products of their metabolism. Unencapsulated adult worms have, however, been noted (Sharp, 1927; van den Berghe, 1936). The microfilariae have been considered to be a cause of an erysipelous condition of the face and head and of disturbances in vision, iritis, punctate keratitis and total blindness. The part that these microfilariae play in the production of these conditions is not clear.

Onchocerciasis is very common along the west coast of Africa from Sierra Leone to the Congo basin and extending eastward through the Congo into Uganda, Anglo-Egyptian Sudan and Kenya. It also occurs endemically in southern Mexico and Guatemala upon the Pacific or southern slopes of the volcanic ranges at altitudes between 2,000 and 4,500 feet (Calderón, 1920). The parasite in Central America was discovered by Robles in 1915 and named *Onchocerca cacucensis* by Brumpt

in 1919, who considered the American form distinct since, in the great majority of cases, the tumor was located upon the scalp or in the region of the face, whereas the tumor in African cases was generally found on the body. Further, the disease in America was observed only in areas mainly inhabited by native Indians and into which regions the negro, apparently, had never been introduced. Later studies by Strong and associates (1934) have shown that the Central American form cannot be separated from the African form on either morphological characters or on biological criteria. The two forms are now generally regarded as belonging to the same species, *O. volvulus*.

Blacklock (1926) demonstrated that the black-fly *Simulium damnosum* is particularly concerned in the transmission of onchocerciasis throughout tropical Africa. *S. nartvi*, however, is said to be the chief, if not the only carrier of the parasite in the Lubilash-Sankuru region in the Province of Lusambo (Kasai). *S. metallicum*, *S. callidum* and *S. ochraceum* are the vectors in endemic regions of Mexico and Guatemala (Strong *et al.*, 1934). The development of the parasite in these flies and its transmission to man are essentially the same as the development and transmission of *Wuchereria bancrofti* in and by mosquitoes.

The control of onchocerciasis in Africa is exceedingly difficult due to its widespread distribution and the general topography of the country, dense vegetation or forests and running streams, ideal environments for the breeding of *Simulium*. Vegetation is usually cleared only in the vicinity of the villages and plantations. This limited clearance of vegetation is probably of little value in control since these flies are capable of flying great distances. The people are attacked by the flies most frequently while defecating at the edges of streams (a common and usual practice), while collecting water for drinking purposes or while engaged in agricultural pursuits, rice, cotton or coffee cultivation which bring them into the immediate environment of the fly.

Measures of individual protection against the bites of these flies, such as wearing of fly-proof clothing and masks, proper screening of houses and bed nets, the use of smudges and repellents, are to be recommended, but are usually not practical and are rarely applied by native populations. It is obvious that effective control of onchocerciasis rests in the eradication of *Simulium* flies but, as yet, there is no practical method known to destroy their eggs, larvae or pupae which abound under stones in the swiftly-flowing streams of the endemic areas.

In Central America attempts have been made to control the human carriers which infect the flies. Surgical removal of the tumors containing adult worms has been a public health procedure of importance, and where a systematic attempt has been made to eradicate the disease in sharply circumscribed areas, the rate of infection has been greatly reduced. It is recommended (Strong *et al.*, 1934) that, under local conditions as in Guatemala, periodic microscopic examinations should be made in each individual after operation to detect the number of microfilariae which may persist and, if large numbers of microfilariae are present, the patient should be regarded as a dangerous carrier and be isolated, or removed to a region where *Simulium* does not occur, until the parasites disappear.

## *Dracunculus medinensis*

W. W. C.

Although the guinea worm, *Dracunculus medinensis*, has been known since ancient times nothing was understood of its life cycle until Fedtchenko (1871) implicated cyclops in its transmission. Since that time various species of cyclops have been infected experimentally and recently Moorthy (1938) has given an adequate account of the developmental stages in this host. Leiper (1907) reported the experimental infection of a monkey and the finding of two immature males, and recently experiments with dogs (Issajev, 1934a; Moorthy and Sweet, 1936 & 1938) have made possible the adequate description of the male (Moorthy, 1937) and added much to our knowledge of all the stages in the definitive host. The researches of Fairley and Liston (1924a), Fairley (1924), and others have served to give a picture of the pathology and symptomatology of dracunculiasis and numerous and widely scattered publications have given the present conception of its geographical distribution, epidemiology, and control. In spite of numerous suggestions no treatment of real value is yet known. Although the extent of the studies on this parasite is impressive, much more needs to be done to bring our knowledge even up to the level of that of the other important human helminths.

Natural infections with worms identified as *D. medinensis* have been reported from a number of mammals including dog, horse, cattle, jackal, wolf, leopard, monkey, deer, baboon, raccoon, mink, and fox (Leiper, 1910; Turklud, 1920; Chitwood,

1933). While some of these records may be due to confusion of closely related species, there is no reason to doubt that in some cases the worms actually were *D. medincusis*. There is no evidence, however, that any of these animals are significant reservoir hosts in the endemic areas. Infection in animals, however, might serve to spread this parasite into new areas as suggested by its presence in animals in China (Hsü and Watt, 1933) and in the United States (Chitwood, 1933) where endemic cases in man have never been reported.

#### INJURY TO HOST

There appears to be no evidence that the guinea worm appreciably injures its host during the developmental period in the deep connective tissues. Just before the female worms reach the skin they secrete toxins which produce general symptoms such as urticaria, nausea, vomiting, diarrhoea, severe dyspnea, and syncope. These symptoms disappear after the worms have established an opening through the skin. Later injury to the host is produced by secondary infection and by tissue reactions to the worms before and after their death. Severe inflammation is produced if the worms are broken in the tissues and the embryos freed. The presence of the guinea worms may also produce permanent joint injuries (Pradhan, 1930).

In endemic areas dracontiasis is often a medical and public health problem of great importance. Large numbers of people are incapacitated for 3 to 6 months of the year; severe suffering and occasionally death are produced; and the economic life of the community is often severely disturbed. For example, Moorthy (1932a) stated that in certain villages in the endemic areas of Mysore a large percentage of the people were more or less completely incapacitated for 5 to 6 months of the year and that outside labor had to be imported. The statements of other authors indicate that this same situation holds in the enormous number of villages in various parts of India where this parasite is endemic. Also over large areas of Tropical Africa the guinea worm is a real scourge.

#### GEOGRAPHICAL DISTRIBUTION\*

Dracontiasis is widely distributed in a number of parts of Tropical Africa and is endemic over large areas of India. It is also found in Arabia, Iran, Afghanistan, and Russian Turkestan. It is supposed to have been introduced into the Western Hemisphere with slaves from the Gulf of Guinea. It was formerly thought to have become endemic in Caracao, Demerara, and Surinam but seems now to have disappeared; it is only in a limited region in the state of Bahia, Brazil, that it is still endemic. There is other evidence that the guinea worm is not easily spread to new regions. Although it has been constantly introduced into the Dutch East Indies it apparently has never been established (Brug, 1930) and no evidence was found of endemic centers along the north coast of Africa and in southern Europe.

In Africa are located the most widespread and perhaps the worst endemic areas of guinea worm infection in the world. In general it can be said that almost all the important endemic centers in Africa are north of the equator and south of the Tropic of Cancer. In West Africa they are in general scattered from Mauritania to Gabon, especially in Mauritania, Senegal, Upper Volta, Ivory Coast, Gold Coast and Northern Territories, Togo, Dahomey, Nigeria, and Cameroon. East of this region endemic areas are known especially East of Lake Chad, over much of the southern part of the Anglo Egyptian Sudan and in Uganda.

In Arabia the guinea worm is present along the shore of the Red Sea and in some places in the interior; it is endemic in certain parts of Russian Turkestan, Afghanistan, and in Iran it appears to be almost entirely limited to certain towns and villages in the province of Laristan, which is located in the south on the Persian Gulf (Lindberg, 1936).

Next to Africa the real home of the guinea worm is India. Important endemic centers in this country are limited to the western half of the peninsula, little infection being found east of Delhi and the Central Provinces. In Rajputana and Central India the infection exists almost everywhere except in a few desert regions; in the Central Provinces it is prevalent in all the districts except a few on the eastern side; in the Bombay Presidency it is widely distributed except in the sea coast area south of Bombay; in the Madras Presidency it is prevalent except for a few districts on the western coast; in Hyderabad 9 out of 16 districts have the infection; in Mysore it is practically limited to one district in the north; it is also present but with lower incidence in the valley of the Indus and the Northwest Frontier Provinces; elsewhere in western and central India there are a few limited centers.

\*Information gathered by questionnaires and summed up in a recent work by E. B. McKinley (1935) supplemented the numerous publications found in the literature on the distribution of dracontiasis in various parts of the world. A personal communication from Dr. V. N. Moorthy gave the latest information on India.

There are certain general points of interest in regard to the geographical distribution of dracontiasis. It extends from the tropics in Africa and southern India well up into the temperate zone in Russian Turkestan, Afghanistan, and the northwestern frontier provinces of India. Even where it is widespread as in tropical Africa north of the equator and in western and central India, its distribution is very discontinuous, and important endemic centers are often separated by wide areas where it is not present. In limited regions too its distribution is very spotted. In Mysore, for example, the infection is almost entirely limited to the Chitaldrug district, and in this district itself there was only a small proportion of infected villages which are widely scattered (Moorthy, 1932a). The same spotted distribution has been noted by other authors in the other endemic areas of India. The same type of distribution has been noted by various workers in different parts of Africa, where guinea worm villages may be close to others where the parasite is absent. It has also been noted that in an infected village itself only part of the families suffer. Even more surprising is the point emphasized by Moorthy (1932a) and Trewn (1937) that in infected families, where there appear to be no differences in habits, some individuals will remain entirely free from infection. All these peculiarities of distribution suggest that the factors involved in the dissemination of the guinea worm are very complicated.

#### EPIDEMIOLOGY

In general it may be said that the guinea worm can only spread where infected individuals wade or bathe in drinking water in which cyclops are present. While most of the endemic areas are in hot countries there is no evidence that temperature *per se* is a determining factor. This parasite is chiefly prevalent in regions where there is a low annual rainfall. This seems to be related to the fact that in such regions the people, during at least part of the year, are forced to depend for their drinking water on open pools, wells, or cisterns in which the population of cyclops becomes concentrated. In a personal communication Dr. V. N. Moorthy recently made the following statement in regard to the distribution of dracontiasis in India: "The most significant fact to note in this distribution is that the intensity of infection appears to vary directly with the scarcity of water supply during the season of infection. In provinces like Bengal and Assam where there is a plentiful supply of water all through the year dracontiasis hardly exists at all." Roubaud (1913) and others have also pointed out that in the forested regions of west equatorial Africa where the rainfall is abundant, as the lower Ivory Coast and the Congo Basin, guinea worm has not been observed.

A number of authors have noted a seasonal relation in guinea worm infection. Since the development of the worms takes about a year, the yearly period when the people are suffering from the disease coincides with the conditions most favorable for its transmission. In India the outbreaks are almost entirely limited to the first half of the year with most of the infection coming in March, April, and May. This is the driest season just before the Monsoon. In Dahomey, Roubaud (1913) found the disease most frequent also in the driest months of the year which are from December to February. The same author noted, however, that in the endemic areas of the Lake Chad region it occurs almost entirely in the rainy season, which is in the middle of winter. He explains this by the use during this period of water from little cisterns and pools which are temporarily filled. Davis (1931) noted that epidemics of dracontiasis occurred in southern Sudan during the rains from April to June. In Iran, Lindberg (1936) found the season of infection to be from March to August with the maximum in June, which are the hottest months of the year just after the rainy season. It is evident, therefore, that the seasonal incidence of dracontiasis varies greatly in different endemic areas and is related to the water supply of the people and not to general climatic conditions.

Little significant information is available on the relation of the distribution of the various species of cyclops to the epidemiology of dracontiasis. These copepods are widely distributed over the world and numerous species occur wherever they are found. In general, therefore, it seems possible that in most regions where the guinea worm would be introduced and where the human habits are favorable for its spread, suitable intermediate hosts would be present. Only certain species of cyclops can serve as intermediate hosts. Chatton (1918) tried to infect four different species of *Cyclops* in Tunis with larvae from introduced cases. One of these, *Cyclops macrurus*, was entirely refractory to infection. In three others, *C. viridis*, *C. prasinus*, and an undetermined species, the larvae were ingested and penetrated into the body cavity but failed to develop although they remained alive for from 40 to 50 days. In India, Lindberg (1935) found that *C. multicolor* dies quickly after

ingesting guinea worm larvae and noted no development after 7 days.

We know little also of the relation of the reactions of the definitive host to this dissemination of this parasite. If any immunity is produced by the presence of worms it must be quickly lost after the completion of development because repeated infection of the same individual year after year is a common phenomenon. Moorthy (1932a) recorded that out of a total of 1,363 patients suffering from dracontiasis 83 percent gave histories of having suffered in previous years. He also noted that certain individuals seem to be entirely lacking in susceptibility to infection and escape the disease year after year, although they live in the same houses and drink the same water as those who become infected. He suggested from *in vitro* studies that in such individuals there might be physiological factors, such as hypo- or hyperchlorhydria, which would prevent the freeing of the infective larvae from the cyclops in the stomach or which would kill them before they could penetrate into the tissues.

As suggested above, the character of the water supply is of the greatest importance in the dissemination of dracontiasis. Infected individuals must have access to drinking water that contains suitable species of *Cyclops*. Absence of this parasite in people who obtain their water supply from rivers or smaller streams can probably be attributed to the absence or scarcity of the proper species of *Cyclops* (Lindberg, 1935). Small open collections of water such as step wells, cisterns, or small pools in which the people frequently wade or bathe are chiefly implicated. For example, in the Gold Coast surface collections of rain water and shallow open wells are considered to be the sources of infection (Leiper, 1907); in the upper Volta, village ponds and hollows made by the natives in obtaining mud for building their huts (LeDentu, 1924); in the Lake Chad basin, temporary cisterns or pools (Roubaud, 1913); in southern Sudan, shallow wells or drinking pools (Davis, 1931); in Iran, cisterns of rain water (birkehls) or washing basins in the mosques (Lindberg, 1936); and in India, the step wells and village pools (Turkhud, 1919; Pradhan, 1930; Moorthy, 1932a; Lindberg, 1936). Such bodies of water only become of considerable danger in spreading the infection when the water is low and the cyclops are present in large numbers and concentrated near the surface (Turkhud, 1912; Pradhan, 1930; Lindberg, 1935). This explains the seasonal cycle of infection in India because the season of greatest infection (March to May) is near the end of the dry season when the water is lowest. It also explains the greater prevalence of guinea worms in those villages with the poorest water supply. The epidemiological data makes clear the difficulty that the guinea worm has in finding conditions in human populations suitable for its spread and goes far to explain the discontinuity of the endemic centers, the failure of the disease to spread readily into new territory, and its spotted distribution over the endemic areas. All these facts on epidemiology suggest obvious methods for control and indicate that any serious attempt to apply control measures should bring rapid and permanent results.

## CONTROL

It is obvious that prophylaxis and control of dracontiasis in the endemic areas can either deal with habits of the individual or with community relations to the water supply. Boiling, filtering, or even straining the drinking water through a cloth would be effective in individual protection. The rapid extraction of the gravid worms from infected individuals and their exclusion from the water supply would help in preventing the infection of the cyclops. However, all the workers who have considered the problem are in agreement that permanent control in an infected community can be achieved only by changing the water supply to eliminate sources of infection. Thus Leiper (1907) pointed out that on the Gold Coast the fencing of the pools, the building of parapets or covering the open wells, and the digging of draw wells would permanently eliminate the disease. Turkhud (1919) argued that the changing of all step wells in the infected villages in India to draw wells would save many times the cost of the pumps by eliminating the economic losses from the disease. Moorthy (1932b) found that where this was done in the Chitaldrug district of Mysore great reduction and in some cases entire elimination of the disease resulted.

Where for some reason it is not possible to change the construction of the wells or pools the employment of methods to kill the cyclops have been suggested. Such measures have to be used repeatedly since they serve only to eliminate the cyclops temporarily. A number of authors have experimented on the use of chemicals to kill cyclops. Davis (1931) recommended lime, either unslacked or slacked, in proportions of 1 to 1,000. In fact in the previous year Pradhan (1930) had

already reported an extensive field experiment in which the use of lime (about 1 drachm per gallon of water) in 27 infected step wells had reduced the incidence of guinea worm in the people using them 21 to 55 percent. Moorthy (1932b) reported that when perchloron (3 lbs. per 100,000 gallons) in combination with copper sulphate (1 lb. per 200,000 gallons) was used in wells they could be rendered completely free of cyclops for about a month. He advocated the use of this method during the infection period (March to June) as a good method of reducing the number of cases in areas where permanent control methods could not be undertaken.

Several authors have suggested the "biological control" of guinea worm infection by the introduction into the wells or ponds of fish that feed on cyclops, but Moorthy and Sweet (1936c) appear to have been the first to report on the successful use of this method. They found a number of cases in which people using wells containing certain species of small fish, particularly of the genus *Barbus*, were entirely free from guinea worm infection. This led to the development of methods for raising and introducing fish into the step wells. Use of this control method in 35 infected villages in 1934 and 1935 caused complete elimination of dracontiasis in six and a marked reduction in four. Their results led to the conclusion that the use of fish was not only cheaper but much more effective than chemical methods.

Finally it seems clear from all the evidence in the literature that prospects for the control of dracontiasis are excellent in any endemic area where a systematic effort can be made. It is very encouraging that Moorthy and Sweet (1936b) were able to report that from 1929 to 1936, by the introduction of draw wells, the use of chemicals, and the introduction of fish, dracontiasis was entirely eliminated from all but 25 of 112 infected villages in the Chitaldrug district of Mysore, India.

## *Enterobius vermicularis*

E. B. C.

The human pinworm or seatworm, *Enterobius vermicularis* (Linn., 1758) Leach, in Baird, 1853, was one of the first of the intestinal helminths to be described from man, a fact easily understood since it comes to the exterior and there produces local symptoms which would lead to its discovery. According to Schmidt, it was discussed by Hippocrates, Aristotle, Galen and others under the name *Ascaris*, before Linnaeus gave it its specific name.

*E. vermicularis* is apparently restricted to man. In view of Cameron's (1929) study indicating that in primates one species of *Enterobius* is restricted to hosts of one genus, reports of *E. vermicularis* from primates other than man must be regarded with suspicion unless supported by unimpeachable evidence. This parasite occurs in the intestine but is not limited in location as are many other intestinal nematodes. It occurs, in various stages of development, from the lower ileum through the rectum and gravid females migrate through the anus to the perianal region to lay eggs.

## SYMPTOMATOLOGY AND PATHOLOGY

Symptoms are extremely variable in nature and degree being apparently absent in some cases and severe in others. There is mechanical stimulation and irritation of the gastro-intestinal tract, occasionally with nausea and vomiting, and of the external surfaces during migration, producing pruritus ani and vulvae, in some cases apparently allergic in nature (Brady and Wright, 1939). By transporting organisms during migrations, the parasites may induce vaginitis and even peritonitis and may cause the formation of cysts in the female genital tubes or in the peritoneal cavity, with resulting irritation (summarized by Africa, 1938). Probably there is slight eosinophilia. The role in appendicitis is debatable (Bachman, 1935; Drüner, 1921; Penso, 1939; and others) but worms apparently may give rise to the syndrome of appendicitis without characteristic histological changes (Botsford, Hudson and Chamberlain, 1939). Restlessness and others secondary effects in behaviorism, including scholastic difficulties, feeling of shame and poor social attitude, may be pronounced.

## DIAGNOSIS

The most reliable method of diagnosis is by the microscopic detection of eggs in scrapings made from the perianal region. This technique has been standardized by the use of a cellophane-tipped swab (Hall, 1937; Folan, 1939), known as the NIH (National Institute of Health) swab (Fig. 201); the cellophane is detachable for mounting and examination under the microscope. Swabs should be made during the night or first thing in the morning, preferable on at least 7 days if first results are negative.

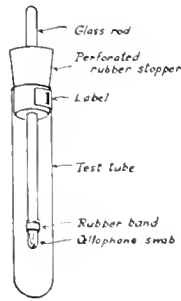


Fig. 201.

N-I-H swab (National Institute of Health) for the detection of *Enterobius* infections.



Fig. 202.

Egg of *Enterobius vermicularis*.

## EPIDEMIOLOGY

Critical investigations, based on examination of perianal scrapings, have been made in European countries, in the United States, including Puerto Rico, in Canada, and in the Philippines. The results of these investigations are summarized in table 11. These results show that examinations of 22,376 persons revealed pinworm infections in 9,703 persons, or 43 percent.

Additional studies, differing in method or scope and not shown in table 11, include stool examination of 495 children of preschool age in Brazil (Moniz de Aragao, 1938); *E. vermicularis* was found in 49 percent, an extremely high figure considering the method of diagnosis. In Spain, Darriba and de Cardenas (1935), from examination of feces, anal scrapings, finger nails and nasal mucus, found 11 of 46 children, or 34 percent, infected with pinworms; in Greece, Pandazis (1937) reported pinworms in 28 percent of infants and 6 percent of adults, apparently from fecal examination supplemented by finger nail examination.

## FACTORS INFLUENCING PREVALENCE

**RACE.** As noted previously, the Negro race has shown a lower incidence than the white race. Although Sawitz, D'Antonio, Rhude and Lob concluded from a small group of boys of the same ages in institutions of similar environments that the incidence was almost identical in the two races, the considerably lower incidence found in Negroes than in white persons in the general population of Washington, D. C., is contrary to what would be expected if environmental conditions are the determining factor. The significance of the racial factor deserves further study; that many persons classed as Negroes in the United States are mulattoes should be kept in mind, in this connection. Of interest are racial differences in the relative frequency of pinworms in 2,317 appendicectomies removed surgically; the incidence was 2.88 percent for the white population, 10.04 percent for Indians of the United States, and 23.91 percent for Eskimos and Aleutians.

**AGE** appears significant only to the extent that in the general

TABLE 11. Incidence of *Enterobius vermicularis*  
By use of various kinds of swabs and scrapers

| Date published | Country  | Population group | Race | Age      | No. exam. | No. positive | % positive |
|----------------|----------|------------------|------|----------|-----------|--------------|------------|
| 1886           | Germany  | Children         | W    | Under 16 | 3,506     | 2,068        | 59         |
| 1925           |          | Militia          | W    | Adult    | 400       | 76           | 19         |
| 1926           | U.S.S.R. | Various          | W    | All      | 7,074     | 4,255        | 60         |
| 1931           |          |                  |      |          |           |              |            |
| 1933           | Sweden   | Asylum           | W    | ?        | 60        | 42           | 70         |
| 1936           |          | Children         | W    | Under 15 | 340       | 123          | 36         |
| 1905           | Finland  | Various          | W    | All      | 2,753     | 81           | 3          |
| 1911           |          | Children         | W    | Under 16 | 300       | 95           | 32         |
| 1935           | U.S.A.   | Ment. hosp.      | W    | 15-60    | 282       | 62           | 22         |
| 1937           |          | Boys inst.       | W    | 12-20    | 213       | 3            | 1.4        |
|                |          | Boys inst.       | N    | 12-20    | 187       | 3            | 1.6        |

### Summary

| Date | Country     | Population group | Race         | Age | No. exam. | No. positive | % positive |
|------|-------------|------------------|--------------|-----|-----------|--------------|------------|
| 1886 | 5 countries | Various groups   | Mostly white | All | 15,115    | 6,808        | 45         |
| 1937 |             |                  |              |     |           |              |            |

### By use of NIH swab—Institutionalized persons

| Date published       | Locality    | Race | Age    | Sex | No. exam. | No. positive | % positive | Swabs per person |
|----------------------|-------------|------|--------|-----|-----------|--------------|------------|------------------|
| <i>United States</i> |             |      |        |     |           |              |            |                  |
| 1937                 | D. C.       | N    | 14-20  | F   | 23        | 0            | 0          | 1                |
|                      |             | W    | 14-20  | F   | 4         | 0            | 0          | 1                |
| 1939-40              | Louisiana   | W    | 6-14   | MF  | 365       | 302          | 83         | 7                |
|                      |             | N    | 6-14   | M   | 63        | 53           | 84         | 7                |
|                      |             | N    | 6-14   | F   | 63        | 10           | 16         | 7                |
| 1939                 | Alabama     | W    | All    | M   | 384       | 317          | 83         | (2 or more)      |
|                      |             | W    | All    | F   | 253       | 98           | 39         |                  |
| 1941                 | Georgia     | W    | Adults | F   | 100       | 31           | 31         | av. 2.9          |
|                      |             | W    | Adults | F   | 65        | 52           | 80         | 6                |
| 1941                 | D. C.       | W    | 6-8    | M   | 17        | 14           | 82         | 4                |
| 1941                 | Puerto Rico | *    | 5-19   | M   | 52        | 6            | 12         | 4                |
|                      |             | *    | 6-19   | F   | 50        | 15           | 30         | 4                |
| <i>Canada</i>        |             |      |        |     |           |              |            |                  |
| 1940                 | Toronto     | W    | 2-14   | MF  | 140       | 98           | 70         | av. 3.7          |

### Summary

| Date      | Locality                | Race | Age | Sex | No. exam. | No. positive | % positive | Swabs per person |
|-----------|-------------------------|------|-----|-----|-----------|--------------|------------|------------------|
| 1937-1941 | 6 localities N. America | WN   | All | MF  | 1,579     | 996          | 63         | 1 to 7           |

### By use of NIH swab—Non-institutionalized persons

| Date published       | Locality                            | Race | Age         | Sex | No. exam. | No. positive | % positive | Swabs per person |
|----------------------|-------------------------------------|------|-------------|-----|-----------|--------------|------------|------------------|
| <i>United States</i> |                                     |      |             |     |           |              |            |                  |
| 1937-41              | D. C.                               | W    | All         | MF  | 2,895     | 1,202        | 42         | (Usually 2 to 4) |
|                      |                                     | N    | All         | MF  | 1,099     | 142          | 13         |                  |
| 1939                 | Sample from various parts of U.S.A. | W    | 12-19       | M   | 166       | 21           | 13         | 1                |
|                      |                                     | N    | 12-19       | M   | 137       | 5            | 4          | 1                |
|                      |                                     | W    | 12-19       | M   | 198       | 40           | 20         | av. 4            |
|                      |                                     | N    | 12-19       | M   | 105       | 9            | 9          | av. 4            |
| 1940                 | Phila.                              | W?   | To 12       | ?   | 144       | 36           | 25         | 1 to 3           |
| 1941                 | Florida                             | W    | 6-12        | MF  | 438       | 71           | 16         | 1                |
| <i>Philippines</i>   |                                     |      |             |     |           |              |            |                  |
| 1939                 | Manila                              | W    | Mostly 6-10 | MF  | 500       | 376          | 75         | 1                |
| Summary              |                                     |      |             |     |           |              |            |                  |
| 1937-41              | U.S.A. and Philippines              | WN   | All         | MF  | 5,682     | 1,902        | 33         | 1 to 4           |

\*Mixed group of white, mulatto and Negro persons.

population the incidence is highest in children of school age, next highest in those of preschool age, and lowest in adults (Cram and Reardon, 1939; Chanceo and Soriano, 1939). There is evidence that the school is the determining factor in these differences; the incidence in children of so-called "preschool" age who attended nursery schools has been found as high as that in older children (Table 12).

**SEX.** The incidence in males has usually been found slightly higher and in an Alabama institution was much higher, but in the Philippines was slightly lower, than in females. Considering both sex and age, Sawitz et al found the peak of infection at 9 years; after that there was a drop in incidence in females, probably correlated with stricter sanitary habits, whereas in males the incidence remained relatively constant up to 15 years of age.

The factor of crowding is important in the spread of pinworms. The familial nature of the infection has been empha-

sized (Schmidt, 1914; Lentze, 1935; Wright and Cram, 1937; Hall and Cram, 1939). From a study of about 300 pinworm-infected families in Washington, D. C., it was apparent that multiple cases are the rule rather than the exception and that frequently all the children of the family, and one or both parents, may be infected. Bozicevich and Brady (1938) found a correlation between the size of the family and the incidence of *Enterobius*, easily explained in that the larger the number of persons in a family, the more chance there is for introduction of the infection into the household and, once introduced, the easier its spread, the infection increasing in a geometrical, not an arithmetical rate. Under institutional conditions Sawitz et al found the incidence of infection much lower among children occupying rooms with one or two beds than where larger groups were quartered in dormitories. Families with pinworm infections are found most numerous in older, comparatively congested residential sections but are by no means confined to those sections (Cram and Reardon, 1939); the social-economic status is not limited to any one level.

TABLE 12.—Incidence of *Enterobius vermicularis* according to age and race of children in camps and nursery schools, Washington, D. C.

| Reported by        | Race                        | Age  | Num- %    |            | Swabs per person |       |
|--------------------|-----------------------------|------|-----------|------------|------------------|-------|
|                    |                             |      | ber       | posi- tive |                  |       |
|                    |                             |      | Sex exam- |            |                  |       |
| Bozicevich         | White <sup>1</sup>          | 6-18 | M         | 230        | 31               | 1     |
| Bozicevich & Brady | White <sup>1</sup>          | 6-18 | M         | 504        | 57               | 2.4   |
| Cram               | White (Jewish) <sup>1</sup> | 6-12 | MF        | 147        | 25               | 4     |
|                    | Negro <sup>1</sup>          | 6-12 | MF        | 63         | 21               | 4     |
| Cram & Nolan       | White <sup>2</sup>          | 2-5  | MF        | 91         | 55               | av. 9 |
| Cram               | White <sup>3</sup>          | 2-5  | MF        | 62         | 52               | 4     |
|                    | Negro <sup>3</sup>          | 2-5  | MF        | 68         | 19               | 4     |

<sup>1</sup>Camps. <sup>2</sup>Private nursery school. <sup>3</sup>Public nursery schools.

#### CONTROL

Control of pinworm infection is extremely difficult. The number of eggs deposited may be enormous, one worm being capable of producing from 5,000 to 17,000 eggs (Reardon, 1938), and the time of development of eggs on the skin of the perianal region is short, as little as 6 hours. The infected individual may contaminate the hands while scratching or when using the toilet and subsequently carry the eggs to the mouth or may contaminate other objects. Eggs which fall off of the person develop more slowly, depending on temperature and humidity; they can pass through cloth and there is considerable evidence that airborne infection is a possibility (Lentze, 1932; Oleinikov, 1929; Nolan and Reardon, 1939; Sondak, 1935). In households and schools with infected members pinworm eggs have been found in dust from a large variety of locations and objects at various levels. The eggs may float on the surface of water and a certain proportion would therefore remain on the sides of wash bowls, bath tubs, laundry tubs and similar containers when they are emptied.

The eggs are very resistant to physical and chemical agents. Temperature and humidity influence the length of their survival. Lentze (1935) found that a temperature of 55° C. and above killed the eggs in a few seconds; at the optimum temperature (36° to 37° C.) on a damp base, as on the human skin, especially under the nails, eggs survived for about 10 days. Jones and Jacobs found that temperatures above 28° C., with humidities below 50 percent, definitely affect the eggs within 24 hours; less than 10 percent of eggs survived after 2 to 3 hours and none survived after 16 hours at a temperature of 36° to 37° C. and relative humidity of 38 to 41 percent. On the other hand, at lower temperatures, 20° to 24½° C., and higher humidity, 62 to 91 percent, 30 percent of eggs survived 6 days; on water at 3° to 5° C. a maximum of 93 percent survived 18 days. According to Sondak (1935), eggs were still viable after drying at room temperatures averaging 10° to 12° C. for 3 weeks but not viable after 35 days. Exposure of eggs to measured quantities of monochromatic ultraviolet radiation (Hollaender, Jones and Jacobs, 1941; Jones, Hollaender and Jacobs, 1941) showed an increased sensitivity of the eggs at wavelengths below 2400Å. As regards the effect of chemicals, Sondak (1935) found that eggs were not killed by formalin in strengths of 1, 2, 5, and 10 percent; by corrosive sublimate 1:1,000; by saturated solution of corrosive sublimate and copper (eupric sulphate); by 5 percent antiformalin; by 1 and 2 percent solutions of carbolic acid or by 1, 2, and 5 percent lysol solutions, but they were killed by 5 percent carbolic acid and by 10 percent lysol.

Because of the large numbers of eggs scattered by an infected individual and because of the resistance of the eggs, hygienic measures alone can not be relied upon to control the spread of

pinworm infection. This was pointed out by Wright and Cram (1937) and was given a practical demonstration by D'Antonio and Sawitz (1940) who put in force a vigorous cleanliness program for 6 weeks in one of the institutions studied by them; at the end of that period swab examination showed an increase from 38 percent to 51 percent in incidence of pinworms. The greatest promise for control lies in medicinal treatment administered over a period which is sufficiently long to cover the period of survival of eggs in the surroundings, thus preventing reinfection of the individual.

#### Bibliography

##### THE HOOKWORMS

- ACKERT, J. E. 1924.—Notes on the longevity and infectivity of hookworm larvae. *Am. J. Hyg.*, v. 4(3):222-225.
- ACKERT, J. E. and PAYNE, F. K. 1923.—Investigations on the control of hookworm disease. XII. Studies on the occurrence, distribution and morphology of *Necator suillus* including descriptions of other species of *Necator*. *Am. J. Hyg.*, v. 2(1):1-25, pls. 1-2.
- ANDREWS, J. 1940.—Hookworm disease and plans for its control in Georgia. *The Georgia Malaria Bull., Hookworm Suppl.*, v. 3(2):64-77.
- ASHFORD, U. K. and GUTIERREZ IGARAYDEZ, P. 1911.—Uncinariasis (hookworm disease) in Porto Rico; a medicinal and economic problem. U. S. 61st Cong., 3d Sess., Senate Doc. 808, 335 pp., pls. and figs.
- AUGUSTINE, D. L. 1922a.—Investigations on the control of hookworm disease. VIII. Experiments on the migration of hookworm larvae in soils. *Am. J. Hyg.*, v. 2(2):162-171.
- 1922b.—Idem. IX. On the position of the infective hookworm larvae in the soil. *Ibid.*, v. 2(2):172-176.
- 1922c.—Idem. X. Experiments on the length of life of infective hookworm larvae in soils. *Ibid.*, v. 2(2):177-187.
- 1923a.—Idem. XIX. Observations on the completion of the second ecdysis of *Necator americanus*. *Ibid.*, v. 3(3):280-285, figs. 1-9.
- 1923b.—Idem. XXII. Further observations on the migrations and the position of infective hookworm larvae in the soils. *Ibid.*, v. 3(4):416-419.
- 1923c.—Idem. XXIII. Experiments on the factors determining the length of life of infective hookworm larvae. *Ibid.*, v. 3(4):420-443, figs. 1-4.
- AUGUSTINE, D. L. and W. G. SMILLIE. 1926.—The relation of types of soils of Alabama to the distribution of hookworm disease. *Am. J. Hyg.*, v. 6 (March Suppl.):36-62.
- BAERMANN, G. 1917a.—Eine einfache Methode zur Auffindung von Ankylostomum (Nematoden) Larven in Erdproben. *Meded. Geneesk. Lab. Waltevrededen, Feestbund 1, Batavia*, pp. 41-47.
- 1917b.—Ueber Ankylostomiase, deren Ausbreitungsbedingungen durch die Bodeninfektion und deren Bekämpfung. *Geneesk. Tijdschr. Nederl. Indië, Afd. 5, Deel 57*, pp. 1-95, charts and maps.
- BARNES, M. E. and O'BRIEN, H. R. 1924.—Final report on hookworm survey and health propaganda work in the Kingdom of Siam. *Health Sec., Siamese Red Cross Soc.*, 26 pp., 2 maps.
- CARR, H. P. 1926.—Observations upon hookworm disease in Mexico. *Am. J. Hyg.*, v. 6 (July Suppl.):42-61.
- CHANDLER, A. C. 1925.—The migration of hookworm larvae in soil. *Indian Med. Gaz.*, v. 60(3):105-108.
- 1926:15-28.—The prevalence and epidemiology of hookworm and other helminthic infections in India. Parts 1-12. *Indian J. Med. Res.*, v. 14(1):185-194; v. 14(1):195-218; v. 14(2):451-480; v. 14(2):481-492; v. 14(2):493-504; v. 14(3):733-744; v. 14(3):745-759; v. 14(3):761-773; v. 14(4):955-971; v. 15(1):143-158; v. 15(1):159-180; v. 15(3):695-745.
- 1926.—Idem. Part V. Tea estates of Assam and Bengal. *Indian J. Med. Res.*, v. 14(2):493-504.
- 1928.—Idem. Part XII. General summary and conclusions. *Ibid.*, v. 15(3):695-743, graphs 1-10, 1 map.
- 1929.—Hookworm disease. Its distribution, biology, epidemiology, pathology, diagnosis, treatment, and control. 494 pp., 33 figs. Macmillan Co., New York.
- COGER, W. W. 1925.—Investigations on the control of hookworm disease. XXXIV. General summary of results. *Am. J. Hyg.*, v. 5(1):49-89.
- 1932.—Variations in hookworm disease. *J. Parasit.*, v. 19(2):142-147.
- 1940.—Research on helminth diseases and public health progress. *Am. J. Trop. Med.*, v. 20(2):183-198.



- CORT, W. W. and co-workers. 1922-1925. Investigations on the control of hookworm disease. I. XXXIV. Am. J. Hyg., vols. 1-5.
- CORT, W. W. and PAYNE, G. C. 1922.—Idem. VI. A study of the effect of hookworm control measures on soil pollution and infestation in a sugar estate. Ibid., v. 2(2):107-148, figs. 1-9.
- CORT, W. W., RILEY, W. A. and PAYNE, G. C. 1923.—Idem. XXIX. A study of the relation of coffee cultivation to the spread of hookworm disease. Ibid., v. 3 (July Suppl.): 111-127.
- CORT, W. W., GRANT, J. B., STOLL, N. R., and other collaborators. 1926.—Researches on hookworm in China. Am. J. Hyg., Monog. Ser. No. 7, 398 pp., figs. & maps.
- CORT, W. W., STOLL, N. R., SWEET, W. C., RILEY, W. A. and SCALAPIRO, L. 1929.—Studies on hookworm, ascaris, and trichuris in Panama. Ibid., Monog. Ser. No. 9, 215 pp.
- CORT, W. W. and OTTO, G. F. 1940.—Immunity in hookworm disease. Rev. Gastroenter., v. 7(1):2-13.
- CRUZ, W. O. 1934.—Pathogenia da anemia na Ancilostome. Parts 1, 2, and 3. Mem. Inst. Oswaldo Cruz, v. 28(3): 391-486; v. 29(2):263-485; v. 29(2):487-561. [Both Portuguese and English.]
- DARLING, S. T. 1920.—Observations on the geographical and ethnological distribution of hookworms. Parasit., v. 12 (3):217-233.  
1922.—The hookworm index and mass treatment. Am. J. Trop. Med., v. 2(5):397-447, figs. 1-3.
- DARLING, S. T., BARBER, M. A. and HACKER, H. P. 1920.—Hookworm and malaria research in Malaya, Java, and the Fiji Islands. Report of the Uncinariasis Commission to the Orient, 1915-1917. Internat. Health Board, Rockefeller Foundation, Publ. 9, pp. 1-191, figs. 1-97.
- DAVIS, N. C. 1925.—Hookworm infection as influenced by the wearing of shoes. Am. J. Trop. Med., v. 5(1):81-86.
- DOCHERTY, J. F. 1926.—Hookworm infestation and reinfestation in Ceylon. A study of high incidence with a moderate degree of infestation. Am. J. Hyg., v. 6 (March Suppl.): 160-171.
- DUBINI, ANGELO. 1843.—Nuovo verme intestinale umano (*Ancylostoma duodenale*) costituente un sesto genere dei nematoidi proprii dell'uomo. Ann. Univ. Med. & Chir., Milano, v. 106:5-13, pls. 1-2.
- EISMA, M. 1932.—Die Differentialdiagnose des dritten Stadiums der Larven von Ankylostomidae bei Mensch, Hund, und Katze. Acta Leidensia, v. 7:1-160, pls. 1-8, figs. 1-87.
- FISCHER, W. O. 1928.—Über eine Methode zum Abtöten von Hakenwurmlarven im Boden. Arch. Schiffs- u. Tropen-Hyg., v. 32(4):163-175, 3 figs.
- FOSTER, A. O. and CORT, W. W. 1932.—The relation of diet to the susceptibility of dogs to *Ancylostoma caninum*. Am. J. Hyg., v. 16(1):241-265.
- FOSTER, A. O. and CORT, W. W. 1935.—Further studies on the effect of a generally deficient diet upon the resistance of dogs to hookworm infestation. Am. J. Hyg., v. 21(2): 302-318.
- FOSTER, A. O. and CROSS, S. X. 1934.—The direct development of hookworms after oral infection. Am. J. Trop. Med., v. 14(6):565-573.
- FOSTER, A. O. and LANDSBERG, J. W. 1934.—The nature and cause of hookworm anemia. Am. J. Hyg., v. 20(2):259-290, graphs 1-6.
- FÜLLEBORN, F. 1928.—Durch Hakenwurmlarven des Hundes (*Uncinaria stenocephala*) beim Menschen erzeugte "Creeping eruption." Hamburg. Univ. Abhand. Geb. Auslandsk., v. 26, Reihe D, Med. u. Vet.-Med., v. 2, Arb. Tropenk. [etc.], pp. 121-133, pls. 12-17, figs. 1-23.  
1929.—Epidemiological observations on hookworm infection. Discussion of the question of immunity and specific reactions of the host to helminth infection. Brit. Med. J., Apr. 27, v. 1:(3564):755-759, figs. 1-6.
- FÜLLEBORN, F., DIOS, R. L. and ZUCCARINI, J. A. 1928.—Bericht über eine im Auftrage der argentinischen Regierung unternommene Reise nach der Provinz Corrientes und nach Paraguay zum Studium der Hakenwurmbekämpfung. Arch. Schiffs- u. Tropen-Hyg., v. 32(9):441-481, figs. 1-6.
- GORDON, R. M. 1925.—The effect of ancylostome, ascaris, and trichuris infections on the health of the West African native. Ann. Trop. Med. & Parasit., v. 19(4):429-460, pl. 7.
- GRASSI, B., PARONA, C. and PARONA, E. 1878.—Intorno all'ancylostoma duodenale (Dubini). Gaz. Med. Ital. Lombardia, Milano, s. 7, v. 38:193-196.
- HALL, M. C. 1921.—Carbon tetrachloride for the removal of parasitic worms, especially hookworms. J. Agric. Res., v. 21(2):157-175.
- HALL, M. C. and SHILLINGER, J. E. 1925.—Tetrachlorethylene, a new anthelmintic. Am. J. Trop. Med., v. 5(3):229-237.
- HILL, R. B. 1925.—Hookworm reinfestation in sanitized and unsanitized areas. South. Med. J., v. 18(9):665-668.  
1926.—Hookworm reinfestation for three years after treatment in a sanitized area in Porto Rico, and its bearing on permanent hookworm control in the group studied. Am. J. Hyg., v. 6 (July Suppl.):103-117.  
1927a.—The amount and distribution of hookworm infestation in Porto Rico. San Juan, P. R., pp. 1-8, reprinted from the Porto Rico Health Rev., v. 2(3): Sept., 1926.  
1927b.—Hookworm infestation in an unsanitized district, after an intensive treatment campaign. J. Prev. Med., v. 1(8):537-545.
- HIRST, L. F. 1924.—Investigations on the epidemiology of hookworm disease in Colombo. Part 2. Observations on the viability of hookworm larvae. Ceylon J. Sc., Sect. D., Med. Sc., pp. 15-31.
- HOWARD, H. H. 1919.—The control of hookworm disease by the intensive method. Internat. Health Board, Rockefeller Foundation, Publ. 8, pp. 1-189.
- HSÜ, H. F. 1938.—Studies on the food and the digestive system of certain parasites. I. On the food of the dog hookworm, *Ancylostoma caninum*. Bull. Fan Mem. Inst. Biol., Zool. Ser., v. 8(2):121-132, pls. 12 & 13.
- INTERNATIONAL HEALTH BOARD, ROCKEFELLER FOUNDATION. 1913-1928.—Annual reports. New York.  
1922.—Bibliography of hookworm disease. Publ. No. 11, xxvi + 417 pp. New York.
- KELLER, A. E., LEATHERS, W. S. and DENSEN, P. M. 1940.—The results of recent studies on hookworm in eight southern states. Am. J. Trop. Med., v. 20(4):493-509.
- KENDRICK, J. F. 1934.—The length of life and the rate of loss of the hookworms, *Ancylostoma duodenale* and *Necator americanus*. Am. J. Trop. Med., v. 14(5):363-379.
- KERR, K. B. 1936.—Studies on acquired immunity to the dog hookworm, *Ancylostoma caninum*. Am. J. Hyg., v. 24(2): 381-406, figs. 1-16.
- KIRBY SMITH, J. L., DOVE, W. E. and WHITE, G. F. 1929.—Some observations on creeping eruption. Am. J. Trop. Med., v. 9(3):179-193, pl. 1.
- KNOWLTON, R. H. 1919.—Hookworm infection among troops, treatment with oil of chenopodium. J. Am. Med. Assoc., v. 72(10):701-703.
- KOBAYASHI, H. 1928.—Morphological differences among mature larvae of *Necator americanus* and three other species of Ankylostomidae. Taiwan Igaku Kwai Zashi, No. 275, pp. 1-19, pl. 1-2. [Japanese with English summary.]
- KORKE, V. T. 1925.—Observations on the life history of hookworm in nature. Indian J. Med. Res., v. 13(2):301-319.
- LAMBERT, S. M. 1928.—Mass treatment for hookworm disease justifies itself. J. Trop. Med. & Hyg., v. 31(10):113-115.
- LANDSBERG, J. W. 1937.—The reticulocyte response in acute, fatal hookworm anemia. Am. J. Hyg., v. 26(1):60-71, graphs 1-2.
- LANDSBERG, J. W. and CROSS, S. X. 1935.—The blood picture in acute fatal infestations with *Ancylostoma caninum*. J. Parasit., v. 21(2):130-132.
- LEICHTENSTERN, O. M. L. 1887.—Einiges über *Ancylostoma duodenale*. Deutsch. Med. Wochenschr., Nos. 26-32.
- LOOSS, A. 1898.—Zur Lebensgeschichte des *Ancylostoma duodenale*. Centrallbl. Bakt. [etc.], 1. Abt., Orig., v. 24(13): 483-488.  
1911.—The anatomy and life history of *Ancylostoma duodenale* Dub. Part II. The development in the free state. Rev. Egypt. Govt. Sch. Trop. Med., v. 4:163-613, Pls. 11-19.
- MCCOY, O. R. 1929.—The suitability of various bacteria as food for hookworm larvae. Am. J. Hyg., v. 10(1):140-156.  
1930.—The influence of temperature, hydrogen-ion concentration, and oxygen tension on the development of the eggs and larvae of the dog hookworm, *Ancylostoma caninum*. Ibid., v. 11(2):413-448, figs. 1-3.
- McVAIL, J. B. 1922.—Report of hookworm research department. Ann. Rpt. Calcutta Sch. Trop. Med., pp. 26-30.
- NISHI, M. 1933.—Experimental observations on the blood-sucking activities of Ankylostomidae, especially *Ancylostoma caninum*. J. Med. Assoc. Formosa, v. 32(5):677-691, 1 pl. [Japanese with English summary.]

- OTTO, G. F. 1940.—A serum antibody in dogs actively immunized against the hookworm, *Ancylostoma caninum*. Am. J. Hyg., v. 31(2) (Sect. D):23-27.
- OTTO, G. F. and KERR, K. B. 1939.—The immunization of dogs against hookworm, *Ancylostoma caninum*, by subcutaneous injections of graded doses of living larvae. Am. J. Hyg., v. 29(1) (Sect. D):25-45, graphs 1-4.
- OTTO, G. F. and LANDSBERG, J. W. 1940.—Dietary deficiencies and iron salts in hookworm infections. Am. J. Hyg., v. 31(2) (Sect. D):37-47.
- PAYNE, F. K. 1922.—Investigations on the control of hookworm disease. XIV. Field experiments on vertical migration of hookworm larvae (Preliminary report). Am. J. Hyg., v. 3(1):46-58.
- 1923.—Idem. XXX. Studies on factors involved in migration of hookworm larvae in soil. Ibid., v. 3(5):547-583, figs. 1-12.
- PAYNE, G. C., CORT, W. W. and RILEY, W. A. 1923.—Investigations on the control of hookworm disease. XX. Human infestation studies in Puerto Rico by the egg-counting method. Am. J. Hyg., v. 3(3):315-338, figs. 1-7.
- PAYNE, G. C. and PAYNE, F. K. 1940.—Relative effectiveness of iron and anthelmintics in the treatment of hookworm anemia. Am. J. Hyg., v. 32(3) (sect. D):125-132.
- PERRONCITO, E. 1909.—La malattia dei Minatori. Dal San Gottard al Sempione. Una questione risolta; edit. Carlo Pasta, Torino, 335 pp., pls. and figs.
- PESSOA, S. B. and PASCALE, H. 1937a.—Pesquisas sobre a Ancylostomose em São Paulo. III. Intensidade do ancylostomose em algumas fazendas de care no município de Ribeirão Preto. Ann. Fac. Med. Univ. São Paulo, v. 13:167-180.
- 1937b.—Idem. IV. Análise da infestação pelo *Necator* em uma fazenda de café e cana no município de Sertãozinho. Ibid., v. 13:181-210.
- RHOADS, C. P., CASTLE, W. B., PAYNE, G. C. and LAWSON, H. K. 1934a.—Observations on the etiology and treatment of anemia associated with hookworm infection in Puerto Rico. Medicine, v. 13(3):317-375, figs. 1-6.
- 1934b.—Hookworm anemia: etiology and treatment with especial reference to iron. Am. J. Hyg., v. 20(2):291-306, figs. 1-5.
- RICE, E. M. 1927.—Mass treatment for hookworm infection on tea estates in Assam. Indian Med. Gaz., v. 62(3):126-129.
- RICKARD, E. R. and KERR, J. A. 1926.—The incidence and intensity of hookworm infestation in the various soil provinces of Tennessee. J. Prev. Med., v. 1(2):185-203.
- SARLES, M. P. 1929.—The reaction and susceptibility of dogs of different ages to cutaneous infection with the dog hookworm, *Ancylostoma caninum*. Am. J. Hyg., v. 10(3):683-692.
- SAWYER, W. A. 1923.—Hookworm disease as related to industry in Australia. Am. J. Trop. Med., v. 3(3):159-176.
- SCHÜFFNER, W. and VERVOORT, H. 1913.—Das Oleum Chenopodii anthelmintici gegen Ankylostomiasis in vergleich zu anderen Wurmmitteln. Tr. Internat. Cong. Hyg. & Demog., 1912, Washington, v. 1:734-739.
- SCOTT, J. A. 1937.—The prevalence and distribution of hookworm infection in Egypt. Am. J. Hyg., v. 26(3):455-503, maps 1-8, graphs 1-3.
- SMILLIE, W. G. 1922.—Studies on hookworm infection in Brazil. Second Paper. Monog. Rockefeller Inst. Med. Res., No. 17, pp. 1-73, charts 1-15.
- SMILLIE, W. G. and AUGUSTINE, D. L. 1925.—Intensity of hookworm infestation in Alabama. Its relationship to residence, occupation, age, sex, and race. J. Am. Med. Assoc., v. 85(25):1958-1963.
- SOPER, F. L. 1927.—The relative egg laying function of *Necator americanus* and *Ancylostoma duodenale*. Am. J. Hyg., v. 7(5):542-556.
- STEKHOVEN, JR. J. H. SCHUERMANS. 1927.—The nemas *Ancylostoma* and *Necator*. II. New researches on the larvae of *Ancylostoma duodenale* Dub. and *Necator americanus* Stiles. K. Akad. Wetensch. Amsterdam, v. 30(5):581-593.
- STILES, C. W. 1902.—A new species of hookworm (*Cuciniaria americana*) parasitic in man. Am. Med., v. 3:777-778.
- 1921.—The hookworm thermometer. J. Parasit., v. 7(4):192-193.
- STOLL, N. R. 1923a.—Investigations on the control of hookworm disease. XV. An effective method of counting hookworm eggs in feces. Am. J. Hyg., v. 3(1):59-70.
- 1923b.—Idem. XXIV. Hookworm cultures with humus, sand, loam, and clay. Am. J. Hyg. (July Suppl.), v. 3:1-36.
- SVENSSON, RUTH. 1925.—Observations on the development and longevity of hookworm larvae in different temperature conditions. China Med. J., v. 39(8):667-673; 4 charts.
- SVENSSON, R. M. and KESSEL, J. F. 1926.—Morphological differences between *Necator* and *Ancylostoma* larvae. J. Parasit., v. 13(2):146-153.
- SWEET, W. C. 1925.—Hookworm re-infection. Ceylon J. Sc., Sect. D, Med. Sc., v. 1(3):129-140, 1 fig.
- WELLS, H. S. 1931.—Observations on the blood sucking activities of the hookworm, *Ancylostoma caninum*. J. Parasit., 17(4):167-182, 1 fig.
- YEAGER, C. H. 1931.—Bored-hole latrine equipment and construction. Philippine J. Sc., v. 46(4):681-749, pls. 1-7, figs. 1-46.
- 1934.—Idem. 108 pp., figs. 1-84. Bureau of Printing, Manila.
- YOKOGAWA, S. and OISO, T. 1926.—Studies on oral infection with *Ancylostoma*. Am. J. Hyg., v. 6(3):484-497.

ASCARIS LUMBRICOIDES AND TRICHRIS TRICHIURA

- ALICATA, J. E. 1934.—Observations on the period required for ascaris eggs to reach infectivity. Proc. Helm. Soc. Wash., v. 1(1):12.
- BAKKER, C. R. 1921.—Over de identiteit van *Ascaris lumbricoides* en *Ascaris suilla*. Tijdschr. Vergelijk. Geneesk., v. 6:160-230, figs. 1-5, 6 pls.
- BARKER, F. D. 1923.—The chromosomes in *Ascaris lumbricoides* of man. [Abstract.] Anat. Rec., v. 24(6):375.
- BROWN, H. W. 1927a.—Human ascaris as a household infection. J. Parasit., v. 13(3):206-212.
- 1927b.—Studies on the rate of development and viability of the eggs of *Ascaris lumbricoides* and *Trichuris trichiura* under field conditions. J. Parasit., v. 14(1):1-15, figs. 1-2.
- 1928a.—A quantitative study of the influence of oxygen and temperature on the embryonic development of the eggs of the pig ascarid (*Ascaris suum* Goeze). J. Parasit., v. 14(3):141-160.
- 1928b.—Further studies on the longevity of the eggs of *Ascaris lumbricoides* and *A. suum*. J. Parasit., v. 15(1):14-22.
- 1934.—Intestinal parasitic worms in the United States. Their diagnosis and treatment. J. Am. Med. Assoc., v. 103(9):651-660.
- BROWN, H. W. and CORT, W. W. 1927.—The egg production of *Ascaris lumbricoides*. J. Parasit., v. 14(2):88-90.
- BUCKLEY, J. J. 1931.—Observations on human resistance to infection with ascaris from the pig. J. Helm., v. 9(2):45-46.
- CALDWELL, F. C. and CALDWELL, E. L. 1926.—Are *Ascaris lumbricoides* and *Ascaris suilla* identical? J. Parasit., v. 13(2):141-145.
- CALDWELL, F. C. and CALDWELL, E. L. 1928.—Preliminary report on observations on the development of ova of pig and human ascaris under natural conditions, and studies of factors influencing development. J. Parasit., v. 14(4):254-264.
- CALDWELL, F. C., CALDWELL, E. L. and DAVIS, G. E. 1930.—Some aspects of the epidemiology of infestation with trichuris and ascaris as revealed in a study at the hospitals for the insane and the home for mentally defective children in the state of Alabama. Am. J. Hyg., v. 11(3):619-651, figs. 1-5.
- CHANDLER, A. C. 1928.—The prevalence and epidemiology of hookworm and other helminthic infections in India. XII. General summary and conclusions. Indian J. Med. Res., v. 15(3):695-743.
- CORT, W. W. 1931.—Recent investigations on the epidemiology of human ascariasis. J. Parasit., v. 17(3):121-144.
- CORT, W. W. and OTTO, G. F. 1937.—*Trichuris trichiura* in the United States. Papers on Helminthology, Jub. Skrzjabin, p. 81-88.
- CORT, W. W., OTTO, G. F. and SPINDLER, L. A. 1930.—Investigations on *Ascaris lumbricoides* and the associated intestinal helminths of man in southwestern Virginia. Am. J. Hyg., v. 9(1):1-55, figs. 1-9.
- CORT, W. W., SCHAPIRO, L., RILEY, W. A. and STOLL, N. R. 1929.—A study of the influence of the rainy season on the level of helminth infestations in a Panama village. Am. J. Hyg., v. 10(3):626-634.
- CORT, W. W., SCHAPIRO, L. and STOLL, N. R. 1929.—A study of

- reinfection after treatment with hookworm and ascaris in two villages in Panama. *Am. J. Hyg.*, v. 10(3):614-625.
- CORT, W. W. and STOLL, N. R. 1931.—Studies on *Ascaris lumbricoides* and *Trichuris trichiura* in China. *Am. J. Hyg.*, v. 14(3):655-689.
- CORT, W. W., STOLL, N. R., SWEET, W. C., RILEY, W. A. and SCHAPIRO, LOUIS. 1929. Studies on hookworm, ascaris, and trichuris in Panama. *Am. J. Hyg., Monog., Ser. No. 9*, pp. 1-215.
- CRAM, E. B. 1924.—The influence of low temperature and of disinfectants on the eggs of *Ascaris lumbricoides*. *J. Agr. Res.*, v. 27(3):167-175.  
1925.—The egg producing capacity of *Ascaris lumbricoides*. *Ibid.*, v. 30(10):977-983, figs. 1-6.
- DAVAINE, C. J. 1858.—Recherches sur le developpement et la propagation du Trichocephale de l'homme et de l'ascaride lombricoide. *Compt. Rend. Acad. Sc., Paris*, v. 46(25):1217-1219.  
1863.—Nouvelle recherches sur le developpement et la propagation de l'ascaride lombricoide et du trichocephale de l'homme. *Compt. Rend. Soc. Biol., Paris*, 3. s., v. 4:261-265.
- DE BOER, E. 1935a.—Experimenteel onderzoek betreffende *Ascaris lumbricoides* van mensch en varken. Proefschrift (Rijks-Univ. Utrecht), 83 pp.  
1935b.—Experimentelle Untersuchungen ueber *Ascaris lumbricoides* des Menschen und des Schweines. *Ztschr. Infektionskrank. Haustiere*, v. 48(4):248-269.
- DENECKE, K. 1937.—Der Befall mit menschlichen Darmhelminthen auf der Elbinsel Finkenwärder und seine Ursachen. *Arch. Hyg. & Bakt.*, v. 117(6):332-362.
- EPSTEIN, A. 1892.—Ueber die Uebertragung des menschlichen Spulwurms (*Ascaris lumbricoides*). *Verhandl. Versamml. Gesellsch. Kinderheilk. Naturf. Aertze*, v. 9:1-16.
- FORSTER, E. 1933.—Askaridenrekord. *Schweiz. Med. Wochenschr.*, May 13, p. 452.
- FÜLLEBORN, F. 1923.—Ueber den "Mundstachel" der trichocephaliden-Larven und Bemerkungen ueber die jungsten Stadien von *Trichocephalus trichiurus*. *Arch. Schiffs- u. Tropen-Hyg.*, v. 27:421-425.  
1927.—Ueber das Verhalten der Larven von *Strongyloides stercoralis*, Hakenwürmern und *Ascaris lumbricoides* im Körper des Wirtes und ein Versuch, es biologisch zu deuten. Beihefte No. 2 *Arch. Schiffs- u. Tropen-Hyg.*, v. 31(2):151-202, figs. 1-5.
- GREGES, R. 1934.—Studies on ascariasis. I. Geographical distribution with special reference to Egypt. *J. Trop. Med. & Hyg.*, v. 37(6):85-90.
- GRASSI, G. B. 1887.—*Trichocephalus* und *Ascaris* entwicklung. Preliminarnote. *Centralbl. Bakt. [etc.]*, v. 1(5):131-132.  
1888.—Weiteres zur Frage der *Ascaris*-entwicklung. *Centralbl. Bakt. [etc.]*, v. 3(24):748-749.
- GUIART, J. 1911.—Les parasites inoculateurs de maladies. Paris.
- HASEGAWA, K. 1924.—Betrag zur Entwicklung von *Trichocephalus* im Wirt. *Arch. Schiffs- u. Tropenkrank.*, v. 28(7):337-340; 4 figs.
- HEADLEE, W. H. 1936.—The epidemiology of human ascariasis in the metropolitan area of New Orleans, Louisiana. *Am. J. Hyg.*, v. 24(3):479-521, figs. 1-2.
- HIRAIISHI, T. 1938.—Experimentelle Infektion junger Schweine mit Ascariden mit Rücksicht auf besondere Beziehungen zu A-avitaminose. In S. Hata's *Neuere Arbeiten über Parasitologie in Japan*. *Arch. Schiffs- u. Tropen Hyg.*, v. 32(10):519-521.
- KELLER, A. E. 1931.—*Ascaris lumbricoides*. Loss of infestation without treatment. *J. Am. Med. Assoc.*, v. 97(18):1299-1300.  
1933.—A study of the occurrence of unfertilized ascaris eggs. *J. Lab. & Clin. Med.*, v. 18(4):371, 1 fig.
- KELLER, A. E., HILLSTRÖM, H. T. and GASS, R. S. 1932.—The lungs of children with ascaris. *J. Am. Med. Assoc.*, v. 99(15):1249-1251, figs. 1-2.
- KERR, K. B. 1938.—The cellular response in acquired resistance in guinea pigs to an infection with pig ascaris. *Am. J. Hyg.*, v. 27(1):28-53, 1 pl.
- KHALIL, M. 1931.—The pail closet as an efficient means of controlling human helminth infection as observed in Tura prison, Egypt, with a discussion on the source of ascaris infection. *Ann. Trop. Med. & Parasit.*, v. 25(1):35-62.
- KOINO, S. 1922.—Experimental infections on human body with ascariades. *Japan Med. World*, v. 2(11):317-320.
- LANE, C. 1934.—The prevention of ascaris infection: A critical review. *Trop. Dis. Bull.*, v. 31(9):605-615.
- LEUCKART, R. 1866.—Zur Entwicklungsgeschichte der Nematoden. *Arch. Ver. Wiss. Heilk.*, v. 2:195-235.
- LUTZ, A. 1888.—Zur Frage der Uebertragung des menschlichen Spulwurms. Weiter Mittheilungen. *Centralbl. Bakt. [etc.]*, v. 3(14):425-428.
- MURRAY, A. 1935.—The extra corporeal hatching of ascaris eggs. *J. Parasit.*, v. 21(3):222-223.
- MANALANG, C. 1928.—Ascariasis: relation between the number of ova per gram of formed stool and the number of female worms harbored by the host. *Philippine J. Sc.*, v. 35(1):23-29.
- MAPLESTONE, P. A. and MUKERJI, P. K. 1936.—An improved technique for the isolation of ascaris eggs from soil. *Indian J. Med. Res.*, v. 23(3):667-672.
- MARTIN, H. M. 1926.—Studies on *Ascaris lumbricoides*. *Univ. Nebraska, Agric. Exper. Sta. Res. Bull.*, 37, pp. 1-78, figs. 1-3.
- MILNER, M. J. 1939a.—The egg count of *Trichocephalus vulpis* infections in dogs. *Proc. Soc. Exp. Biol. & Med.*, v. 42(1):301-303.  
1939b.—*Trichocephalus* and *Trichocephalosis*. *Canad. J. Comp. Med.*, 3:282-287.  
1941.—Quantitative studies on *Trichocephalus vulpis* infections in dogs. *Am. J. Hyg.*, v. 32(2), Sect. D:58-70.
- MILLS, R. G. 1927.—Parasites, chiefly metazoan, observed in 7,000 specimens of feces from Koreans, with an attempt to interpret the findings. *Am. J. Hyg.*, v. 7(3):222-263.
- MORGAN, D. O. 1931.—Some observations on experimental ascariasis in pigs. *J. Helmin.*, v. 9(3):121-127.
- NAIR, P. K. 1935.—An epidemiological study of ascaris, trichuris, and hookworm in a coastal village in Puerto Rico. *Puerto Rico J. Pub. Health & Trop. Med.*, v. 11(1):118-138, figs. 1-4.
- NOLF, L. O. 1932.—Experimental studies on certain factors influencing the development and viability of the ova of the human trichuris as compared with those of ascaris. *Am. J. Hyg.*, v. 16(1):288-322, figs. 1-8.
- OGATA, S. 1925.—The destruction of ascaris eggs. *Ann. Trop. Med. & Parasit.*, v. 19(3):301-304.
- ONORATO, A. R. 1932.—The effects of temperature and humidity on the ova of *Toxocara canis* and *Trichuris vulpis*. *Am. J. Hyg.*, v. 16(1):266-287.
- OTTO, G. F. 1929.—A study on the moisture requirements of the eggs of the horse, the dog, human, and pig ascariids. *Am. J. Hyg.*, v. 10(2):497-520.  
1930.—*Ascaris lumbricoides*. Treatment, loss of worms, and reinfestation. *J. Am. Med. Assoc.*, v. 95(3):194-196.  
1932.—The appearance and significance of the unfertilized eggs of *Ascaris lumbricoides* (Linn.). *J. Parasit.*, v. 18(4):269-273, 1 pl.  
1935.—Blood studies on trichuris-infested and worm-free children in Louisiana. *Am. J. Trop. Med.*, v. 15(6):693-704.
- OTTO, G. F. and CORT, W. W. 1934a.—The distribution and epidemiology of human ascariasis in the United States. *Am. J. Hyg.*, v. 19(3):657-712.  
1934b.—Further studies on post-treatment reinfection with ascaris in the United States. *J. Parasit.*, v. 20(4):245-247.
- OTTO, G. F., CORT, W. W. and KELLER, A. E. 1931.—Environmental studies of families in Tennessee infested with ascaris, trichuris, and hookworm. *Am. J. Hyg.*, v. 14(1):156-193.
- PAYNE, F. K., ACKERT, J. E. and HARTMAN, E. 1925.—The question of human and pig ascaris. *Am. J. Hyg.*, v. 5(1):50-101.
- RANSOM, B. H.—1920. Pig parasites and thumps. *U. S. Dept. Agric. Yearbook, Sep. No. 837*, pp. 175-180, figs. 1-3.
- RANSOM, B. H. and CRAM, E. B. 1921.—The course of migration of ascaris larvae. *Am. J. Trop. Med.*, v. 1(3):129-156, figs. 1-5, pls. 1-2.
- RANSOM, B. H. and FOSTER, W. D. 1920.—Observations on the life history of *Ascaris lumbricoides*. *U. S. Dept. Agric. Bull. No. 817*, pp. 1-47, figs. 1-6.
- REDI, FRANCESCO. 1684.—Osservazioni—intorno agli animali viventi che si trovano negli animali vivendi. 253 pp., 26 pls., Firenze.
- ROBERTS, F. S. S. 1934.—The large roundworm of pigs, *Ascaris lumbricoides* L., 1758. Its life history in Queensland, economic importance, and control. *Dept. Agric., Queensland*,

- Austral. Anim. Health Sta. Yeerongpilly. Bull. No. 1, figs. 1-11, pls. 1-2.
- ROBERTSON, R. C. 1936.—The transmission in China of helminths by vegetables. Chinese Med. J., Suppl., v. 1:418-422.
- SCOTT, J. A. 1939.—Observations on infection with the common roundworm, *Ascaris lumbricoides*, in Egypt. Am. J. Hyg., v. 30(3), Sect. D:83-116, maps 1-7, graphs 1-2.
- SCHWARTZ, B. 1920.—The biological relationship of ascarids. J. Parasit., v. 6(3):115-123.
- SPINDLER, L. A. 1929a.—On the use of a method for the isolation of ascaris eggs from the soil. Am. J. Hyg., v. 10(1):157-164.
- 1929b.—The relation of moisture to the distribution of human trichuris and ascaris. Am. J. Hyg., v. 10(2):476-496, figs. 1 and 2.
- 1929c.—A study of the temperature and moisture requirements in the development of the eggs of the dog trichuris (*Trichuris vulpis*). J. Parasit., v. 16(1):41-46.
- STEWART, F. H. 1916.—On the life history of *Ascaris lumbricoides*. Brit. Med. J. (July 1), v. 2:5-7.
- SUZUKI, J. 1934.—Experimental studies on repeated infections of *Trichuris*. Keio-Igaku, v. 14:1079-1117.
- SWARTHWELDER, J. C. 1939.—Clinical *Trichocephalus trichuris* infection. An analysis of 81 cases. Am. J. Trop. Med., v. 19(5):473-481.
- SWEET, W. C. 1924.—The intestinal parasites of man in Australia and its dependencies as found by the Australian hookworm campaign. Med. J. Austral., v. 1(17):405-407.
- TUBANGUL, M. A., BASACA, M. and PASCO, A. M. 1934.—Human infestations with ascaris and trichuris in different parts of the Philippine Islands. Philippine J. Sci., v. 55(2):91-113, figs. 1-4.
- TYSON, EDWARD. 1683.—*Lumbrieus teres* or some anatomical observations on the round worm bred in human bodies. Phil. Tr. Lond., v. 13:154-161, 1 pl., figs. 1-4.
- WALKER, J. H. C. 1927.—*Ascaris lumbricoides* in the Singapore garrison. J. Roy. Army Med. Corps, v. 49(1):49-53.
- WINFIELD, G. F. 1937a.—Studies on the control of fecal-borne diseases in North China. II. The distribution of *Ascaris lumbricoides* infestations in a rural population. Chinese Med. J., v. 51(4):502-518.
- 1937b.—Studies on the control of fecal-borne diseases in North China. III. Family environmental factors affecting the spread of *Ascaris lumbricoides* in a rural population. Chinese Med. J., v. 51(5):643-658.
- WINFIELD, G. F. and CHIN, T. H. 1938.—Studies on the control of fecal-borne diseases in North China. VI. The epidemiology of *Ascaris lumbricoides* in an urban population. Chinese Med. J., v. 54(3):233-254.
- WINFIELD, G. F. and YAO, T. N. 1937.—Studies on the control of fecal-borne diseases in North China. IV. Vegetables as a factor in the spread of *Ascaris lumbricoides*. Chinese Med. J., v. 51(6):919-926.
- YOKOGAWA, S. 1923.—On ascariasis and life history of ascaris. (Investigations on helminthiasis in Formosa, IV. Report). [Japanese text; English summary], pp. 1-18.
- YOSHIDA, S. 1919.—On the development of *Ascaris lumbricoides* L. J. Parasit., v. 5(3):105-115, figs. 1-14.
- 1920.—On the resistance of ascaris eggs. J. Parasit., v. 6(3):132-139.
- 1925.—Wichtige resultate Japanischer Ascaris-forschungen der letzten Zeit. Reprinted from Jap. Deutsch. Ztschr. Wiss. & Tech., 3. Jahrgang, Heft 5, Mai 1925.
- pathology of dracontiasis. Part I. Indian J. Med. Res., v. 11(3):915-932.
- 1924b.—Studies in the transmission of *Dracunculus medinensis*—a negative experiment. Indian J. Med. Res., v. 12(1):93-104.
- FEDTSCHENKO, B. A. 1871.—(On the formation and increase of *Filaria medinensis* L.) (Russian). Izviest. Imp. Obsh. Ljub. Estestvozn., Antrop., Moskva, v. 8(1):71-82. (Cited from Moorthy, 1938).
- HSÜ, H. F. and WATT, J. Y. C. 1933.—*Dracunculus medinensis* infection in two dogs in Peiping. Experimental infection of cyclops. Chinese Med. J., v. 47:1326-1330.
- ISSAJEV, L. 1934a.—Experimentelle Dracunculosis beim Hunde. Med. Parasit. & Parasitic Dis., Moscow (Russian), v. 3(3):231-238. (Cited from Moorthy, 1938.)
- 1934b.—Ueber die Eindringung den *Dracunculus medinensis*-Larven in Cyclops. Med. Parasit. u. Parasitic Dis., Moscow (Russian), v. 3(3):212-230. (Cited from Trop. Dis. Bull.)
- LEDENTU, R. 1924.—Paludisme et ver de Guinee en Haute-Volta. Bull. Soc. Path. Exot., v. 17(1):104-107.
- LEIPER, R. T. 1907.—The etiology and prophylaxis of dracontiasis. Brit. Med. J., v. 1(1907):129-132.
- 1910.—Guinea-worm in domesticated animals. J. Trop. Med. & Hyg., v. 13(5):65-66.
- LINDBERG, K. 1935.—Remarques sur l'épidémiologie de la dracontulose dans l'Inde britannique. Un plaidoyer en faveur de recherches. Bull. Soc. Path. Exot., v. 28(9):866-875.
- 1936.—Dracontulose en Iran. Arch. Schiffs- u. Tropen-Hyg., v. 40(7):330-342.
- LISTON, W. G. 1913.—Report of the Bombay Bacteriological Laboratory for 1912, Section VI, Dracontiasis, Guinea worm disease, pp. 32-36. (Cited from Fairley and Liston, 1924.)
- McKINLEY, E. C. 1935.—A geography of disease. Am. J. Trop. Med., v. 15(5) (Suppl.):1-494.
- MOORTHY, V. N. 1932a.—An epidemiological and experimental study of dracontiasis in Chitaldrug district. Indian Med. Gaz., v. 67(9):498-504.
- 1932b.—Treatment and prophylaxis of dracontiasis. Indian Med. Gaz., v. 67(11):617-619.
- 1937.—A redescription of *Dracunculus medinensis*. J. Parasit., v. 23(2):220-224, pls. 1-3.
- 1938.—Observations on the development of *Dracunculus medinensis* larvae in cyclops. Am. J. Hyg., v. 27(2):437-460.
- MOORTHY, V. N. and SWEET, W. C. 1936a.—A note on the experimental infection of dogs with dracontiasis. Indian Med. Gaz., v. 71(8):437-442, figs. 1-8.
- 1936b.—Guinea-worm infection in nature. Indian Med. Gaz., v. 71(10):568-570.
- 1936c.—A biological method for the control of dracontiasis. Indian Med. Gaz., v. 71(10):565-568.
- 1938.—Further notes on the experimental infection of dogs with dracontiasis. Am. J. Hyg., v. 27(2):301-310, pls. 1-3.
- PRADHAN, Y. M. 1930.—Observations on experiments designed to combat dracontiasis in an endemic area by Col. Morrison's method of "liming wells." Indian J. Med. Res., v. 18(2):443-465, pl. 29.
- RAO, S. S. 1936.—The effect of gastric juice and bile on cyclops infected with guinea-worms larvae. Indian J. Med. Res., v. 24(2):535-554.
- ROUBAUD, E. 1913.—Observations sur le biologie du Ver de Guinee. Infection intestinale des cyclops. Bull. Soc. Path. Exot., v. 6(4):281-288.
- TREWN, H. S. 1937.—Guinea worm. Indian Med. Gaz., v. 72(10):606-609.
- TURKHUDD, D. A. 1912.—Report of the Bombay Bacteriological Laboratory for the year 1911. Govt. Cent. Printing Press, Bombay, p. 40. (Cited from Trop. Dis. Bull.)
- TURKHUDD, D. A. 1919.—Prophylaxis in dracontiasis. Indian J. Med. Res., Spec. Indian Se. Cong. No., pp. 217-225.
- 1920.—Dracontiasis in animals; with notes on a case of guinea worm in a cobra. Indian J. Med. Res., v. 7(4):727-734, figs. 1-3.

DRACUNCULUS MEDINENSIS

THE FILARIAE

- BRUG, S. L. 1930.—*Dracunculus medinensis* in the Dutch East Indies. Meded. Dienst. Volksgezondheid in Nederl. Indië, v. 19(1):153-157.
- CHATTON, E. 1918.—Observations sur le ver de Guinee. Preuve experimentale de l'infestation des Cyclops par voie digestive. Bull. Soc. Path. Exot., v. 11(4):339-348.
- CHITWOOD, B. G. 1933.—Does the guinea-worm occur in North America? J. Am. Med. Assoc., v. 100:802-804.
- DAVIS, L. J. 1931.—A note on some experiments with agents lethal to cyclops. Tr. Roy. Soc. Trop. Med. & Hyg., v. 24(6):631-633.
- FAIRLEY, N. H. 1924.—Studies in dracontiasis. Part IV. The clinical picture—an analysis of 140 cases. Indian J. Med. Res., v. 12(2):351-367.
- FAIRLEY, N. H. and LISTON, W. G. 1924a.—Studies in the
- ANDERSON, J. 1924.—Filariasis in British Guiana. Res. Mem., London Sch. Trop. Med., v. 5 (7): 122 pp., 20 pls. London.
- AUGUSTINE, D. L., FIELD, M. E. and DRINKER, C. K. 1936.—Observations on living *Microfilaria immitis* in the capillary

- circulation of bats. Tr. Roy. Soc. Trop. Med. & Hyg., v. 30(2):231-232.
- AUGUSTINE, D. L. 1937.—Observations on living "sheathed" microfilariae in the capillary circulation. Tr. Roy. Soc. Trop. Med. & Hyg., v. 31(1):55-60.
- BAHR, P. H. 1912.—Filariasis and elephantiasis in Fiji, a report to the London School of Tropical Medicine. viii + 192 pp., 17 figs., 34 pls. London.
- BLACKLOCK, D. B. 1926.—The development of *Onchoecera volvulus* in *Simulium damnosum*. Ann. Trop. Med. & Parasit., v. 20(1):1-40, pls. 1-4, figs. 1-10.
- BRUG, S. L. 1927.—Een nieuwe filaria soort (*Filaria malayi*), parasiteerende bij den mensch. Geneesk. Tijdschr. Nederl.-Indië, v. 67(5):750-754, 1 pl. (6 figs.).
- CALDERÓN, V. M. 1920.—Contribución al estudio del Filárido onchoecera sp. Dr. Robles—1915 y de las enfermedades que produce. 107 pp., 36 figs. Guatemala. (Junio.)
- DRINKER, C. K., FIELD, M. E. and HOMANS, J. 1934.—Experimental production of edema and elephantiasis as a result of lymphatic obstruction. Am. J. Physiol., v. 108(3):509-520, figs. 1-6.
- DRINKER, C. K., AUGUSTINE, D. L. and LEIGH, O. 1935.—On filtration of microfilariae by lymph nodes. Tr. Roy. Soc. Trop. Med. & Hyg., v. 29(3):303-306, figs. 1-3.
- DRINKER, C. K. 1936.—The relation of lymph circulation to streptococci infection. Medical papers dedicated to Dr. Henry A. Christian, pp. 705-710, figs. 1-3. Boston.
- FENG, LAN-CHOU. 1931.—Filariasis in China with special reference to its distribution and transmission. Natl. Med. J. China, v. 17(4/5):464-474. 1 pl. (4 figs.).
- FRANCIS, E. 1919.—Filariasis in Southern United States. U. S. Pub. Health Service, Hyg. Lab., Bull. No. 117, 36 pp., 10 pls. Washington.
- FÜLLEBORN, F. 1929.—Filariosen des Menschen. Handb. Path. Mikroorg., v. 6(28):1043-1224, pls. 1-3, figs. 1-77. Jena.
- GERVAIS, P. and VAN BENEDEEN, P.-J. 1859.—Zoologie Médicale. Exposé méthodique du règne animal. v. I, xii + 504 pp., 97 figs. Paris.
- GOLDEN, R. and O'CONNOR, F. W. 1934.—The roentgen treatment of filariasis. I. Chyluria. II. Filarial lymphangitis. Tr. Roy. Soc. Trop. Med. & Hyg., v. 27(4):385-398.
- HARLEY, G. W. 1932.—A theory regarding the rôle of insect saliva in filarial periodicity. Tr. Roy. Soc. Trop. Med. & Hyg., v. 25(6):487-491.
- HILLARY, W. 1766.—Observations on the changes of the air and the concomitant epidemical diseases in the Island of Barbadoes. 2nd ed., 8 + xiv + 15-136 pp. London.
- IYENGAR, M. O. T. 1938.—Studies on the epidemiology of filariasis in Travancore. Indian Med. Res. Mem., Suppl. to Indian J. Med. Res., No. 30, iv + 179 pp., 12 pls. (27 figs.).
- KHALIL, M. 1938.—The site of the adult filaria in the human body is the determining factor in the microfilaria being periodic or non-periodic even in the same species. J. Egypt. Med. Assoc., v. 21(8):502-505.
- LANE, C. 1929.—The mechanism of filarial periodicity. Lancet, Lond. (5521), v. 216, v. 1(25):1291-1293, 1 fig.  
1937.—Bancroftian filariasis and the reticulo endothelial system. Tr. Roy. Soc. Trop. Med. & Hyg., v. 31(1):61-80, figs. 1-4.
- LEE, C. U. 1926.—Filariasis investigations in the Province of Kiang Su, China. Tr. Roy. Soc. Trop. Med. & Hyg., v. 20(4):279-287, figs. 1-5.
- LEWIS, T. R. 1879.—The microscopic organisms found in the blood of man and animals, and their relation to disease. v + 91 pp., 27 figs., 3 pls. Gov't. Printing Office. Calcutta.
- LOW, G. C. 1913.—Discussion on filariasis. Brit. Med. J., v. 2:1298-1302.
- MENON, T. 1935.—Maharaja of Travancore Curzon Lectures. (Univ. Madras, 1934-35). Problems in filariasis. ii + 67 pp., 16 pls., 22 figs. Madras.
- O'CONNOR, F. W. 1923.—Researches in the Western Pacific. Res. Mem., Lond. Sch. Trop. Med., v. 4:57 pp., 8 pls.
- O'CONNOR, F. W. and HULSE, C. R. 1935.—Studies in filariasis. Puerto Rico J. Pub. Health & Trop. Med., v. 11(2):167-272, 16 pls., 26 figs.
- O'CONNOR, F. W. and BEATTY, H. A. 1938.—*Wuchereria bancrofti* in mosquitoes of St. Croix. Tr. Roy. Soc. Trop. Med. & Hyg., v. 31(4):413-430, figs. 1-5.
- POYNTON, J. O. and HODGKIN, E. P. 1938.—Endemic filariasis in the Federated Malay States. Inst. Med. Res., Federated Malay States, Bull. No. 1, 67 pp., 5 pls. Kuala Lumpur.
- ROHMANN, J. 1937.—Les localisations tissulaires de *Microfilaria volvulus* (Lorenkart). Sonderabdruck aus der Festschr. Nocht, 1937, pp. 513-516, pl. 21, figs. 1-2. Hamburg.
- SHARP, N. A. D. 1927.—A new site for *Onchoecera volvulus*. Lancet, Lond. (5442), v. 213, v. 2(25):1290.
- STRONG, R. P., SANDGROUND, J. H., BEQUAERT, J. C. and OCHOA, M. M. 1934.—Onchoecereiasis with special reference to the Central American form of the disease. xiv + 234 pp., 103 figs., 6 pls. Cambridge, Mass.
- STRONG, R. P., HISSETTE, J., SANDGROUND, J. H. and BEQUAERT, J. C. 1938.—Onchoecereiasis in Africa and Central America. Am. J. Trop. Med., Suppl., v. 18(1):136 pp., 68 figs., 1 pl., 4 figs.
- SWEET, W. C. and PILLAI, V. M. 1937.—Clearance of *Pistia stratiotes* as a control measure for *F. malayi* infection. Indian Med. Gaz., v. 72(12):730-734, figs. 1-4.
- VAN DEN BERGHE, L. 1936.—Note préliminaire sur la localisation extranodulaire de "*Onchoecera volvulus*" chez l'homme. Ann. Soc. Belge Med. Trop., v. 16(4):549-551.

TRICHINELLA SPIRALIS

- ALICATA, J. E. 1938.—A study of *Trichinella spiralis* in the Hawaiian Islands. Pub. Health Rpts., U. S. Pub. Health Service, v. 53(10):384-393, figs. 1-2.
- AUGUSTINE, D. L. 1933.—Experimental trichinosis in chicks. Science, n.s., v. 78(2035):608-609.
- AUGUSTINE, D. L. and THEILER, H. 1932.—Precipitin and skin tests as aids in diagnosing trichinosis. Parasit., v. 24(1):60-86, fig. 1, pl. 8, figs. 1-2.
- BACHMAN, G. W. 1928.—A precipitin test in experimental trichinosis. J. Prev. Med., v. 2(1):35-48.  
1928.—An intradermal reaction in experimental trichinosis. Ibid., v. 2(6):513-523, fig. 1, pl. 1.  
1935.—Helminths as agents of disease. Reprinted from "Agents of disease and host resistance," Chap. 57, 1324-1409, figs. 160-207.
- BAYLIS, H. A. 1929.—A manual of Helminthology, medical and veterinary. 303 pp., 200 figs. London.
- BOZICEVICH, J. 1939.—Studies on trichinosis. XII. The preparation and use of an improved trichina antigen. Pub. Health Rpts., U. S. Pub. Health Service, v. 53(48):2130-2138, fig. 1.
- CH'IN, YAO-TING. 1937.—*Trichinella* infection in a cat in Mukden. Chinese Med. J., v. 51:500-501.
- CHITWOOD, B. G. 1930.—[Notes on the esophagus of the Trichinelloidea.] J. Parasit., v. 16(3):165-166.
- FERENBAUGH, T. L., SEGAL, L. and SCHULZE, H. A. 1938.—A trichinosis epidemic of sixty-four cases. J. Am. Med. Assoc., v. 110:1434-1436, 2 figs.
- GERGER, J. C. and HOBMAIER, M. 1939.—Trichinelliasis and carnivorous mammals (Bears). Calif. & Western Med., v. 51(4):249-250.
- GLAZIER, W. C. W. 1881.—Report on trichinae and trichinosis. Prepared under the direction of the Supervising Surgeon-General (U. S. Marine Hospital Service). 212 pp., 87 figs. Washington.
- HALL, M. C. 1937a.—Studies on Trichinosis. III. The complex clinical picture of trichinosis and the diagnosis of the disease. Pub. Health Rpts., U. S. Pub. Health Service, v. 52(18):539-551.  
1937b.—Idem, IV. The role of the garbage-fed hog in the production of human trichinosis. Ibid., v. 52(27):837-886, 1 fig.  
1938a.—Idem, VII. The past and present status of trichinosis in the United States, and the indicated control measures. Ibid., v. 53(33):1472-1486.  
1938b.—Idem, VI. Epidemiological aspects of trichinosis in the United States as indicated by an examination of 1,000 diaphragms for trichinae. Ibid., v. 53(26):1086-1150, 1 fig.
- HALL, M. C. and COLLINS, B. J. 1937.—Studies on trichinosis. I. The incidence of trichinosis as indicated by post-mortem examinations of 300 diaphragms. Pub. Health Rpts., U. S. Pub. Health Service, v. 52(16):468-490.
- KAUFMAN, R. E. 1940.—Trichiniasis: Clinical considerations. Ann. Int. Med., v. 13(8):1433-1460.
- KERR, K. B. 1940.—Public Health Aspects of the trichinosis problem in the South. South. Med. J., v. 33(5):511-516.

- KERR, K. B., JACOBS, L. and CUVILLIER, E. Studies on trichinosis. XIII. The incidence of human infection with trichinae as indicated by post-mortem examination of 3,000 diaphragms from Washington, D. C., and 5 eastern seaboard cities. Pub. Health Rpts., U. S. Pub. Health Service, v.56(16):836-855.
- LICHTERMAN, A. and KLEEMAN, I. 1939.—Detection of *Trichinella* infestation in hogs by the intradermal test. Am. J. Pub. Health, v. 29(10):1098-1102.
- McCoy, O. R. 1932.—Experimental trichiniasis infections in monkeys. Proc. Soc. Exper. Biol. & Med., v. 30:85-86.
- MAGATH, T. B. 1937.—Encysted trichinae. Their incidence in a private practice and the bearing of this on the interpretation of diagnostic tests. J. Am. Med. Assoc., v. 108 (June 5):1964-1967.
- MATOFF, K. 1936.—Bei Tauben auf enteralem Wege erzeugte Muskeltrichinellose. Rpt. Tierärztl. Rundschau, v. 42:401-404.
- 1938.—Zur Frage der Muskeltrichinellose beim Geflügel. Ztschr. Infektionskrank. Haustiere, v. 54(1/2):116-134.
- MAUSS, E. A. 1940.—Transmission of immunity to *Trichinella spiralis* from infected animals to their offspring. Am. J. Hyg., v. 32, Sect. D (2):75-79.
- NELSON, T. C. 1939.—A practical plan for the control of trichinosis. J. Parasit., v. 25(6) (suppl.):33-34.
- NOLAN, M. O. and BOZICEVICI, J. 1938.—Studies on trichinosis. V. The incidence of trichinosis as indicated by post mortem examinations of 1,000 diaphragms. Pub. Health Rpts., U. S. Pub. Health Service, v. 53(17):652-673.
- OTTO, G. F. and ABRAMS, E. 1939.—Quantitative studies on the effect of heat on trichina (*Trichinella spiralis*) larvae. Am. J. Hyg., v. 29, Sect. D (3):115-120.
- PAVLOV, P. 1937a.—Recherches expérimentales sur la trichinose des volailles. Ann. Parasit., v. 15: 434-439.
- 1937b.—Recherches expérimentales sur la trichinose des volailles et des vertébrés à sang froid. Ibid: 440-447.
- 1940.—Le rôle de l'avitaminose dans l'infestation du pigeon par le *Tacnia chinococcus* (les pigeons âgés sont-ils susceptibles de servir d'hôte au parasite?). Bull. Soc. Path. Exot., v. 33(2):93-96.
- RANSOM, B. H. 1915.—Trichinosis. Rpt. 18th Ann. Meeting U. S. Live Stock Sanit. Assoc., pp. 1-19.
- 1916.—Effects of refrigeration upon larvae of *Trichinella spiralis*. J. Agric. Res., v. 5:819-854.
- RANSOM, B. H. and SCHWARTZ, B. 1919.—Effects of heat on trichinae. J. Agric. Res., v. 17:201-221.
- RANSOM, B. H., SCHWARTZ, B. and RAFFENSPERGER, H. B. 1920.—Effects of pork-curing processes on trichinae. U. S. Dept. Agric. Bull. 880, 37 pp.
- ROTH, H. 1935.—Ein Beitrag zur Frage der pränatalen Trichineninfektion. Acta Path. Microbiol. Scand., v. 12(1-2):203-215.
- 1936.—Ueber das Vorkommen pränatalen Trichinenübertragung bei künstlich infizierten Meerschweinchen. Zentrabl. Bakt. [etc.], Abt. 1, Orig., v. 136:279-284.
- 1938a.—On the localization of adult trichinae in the intestine. J. Parasit., v. 24(3):225-231.
- 1938b.—Experimental studies on the course of trichina infection in guinea pigs. I. The minimum dose of trichina larvae required to produce infestation of the muscles; with an account of the potential productiveness of the female trichina. Am. J. Hyg., v. 28(1):85-103.
- 1939.—Idem. II. Natural susceptibility of the guinea pig to experimental trichina infection. Ibid., v. 29, Sect. D. (3):89-104.
- RUBLI, H. 1936.—Trichinose beim sumpflüher, *Myocastor coypus*. Schweiz. Arch. Tierheilk., v. 78(9):420-424.
- SAWITZ, W. 1937.—Are post-mortem statistics on trichinosis valid for the living population? Am. J. Pub. Health, v. 27(10):1023-1024.
- 1938.—Prevalence of trichinosis in the United States. Pub. Health Rpts., U. S. Pub. Health Service, v. 53(10):365-383, figs. 1-6.
- SCHIAPIRO, M. M., CROSBY, B. L. and SICKLER, M. M. 1938.—The correlation of clinical diagnosis and post-mortem findings in trichinosis. J. Lab. & Clin. Med., v. 23(7):681-687.
- SCHWARTZ, B. 1938.—Trichinosis in swine and its relationship to public health. J. Am. Vet. Med. Assoc., n.s., v. 45(3):317-344.
- SOBEL, I. P. 1936.—Sporadic trichinosis in children. Am. J. Dis. Children, v. 51:367-388.
- SPINDLER, L. A. and CROSS, S. X. 1939.—Intra-cutaneous tests for the detection of trichina infections experimentally and naturally acquired by swine. Proc. Helm. Soc. Wash., v. 6(2):37-42.
- STILES, C. W. 1901a.—*Trichinella spiralis*, trichinosis, and trichina-inspection: A zoological study in public hygiene. Proc. Path. Soc. Phila., n.s., v. 4(7):137-153.
- 1901b.—Trichinosis in Germany. U. S. Dept. Agric. Bull. 30, 211 pp.
- VAN SOMEREN, V. D. 1937.—The occurrence of subclinical trichinosis in Britain. Brit. Med. J. (4014):1162-1165.
- WALKER, A. T. 1932.—Trichiniasis. Report of an outbreak caused by eating trichinous bear meat in the form of "jerky." J. Am. Med. Assoc., v. 98:2051-2053.
- WRIGHT, W. H. 1939a.—Studies on trichinosis. XI. The epidemiology of *Trichinella spiralis* infestation and measures indicated for the control of trichinosis. Am. J. Pub. Health, v. 29(2):119-127, figs. 1-2.
- 1939b.—Idem. IX. The part of the veterinary profession in the control of human trichinosis. J. Am. Vet. Med. Assoc., n.s., v. 47(6):601-608.
- 1940.—Idem. XIV. A survey of municipal garbage disposal methods as related to the spread of trichinosis. Pub. Health Rpts., U. S. Pub. Health Service, v. 55(24):1069-1077.

ENTEROBIUS VERMICULARIS

- AFRICA, C. M. 1938.—On some possible hazards of *Enterobius* infection. Modern Med. (August), pp. 13-19, 2 figs.
- BACHMAN, G. W. 1935.—Helminths as agents of disease. Reprinted from "Agents of disease and host resistance," Chap. 57, 1324-1409, figs. 160-207.
- BAYLIS, H. A. 1936.—The fauna of British India, including Ceylon and Burma. Nematoda, v. 1. (Ascaroidea and Strongyloidea.) London, 408 pp., 182 figs.
- BOTSFORD, T. W., HUDSON, H. W. and CHAMBERLAIN, J. W. 1939.—Pinworms and appendicitis. New England J. Med., v. 221(24):933-936, 2 figs., 2 tables.
- BOZICEVICI, J. and BRADY, F. J. 1938.—Studies on oxyuriasis. XV. A study of five hundred and four boys in a boy's camp. Med. Ann. District of Columbia, v. 7(6):187-190.
- BRADY, F. J. 1941.—The incidence of oxyuriasis in two institutions in Puerto Rico. Proc. Helm. Soc. Wash., v. 8(1):10.
- BRADY, F. J. and WRIGHT, W. H. 1939.—Studies on oxyuriasis. XVIII. The symptomatology of oxyuriasis as based on physical examinations and case histories on 200 patients. Am. J. Med. Sc., v. 198(3):367-372.
- CALANDEBRUCCIO, S. 1890.—Animali parassiti dell'uomo in Sicilia. Atti Accad. Gioenia Sc. Nat. Catania (1889-90), v. 2:95-135.
- CAMERON, T. W. M. 1929.—The species of *Enterobius* Leach, in primates. J. Helm., v. 7(3):161-182, 28 figs.
- CHANCO, P. P. and SORIANO, L. J. 1939.—The incidence of *Enterobius vermicularis* among Filipino school children: A preliminary report. Acta Med. Philippina, v. 1(1):81-89.
- COBB, N. A. 1890.—Oxyuris larvae hatched in the human stomach under normal conditions. Proc. Linn. Soc. N. S. Wales, Sydney, 2. s., v. 5(1):168-185.
- CRAM, E. B. 1940.—Studies on oxyuriasis. XXIV. Comparative findings in the white and Negro races. Proc. Helm. Soc. Wash., v. 7(1):31-35.
- 1941.—Idem. IX. The familial nature of pinworm infestation. Med. Ann. District of Columbia, v. 10(2):39-48, 77.
- CRAM, E. B. and FOLAN, J. P. 1939.—Intestinal helminths found in boys recently arrived in Washington, D. C. from various parts of the United States. Rev. Med. Trop. y Parasit., v. 5(5):243-256.
- CRAM, E. B., JONES, M. F. and REARDON, L. The incidence of pinworm (*Enterobius vermicularis*) in various population groups. Rev. Med. Trop. y Parasit., v. 7(1-2):4-6.
- CRAM, E. B., JONES, M. F., REARDON, L. and NOLAN, M. O. 1937.—Studies on oxyuriasis. VI. The incidence of oxyuriasis in 1,272 persons in Washington, D. C., with notes on diagnosis. Pub. Health Rpts., U. S. Pub. Health Service, v. 52(43):1480-1504.
- CRAM, E. B. and NOLAN, M. O. 1939.—Studies on oxyuriasis. XIX. Examinations of children in a private nursery school over an 18-month period. Pub. Health Rpts., U. S. Pub. Health Service, v. 54(14):567-574.
- CRAM, E. B. and REARDON, L. 1939.—Studies on oxyuriasis.

- XII. Epidemiological findings in Washington, D. C. *Am. J. Hyg.*, v. 29(1) Sec. D: 17-24.
- D'ANTONI, J. S. and SAWITZ, W. 1940. The treatment of oxyuriasis. *Am. J. Trop. Med.*, v. 20(3):377-383.
- DARRIBA, A. R. and DE CARDENAS, J. 1935. Ueber Oxyuren (*Enterobius vermicularis*). [Abstract of 1932. Pub. Of. Comision Perm. de Inv. San. 30 pp., 4 figs. Madrid.] *Centralbl. Bakt. [etc.]*, Abt. I, Orig., v. 116(5-6): 140.
- DRÜNER, L. 1921. Ueber die Oxyuren im Wurmfortsatz. *Brunns' Beitrag.* *Klin. Chir.*, v. 122(2):438-454.
- FOLAN, J. P. 1939. The preparation and cleaning of the NIH anal swab used in the diagnosis of oxyuriasis. *Pub. Health Rpts.*, U. S. Pub. Health Service, v. 54(30):1392-1395, 3 figs.
- GAULT, ED. S. 1940. A method for the laboratory diagnosis of oxyuriasis. *Gradwohl Lab. Digest*, v. 3(11):6.
- GRASSI, G. B. 1881. Note intorno ad alcuni parassiti dell'uomo. *Gazz. Osp.*, v. 2(10-12):433-439.
- HALL, M. C. 1937. Studies on oxyuriasis. I. Types of anal swabs and scrapers, with a description of an improved type of swab. *Am. J. Trop. Med.*, v. 17(3):445-453.
- HALL, M. C. and CRAM, E. B. 1939. Studies on oxyuriasis. XVII. The special and peculiar nature of oxyuriasis. Vol. Jub. pro Prof. Sadao Yoshida, Osaka, v. 2:249-267.
- HEADLEE, W. H. 1935. Studies on infections of human parasitic worms under institutional conditions. *J. Lab. & Clin. Med.*, v. 20(10):1069-1077.
- HOLLAENDER, A., JONES, M. F. and JACOBS, L. 1940. The effects of monochromatic ultraviolet radiation on eggs of the nematode, *Enterobius vermicularis*. I. Quantitative response. *J. Parasitol.*, v. 26(5):421.
- JACOBS, L. and JONES, M. F. 1939. Studies on oxyuriasis. XXI. The chemistry of the membranes of the pinworm egg. *Proc. Helm. Soc. Wash.*, v. 6(2):57-60.
- JONES, M. F., JACOBS, L. and HOLLAENDER, A. 1940. The effects of monochromatic ultraviolet radiation on eggs of the nematode, *Enterobius vermicularis*. II. Sublethal effects. *J. Parasitol.*, v. 26(6):435-445, figs. 1-3.
- JONES, M. F. and JACOBS, L. 1941. Studies on oxyuriasis. XXIII. The survival of eggs of *Enterobius vermicularis* under known conditions of temperature and humidity. *Am. J. Hyg.*, Sect. D, v. 33(3): 88-102.
- KITTUNEN-EKBAUM, E. 1940. The incidence of Enterobiasis in children in a convalescent home in Toronto. *Canad. Pub. Health J.* (June), pp. 287-290.
- LENTZE, F. A. 1932. Ueber die Verbreitung von Spul- und Madenwürmer und über die Massnahmen zu ihrer Bekämpfung vom Standpunkte der öffentlichen Gesundheitspflege. Veröffentlichungen aus dem Gebiete der Medizinalverwaltung, v. 37(2):79 pp.
1935. Zur Biologie des *Oxyuris vermicularis*. *Centralbl. Bakt. [etc.]*, Abt. I, Orig., v. 135(1-3):156-159, fig. 1.
- LEUCKART, K. G. F. R. 1868. Die menschlichen Parasiten and die von ihnen herrührenden Krankheiten. Ein Hand und Lehrbuch für Naturforscher und Aerzte, v. 2:257-512.
- MONIZ DE ARAGAO, R. 1938. Incidencia verminotica na populacã de João Pessoa. (Incidence of worms in João Pessoa.) *Brasil Med.*, v. 52(30):679-680.
1939. Idem (Abstract). *Trop. Dis. Bull.*, v. 36(7): 593.
- NOLAN, M. O. and REARDON, L. 1939. Studies on oxyuriasis. XX. The distribution of the ova of *Enterobius vermicularis* in household dust. *J. Parasit.*, v. 25(2):173-177.
- OLENIKOV, S. V. 1929. (Diagnosis and epidemiology of enterobiasis). In Russia. *Russ. J. Trop. Med., Med. & Vet. Parasit.*, v. 7(6):393-402.
1932. Idem [Abstract]. *Biol. Abstr.*, v. 6(4):1123.
- PANDAZIS, G. 1937. Les helminthes parasites de l'Homme en Grèce. 12th Internatl. Cong. Zool., Lisbon, 1935, *Compt. Rend.*, v. 3:2006-2012.
- PENSO, G. 1939. Il ciclo di sviluppo degli ossinuri. Vol. Jub. pro Prof. Sadao Yoshida, Osaka, v. 2:279-283.
- REARDON, L. 1938. Studies on oxyuriasis. XVI. The number of eggs produced by the pinworm, *Enterobius vermicularis*, and its bearing on infection. *Pub. Health Rpts.*, U. S. Pub. Health Service, v. 53(24):978-984.
1941. Incidence of *Endamoeba histolytica* and intestinal nematodes in a Georgia State institution. *J. Parasitol.*, v. 27(1):89-90.
- SAWITZ, W., D'ANTONI, J. S., RHUDE, K. and LOB, S. 1940. Studies on the epidemiology of oxyuriasis. *South. Med. J.*, v. 33(9):913-922.
- SCHMIDT, W. TH. 1914. Welche verschiedenen Methoden zur Diagnostizierung der Oxyuriasis gibt es und welche ist wegen der Leichtigkeit der Ausführung und ihrer Zuverlässigkeit die allein empfehlenswerte? Inaugural-Dissertation, Rostock, Germany.
- SMITH, W. H. Y., GILL, D. G. and McALPINE, J. G. 1939. Intestinal parasite survey in Alabama. *South. Med. J.*, v. 32(11):1094-1105.
- SONDAK, V. 1935. Resistance of the eggs of the broad tapeworm and *Enterobius vermicularis* to chemicals. [In All-Union Institute of Exper. Med. (Viem). Parasites, Transmetteurs, animaux venimeux. Recueil des travaux dédiés . . . Prof. Eugene Pavlovsky.] Moscow, pp. 307-315.
- WRIGHT, W. H. and CRAM, E. B. 1937. Studies on oxyuriasis. IV. Some aspects of the problem of therapy. *Am. J. Dis. Children*, v. 54:1276-1284.
- ZAWADOWSKY, M. M. 1931. Nochmals zur Frage über die Bedingungen, unter denen eine Autoinfektion bei Oxyuriasis unmöglich ist (On the question of the conditions under which autoinfection with Oxyuriasis is impossible.) *Tr. Dynam. Develop. Moscow*, v. 6:213-219. Idem, *Zool. Bericht*, v. 29:144.
- ZAWADOWSKY, M. M. and SCHALIMOV, L. G. 1929. Die Eier von *Oxyuris vermicularis* und ihre Entwicklungsbedingungen, sowie über die Bedingungen, unter denen eine Autoinfektion bei Oxyuriasis unmöglich ist. *Ztschr. Parasitenk.*, v. 2(1):12-43.

## CHAPTER IX

### ANTHELMINTIC MEDICATION FOR NEMIC DISEASE OF DOMESTIC ANIMALS AND MAN

WILLARD H. WRIGHT, Washington, D. C.

and

PAUL D. HARWOOD, Ashland, Ohio

#### History

The use of remedies for the removal of worms dates far back into antiquity. As primitive man became aware of his intestinal parasites by observing the passage of such a large nematode as *Ascaris* or the proglottids of large cestodes such as *Taenia saginata* or *Taenia solium*, he no doubt sought from his limited armamentarium weapons for the removal of these undesirable boarders. Since most of his medicines were derived from the plants found in his circumscribed environment, he turned to them for his worm treatments. He chose so well that derivatives of some of these plants in one form or another are still in use as anthelmintics. Thus male fern, a frequently employed taeniafuge, was known to the early Greek physicians, if not before them; Jerusalem Oak, *Chenopodium anthelminticum*, was used as a worm remedy by the North American Indians; and a decoction of the leaves of *Mallotus philippinensis*, from which the taeniafuge kamala is obtained, was employed by the early Ethiopians.

Developments in anthelmintic medication have been divided aptly into three epochs: The first, comprising centuries of uncritical empiricism; the second, comprising several decades of critical empiricism; and the third and last, comprising a relatively few years of critical experimentation.

The first epoch marked the period of primitive groping and the centuries of acceptance of its empirical findings without any marked advance being registered in the field.

The second epoch followed the discovery of the Old World hookworm, *Ancylostoma duodenale*, by Dubini in 1843, and the gradual unfolding of knowledge regarding the importance of the parasite and the recognition of ancylostomiasis as a disease entity. Griesinger's association of the hookworm with Egyptian chlorosis, Wucherer's work which showed its relation to tropical anemia in Brazil and Perroncito's discovery of hookworm as the cause of the St. Gothard tunnel disease stimulated interest in the hookworm problem. These discoveries, followed by Sossino's classical observations, demonstrated the need for specific therapeutics and prepared the way for the development of a number of anthelmintics which, if not thoroughly efficient, provided useful treatments; these held their place for a period of four decades and until the epoch of critical testing provided more specific and more effective drugs. The year 1881 marked Perroncito's proposal of male fern as a hookworm treatment, the introduction by Bozzolo of thymol, and Baumlér's unfavorable report on oil of chenopodium for this purpose. Male fern had only limited use as a hookworm treatment but thymol proved to have considerable efficacy and enjoyed a long vogue. In fact, the latter drug was used more extensively than any other until Schuffner and Verwoort reintroduced oil of chenopodium in 1913 and showed that Baumlér's conclusions, which were apparently based on the treatment of only one case, were erroneous. In the meantime, Bentley in 1904 reported his findings with betanaphthol and advocated its use in hookworm disease.

In 1905 Herman introduced a mixture of chloroform, eucalyptus and castor oil as a treatment for ancylostomiasis in miners at Mons, Belgium. The mixture was later modified by Phillips and others and was subsequently employed extensively in the treatment of hookworm disease in many parts of the world. Schultz later found chloroform to be the active ingredient of Herman's Mixture and reported the drug to be effective against hookworms in the dog.

In the meantime progress was being made also in the field of anthelmintics for veterinary use. As early as 1894 Perroncito and Bosso discovered the efficacy of carbon disulphide for the removal of bots, *Gasterophilus* spp., from the horse. In fact, the first critical testing of anthelmintics was actually carried out by Grassi and Calandrucio in 1884 and 1885 and by Perroncito in 1885 and 1886 in establishing the value of male fern for the destruction of liver flukes in sheep by post-mortem examination of treated animals. However, this method of testing found no further advocates for a quarter of a century.

A work of far reaching economic importance was the discovery by Hutcheon in South Africa in 1891 of the efficacy of copper sulphate solution for the removal of the common sheep

stomach worm, *Haemonchus contortus*. The wireworm remedy of copper sulphate and sodium arsenite worked out by Theiler in 1912 and Veglia in 1920 has also been used extensively in South Africa and was an important contribution to anthelmintic therapy. In the United States, Lewis and Guberlet added a tobacco infusion to the copper sulphate solution; Lamson introduced nicotine sulphate solution; and Curtice combined copper sulphate and nicotine sulphate into the "Cu-Nic" solution with an increase in efficacy against the common stomach worm and some other gastro-intestinal parasites of ruminants. In general, however, it may be said that the four decades of critical empiricism produced less progress in the development of veterinary anthelmintics than in anthelmintics for human use. It was not until 1915 that substantial progress was achieved in the former field.

The year 1915 marked the practical beginning of the epoch of the critical testing of anthelmintics. Hall laid down the basic principles of this method and together with his associates, including Foster, Avery, Snead, Wolf, Wilson, Wigdor and Shillinger, checked critically the efficacy of empirical anthelmintics and developed new compounds of far reaching and fundamental importance in both human and veterinary medicine.

The method which Hall adopted was to administer known doses of drugs to test animals of various species, collect all worms passed in the feces for a given period of time, identify and count these worms, sacrifice the test animals and make thorough post-mortem examinations with the recovery, the identification and the counting of all worms remaining. This method gave specific information concerning the number of worms present, the number removed and the number left after treatment and provided an accurate index concerning the efficacy of the drug tested. The method was relatively ponderous and time consuming compared to the favored process of dropping ascarids or some other easily collected invertebrate into solutions of drugs and calculating the anthelmintic efficacy of the drug by observing the ultimate fate of the animal in the solution. However, critical testing developed precise information whereas *in vitro* tests were often entirely valueless.

The method of critical testing was of particular value in veterinary medicine. It enabled an accurate assay of drugs whose value was often more traditional than real, and its use confirmed in many cases the efficacy of empirically selected anthelmintics and enabled dependable information to be obtained concerning their therapeutic dose rate, their margin of safety, the contraindications for their use and the type and mode of purgation most suitable to promote the efficacy of the drug and to protect the patient.

Many of the tests, especially those on dogs, provided results which were applicable with but slight modification to human medicine. The outstanding discovery in this connection was that by Hall in 1921 of the value of carbon tetrachloride for the removal of hookworms from the dog. Hall immediately suggested the use of the drug in the treatment of human hookworm disease, a suggestion which was forthwith adopted by a number of investigators particularly Lambert and other physicians on the staff of the International Health Board. It was soon found that the efficacy of carbon tetrachloride exceeded that of all other drugs in this condition and it was adopted practically as a standard treatment and used in millions of cases in various parts of the world. Another discovery less spectacular but actually of greater importance was that of Hall and Shillinger in 1925 of the value of tetrachlorethylene for the removal of hookworms. Because of its greater safety and the fact that it produces little or no hepatic or renal damage, tetrachlorethylene is replacing carbon tetrachloride in human ancylostomiasis and for many parasitic infections of domesticated animals.

Using the method of critical testing, Hall and his coworkers established or confirmed the value of many anthelmintics including copper sulphate for *Haemonchus contortus*, oil of chenopodium for ascarids in dogs and swine and for strongyles, cyclicostomes, and pinworms in horses; carbon disulphide for bots and ascarids in equines; carbon tetrachloride for stomach worms and other worms in sheep and for ascarids and



strongyles in horses; tetrachlorethylene for certain sheep parasites; and other treatments.

The method devised by Hall has since been widely adopted and employed by numerous investigators in establishing the value of many other anthelmintics.

Except in the case of condemned criminals who will voluntarily submit to test, the method of critical testing cannot be used in man. Other methods have necessarily been adopted for evaluating the efficiency of anthelmintics for nematode infections in this host. One of these, the use of a so-called standard treatment, was based on the administration of the test drug, the screening of the stools and the recovery of worms, followed within a suitable period by the administration of the standard treatment, the relative efficiency of which was known. Worms passed following this treatment were collected and counted and a comparison between the results obtained with both treatments enabled the investigator to arrive at some evaluation of the efficacy of the test drug. While the method necessarily had its limitations, its use led to findings of value particularly when applied to relatively large numbers of persons to obviate the margin of error in individual differences. Under these conditions, the standard treatment method was used effectively by Caius and Mhaskar in their extensive investigations in connection with the hookworm injury in the Madras Presidency and similarly by Darling, Barber, Hackett, Smillie and other physicians on the staff of the International Health Board in their far flung search for the most effective treatment for hookworm disease.

Following the discovery by Stoll in 1923 and Stoll and Hausheer in 1926 of a method of counting nematode ova in feces with some degree of accuracy, the Stoll count has been used extensively in evaluating anthelmintic treatments in man. With due regard for the limitations imposed upon it by the varying factors involved, the method has been of marked value in gauging the efficiency of certain anthelmintics particularly those designed for the treatment of *Ascaris* and hookworm infections in man. Because of its greater reliability and its ease of application, this method has replaced largely the use of the standard treatment method. Hall and Augustine in 1929 supplemented the Stoll count with a count of worms passed following treatment in evaluating certain anthelmintic treatments for man.

At times it is profitable to employ several different methods of research. Lamson and his associates used *in vitro* testing, critical testing and the Stoll egg-counting method in their extensive investigations into the anthelmintic value of the alkyl hydroxy benzenes and related compounds. In this case, comparable *in vitro* tests on *Ascaris lumbricoides* with large numbers of compounds gave leads which could be developed further by the employment of other methods.

### Mode of Action

Little information is available concerning the manner in which anthelmintics act on worms. An extensive use of *in vitro* tests in this field of investigation may yield some data but, since it is difficult, if not impossible, to simulate *in vitro* the environmental conditions of the parasite in its natural host, results obtained in this manner must be used with great caution. The physiology of nematodes in itself is an almost totally unexplored field. In the absence of precise knowledge concerning the life processes of a parasite, it is not likely that we shall know in what manner toxics act on the organism. The meager information which is available throws little light on the problem in hand.

According to their mode of action, anthelmintics may be divided roughly into the following groups:

1. Narcotizing or paralyzing agents.
2. Compounds exhibiting a destructive action on protein.
3. Compounds containing enzymes capable of digesting nematode tissues.
4. Anthelmintics of unknown action.

The first group contains such well known anthelmintics as santonin and the chlorinated hydrocarbons. Worms eliminated following the administration of these anthelmintics may exhibit more or less movement. This characteristic is so marked with santonin that earlier authors were led to describe this drug as a vermifuge, a term which originally designated an anthelmintic that irritated the parasites and drove them into the colon where they might be removed with a brisk purge. (Trendelenburg, 1915.) However, as Lo Monaco demonstrated in 1896 and as Chopra and Chandler (1928) have pointed out, santonin is highly toxic to ascarids *in vitro* if the test solutions are properly prepared. If santonin is partially dissolved in suitable quantities of normal hexane, a chemical which in itself is innocuous, the drug causes *in vitro* successive stages of

stimulation and profound paralysis. Therefore, it is now considered that santonin partially paralyzes the parasites, which in that condition are unable to maintain their position in the alimentary canal. The prompt elimination of the parasites by the action of a purgative may increase the efficacy of santonin as shown by Morris and Martin (1931) and by others.

The chlorinated hydrocarbons contain several well known compounds which exert an anesthetic action on worms. *In vitro* the parasites gradually lose their motility and, if exposure to the drug is continued after immobility sets in, the parasite may be killed. However, if removed from the solution promptly, it may recover. The authors have observed hookworms and ascarids moving feebly when removed from dogs with drugs belonging to this series.

On the other hand, oil of chenopodium belonging in the first group apparently has a paralyzing action on the musculature, an effect which almost always results in the death of the nematode.

The large group of hydroxy benzenes are examples of those anthelmintics in the second group. If solutions of egg albumin are treated with these compounds, the proteins are promptly precipitated. With the more water soluble compounds of this series, such as phenol, the precipitation is relatively complete; with compounds such as thymol and hexylresorcinol, the precipitation is partial; while the extremely insoluble compounds precipitate only small quantities of the protein. *In vitro*, hexylresorcinol exerts a searing effect on the cuticle of *Ascaris lumbricoides*, resulting in the destruction of tissue; if the exposure is closely controlled, blisters may be formed. In solutions of hydroxy benzenes which are not quickly fatal, *Ascaris* exhibits a marked stimulation of activity greater than that observed in solutions of santonin or of the halogenated hydrocarbons. Since Lamson and Ward (1932) have described a blistered condition of the cuticle of ascarids removed from patients treated with hexylresorcinol, the mode of action *in vivo* may be identical with the action observed *in vitro*.

In connection with the third group, Robbins (1930) has shown that the anthelmintic activity of leche de higuera, the sap of the Central and South American fig tree, *Ficus lanrifolia*, is correlated with the presence of a proteolytic enzyme, which he has named "ficin." Asenjo (1940) has shown recently that the destructive effect of fresh pineapple juice on *Ascaris lumbricoides in vitro* is probably associated with the action of the proteolytic enzyme, bromelin. However, there is no evidence as yet that the above-mentioned juice has any anthelmintic value.

The fourth group probably includes the majority of anthelmintics. Any comments regarding the mode of action of these drugs would be speculative for the most part. For instance, we do not know how trivalent antimony compounds act on somatic nematode parasites, although in the case of "Fouadin" the action of the drug is cumulative on *Dirofilaria immitis* and the adult worms succumb very gradually. Some observations of the senior author seemed to indicate that sterilization of the adult female worms is due to fatty degeneration and necrosis of the reproductive cells of the ovary and perhaps the drug acts similarly on the somatoplasm.

Little is known concerning the nature of the anthelmintic activity of various dyes. Gentian violet stains the tissue of such nematodes as *Strongyloides* and *Enterobius*, against which it is effective. In *Enterobius* passed following treatment, the cuticle is usually slightly stained, the digestive tract more so, and the reproductive organs, particularly in the female, are intensely stained. The dye no doubt has a cumulative action since some stained gravid female pinworms will migrate in the early stages of treatment. Furthermore, the prolonged course of treatment necessary to eradicate infections with both of these nematodes supports the view that the anthelmintic is not one of the contact type.

Similarly, worms eliminated following the administration of phenothiazine are stained reddish, but there is little reason for thinking that the action of this drug is in anyway cumulative, as prolonged treatment seems to be relatively less effective than a single large dose. Many worms eliminated following treatment with this drug are alive and move feebly, a circumstance which suggests that phenothiazine should be classed with the narcotics and paralyzants.

The manner in which anthelmintics reach the tissues of the parasite is as little known as is the action of drugs on these tissues. One assumption has been that nematode parasites with their well developed digestive tract ingest the anthelmintic in solution with the food and absorption therefore takes place from the oesophagus or through the cells lining the wall of the intestine. However, evidence for such a hypothesis is not convincing. For instance, Well's (1931) striking demonstration of the blood sucking proclivities of the dog hookworm has shown that the parasite may take up as much as 0.84 cc. of the

host's blood in 24 hours. The blood passes rapidly through the digestive tract and apparently is not subjected to any material amount of digestion. Consequently, the worm probably uses as food diffusible substances in the plasma. In spite of the marked diffusibility of carbon tetrachloride, the drug has little or no action on hookworms when injected intravenously. Wright and Underwood (1934) cite work with Bozicevich in which repeated intravenous injections of carbon tetrachloride failed to have any effect on the microfilariae or adults of *Dirofilaria immitis*. While it is probable that this parasite takes only a limited amount of nourishment orally because of the atrophied digestive tract, yet it is bathed continuously in the blood plasma. Even under these conditions of intimate contact, the anthelmintic had no effect.

The studies of Mueller (1929) indicated that carbon tetrachloride penetrated the cuticle of *Ascaris* and was not taken in through the digestive tract. Mueller believed that fat soluble compounds such as chloroform and carbon tetrachloride exert an anthelmintic effect by reason of their action on the fat content of the muscle cells.

Brown (1937) has reported the results of some ingenious experiments designed to ascertain the manner in which certain anthelmintics reach the tissues of *Ascaris lumbricoides*. While the results were in part inconclusive because of the difficulty of handling worms without injuring them traumatically, the evidence seemed to indicate that oil of chenopodium and carbon tetrachloride in solution are absorbed through the body wall. Brown was able to show that *Ascaris* will ingest solutions of carbon tetrachloride in mineral oil but will refuse to ingest dilute solutions of chenopodium. Brown did not believe that the drugs tested acted directly on the nervous system of the parasite even when injected in the region of the nerve ring.

The experiments of Strong (1918), Lambert (1923), Hall and Shillinger (1925) and Fernán-Núñez (1927) may be cited as bearing on this problem. Strong injected oil of chenopodium intramuscularly for the removal of whipworms without success. Lambert was equally unsuccessful with intramuscular injections in two cases in man but obtained better results in a third case. However, when used intravenously, the drug had a marked vermifugal action against *Trichuris*, little action on *Ascaris* and none on hookworms. Hall and Shillinger obtained very indifferent results on dogs but Fernán-Núñez reported marked success against *Trichuris* in man with intramuscular and intravenous injections. The manner in which whipworms derive their nourishment is still a debatable point although Garin (1913) was able to demonstrate blood in the intestinal contents and Schwartz (1921) reported a hemolysin from *Trichuris vulpis*. The evidence from the above-mentioned experiments would seem to indicate, however, that the drug was possibly absorbed through the digestive tract in view of the little likelihood that under these conditions it would have been in adjacent tissues in sufficient concentrations to have been absorbed through the cuticle. Recently Trum (1938) found that oil of chenopodium injected intravenously was very toxic for horses, but had very little effect on the blood sucking strongyles present in these animals.

### Correlation Between Chemical Structure and Anthelmintic Efficacy

Compounds comprising the group of effective anthelmintics and those for which some anthelmintic efficacy has been reported are associated with such widely divergent chemical groups that no general correlations can be drawn between anthelmintic efficacy and chemical composition. As a rule, anthelmintics are very specific in their action, exhibiting their optimum efficacy against one species of parasite or at best against closely allied species of parasites. Drugs specific for the removal of nematodes are seldom effective for the removal of cestodes. The one glaring exception to this rule is carbon tetrachloride which is a fairly effective treatment for *Taenia* and *Diphyllobothrium latum* infections of man although of little or no value against other cestodes. On the contrary, there is no taeniaefuge which is effective for the removal of nematode parasites.

The specificity of anthelmintics is conditioned not only by the anatomy and physiology of the parasite but also in part by the anatomy and physiology of the host. In this connection, drugs effective for the removal of strongylid parasites from carnivores fail to a great extent when employed against similar parasites of ruminants. Such drugs are frequently held in the rumen with a resultant dissipation of their action long before they reach the object of their attack farther down in the complicated digestive tract. On the other hand, the anthelmintic value of copper sulphate solution against *Haemonchus contortus* in ruminants is associated with the peculiar stimulus which the drug exerts in bringing about the closure

of the oesophageal groove thus permitting the solution to be diverted directly into the abomasum.

The optimum action of two effective drugs is often lost or markedly reduced when such drugs are combined in a single dose. Frequent efforts have been made to develop a single method of treatment which would be effective against both intestinal nematodes and cestodes. These efforts have nearly always resulted in failure. In such cases, drugs which are effective against nematodes and cestodes, respectively, lose much of their efficacy when combined. Under these varied circumstances, attempts to correlate anthelmintic efficacy and chemical structure must be made on the basis of a selection of closely related compounds on a single species of parasite.

Hall and Wigdor (1926) were apparently the first to carry out studies of this sort. Their work was carried on in 1917 and 1918 but was interrupted by military service. Their limited study was made with terpenes and certain other aromatic hydrocarbons. Unfortunately, the study provided little information of value partly because of the divergent structure of the compounds tested and partly because of the feeble anthelmintic activity of many of them.

Caius and Mhaskar's extensive investigation into the value of hookworm remedies was a thorough piece of work. However, here again too many compounds (70 in all) of too divergent a character were employed. In the summary and conclusions of their work, Caius and Mhaskar (1923) stated that the effective hookworm treatments studied by them differed so much in molecular composition and structure that no general correlations could be said to exist between anthelmintic properties and chemical composition. They concluded that anthelmintic action on hookworms is specific.

Wright and Schaffer (1932) selected a series of chlorinated alkyl hydrocarbons, a few of which had been studied by Hall and his associates. The previously unstudied compounds were tested critically for their anthelmintic efficacy against hookworms and general correlations were drawn between anthelmintic efficacy, chemical structure and physical properties.

In the homologous series, there was a rise in anthelmintic efficacy against *Ancylostoma caninum* with an increase in the length of the hydrocarbon chain from the low member of each group to the next higher member. In each case, there was an accompanying decrease in solubility from above the optimum solubility range to a solubility within that range. In one homologous series (normal monochlor compounds) a peak of anthelmintic efficacy was reached. It was pointed out that in a similar way other homologous series would no doubt each have a peak of anthelmintic efficacy, for the reason that a point will be reached where the solubility of a higher member will be so slight as to result in little or no anthelmintic efficacy. It was concluded that although the addition of  $-\text{CH}_2-$  groups to the hydrocarbon chain results in a progressive change in solubility from one member to the next in homologous series, a progressive change in anthelmintic efficacy does not necessarily follow.

An increase in anthelmintic efficacy against *A. caninum* did not always result with an increase in the number of chlorine atoms in the molecule or with an increase in the relative percentage weight of chlorine in the molecule. In those cases where a high degree of anthelmintic efficacy was associated with increase in the chlorine content, the resulting compounds without exception possessed a solubility within the optimum range. Further, the spreading of the chlorine atoms in the hydrocarbon molecule did not invariably result in an increase or decrease in anthelmintic efficacy.

Differences in position of the chlorine atom in the molecule resulted in changes in anthelmintic efficacy and the accompanying change in water solubility was an important factor in determining anthelmintic efficacy. Even among compounds with the same number of carbon, hydrogen and chlorine atoms, changes in the position of the chlorine atom resulted in compounds showing marked differences in anthelmintic efficacy for hookworms. In addition, differences in anthelmintic efficacy were exhibited when the methyl radical was introduced in different positions in the chlorinated hydrocarbon molecule.

Wright and Schaffer concluded that anthelmintic efficacy of chlorinated alkyl hydrocarbons against *A. caninum* is intimately linked with water solubility which varies with the chemical structure of the molecule and that the anthelmintic efficacy is not solely dependent on the halogen concentration or on the position of the chlorine atom or atoms in the molecule. With a single exception those compounds having water solubilities between 1:1250 and 1:5300 showed a high degree of anthelmintic efficacy for hookworms in the dog regardless of the halogen concentration or the position of the chlorine atom or atoms in the molecule. Water solubility is, therefore, the factor most definitely correlated with the anthelmintic efficacy of chlorinated alkyl hydrocarbons for hookworms.

It is interesting to note that the above mentioned conclusions did not apply in the case of anthelmintic efficacy of the compounds against *Toxocara canis* and *Toxascaris leonina*.

In critical tests on dogs with monobrom hydrocarbons, Wright, Schaffer, Bozicevich and Underwood (1937) found that an increase in the hydrocarbon chain was associated with a progressive decrease in water solubility from one member to the next without a progressive change in anthelmintic efficacy against *Ancylostoma caninum*. The peak of anthelmintic efficacy against hookworms was reached with n butyl bromide, the efficacy thereafter declining. This compound was the only member of the series possessing a water solubility lying within the optimum solubility range of chlorinated hydrocarbons. It appeared probable that an optimum solubility range similar to that for chlorinated hydrocarbons exists among brominated hydrocarbons so far as anthelmintic efficacy against hookworms is concerned. These authors concluded that water solubility appeared to be the factor most definitely correlated with anthelmintic efficacy of brominated hydrocarbons for hookworms, as it is with chlorinated hydrocarbons. Wright and Schaffer (1931) came to similar conclusions in connections with mono iodated compounds.

Lamson and his associates (1934, 1935) studied extensively the anthelmintic value of a large number of phenolic compounds, most of the comparable tests having been carried out on *Ascaris lumbricoides in vitro*. These compounds included (1) alkyl resorcinols, (2) alkyl phenols, (3) alkyl cresols, (4) polyalkyl phenols, and (5) phenols with other than normal alkyl side chains. The authors concluded that the ascaricidal activity of phenolic compounds is related to the local irritating action although all phenols exhibiting such action are not necessarily active ascaricides. To be effective as an ascaricide, it was found that a phenol should be a liquid or a substance which will liquify or emulsify in the intestinal tract. Such substances were found to have a melting point of not over 75° C. The solubility range of ascaricidal phenols was found to lie between 1:1,000 to 1:35,000, although the most effective anthelmintic of this type in the large number of compounds studied was hexylresorcinol with a water solubility of 1:2,000.

It was found that the ascaricidal properties of phenols and resorcinols are increased by the introduction of alkyl radicals. Such properties become more marked with the lengthening of the alkyl chain and reach a maximum which differs in different series, thereafter declining rapidly. The ascaricidal value of dihydroxybenzenes was not strikingly different from that of monohydroxybenzenes. No significant differences were found between ortho and para alkyl phenols. The introduction of single normal chains into the nucleus was more effective than the introduction of multiple chains with the same total number of carbon atoms. Normal chains in general were more effective than branched chains, although exceptions were noted, such as the increased efficacy of thymol over that of n propyl-meta-cresol. Some of the differences in activity were thought to be accounted for by the higher melting point of the branched chain compounds over that of normal compounds. Cyclic side chains behaved similarly to forked chains.

From the evidence at hand it may be concluded that little or no correlation can be drawn between the anthelmintic efficacy and the chemical constitution of compounds differing widely in their chemical structure. When closely allied compounds have been tested against a single species of parasite, the results have indicated generally that there is a rise and fall in anthelmintic activity within the homologous series, the activity reaching a peak and then declining. In the case of liquids, the anthelmintic efficacy is definitely linked with the water solubility and in the case of solid compounds with the water solubility and the melting point. In general, in homologous series compounds with normal chains are usually more effective than those with branched chains. Finally, the evidence, meager as it is, emphasizes almost dramatically the extreme specificity of anthelmintics.

### Chemical Classification

The following classification showing the various chemical groups to which anthelmintics belong is taken mainly from the excellent summary of Lamson and Ward (1932). The listing includes for the most part the compounds more commonly employed against nematode parasites and contains mainly those drugs which have been shown by adequate test to possess marked anthelmintic properties. For information concerning the chemical grouping of other drugs, including those employed in cestode and trematode infections, the reader is referred to the more detailed classification of Lamson and Ward.

1. *Inorganic substances*
  - Bismuth subcarbonate
  - Copper sulphate

- Antimony potassium tartrate
- Colloidal iodine
- Sodium arsenite
- Carbon disulphide
- Hydrogen peroxide
- 2. *Halogenated hydrocarbons*
  - a. *Aliphatic*
    - (1) *Saturated*
      - Chloroform
      - Bromoform
      - Carbon tetrachloride
      - n Butyl chloride
      - n Butylidene chloride
      - n-Butyl bromide
    - (2) *Unsaturated*
      - Tetrachlorethylene
  3. *Phenols*
    - a. *Monohydric phenols*
      - n Hexyl m cresol
      - Thymol
      - Carvaerol
      - Betanaphthol
    - b. *Dihydric phenols*
      - n Hexylresorcinol
      - n-Heptylresorcinol
  4. *Organic acids and their salts or esters*
    - Aluminum subacetate
  5. *Organic dioxides*
    - Disuccinyl peroxide
  6. *Organic antimony compounds*
    - Sodium antimony III pyrocatechin disulphonate of sodium
    - “Filsol”
    - “Stibsol”
  7. *Terpenes*
    - a. *Bridged ring*
      - (1) *Peroxides*
        - Ascaridol
    - b. *Sesquiterpenes*
      - Santonin
  8. *Alkaloids*
    - Nicotine
    - Pyrethrine
  9. *Enzymes*
    - Ficin
    - Bromelin
  10. *Plant products*
    - Leeche de higueron
    - Digenca simplex*
    - Oleum chenopodii
    - Oleum eucalypti
    - Oleum terebinthinac
    - Quassia
    - Tobacco
  11. *Dyes and similar compounds*
    - a. *Thiazin*
      - Phenothiazine
    - b. *Triamino-triphenyl methane*
      - Gentian violet
    - c. *Phthalein*
      - Mercurochrome

### General Principles of Anthelmintic Medication

Elsewhere in this discussion we have emphasized the specificity of anthelmintics, a thing which is of prime importance from a medical standpoint. It is not only a waste of time and effort to employ a nonspecific treatment against a given parasitic infection but it is a hazard to the safety and well being of the host. Specific treatments cannot be chosen unless an accurate diagnosis is made. Hence any anthelmintic medication should be predicated on such a diagnosis. Even today when the average physician or veterinarian is far better qualified than formerly in the field of parasitology, we find practitioners administering anthelmintic treatment on the basis of a clinical diagnosis without proper laboratory checks. No parasitic infection is characterized by pathognomonic symptoms and the shifting sand of the clinical picture is not a sufficiently firm foundation upon which to base treatment with drugs which at best have only a small margin of safety.

In the past, mass treatment of large population groups has been a popular method of attack against a given parasite. The benefits anticipated from such a procedure have not been generally realized for all too frequently the important subject of prophylaxis has not been given sufficient attention. Under such circumstances, the population groups involved have continued to indulge in the habits responsible for their parasitic

infection and after a suitable period of time are again ready for further treatment.

With improved techniques for determining the presence of most parasites and for evaluating the relative degree of infection with many of them, mass treatment is no longer justified in the field of medicine. Even in veterinary medicine it can be condoned only in the case of large flocks or herds in which individual diagnosis would be economically unsound.

The question is frequently raised as to whether an infection with a given number of worms is of clinical importance and thus warrants treatment. No categorical answer can be given to such a question. An infection with a certain number of worms might be injurious to the health of one individual without affecting in any appreciable degree the well being of another individual. No one has been able to define the line of demarcation between a clinical and a sub-clinical infection. In mass treatment such finess of judgment is not required or at least is not exercised but in medical practice it is best that due cognizance be taken of the relative degree of parasitism. If the patient has only a few worms, such as hookworms, he had better go without treatment rather than be subjected to the potential hazards of anthelmintic medication. However, with such a circumscribed environmental parasite as *Enterobius vermicularis*, it is necessary from a control standpoint to treat simultaneously all infected individuals in the household regardless of the degree of infection or the presence or absence of clinical symptoms. Otherwise, untreated individuals provide direct avenues of reinfection for treated individuals.

**METHODS OF APPLICATION.** Anthelmintics are administered in a great many different ways, depending on the kind of parasite, its location within the host and the species of host animal. In man, palatability is a matter of some importance and it is desirable to administer the drug in a manner least distasteful to the patient. While the esthesia of taste is not usually considered in the case of lower animals, palatable doses of drugs are more apt to be retained by dogs and cats in which the vomiting reflex is acutely sensitive. Many of the anthelmintics now on the market are dispensed in soft gelatin capsules. Hard gelatin capsules are still employed by some practitioners who prefer to fill the capsules at the time they are used.

For certain parasites located far down in the digestive tract, the use of enteric-coated tablets is an advantage. However, most of the enteric-coatings employed become harder with age and are less apt to dissolve in the digestive tract. A new type of water-soluble coating has recently been devised to obviate the disadvantages of the usual enteric coating. The new coating permits timed disintegration of the tablet within certain definite periods after administration and radiographic evidence in support of this has been furnished by Worton, Kempf, Burin and Bibbins (1938).

For ruminants, certain anthelmintics such as solutions of copper sulphate and nicotine sulphate are given as a drench. In fact, Ortlepp and Mönning (1936) have shown that the administration of a dose of copper sulphate solution immediately prior to the use of other drugs has the effect of closing the oesophageal groove and permitting the drug to reach the abomasum directly. This is of marked advantage in connection with some treatments against ruminant parasites. On the other hand, some anthelmintics, such as the sodium arsenite-bluestone mixture for the common sheep stomach worm, are given in powdered form.

The duodenal tube method of administration is an advantage in some instances and is particularly valuable in stubborn cases of strongyloidosis in man in which ordinary methods of administration fail.

Somatic helminths, when they can be reached at all, are usually attacked through the intramuscular or intravenous route. Lungworms in domestic animals are susceptible to some extent to anthelmintics introduced intratracheally and good results have been reported in this connection by certain workers in the Soviet Union. The inhalation method was used by Wehr, Harwood and Schaffer (1938) in the attack against *Syngamus trachea* in chickens with barium antimonyl tartrate dust.

Parasites in the lower bowel are subject to attack per rectum. The employment of enemas is a common practice against *Enterobius vermicularis* in man. The method has been used by Mönning in South Africa and by others in removing nodular worms from sheep, while intrarectal injections have been advocated and employed with some success for the expulsion of whipworms from the dog. In a like manner, *Heterakis gallinae* can be reached with anthelmintics injected by way of the cloaca.

The individual anthelmintic treatment of farm animals has never appealed to the livestock owner and there has always been demand for an anthelmintic which could be given

with the feed. Other than the tobacco dust or nicotine treatment for *Ascaridia* in poultry, anthelmintics administered in the feed are generally ineffective. The method has the disadvantage that some animals ingest too much and others too little of the drug. More recent tests with phenothiazine seem to indicate that for some parasites this drug may be of value when given with the feed. If results are substantiated in further trials, the method will no doubt find widespread use.

While most anthelmintic therapy is based on the use of single dose treatments, it is sometimes of advantage to employ divided doses. The dose of chenopodium for man is occasionally divided into two or three parts administered at one-half to one hour intervals. When given in this way, the efficacy of the drug against hookworms is believed by some workers to be slightly enhanced. If toxic symptoms are manifested by individuals having an idiosyncrasy for the drug, dosage can be discontinued. However, the purgative is usually withheld until the last portion of the dose has been administered and under these conditions increased absorption of the anthelmintic is apt to occur.

Repeated treatment over a period of time is required for the eradication of such parasitic nematodes as *Strongyloides stercoralis* and *Enterobius vermicularis*. Likewise some degree of efficacy can be secured against whipworms by repeated dosing with a drug such as santonin which exerts little or no action against these parasites when given in a single dose.

The above citations will be sufficient to indicate to the reader that anthelmintic warfare against parasites, whether in man or the lower animals, requires the employment of varied methods of attack based on the nature of the host terrain and the accessibility of the parasite to the range of the weapon or weapons available. Some parasites can be overcome by a single anthelmintic onslaught but others are expelled from their position only after repeated attacks. The method of applying treatment is therefore an important factor in anthelmintic medication.

**PRELIMINARY FASTING.** It is customary usually to fast the patient before the oral administration of most anthelmintics with a view of emptying the stomach and reducing the bulk of the intestinal contents. In the treatment of *Ascaris* and hookworm infections in man, the patient is usually given a light supper the night before and the anthelmintic administered in the morning, no food being permitted until adequate purgation has ensued. Dogs and cats are usually fasted overnight. Various periods of fasting are prescribed for larger domestic animals. Swine should be fasted for 24, and preferably, 36 hours. For equines it is advisable to withhold feed for 18 hours prior to anthelmintic medication for parasites in the stomach and small intestine and 36 hours for parasites in the large intestine. Conditions are somewhat different in the case of ruminants. Even prolonged fasting will not entirely reduce the bulk of the contents of the rumen. Formerly, it was customary to fast animals for 12 to 18 hours but more recently Clinies, Ross and Gordon (1934, 1935) have shown that there is no increase in the efficacy of a number of drugs used for the removal of the common sheep stomach worm in animals fasted for 24 hours as compared to the efficacy of the same drugs in unstarved sheep.

**CONSIDERATION OF THE PATIENT.** Since the safety of the patient is of paramount importance, it is the duty of the practitioner to satisfy himself that no contraindications for anthelmintic treatment are present. This calls for an adequate physical examination to rule out general contraindications and a suitable inquiry to ascertain the possible presence of specific contraindications for the drug of choice. General contraindications include febrile conditions, extreme youth or old age, chronic debilitating diseases, pregnancy, gastro-intestinal disturbances, chronic constipation and alcoholism. The presence of one or more of these conditions does not necessarily mean that treatment should be withheld but it does mean that due regard should be taken with respect to the type of drug and the dosage employed. The practitioner must decide whether the injury from parasitism is sufficient to warrant the risks attendant on treatment and must weigh the advisability of substituting a less specific but safer drug for a more specific but more dangerous drug. In patients who are poor risks for adequate doses of specific drugs, it is advisable to reduce the dose and remove a few worms at a time rather than hazard injury to the patient. In persons with severe hookworm disease, it is questionable whether anthelmintic treatment should be resorted to until the anemia has been corrected by suitable doses of iron.

In particular, doses of anthelmintics for children should be computed very carefully and apparent age rather than chronological age should form the basis of computation. Since the evacuation habits of children are not always regular, the administration of a high soap-suds enema on the morning of treatment often helps to prevent reactions to such anthelmintics as the chlorinated hydrocarbons.

The practitioner's obligation to the patient has not been fulfilled until a suitable check is made on the results of the treatment. In the case of most parasites, it is advisable to wait two weeks before a reexamination since some anthelmintics definitely inhibit egg production in some parasites. With such a specialized parasite as *Enterobius vermicularis* a longer period of time is needed to determine freedom from infection following treatment. In evaluating the efficacy of any treatment due cognizance should be taken of the possibilities of migrating larvae developing to maturity and also of possible exposure to reinfection following treatment.

**CHOICE OF THE ANTHELMINTIC.** An ideal anthelmintic would be one which could be given with complete safety to the patient; would be nontoxic in all cases; would be effective in removing all of the particular kind or kinds of worms against which it was directed; could be easily administered even in large scale treatments; and would be sufficiently cheap that cost would be no obstacle to its use.

In spite of the exuberant enthusiasm of some investigators, the ideal anthelmintic has yet to be discovered. Drugs which on first test seem to fulfill such specifications are usually found wanting in some respect when submitted to adequate field trials on large numbers of individuals.

Keeping in mind the general specificity of anthelmintics, it is best to select the most effective drug available provided no general or specific contraindications exist for the use of that specific drug. If contraindications are present, they usually modify either the selection of the anthelmintic or the dose employed. The presence of more than one nematode parasite or concomitant infections with cestode or trematode parasites frequently changes the picture. In the latter case the administration of a single drug will seldom be effective in eradicating such diverse helminths. Even in multiple nematode infections treatment with a single anthelmintic may not be effective. In the case of certain parasites, a combination of two drugs may be of value such as the chenopodium-tetrachlorethylene mixture in concomitant ascariid and hookworm infections in man. In other cases, different kinds of parasites have to be attacked by means of separate treatments.

One method of attack has been suggested as being of value for the removal of all intestinal helminths in certain animals. DeRivas (1926, 1927, 1936) advocated the use of trans-duodenal lavage with hot water or hot saline for parasites in the small intestine and colonic lavage with 1:5000 copper sulphate solution for parasites in the large bowel. He carried out experiments on dogs and man and reported that the use of two liters of hot saline at temperatures of 45° to 47° C. resulted in the elimination of worms with little discomfort to the patient. Hall and Schilling (1926) used the method on dogs with water having an initial temperature of 49° to 52° C. in the container and cooled to 47° to 48° C. at the time of administration. The use of 2 to 4 gallons of fluid resulted in an efficacy of 97.7 percent against ascariids, 77 percent against hookworms and 51.6 percent against tapeworms. However, the treatment resulted in the death of half the experimental dogs and was responsible for hemorrhage, enteritis and intestinal edema in those surviving. The safety of this method of treatment does not seem to be well established and perhaps for this reason the technique has never become popular.

Somewhat the same method of treatment was used by Whitney (1939) for removing various species of intestinal parasites from dogs. He employed a 1.5 percent solution of hydrogen peroxide in warm water and injected this solution per rectum under pressure until the act of vomiting indicated that the material had passed through the entire gastro-intestinal tract. The treatment was said to be highly effective against all of the helminth parasites commonly found in the gastro-intestinal tract of the dog. Reactions were encountered in some of Whitney's cases. Serious after effects in the form of gastro-enteritis and paralysis have since been reported by some veterinarians following the use of the treatment. Apparently, the treatment does not have an adequate margin of safety.

**PURGATION.** The administration of a purgative in connection with anthelmintic medication is of the utmost importance in the case of most drugs. Usually the purgative acts to promote the efficiency of the anthelmintic by distributing it throughout the intestinal tract and by aiding in the prompt expulsion of the parasites. In most cases, purgation is of marked value in safeguarding the patient by reducing the absorption of the anthelmintic. Some purgatives also give local protection against the irritating action of certain drugs.

The choice of the purgative is conditioned by the method of treatment and the drug or drugs employed. The use of the chlorinated hydrocarbon group of anthelmintics requires the administration of saline purgatives, since fats and oils tend to increase the absorption of such compounds, a thing which results in more marked reactions to the treatment. In the case

of oil of chenopodium, castor oil is the purgative of choice even though saline purgatives have been used with this drug. Castor oil not only promotes prompt expulsion of the drug and reduces absorption but it also exerts a local emollient action and protects the intestinal mucosa against the irritating properties of chenopodium.

Purgatives are usually administered concomitantly with the anthelmintics but practice in this regard varies with the host, the parasite and the drug employed. In treating large numbers of hookworm patients at one time, it is customary to give carbon tetrachloride or tetrachlorethylene in a solution of magnesium or sodium sulphate. However, in this case the drug may be given in gelatin capsules and immediately preceded or followed by the purgative. In the treatment of *Ascaris* infections in man with hexylresorcinol, it is the usual practice to administer the purgative 24 hours after the drug. Calomel has always been the time honored purgative for use with santonin but it is probable that better results would follow the employment of a saline purgative.

Adequate protection presupposes the administration of full doses of the purgative. Perhaps more injury has followed the use of inadequate doses of purgatives in connection with anthelmintics than has come from over dosing with the anthelmintics themselves. By this we mean that over doses of anthelmintics will frequently be tolerated if accompanied by adequate doses of purgatives whereas many fatalities have resulted from standard doses of certain anthelmintics used without adequate purgation. Therefore, in using nearly all anthelmintics, attention should be given to gauging accurately both the dose of the anthelmintic and the dose of the purgative.

In event that adequate purgation does not ensue within a reasonable time, prompt measures must be taken to protect the patient. High enemas should be resorted to and, if necessary, an additional dose of the purgative should be given by duodenal tube. Warm applications to the lower extremities and to the abdomen will hasten evacuation. The point of most importance in such circumstances is the rapid institution of corrective measures. Every effort should be made to stimulate bowel movements and promote prompt expulsion of the anthelmintic. If the patient is permitted to go unaided, increased absorption of the anthelmintic will ensue and the life of the individual may be endangered.

No doubt much of the distress following the administration of many anthelmintics is caused by the purgative and not by the anthelmintic. Malloy (1926) showed that the nausea, dizziness, headache and abdominal pain following the administration of carbon tetrachloride in magnesium sulphate solution was due in most cases to the purgative and not to the anthelmintic. Wright, Bozicevich and Gordon (1937) found that reactions to the tetrachlorethylene treatment in children were markedly reduced when magnesium citrate solution, a more pleasant and palatable purgative, was used instead of magnesium sulphate. In most cases, the symptoms described above are not alarming and usually pass off rapidly after the bowels move.

## Anthelmintic Medication for Nematode Parasites of Man

### TREATMENT FOR ASCARIS LUMBRICOIDES INFECTION

**HEXYLRESORCINOL.** This is the drug of choice since it is highly effective and is safer than other drugs formerly employed for this purpose.

Proper fasting is important since hexylresorcinol combines with protein and is rendered inert insofar as its anthelmintic action is concerned. The patient should be given a light supper on the evening before treatment and the drug should be administered on an empty stomach the following morning. Hexylresorcinol is used in the form of Caprokol pills, each of which contains 0.2 gram of the drug. The dosage for adults consists of 5 pills or a total of 1.0 gram. The dosage for children is, as follows: Under six years, 2 pills; six to eight years, 3 pills; eight to twelve years, 4 pills; over twelve years, 5 pills.

The pills should be swallowed with a little water; special care should be taken that they are not chewed since the drug is a local irritant and produces annoying burns. Children in particular should be observed closely to make sure that the pills are properly swallowed. Food should be withheld for 4 hours following administration of the drug. A saline purgative should be given 24 hours after treatment to sweep out the dead worms.

As a usual thing there is little or no discomfort from the drug although some patients may complain of nausea and slight abdominal pain. Occasionally a slight burning sensation in the epigastrium is noted but this soon passes off.

There are no well established contraindications for hexylresorcinol therapy. However, it is advisable for the patient to abstain from alcohol immediately before and after treatment. As a precautionary measure, it is probably well to avoid treating persons suffering from gastric or duodenal ulcer and any form of gastro-enteritis.

**OIL OF CHENOPODIUM.** This drug has had widespread application in the treatment of ascariasis and hookworm disease but its margin of safety is small and it has probably been responsible for more fatalities than any other single anthelmintic. However, its efficacy against ascarids is very high.

The active principle of chenopodium is ascaridol which varies in content with different oils. Effort has been made to standardize the ascaridol content at 70 percent in order to have available a uniform product but various oils on the market may vary in the content of the active principle.

In using chenopodium, the patient should be given a light evening meal. If constipated, a saline purge is indicated followed by a high soapsuds enema the next morning. These precautions are important in the case of constipated individuals since chenopodium itself tends to produce constipation.

The drug is given on an empty stomach and no food should be allowed until the bowels move. The adult dose should not exceed 1.5 cc. The dose for children is based on 0.05 cc. for each year of apparent (not chronological) age. The drug may be given in gelatin capsules and immediately preceded or followed by adequate dose of a saline purgative. Some authorities recommend dividing the dose into two parts and administering the doses 2 hours apart, in which case the purgative is given immediately after the last dose. If the patient shows any signs of reaction, the second half of the dose should be omitted and the purgative given immediately. The advisability of the split dose method is problematical since increased absorption and toxicity may result when the purgative is thus delayed.

The preferred method of administering chenopodium is to mix it with castor oil and give as a single dose. One to 2 cc. of castor oil should be given for each year of apparent age in children. The larger dose provides more adequate protection. The oil not only produces adequate purgation but protects the intestinal mucosa against the irritating action of the drug.

When chenopodium is measured by the drop method, there is a wide variation in dosage. Measurement should be made by a standard 1 cc. pipette graduated into tenths in order to avoid errors in dosage.

Toxic symptoms manifested in chenopodium poisoning are nausea, vomiting, dizziness, a tingling sensation of the extremities, muscular incoordination, stupor, profound collapse, cyanosis and respiratory failure followed by death. Severe and even permanent deafness may result. If purgation does not ensue within a reasonable time, strenuous efforts should be made to evacuate the bowels as promptly as possible. Any delay in instituting rigorous measures may seriously endanger the life of the patient.

Contraindications for chenopodium therapy include gastro-enteritis, chronic constipation, alcoholism, pregnancy, debilitating diseases, and moderate to severe cardio-vascular-renal disease. Very young children or aged individuals are poor risks for treatment.

**SANTONIN.** Santonin is a time honored remedy for the removal of large intestinal roundworms, although its efficacy in single doses does not approach that of either hexylresorcinol or oil of chenopodium. However, it is non-irritating and easily administered and can be used to advantage when there are definite reasons for avoiding the two other drugs.

The patient should be given a light evening meal and the dose of santonin administered with an equal amount of calomel at 10.00 p.m. The next morning before breakfast, a saline purgative should be given. The dose of santonin for adults is 3 to 5 grains (0.2 to 0.3 gram). For children, the dose rate is based on 1/6 grain (0.01 gram) for each year of apparent age.

Santonin is more effective when given in repeated treatments over a period of time. A satisfactory routine is to give 1 to 2 grains (0.06 to 0.12 gram) for adults and 1/4 to 1/2 grain (0.015 to 0.03 gram) for children daily over a period of 7 days. The drug is given with an equal amount of calomel and no other purgative employed. With continued treatment, the patient should be observed carefully for any evidence of toxicity.

Santonin is responsible in some cases for disturbances in perception and there may result yellow, green, and occasionally, blue vision. Symptoms of toxicity are evidenced by nausea, vomiting, dizziness, diarrhea, hematuria and convulsions. The drug is contraindicated in nervous disorders such as epilepsy. Fats and oils should be avoided as they increase absorption. The factor of safety for santonin is considerably greater than that for chenopodium but the drug is not without its hazards. Some authorities recommend that a single dose of 3 grains for adults be not exceeded.

#### TREATMENT FOR THE REMOVAL OF HOOKWORMS, ANCYLOSTOMA DUODENALE AND NECATOR AMERICANUS

**TETRACHLORETHYLENE.** Because of its greater safety, this drug is largely replacing carbon tetrachloride and other treatments for hookworm disease.

The patient should be given a light evening meal and should receive the drug on an empty stomach the following morning. No food should be allowed until after the bowels move. The dose for adults is 3.0 cc. and for children 0.1 to 0.2 cc. for each year of apparent (not chronological) age. Better results are obtained with a dose of 4.0 cc. for adults but the larger dose is apt to be followed by more severe reactions. The drug may be administered in gelatin capsules followed immediately by an adequate dose of magnesium or sodium sulphate. In mass treatment, tetrachlorethylene is given with the purgative. In such cases, the mixture should be stirred while the patient is drinking it so that the tetrachlorethylene will be distributed evenly throughout and not sink to the bottom of the container. The purgative should be dissolved in a liberal amount of water. One of the preferred methods is to use 30 cc. of a saturated solution of the saline purgative plus 60 cc. of water for an adult patient. As previously noted, a solution of magnesium citrate meets with less objection on the part of children and apparently causes less disagreeable reactions. In constipated individuals, it is best to give a saline purgative the night before treatment followed the next morning by a high soapsuds enema.

In the hands of various investigators, tetrachlorethylene has shown a degree of efficacy varying between 75 and 95 percent. Like carbon tetrachloride, it is more effective against *Necator* than against *Ancylostoma*.

Following treatment, patients frequently complain of dizziness, headache, nausea, vomiting and abdominal pain. Experience indicates that these reactions are less severe if the patient remains quietly in bed and for safety's sake it is best to insist on his doing so. Reactions usually disappear rapidly following action of the purgative. If the bowels do not move within the expected period of time or if minatory symptoms develop, prompt measures should be taken to hasten evacuation.

Tetrachlorethylene is contraindicated in cases of gastro-enteritis, chronic constipation and concomitant infections with *Ascaris lumbricoides*. Fats and oils should be withheld from the diet for 48 hours prior to the administration of the drug since they increase absorption and add to the toxicity. Patients receiving arsenical treatments are poor risks.

**Hexylresorcinol:** This drug, administered as for *Ascaris*, is about 50 to 60 percent effective for the removal of hookworms. Because of its relatively wide margin of safety, it can be used to advantage in cases in which the physician might hesitate to employ tetrachlorethylene.

#### TREATMENT FOR CONCOMITANT ASCARIS AND HOOKWORM INFECTIONS

**TETRACHLORETHYLENE AND OIL OF CHENOPODIUM.** A mixture of these two drugs can be used in cases in which both kinds of parasites are present. By itself, tetrachlorethylene should not be given when *Ascaris* is present because the drug tends to stimulate clumping of the worms with possible intestinal obstruction.

The dosage of the mixture for adults is 1.0 cc. of oil of chenopodium plus 2.0 cc. of tetrachlorethylene. For children, the dose rate is based on 0.05 cc. of chenopodium and 0.1 cc. of tetrachlorethylene for each year of apparent (not chronological) age. The mixture is given in one dose and followed immediately by a saline purgative as outlined for tetrachlorethylene. The contraindications and precautions are those noted in connection with the use of chenopodium for *Ascaris*.

**HEXYLRESORCINOL.** Because of its greater safety, this drug is to be preferred over the above-mentioned mixture for the treatment of combined hookworm and *Ascaris* infections. The method of administration is the same as that for the latter parasite.

#### TREATMENT FOR TRICHURUS TRICHURA INFECTION

While various anthelmintics in single doses will remove a small percentage of these worms, treatment is generally unsatisfactory. Repeated doses of santonin, as outlined under therapy for *Ascaris lumbricoides*, represent the most practical treatment at the present time. Even this regimen of treatment may have to be repeated on several different occasions to approach any considerable degree of efficacy.

Hexylresorcinol and tetrachlorethylene each will remove small numbers of worms, as will oil of chenopodium. Leche de bigueron, the sap of the Central and South American fig tree, *Ficus lanrifolia*, is a fairly effective treatment when given in doses of 30 to 60 cc. However, this material is not usually

available outside of the native habitat of the tree since the sap undergoes rapid fermentation and becomes very unpalatable at ordinary temperatures. Effort is being made to preserve the material in a way which will permit of its transportation and storage. Plein, the proteolytic enzyme isolated from the sap by Robbins, cannot be used safely in man because of its marked property of digesting the mucosa of the gastrointestinal tract in the presence of abrasions.

#### TREATMENT FOR STRONGYLOIDES STERCORALIS INFECTION

Kwa Tjaon Sioe (1928) and de Langen (1928) introduced gentian violet for the treatment of infections with this parasite and the treatment was further developed by Faust (1930). For adults, Faust recommends a dose of 1 grain (64 mgm.) three times a day before meals over a period of 16 $\frac{2}{3}$  days or a total dose of 50 grains. For children, the drug may be given at the rate of 1/6 grain (10 mgm.) per day for each year of apparent age or approximately 1/2 grain (32 mgm.) for each 3 years of apparent age, given over a similar period of time. Gentian violet is procurable in 1/2 grain and 3/20 grain enteric-coated or water soluble coated tablets.

Some *Strongyloides* cases are refractory to oral therapy with gentian violet and for such cases Faust recommends the duodenal intubation of 25 cc. of a 1 percent solution of the dye. The patient should remain quietly in bed after this treatment as nausea and vomiting are apt to ensue.

About one-third of the patients treated with gentian violet experience reactions consisting of one or more of the following symptoms: Nausea, vomiting, diarrhea, headache, dizziness and abdominal pain. These reactions are usually not of a serious character and can be controlled by reducing the dosage for a short time or discontinuing treatment for a day or two.

Contraindications for gentian violet are not clearly defined but as a precautionary measure the drug should not be given to patients suffering from gastro-enteritis, moderate to severe cardiac, hepatic or renal disease and concomitant infections with *Ascaris lumbricoides*. Pregnant women are apt to be markedly nauseated by the treatment. The consumption of alcohol should be prohibited during the period of treatment.

#### TREATMENT FOR ENTEROBIUS VERMICULARIS INFECTION

The ease with which many individuals become constantly reinfected with pinworms makes eradication of the parasite an extremely difficult matter. The failure in many cases to achieve control by the rigid application of hygienic measures calls for supplementing such measures in most cases with suitable therapeutic procedures.

It is probable that many of the failures to control pinworm infection are attendant on the fact that treatment is usually administered only to those persons in the household who show clinical symptoms. Frequently, other members of the family may be infected without being aware of the fact. Under such circumstances, these persons serve as reservoirs of infection which is again acquired by the treated individuals. Wright and Cram (1937) have emphasized the importance of carrying out adequate diagnostic tests on all members of a household and treating all infected individuals simultaneously with the view of eliminating at one time all sources of infection within the home.

The literature probably contains a greater array of drugs recommended for the removal of pinworms than for any other parasite. Single dose treatments are not well adapted for combating this parasite. Tetrachlorethylene, probably the best of these, is less than 50 percent effective. In general, better results follow the employment of repeated doses of drugs over a period of time sufficient to allow for desiccation of ova in the patient's surroundings and thus reduce opportunities for reinfection.

Santonin in repeated doses as for *Ascaris* has been used frequently, although its efficacy is somewhat less than 50 percent. Enemas, medicated or non-medicated, are of value particularly in young children but they must be carried over a period of time sufficient to care for the possibilities of reinfection.

Brown (1932) obtained good results in a small series of patients with hexylresorcinol enemas administered at varying intervals and supplemented by oral therapy with Caprokol pills. Wright, Brady and Bozicevich (1939) treated 27 patients without oral therapy and found 18 negative on post-treatment swabs, although some of the negative patients failed to furnish an adequate number of such swabs. A preliminary soap-suds enema was given at bedtime followed immediately after its expulsion by an enema consisting of a 1:2000 solution of hexylresorcinol in water. The above-mentioned workers found that satisfactory results in most cases required the administration of at least 10 such enemas over a period of 3 weeks. No doubt more consistent results would follow more prolonged

treatment. It is possible that Caprokol orally once or twice during the period of treatment would add to the efficacy of the regimen, although the preparation in single doses is not effective in eradicating the worms.

It would appear that the drug coming closest to fulfilling the requirements for a satisfactory treatment for oxyuriasis is gentian violet as reported by Wright, Brady and Bozicevich (1938) and Wright and Brady (1940). These investigators completed experimental treatment on 224 individuals, of whom 84 percent were negative for pinworm ova on 7 consecutive daily anal swab examinations taken at various intervals after the end of the treatment.

The dosage for gentian violet is the same as that used for the treatment of strongyloidosis. However, the regimen of treatment is somewhat different, the patient being given the drug over a period of 8 days, followed by a rest period of one week and then another course of treatment for 8 days. The contraindications and precautions are the same as those outlined under therapy for strongyloidosis.

Recently Manson-Bahr (1940) reported good results in the treatment of pinworm infection with phenothiazine. Of 6 children and 3 adults, clinical cures were said to have been obtained in all cases, although 3 individuals required a second course of treatment. The following dosage was recommended: For children under 8 years of age, 2 grams daily for 7 days; for children under 4 years of age, one-half of the above-mentioned dose; and for adults, 8 grams daily for at least 5 days. In the cases in question, results of treatment were not checked by swab technique or other methods to determine disappearance of infection. Nothing is said in Manson-Bahr's paper concerning the dangers of blood dyscrasias from the use of phenothiazine, although DeEds, Stockton and Thomas (1939) reported the occurrence of secondary anemia in 3 of 49 patients given phenothiazine as a urinary antiseptic. The maximum total dose recommended by Manson Bahr is greatly in excess of that specified by DeEds, Stockton and Thomas as being within the limits of safety. It would seem that this treatment should be used with considerable caution.

#### TREATMENT FOR WUCHERERIA BANCROFTI INFECTION

There is no specific medication for this condition. Various drugs have been reported as being of value for the destruction of the microfilariae or preventing their appearance in the peripheral circulation. However, evidence for the efficiency of such drugs is meager as in many cases the larvae reappear later. There is no known drug effective for the destruction of the adult worms.

Chopra and Sundar Rao (1939) have reported on tests extending over 10 years with patients treated with a large number of different drugs at the Calcutta School of Tropical Medicine. None of the compounds employed was of value in effecting the destruction of adult or larval worms. Soaquin, an arsenical preparation, reduced the number of febrile and inflammatory attacks. Fouadin had a temporary sterilizing effect on the parasite but microfilariae reappeared in the blood after several days. However, the drug was said to be very useful in controlling inflammation and fever over comparatively long periods of time. In a few cases, chyluria disappeared even after a single dose. Prontosil and its derivatives were found of value in the treatment of secondary infection.

Roentgen-ray therapy has been advocated as being of value in filariasis but Golden and O'Connor (1934) were unable to obtain consistently promising results.

In filarial lymphangitis and elephantiasis, surgical intervention by means of the Auchincloss technique or one of its modifications will bring some temporary relief. Knott (1938) has advocated prolonged tight bandaging. The use of the method on 105 unselected patients in his series indicated apparently that it is of value for the gradual removal of the lymphoedema and in the prevention of the recurrent attacks of lymphangitis.

Anti-streptococcal vaccines have been reported to be effective in some cases but O'Connor (1932) pointed out that the relief is only temporary and that any serum or vaccine produces similar relief, indicating probably that temporary cure is due to protein shock rather than to specific anti-bacterial action.

#### Anthelmintic Medication for Nematode Parasites of Dogs, Cats and Related Carnivores

##### TREATMENT FOR ASCARID INFECTIONS

TETRACHLORETHYLENE. This drug in a dose of 0.2 cc. per kilogram (2.2 pounds) of body weight is effective for the removal of dog ascarids. In using chlorinated hydrocarbons in the presence of heavy ascarid infections, particularly in puppies and young dogs, it is advisable to follow the anthelmintic in 3 or 4 hours by an adequate dose of castor oil, or to give

a saline purgative immediately following the treatment. The purpose of this is to prevent clumping of the ascarids, which are inordinately stimulated by these compounds, and a possible intestinal obstruction which sometimes causes enteritis, necrosis and death. Tetrachlorethylene may be given to cats at the same dose rate and in the same manner as for dogs. The drug in doses of 1 cc. has been reported to be of value for the removal of ascarids from foxes. In these animals, it is said to cause a slight enteritis which is not of serious consequence.

**OIL OF CHENOPODIUM.** Numerous experiments have shown that this drug is very effective for the removal of ascarids from dogs. The rate of dosage is 0.1 cc. per kilogram of body weight or 1.0 cc. for a 10-kilogram (22-pound) dog. For practical purposes, this can be regarded as equivalent to the following doses: For dogs weighing 10 pounds or less (except toy dogs), 5 minims; for dogs weighing 10 to 20 pounds, 10 minims; for dogs weighing 20 to 30 pounds, 15 minims; and for dogs weighing over 30 pounds, 20 minims. Toy dogs require small doses and considerable precaution should be exercised in treating such animals; a dose of 2 or 3 minims is advisable. The dog should be fasted from the afternoon of the day previous to treatment and should be dosed the following morning. The chenopodium should be accompanied by at least an ounce (30 cc.) of castor oil. It is not advisable to give the chenopodium in the castor oil, as chenopodium is salivating and the combination produces a disagreeable slobbering effect. For choice, the chenopodium should be given in gelatin capsules and the castor oil administered immediately before or after the capsules. The animal should not be fed until 3 hours after treatment. If dogs show serious toxic effects, large additional doses of castor oil should be given and ememas used to insure prompt purgation. The contraindications for chenopodium have been discussed in connection with the treatment of ascariasis in man.

Chenopodium is very effective for the removal of ascarids from cats but the drug is more toxic for these animals than it is for dogs. The dose for the cat should not exceed 0.05 cc. per kilogram (2.2 pounds) of body weight, immediately preceded or followed by an adequate dose of castor oil.

For fox pups, Young (1930) recommended 1-minim doses of oil of chenopodium in castor oil and found this safe for pups 3 weeks old and effective for pups up to 8 weeks of age. He preferred not to treat them until they were 4 weeks old.

**SANTONIN.** When there are contraindications for other treatments, santonin in repeated doses may be used to remove ascarids from dogs. Experiments show that single doses of santonin, even very large doses, such as  $\frac{1}{2}$  grain for each pound of body weight, are less effective than a single therapeutic dose of chenopodium, but that smaller doses of santonin daily for several days gives very good results. Small dogs may be given  $\frac{1}{2}$  grain of santonin and an equal amount of calomel, and large dogs double this dose, daily for a week. This should be given early in the morning and the animal not fed for 2 or 3 hours. As previously stated, a saline purge following single doses of santonin seems to increase materially the efficacy of the drug.

**N-BUTYL CHLORIDE.** The administration of tetrachlorethylene to dogs is frequently followed by a temporary narcosis which often embarrasses the veterinarian and alarms the client. Harwood, Jerstad, Underwood and Schaffer (1940) are of the opinion that n-butyl chloride does not produce such reactions. For the removal of ascarids, these investigators recommend the following dosages: For dogs weighing 2.3 to 4.5 kilos (5 to 10 pounds), 2 cc.; 4.5 to 9 kilos (10 to 20 pounds), 3 cc.; 9 to 18 kilos (20 to 40 pounds), 4 cc.; and 18 or more kilos (40 or more pounds), 5 cc.

**HEXYLRESORCINOL.** Lamson, Brown and Ward (1930) have reported that hexylresorcinol is very effective for the removal of dog ascarids. The drug is given in doses of 0.5 to 1 gram. With hexylresorcinol, it is necessary to withhold food for 12 to 18 hours before treatment. Animals should not be permitted to crush or chew capsules or pills of hexylresorcinol since, as previously stated, the drug is irritant to the mucosa of the mouth.

#### TREATMENT FOR HOOKWORM INFECTION

**TETRACHLORETHYLENE.** At the present time, this is the drug of choice having largely replaced carbon tetrachloride because of the toxicity of the latter. The therapeutic dose rate of tetrachlorethylene for dogs and cats is 0.2 cc. per kilogram (2.2 pounds) of body weight, or 2 cc. for a 10-kilogram or 22-pound animal. It is usually not necessary to give a purgative in connection with tetrachlorethylene, but a purgative is advantageous as it helps to sweep out worms killed by the treatment and to eliminate the drug rapidly from the intestinal

tract. It is advisable in concomitant heavy ascarid infections, particularly in puppies, to follow tetrachlorethylene with a suitable dose of purgative. However, castor oil or other oils, or fats, should not be given immediately preceding or following tetrachlorethylene as they aid in the absorption of the drug. Tetrachlorethylene has the disadvantage of causing in some cases a transient vertigo or dizziness, which may be alarming to the owner of the animal, but which in fact is not serious and which soon passes off. For this reason, as mentioned under the section on the treatment of ascarid infection, n-butyl chloride may be used in place of tetrachlorethylene. The dosages suggested for the removal of hookworms are the same as those suggested for the removal of ascarids from dogs.

Tetrachlorethylene can be used to advantage in the removal of *Uncinaria stenocephala* from foxes. The dosage is the same as that for dogs. However, foxes do not tolerate anthelmintic treatment as well as do dogs, and particular care should be taken to judge accurately the dosage of the drug and to appraise closely the possible presence of contraindications for treatment. Care should be taken to see that capsules are not broken in the mouth, as inhalation of tetrachlorethylene may lead to serious complications, particularly in fox pups.

**HEXYLRESORCINOL.** This drug can be used to advantage when contraindications for other treatments are present. However, its efficacy falls below that of tetrachlorethylene and many other halogenated hydrocarbons. The dosage is the same as that given under treatments for the removal of ascarids.

#### TREATMENT FOR TRICHURIS VULPIS INFECTION

Numerous experiments on dogs indicate that a large number of anthelmintics are potent in the removal of whipworms but that a single dose of such drugs will rarely remove many whipworms. The failure of single dose treatments is no doubt due in part to the fact that the anthelmintic fails to enter the cecum or enters it only in insufficient amounts. It is, therefore, necessary to give a drug from day to day, until it does come in contact with the worms in effective doses, or to give large doses of relatively non-toxic drugs to ensure the entry of the drug into the cecum.

**SANTONIN.** For the purposes of repeated treatments, santonin is a very satisfactory drug since it does not cause gastrointestinal irritation even when given over a period of time. The drug may be given to dogs in a dose of  $\frac{1}{2}$  to 1 grain each of santonin and calomel, according to the size of the animal, daily for 7 days. The treatment may then be discontinued and repeated after an interval of a week.

**LECHE DE FIGUERON.** This drug has been described under treatment for whipworms in man. While adequate tests have not been carried out to establish its efficacy for the removal of *T. vulpis*, it seems probable that it would be effective for that purpose. However, until the material becomes more generally available, its use will be restricted to the geographical areas in which the tree is indigenous.

**N-BUTYL CHLORIDE.** Harwood, Jerstad, Underwood and Schaffer (1940) showed n-butyl chloride to be over 50 percent effective for the removal of whipworms. While this degree of efficacy is certainly not satisfactory, these workers pointed out that the drug is superior nevertheless to any other single dose treatment known at present. As it is highly effective for the removal of ascarids and hookworms, it seems worthy of trial in whipworm infections. For whipworms, the above-mentioned investigators recommend a dose of 3 to 5 cc. for dogs weighing 5 pounds or less; 6 to 8 cc. for 5 to 10-pound dogs; 10 to 12 cc. for 10 to 20-pound dogs; 15 cc. for 20 to 40-pound dogs; and 25 cc. for dogs weighing over 40 pounds. If the dog is infected with ascarids, a saline purgative should be given immediately following the anthelmintic.\*

**OTHER METHODS.** Hall and Shillinger (1926) found that mercurochrome gave fairly satisfactory results for the removal of whipworms from dogs when the drug was given in doses of 2 to 5 tablets each containing 1.5 grains (96 mgm.) daily for 5 to 11 days. The drug removed 273 of 311 whipworms from 9 dogs, or 88 percent, and removed all whipworms from 4 of 6 infected animals. The safety of this treatment has not been established. Although it has never come into general use, it would seem worthy of trial.

The use of drugs injected into the cecum by means of a catheter passed per rectum has been advocated for the removal of whipworms. However, it is extremely difficult to pass a flexible rubber tube in such a way that the operator has any assurance that the orifice of the catheter is opposite the orifice of the cecum and that the drug actually enters that organ. In critical tests, Underwood, Wright and Bozicevich (1931),

\*Chitwood (personal communication) has obtained 100 percent efficacy for whipworms when n-butyl chloride was administered in ice hard gelatin capsules at the rate of 1cc per kilo body weight and with no purgative. Purgatives appear to lower the efficacy of this drug.



using tetrachlorethylene, oil of chenopodium or ethylidene chloride, obtained an efficacy of 100 percent in one dog, 3.1 percent in a second dog but complete failure in 11 other animals.

Surgical intervention with the removal of the cecum is practiced by some veterinarians who report very good results in cases in which it is impossible to remove the worms by anthelmintic treatment. Symptoms of abdominal distress with alternating constipation and diarrhea associated with whipworm infection are said to be relieved permanently following removal of the cecum. While this method will not obviate reinfection, subsequent infections in the colon are usually of very light degree and not associated with clinical symptoms.

#### TREATMENT FOR CAPILLARIA AEROPHILA AND CRENOSOMA VULPIS INFECTIONS

Intratracheal injection of various medicinal substances has been advocated in the treatment of these very serious parasites of foxes on fur farms but it is doubtful whether any great benefit has resulted. On the other hand, the development within recent years of the tracheal swab-syringe and the tracheal brush for the mechanical removal of lungworms from the trachea of the fox has provided a fairly satisfactory method for the removal of worms which are actually in the trachea, the instruments owing to mechanical difficulties being of little value for the removal of worms from the bronchi or bronchioles. Hanson (1933), who was largely instrumental in developing this method of treatment to its present satisfactory state, has published results of critical tests with the instruments and detailed information concerning their use. This method of treatment is more effective in the case of *C. aerophila* than with *Crenosoma vulpis*, since the latter parasite is more frequently located in the bronchi and bronchioles, where it cannot be reached by the tracheal brush or swab.

Recently Russian investigators have reported that a solution consisting of iodine, 1 gram; potassium iodide, 2 grams; and water, 1,500 cc. is effective for the destruction of these parasites when injected intratracheally. The animal is placed on its back with the head elevated at an angle of 30 degrees. One-half the dose is injected while the animal is rolled slightly to one side; then the animal is rolled slightly to the other side and the remainder of the dose injected. The treatment is repeated after 3 days. Maximum doses are 3 cc. of the solution. It is reported that maximum doses remove 80 percent of the lungworms.

#### TREATMENT FOR SPIROCERCA LUPI INFECTION

There is no anthelmintic treatment of value in this condition. Treatment is symptomatic with the view of relieving the cough and nausea and maintaining the condition of the animal. Oil of chenopodium has been suggested but it is unlikely that worms in the tumors would be affected. On theoretical grounds, chlorinated hydrocarbons, such as carbon tetrachloride, should be more penetrating and more effective than chenopodium. Suchanek (1932) reported a case of spirocerosis in a dog which was diagnosed by means of X ray and the esophagoscope. The dog was placed under chloral hydrate narcosis, the blade of a scalpel was fixed in a pair of forceps which were passed through a tube and, with the aid of the esophagoscope, the tumor was removed.

#### TREATMENT FOR PHYSALOPTERA SPP.

Ehlers (1931) reported on the anthelmintic treatment of infections with *Physaloptera* sp. in badgers (*Taxidea taxus*) and it is probable that the treatments found effective can be used also on other animals. Tetrachlorethylene in doses of 0.5 to 1 cc. (8 to 16 minims) failed to remove the worms but a dose of 5 cc. killed all physalopterids although it proved fatal to one animal. Ehlers stated that the drug deserves further trial in doses of 1.3 to 2 cc. (20 to 32 minims). Carbon disulphide was found to be very effective in doses of 0.8 to 1 cc. (12 to 16 minims), administered after a period of fasting for 18 to 24 hours, and followed in 6 hours by a table spoonful (15 cc.) of castor oil mixed with honey, a mixture which badgers will eat readily out of a spoon. While the administration of a purgative is desirable, no ill effects were observed in those animals to which a purgative was not given.

#### TREATMENT FOR DIROFILARIA IMMITIS INFECTION

Fouadin (sodium antimony III pyrocatechin disulphonate of sodium) has been used more extensively than any other drug for this condition. As shown by Wright and Underwood (1934), a suitable course of treatment results usually in the permanent disappearance of microfilariae from the peripheral circulation, in the sterilization of female worms, and in the eventual destruction of some or all of the adult worms in the heart and

pulmonary artery. The action of Fouadin on adult worms is cumulative and is exerted over a relatively long period of time. The destruction of any considerable number of adult worms at any one time may result in embolic pneumonia or in an acute toxemia with consequent danger to the life of the patient. Consequently, heavily infected animals should be treated with caution and in such animals treatment should not be administered rapidly, or in large doses, or at too frequent intervals. The administration of moderate doses of the drug over a period of time results in a central necrosis of the liver and in an acute toxic nephrosis. The liver damage may lead to guanidine retention with a lowering of the blood calcium level. Symptoms of calcium tetany should be combated through the use of calcium gluconate. Considerable judgment must be exercised in the administration of this treatment and due weight should be given to the presence of chronic or acute disease conditions which might influence the tolerance of the animal for the drug. Wright and Underwood recommended the following dose rates for intramuscular and intravenous injections for dogs in good physical condition and not suffering from cardiac, hepatic or renal disease; these dose rates have been generally followed by most veterinarians.

#### Intramuscular injections

| Body weight of dog             | Daily dose       | Daily dose        | Daily dose          |
|--------------------------------|------------------|-------------------|---------------------|
|                                | for first 6 days | for second 6 days | after second 6 days |
| Under 10 kgms. (22 lbs.)       | 0.5 cc.          | 1.0 cc.           | 1.0 cc.             |
| 10 to 15 kgms. (22 to 33 lbs.) | 1.0              | 1.5               | 1.5                 |
| 15 to 20 kgms. (33 to 44 lbs.) | 1.0              | 1.5               | 2.0                 |
| 20 to 25 kgms. (44 to 55 lbs.) | 1.5              | 2.0               | 2.0                 |
| Over 25 kgms. (55 lbs.)        | 2.0              | 2.5               | 2.5                 |

#### Intravenous injections

| Body weight of dog             | Days of treatment |     |     |     |     |     |      |      |  |  |
|--------------------------------|-------------------|-----|-----|-----|-----|-----|------|------|--|--|
|                                | 1st               | 3rd | 5th | 7th | 8th | 9th | 10th | 12th |  |  |
| Under 10 kgms. (22 lbs.)       | 0.5               | 0.5 | 0.5 | 1.0 | 1.0 | 1.5 | 1.5  | 1.5  |  |  |
| 10 to 15 kgms. (22 to 33 lbs.) | 1.0               | 1.0 | 1.0 | 1.5 | 1.5 | 2.0 | 2.0  | 2.0  |  |  |
| 15 to 20 kgms. (33 to 44 lbs.) | 1.5               | 1.5 | 1.5 | 2.0 | 2.0 | 2.5 | 2.5  | 2.5  |  |  |
| 20 to 25 kgms. (44 to 55 lbs.) | 2.0               | 2.0 | 2.0 | 2.5 | 2.5 | 3.5 | 3.5  | 3.5  |  |  |
| Over 25 kgms. (55 lbs.)        | 2.5               | 2.5 | 2.5 | 3.5 | 3.5 | 5.0 | 5.0  | 5.0  |  |  |

Intramuscular injections are without appreciable unfavorable local reaction and are particularly applicable for use in small dogs in which the subcutaneous veins are so small as to make intravenous injections difficult. However, intravenous administration permits the use of a smaller total dose in most cases, and the results desired are obtained in a shorter period of time.

In connection with other treatments, Hayes (1933) recommended the use of an antimony preparation called "Filsol," the chemical composition of which has never been made public. This preparation appears to be more toxic than Fouadin and should be used with even greater caution.

Brown and Austin (1939) have published case reports on the use of "Stibsol," said to be antimonial-3-catechol-thiosalicylic acid-sodium, and to contain 30 percent of antimony. The solution contains approximately 8.5 mg. of trivalent antimony per cubic centimeter. These investigators recommend for this compound the same dose rates as recommended by Wright and Underwood for the intravenous injection of Fouadin. Evaluation of the efficacy of this compound must await either the publication of more extensive and more critical tests or the results of field trials in relatively large numbers of cases.

Simonelli (1936) and Lucas (1937) have reported successful results in the treatment of canine filariasis following the use of emetine hydrochloride at dose rates varying from 10 to 60 mgm. per day, but more critical evidence is needed before this treatment can be evaluated. The drug had been previously used by MacCallum (1921) for this purpose.

### Anthelmintic Medication for Nematode Parasites of Swine

#### TREATMENT FOR ASCARIS SUUM INFECTION

OIL OF CHENOPodium. This is probably the most effective treatment available at the present time. The drug is given at a dose rate of 1/2 to 1 fluid dram (2 to 4 cc.) for a 100-pound (45.5-kilogram) animal, immediately preceded or followed by

at least 2 fluid ounces (60 cc.) of castor oil, or the drug may be administered with the oil. Doses for animals of various sizes should be computed on a weight basis, though it is likely that a dose of 2 fluid drams is adequate for animals weighing 300 to 400 pounds (136.4 to 181.8 kilograms). The drug may be given with a dose syringe or by stomach tube. The animals should be fasted for 18 to 24 hours prior to treatment and should not be fed or watered for 3 hours after treatment. Oil of chenopodium should not be given to animals suffering from gastro-enteritis, constipation or febrile conditions, or to very young animals or sows in advanced pregnancy. If a herd is to be treated without regard to possible contraindications in individuals, the lower dose rate of chenopodium should be used.

**SANTONIN.** Santonin has been widely recommended as a treatment for the removal of ascarids from swine. At various times, it has been tested critically by Mote, Vадja, Shillinger, and others, all of whom have found that santonin in the doses commonly recommended and given in the manner usually recommended exhibits a relatively low efficacy for the removal of these worms. Under these conditions, the efficacy of santonin does not compare favorably with that of oil of chenopodium. More recently, Morris and Martin (1931) as well as Sheherbovich (1935) have found that santonin administered in relatively large doses and followed by an adequate dose of an active purgative, such as magnesium sulphate or castor oil, will remove a large percentage of the ascarids from swine. Morris and Martin administered santonin at dose rates varying between 1/6 to 2/3 grain (10.7 to 43 mgm.) per pound (45 cgm.) of body weight, followed in 12 hours by 1 dram (4 grams) of magnesium sulphate per pound of body weight. It would seem that adequate purgation is necessary and relatively large doses required if satisfactory results are to follow the use of this drug.

**PHENOTHIAZINE.** Swanson, Harwood and Connelly (1940) have recently reported on the use of this drug for swine and it appears to have considerable efficacy for the removal of ascarids. However, better results were obtained in the removal of mature ascarids than in the removal of immature forms. In view of the marked efficacy of the drug for the removal of nodular worms from swine, it could probably be used to advantage in animals in which both kinds of worms are present. The above mentioned investigators have suggested dose rates of phenothiazine for experimental use in swine and these may be found under the treatment for *Oesophagostomum* spp.

#### TREATMENT FOR THE REMOVAL OF HOOKWORMS

Satisfactory medication has not been established. On theoretical grounds, some of the chlorinated hydrocarbons would seem to be promising. However, Raffensperger, as reported by Wright and Raffensperger (1930), did not find carbon tetrachloride in a dose of 25 cc. in 75 cc. of castor oil for pigs weighing 125 pounds effective for the removal of *Globocephalus urosulatus*. Tetrachlorethylene or n-butyl chloride might be more promising since carbon tetrachloride is not well tolerated by swine and is more soluble.

#### TREATMENT FOR THE REMOVAL OF SWINE STOMACH WORMS

Bozicevich and Wright (1935) found that carbon disulphide, administered in capsules or by stomach tube, at a dose rate of 0.1 cc. per kilogram (2.2 pounds) of body weight, or 4.5 cc. for a 100 pound pig, was approximately 90 percent effective for the destruction of *Hyostrongylus rubidus* and even more effective for the removal of *Ascarops strongylina*. Food must be withheld for 36 to 44 hours prior to treatment, as the presence of food in the stomach interferes with the action of the carbon disulphide and acts to reduce the efficacy of the treatment. Lower doses of carbon disulphide were less effective. Pigs killed 2 hours after treatment showed a slight to moderate gastritis but, as in the administration of carbon disulphide to horses, this gastritis does not constitute a marked objection to the use of the treatment as it probably clears up rather quickly. It appears that this treatment should be effective also for the removal of *Physoccephalus scroalatus* and other nematodes occurring free in the stomach of swine.

#### TREATMENT FOR THE REMOVAL OF NODULAR WORMS, OESOPHAGOSTOMUM SPP.

Of a number of drugs tested for the removal of these worms, none showed a high efficacy until Harwood, Jerstad and Swanson (1938) and Swanson, Harwood and Connelly (1940) demonstrated the marked efficiency of phenothiazine for this purpose. In experiments reported by the latter investigators, conditioned phenothiazine removed 4,753, or 92.1 percent, of 5,162 nodular worms from 22 pigs. In other tests, recrystallized phenothiazine showed approximately the same degree of effi-

cacy. Swanson, Harwood and Connelly recommended the following dose rates for phenothiazine for experimental use in swine:

| Weight of pig                       | Size of dose       |
|-------------------------------------|--------------------|
| Up to 11.4 kgm. (25 lbs.)           | 5 gm. (1.2 drams)  |
| 11.4 to 22.8 kgm. (25 to 50 lbs.)   | 8 gm. (2.0 drams)  |
| 22.8 to 45.5 kgm. (50 to 100 lbs.)  | 12 gm. (3.0 drams) |
| 45.5 to 91.0 kgm. (100 to 200 lbs.) | 20 gm. (5.0 drams) |
| Over 91 kgm. (200 lbs.)             | 30 gm. (7.5 drams) |

Phenothiazine may be administered to swine in hard gelatin capsules if the operator is sufficiently skilled to avoid lodging the capsules in the pharyngeal pouch, or it may be administered mixed with any ground feed to which the pigs are accustomed. Pigs varying greatly in size should not be treated at one time in the latter manner, and the chemical should not be offered to the animals except when they are sufficiently hungry to consume the medicated food at once. The efficacy of the drug when administered with the feed needs further investigation but this promises to be a very valuable method of treatment.

#### TREATMENT FOR STEPHANURUS DENTATUS INFECTION

No effective treatment is known for the destruction of swine kidney worms. Turpentine has been recommended on the ground of the great diffusibility of the drug but it has not been established that the drug could reach the adult worms in the perirenal fat. Kauzal (1932) interpreted his experimental results with carbon tetrachloride as indicating that the treatment was of some benefit, as no worms were found in the liver of one of the treated animals, while worms in the liver of a second animal were encapsulated. It is possible that this drug might check the migration of worms or destroy migrating worms in the liver, although it is probable that the drug would have no effect on adult worms in the perirenal tissue.

#### TREATMENT FOR LUNGWORM INFECTIONS

Freeborn (1916) recommended the injection into the nostrils of swine of 5 cc. of chloroform repeated at intervals of 3 to 5 days until the infection is controlled. However, there is no critical evidence that this treatment is effective in the destruction of the worms.

Skrjabin and Schul'ts (1936) reported that one part of chlorine in 30,000 parts of air had little effect on the host after one hour and claimed that this exposure destroyed 73.3 percent of the lungworms present. The same authors also recommended intratracheal injections of the iodine solution described under the treatment of lungworms of carnivores. The doses employed for swine are 0.25 cc. per kilogram of body weight for small pigs and 0.5 cc. per kilogram of body weight for average-sized pigs.

The treatment which appears to be safest and best is good nursing in connection with an abundance of good feed and adequate shelter. In the absence of specific therapy, emphasis should be placed on prevention and animals should be isolated and removed from areas where the intermediate hosts are prevalent.

#### TREATMENT FOR TRICHURIS SUI INFECTION

Medication for whipworm infection in swine is entirely uncertain and no effective treatment is known at present. Single doses of various anthelmintics will remove a few whipworms at times but consistent results are not obtained with any of them. In the absence of more information concerning the pathogenicity of this parasite, chemotherapy does not seem to be a matter of any considerable importance.

### Anthelmintic Medication for Nematode Parasites of Equines

#### TREATMENT FOR PARASCARIS EQUORUM INFECTION

**CARBON DISULPHIDE.** This drug is probably the most effective treatment available. It should be administered in a dose of 6 fluid drams (24 cc.) for a 1,000 pound animal, after a fast of 18 hours, or at a dose rate of 1.5 fluid drams (6 cc.) for each 250 pounds of body weight. No purgative is needed but a saline purgative may be advisable in the case of heavy infections; oils should be avoided as they increase absorption and add to the toxicity of the drug. Carbon disulphide should be administered by stomach tube; if capsules containing the drug are broken in the mouth, asphyxiation and death may result. Capsules containing carbon disulphide adsorbed on various kinds of powdered material are available; these capsules undoubtedly are safer to administer but fail to provide the same high efficacy as exerted by the liquid drug. Carbon disulphide produces a well marked inflammatory reaction in the stomach and upper duodenum. This inflammation usually clears up in a short time however. Carbon disulphide is contraindicated in

the presence of gastro enteritis; it is not indicated in the treatment of pregnant mares.

**CARBON TETRACHLORIDE.** This is also an effective treatment for the removal of ascarids from horses. It is given in the same manner as for the removal of strongyles, but in heavy ascarid infections it is advisable to follow the drug by a saline purgative in order to sweep out dead worms and prevent their clumping in the small intestine. This is particularly indicated in the case of foals.

**PHENOTHIAZINE.** Experiments indicate that this drug will remove some ascarids but more data are needed before it can be established as a satisfactory treatment.

#### TREATMENT FOR THE REMOVAL OF LARGE AND SMALL STRONGYLES, *STRONGYLUS* SPP., *TRICHONEMA* SPP. AND RELATED GENERA

**OIL OF CHENOPodium.** Chenopodium is very effective for the removal both of large and small strongyles. Animals should be fasted for 36 hours and oil of chenopodium administered in a dose of 4 to 5 fluid drams (16 to 20 cc.) for a 1,000 pound animal, or at a dose rate of 1 fluid dram (4 cc.) for each 250 pounds of body weight, immediately preceded or followed by 1 quart of raw linseed oil. Cases of excessive purgation have been reported in some instances following the use of raw linseed oil. It is possible that this undesirable action is due to impurities in the product; consequently a good grade of oil should be used. Veterinarians of the U. S. Army have proposed a substitute purgative of castor oil and mineral oil, claiming that this mixture provides suitable purgation following treatment with chenopodium and is without undesirable effects. The following are the doses of the mixture recommended: For weanlings, castor oil 4 to 6 ounces and mineral oil 1 pint; for yearlings and 2-year-olds, castor oil 6 to 8 ounces and mineral oil 1 pint; for 3-year-olds and older, castor oil 8 to 10 ounces and mineral oil 1½ pints. Oil of chenopodium is contraindicated in the presence of constipation, gastro-enteritis and febrile conditions, and in pregnant mares.

**CARBON TETRACHLORIDE.** This drug is effective for the removal of large strongyles but only about 50 percent effective against small strongyles. It should be given in capsule or by stomach tube in a dose of 6 to 12 fluid drams (25 to 50 cc.) for a 1,000-pound animal after fasting for 24 to 36 hours. The drug need not be accompanied by a purgative but if one is used, sodium sulphate is to be preferred. If linseed oil is used, it should be given 4 to 5 hours after carbon tetrachloride. The administration of carbon tetrachloride to equines is followed by a fall in the blood calcium level and by a marked increase of bilirubin in the blood. In carbon tetrachloride intoxication, it is advisable to use calcium gluconate. The drug is contraindicated in animals suffering from hepatic disease or from calcium deficiencies, such as rickets or osteomalacia.

**N-BUTYLIDENE CHLORIDE.** In a dose of 0.2 cc. per kilogram of body weight, this compound is very effective for the removal both of large and small strongyles. It is probable that the dose could be reduced to 0.15 cc. per kilogram without materially affecting the efficacy of the treatment; this dose is equivalent approximately to 70 cc. for a 1,000-pound animal. As n-butylidene chloride is constipating, it is advisable to follow the drug in 5 hours by raw linseed oil in a dose of 1 quart for a 1,000 pound animal.

**N-BUTYL CHLORIDE.** Because of the relatively higher cost of n-butylidene chloride, Harwood, Underwood and Schaffer (1938) tried n-butyl chloride for the removal of strongyles. In a dose of approximately 0.2 cc. per kilogram of body weight, the compound proved very effective for the removal of small strongyles and reasonably effective for the removal of large strongyles. In the tests in question, the drug was given in 10 times its volume of raw linseed oil. In tests by the above-mentioned workers, two horses succumbed to treatment with doses ten times the therapeutic dose. It would appear that the compound is not as safe as is n-butylidene chloride and that further tests are needed to clarify this point.

**PHENOTHIAZINE.** This is a very effective drug for the removal of strongyles from horses. Several authors have reported excellent results following the use of doses varying from 30 to 100 grams for adult animals. However, the only critical experiments reported thus far were by Harwood, Habermann, Roberts and Hunt (1940) and Habermann, Harwood and Hunt (1941). In the tests described in the latter paper, it was found that this drug in doses varying from 50 to 100 grams per equine removed practically all of 362,797 cyclostomes and 96 percent of 137 *Strongylus* spp. These authors concluded that the dose per adult equine should be held at 50 grams pending additional critical experimentation with smaller dosages. In some instances, a dose of 50 grams failed to remove a few of the *Strongylus* spp. present in the treated animals and, since these are the most pathogenic of the nematodes present in equines, it does not seem advisable to employ dos-

ages of 30 grams per horse such as recommended by Taylor and Sanderson (1940) on the basis of tests checked by the egg count method alone.

Single doses of phenothiazine as high as 500 grams have been given to horses without producing alarming symptoms, but these dosages may cause pronounced cloudy swelling of the liver, the formation of methaemoglobin, and anemia (Lapage, 1940; Habermann, Harwood and Hunt, 1941). Single doses of 1,000 grams have produced fatalities in horses. Since the toxic manifestations appear to be associated with the destruction of the erythrocytes, it is advisable to administer the drug cautiously to horses suffering from anemia. The Bureau of Animal Industry has issued a press release terming poor, weak animals and those suffering from infectious anemia as bad risks for treatment and it would appear that the drug should be employed with considerable caution in such cases.

Hatcher (1941) reported the death of 5 of 12 horses, each of which was given a dose of approximately 120 grams of phenothiazine as an anthelmintic. Under experimental conditions, doses of this size have caused no symptoms other than discoloration of the mucous membranes, transient loss of appetite and a temporary anemia. Grahame, Morgan and Sloane (1940) administered 100 grams to each of 35 horses without accident, and others have reported similar results. Possibly under certain conditions horses may prove more sensitive to phenothiazine than present experimental evidence suggests. As a measure of precaution, wholesale treatment should be avoided. The Bureau of Animal Industry recommends that when large numbers of animals are to be treated with this drug, one or two, the least valuable of the lot, be treated first to determine tolerance for the drug. Such animals should be kept under observation for a week before others are treated. If no bad results are observed, the remaining animals should be treated a few at a time and the observations repeated. This procedure should be followed until the entire group has been treated.

Phenothiazine may be administered to horses in gelatine capsules or in a suitable suspension. In order to make a suspension of phenothiazine suitable for administration to animals, it is necessary to use some chemical as a dispersing agent. Numerous dispersing agents are known and many of these have been employed; however, few of these suspensions have been given critical test. It is known that certain agents will greatly reduce the efficacy of phenothiazine when such chemicals are employed as suspending agents. Therefore, it is not advisable to employ such mixtures unless they have been tested critically. A formula which has been found satisfactory consists of phenothiazine 50 grams (1.67 ounces), molasses 20 cc. (0.67 fluid ounces), and water to make 90 cc. (3 fluid ounces). The molasses is thoroughly mixed with the phenothiazine, then a small portion of water is added and thoroughly stirred in. The process of alternately adding water in small quantities and of stirring is repeated until a smooth suspension of the required volume results. Also, phenothiazine may be mixed with almost any ground feed. Since certain animals do not take readily feed medicated with phenothiazine, the following regimen may be employed:

For one week prior to the administration of the drug the horse should receive no salt. During this time it should receive daily one pint of a mixture containing equal parts of oats and bran to which 50 grams (about 2 fluid ounces) of molasses have been added. For administration of the drug, 50 grams (1.67 ounces) of phenothiazine is incorporated in about 150 grams (about 5 fluid ounces) of molasses, and this mixture is mingled thoroughly with 2 quarts of an oats bran mixture. Two ounces of salt are added to this formula. While fasting is unnecessary, the medicated mixture should not be placed before the horse until the animal is hungry. If the horse hesitates to eat the medicated mixture, it may often be encouraged to do so by sprinkling a small quantity of untreated oats or corn over the surface of the mixture. Even if the drug is administered in the feed, it is better to treat horses individually rather than to attempt mass treatment.

#### TREATMENT FOR LUNGWORM INFECTION

Skrjabin and Schul'ts (1936) and Kulikov and Tamarin (1937) have advocated the use of iodine in a solution of potassium iodide for the removal of lungworms from horses. The material is injected intratracheally while the horse is on its back, slightly inclined to one side for the first half of the injection, and to the other side for the remainder. The dose is 250 to 300 cc. of a 0.1 percent solution of iodine in a 0.2 percent solution of potassium iodide.

#### TREATMENT FOR OXYURIS EQUI INFECTION

Oil of turpentine is an effective treatment for the removal of pinworms from the horse. Animals should be fasted for

36 hours and the drug administered in a dose of 2 fluid ounces (60 cc.) for a 1,000 pound animal, immediately preceded or followed by 1 quart of raw linseed oil. The drug should not be given to animals already suffering from renal disorders.

Oil of chenopodium, as administered for large and small strongyles, is effective also for the removal of pinworms.

#### TREATMENT FOR THE REMOVAL OF STOMACH WORMS

Carbon disulphide in a dose of 6 fluid drams (24 cc.) for a 1,000-pound animal, preceded by gastric lavage with 8 to 10 liters of a 2 percent solution of sodium bicarbonate, is very effective for the destruction of *Habronema muscae* and *H. microstoma*, as determined by Wright, Bozicevich and Underwood (1931). Without preliminary lavage, the drug gave less favorable results in the tests of these investigators. Apparently the alkaline solution serves to remove excess mucus from the stomach wall and permits the drug to reach the parasites more effectively. Furthermore, the solution seems to give some protection against the irritating action of the carbon disulphide. In the above-mentioned experiments, *Draschia megastoma* in stomach tumors was not affected by the treatment. It is advisable, though not necessary, to siphon off the sodium bicarbonate solution 5 to 10 minutes after its administration. The contraindications for the treatment are the same as those listed under the discussion of this drug for the removal of ascarids.

While not determined by critical tests, it would appear that carbon disulphide would be a fairly satisfactory treatment for the destruction of *Trichostrongylus axei* in the stomach of the horse.

### Anthelmintic Medication for Nematode Parasites of Ruminants

#### TREATMENT FOR STRONGYLOIDES PAPILLOSUS INFECTION

There is no established treatment for strongyloidosis. The parasite appears to be resistant to most of the anthelmintics commonly employed in sheep and even prolonged dosage with some of these drugs fails to eradicate it. Gentian violet, the only known drug which has shown any specificity against worms of this genus, has not been tried in ruminants.

#### TREATMENT FOR INFECTION WITH TRICHURIS SPP.

Like whipworms in other animals, those forms occurring in ruminants are difficult to remove. Occasional whipworms will be removed by many of the anthelmintics used for the removal of other worms from sheep but there is no specific treatment available at the present writing. The enema treatment described under therapy for oesophagostomiasis is said to be fairly effective against *T. oris*. However, in view of the lack of evidence concerning the pathogenicity of the parasite, there would be little need for the use of the treatment in uncomplicated infections.

#### TREATMENT FOR INFECTIONS WITH OESOPHAGOSTOMUM SPP.

**PHENOTHIAZINE.** The introduction of phenothiazine by Harwood, Habermann and Jerstad (1939), provided the first anthelmintic which is useful for the removal of *O. columbianum* when administered orally in a single dose. These investigators found that the conditioned drug administered as 20 percent of a meal of concentrates after a period of fasting at a dose rate of 0.5 gram per pound of body weight removed 90 percent of the nodular worms, almost 80 percent of the *Hacmonchus*, 76.7 percent of the hookworms, and apparently 100 percent of the *Ostertagia*. Subsequent investigations in the U. S. Bureau of Animal Industry (Habermann and Harwood, 1939; Habermann, Harwood, and Hunt, 1940) demonstrated that either recrystallized phenothiazine or the crude non-conditioned drug was even more effective than the product which had been conditioned for use as an insecticide and which was employed in the earlier tests. A dose of 25 grams has been recommended for adult sheep. These results have been confirmed by a number of other workers, including Swales (1939), Roberts (1939) and Gordon (1939).

Swales administered phenothiazine in an enema without obtaining any efficacy for the removal of *Chabertia ovina* or *O. columbianum*. This finding would seem to confirm the view of Harwood and his associates that the drug probably undergoes some chemical change in the digestive tract of the host which acts to promote its efficacy. Failing to find that sheep regularly consumed mixtures of the anthelmintic with the feed, Swales reduced the bulk of the commercial product by preparing compressed tablets according to the following formula:

Commercial phenothiazine (pulverized) . . . . . 80 parts  
Starch (pulverized) . . . . . 8 parts

Effervescent salt (sodium bicarbonate, 50 parts, dehydrated tartaric acid, 45 parts) . . . . . 9 parts  
Dried ox gall . . . . . 2 parts  
Phenolphthalein . . . . . 1 part

The individual dosage of sheep at the rate of 0.3 gram of phenothiazine for each pound of body weight in Swales' experiments was very effective for the removal of *H. contortus*, *Bunostomum trigonocephalum*, *O. columbianum*, *Chabertia ovina*, *Nematodirus* sp., *Cooperia* sp. and *Ostertagia* sp. The treatment was approximately 50 percent effective for the removal of *Trichostrongylus* spp. but was apparently ineffective against *Strongyloides* and *Capillaria longipes*.

Roberts (1939) tested Thiox, a commercial preparation containing 93 percent phenothiazine, on a large number of animals and found that satisfactory results were secured against *O. columbianum* with a dose rate of 0.15 gram per pound of body weight given immediately after previous stimulation of the oesophageal reflex with 2 cc. of a 10 percent copper sulphate solution and following a 24-hour fast. Without fasting or the use of the copper sulphate, a dose of 0.4 gram per pound was effective. Good results were obtained also against *H. contortus* but the treatment failed to remove *Trichuris parvispiculum* and *T. globulosa*. In certain of Roberts' tests some of the sheep failed to respond to treatment even with the higher dose rate of the drug.

Gordon (1939) confirmed also results against *H. contortus* and nodular worms. He found also that phenothiazine in a dose of 0.6 gram per kilogram (2.2 pounds) of body weight reduced the egg counts of *Trichostrongylus* spp. by 90 percent or more. Small daily doses of the drug (1 gram daily for 5 days) were effective against the common stomach worm and the nodular worm.

**INSOLUBLE COPPER SALTS.** A treatment which was developed by Mönnig (1935) in South Africa is of considerable value against *O. columbianum* but does not approach the efficacy of phenothiazine. This treatment consists in the administration of certain insoluble salts of copper in the proportions and doses given below:

| Drug                | Parts | Dosage in grams for sheep<br>of various ages |                        |                   |
|---------------------|-------|--|------------------------|-------------------|
|                     |       | Over 6                                       |                        |                   |
|                     |       | 3 to 6<br>months                             | and under<br>18 months | Over<br>18 months |
| Copper arsenate     | 2     | 0.2  | 0.36                   | 0.5               |
| Calcium hydroxide   | 3     | 0.3  | 0.54                   | 0.75              |
| Copper tartrate     | 5     | 0.5  | 0.9                    | 1.25              |
| Total dose in grams |       | 1.0  | 1.8                    | 2.5               |

In an effort to deliver the mixture into the abomasum, the sheep are given 2.5 cc. each of a 10 percent solution of copper sulphate and this is followed immediately by the appropriate dose of the mixture. Sheep should be watered immediately before treatment but should be fasted for 48 hours prior to dosing and 24 hours after dosing. Water should be withheld for 1 to 2 hours after treatment. Recommendations call for repeating the treatment on the following day. The treatment is said to have a fair degree of efficacy against *H. contortus* and *Moniezia expansa*.

**ENEMA TREATMENT.** This treatment, originally recommended by Brumpt 35 years ago, has more recently been developed further by South African and Australian workers. The treatment consists of a solution of 2 grains (125 mgm.) of sodium arsenite per liter of water. The solution is administered by enema in the following doses:

Lambs up to 4 months of age . . . . . 1 pint  
Lambs 4 to 6 months . . . . . 1.5 pints  
Six months old to 2-tooth sheep . . . . . 1 quart  
Aged sheep . . . . . 1.5-2 qts.

Sheep should be fasted for 24 hours before treatment. The solution may be allowed to flow by gravity, may be given by syringe, or may be injected by a specially devised apparatus. The forced injection of the solution involves some risk and deaths have followed its use. This method of treatment is time consuming and ill adapted to large scale application.

Swanson, Porter and Connelly (1940) found that unconditioned phenothiazine removed 99.8 percent of *O. radiatum* from calves when the drug was administered in doses varying from 50 to 80 grams (0.44 to 1.1 gram per kilogram of body weight). While it is not possible to make definite therapeutic recommendations on the basis of these preliminary experiments, it would appear that phenothiazine is of considerable promise for the removal of nodular worms from cattle.

#### TREATMENT FOR CHABERTIA OVINA INFECTION

As previously stated, Swales found phenothiazine 100 percent effective for the removal of these worms from a limited number of animals. Habermann and Harwood (1939) reported

later that non-conditioned phenothiazine in doses of 9.7 to 12 grams removed 26.7 percent of 126 *Chabertia* from 6 sheep; in doses of 15 to 20 grams, 47.9 percent of 48 worms from 5 sheep; and in doses of 22 to 26 grams, 93.8 percent of 32 worms from 3 sheep. The enema treatment described under *O. columbianum* is said to be satisfactory also.

#### TREATMENT FOR GAIGERIA PACHYSCELIS INFECTION

Ortlepp (1935) found tetrachlorethylene effective for the removal of these worms when given in a dose of 10 cc. in 10 to 20 cc. of liquid paraffin and immediately preceded by a dose of 2.5 cc. of a 10 percent solution of copper sulphate to stimulate closure of the oesophageal groove. Smaller doses should be used for lambs and young sheep. Fasting is not necessary. Treatment should be repeated twice at intervals of 10 to 14 days. Some reactions may be encountered with this relatively large dose of tetrachlorethylene.

To reduce the number of reactions, Ortlepp and Mönning (1936) investigated a number of preparations containing tetrachlorethylene. The results of a limited number of experimental trials suggested that an emulsion consisting of 25 cc. of an aqueous solution of 7.7 grams of soft soap, 37.5 cc. of tetrachlorethylene and 37.5 cc. of liquid paraffin might be satisfactory. Later croton oil was added in a dose of 1 cc. to each 40 cc. of the emulsion which contained 10 cc. of tetrachlorethylene, this amount of the emulsion being the dose for an adult animal.

Subsequently, certain disadvantages were encountered in the use of this emulsion and Mönning and Ortlepp (1939) conducted further experiments in order to devise a more satisfactory vehicle for the tetrachlorethylene. The formula finally worked out was made up, as follows: To 500 cc. of tapwater heated to 70° C. are added 6 grams NaOH and then 40 grams of casein is rapidly stirred in. The solution is then heated to 85° C. and 40 grams of ground resin is stirred in rapidly. A highly saponifiable resin should be employed and this should be ground to a fine powder. The solution should be kept at 85° C. and stirred for about 15 to 20 minutes until complete combination of the alkali with the other ingredients has occurred. It is then made up to 800 cc. with cool water. The tetrachlorethylene is added in successive small quantities while mixing proceeds in the proportion of 75 cc. of tetrachlorethylene to 25 cc. of the emulsifier. The emulsion is issued in concentrated form and is diluted with an equal quantity of water before use. The dose for an adult sheep is 20 cc. of the diluted emulsion (i.e. 7.5 cc. of tetrachlorethylene) for adult animals; 15 cc. for lambs 6 to 12 months old, and 10 cc. for lambs 3 to 6 months old. The remedy is given after a preliminary dose of 2.5 cc. of 10 percent copper sulphate solution. This treatment is said to be effective against *Haemonchus contortus*, *Trichostrongylus* spp., *Nematodirus* spp., *Gaigeria pachyscelis* and *Bunostomum trigonocephalum*.

While no tests are on record, it would seem that phenothiazine would be effective against *Gaigeria*.

#### TREATMENT FOR BUNOSTOMUM TRIGONOCEPHALUM INFECTION

The phenothiazine treatment, as described under nodular worms, is the most effective available at the present time. Tetrachlorethylene, as used against *Gaigeria*, is also quite effective but is probably more hazardous. The "Cu-Nic" solution, described under treatment for the common stomach worm, will usually remove a high percentage of hookworms.

Little is known concerning removal of *B. phlebotomum* from cattle but it is probable that the "Cu-Nic" solution would be of value. For dosage, the reader is referred to treatment for *H. contortus*. Swanson, Porter and Connelly (1940) reported that phenothiazine in dosages of 50 to 80 grams removed 1,160 of 1,729 *B. phlebotomum* from 6 calves. In these limited tests, the unconditioned drug at a dose rate of 0.44 to 0.55 gram per kilogram of body weight in calves weighing over 91 kilograms (200 pounds) was less effective against this species than when given to lighter calves at a dose rate of 0.66 to 1.1 grams per kilogram of body weight. The drug is promising but more information is needed concerning its exact efficacy and its safety for cattle.

#### TREATMENT FOR HAEMONCHUS CONTORTUS INFECTION

**COPPER SULPHATE.** The treatment which has been used most extensively is the copper sulphate solution devised by Hutecheon (1891) in South Africa. In the United States a 1 percent solution has been employed most commonly and this is administered in a dose of 50 cc. for sheep up to a year old and 100 cc. for mature sheep. The dose for calves is 100 cc., for yearlings up to 200 cc., and for mature cattle up to 1 liter. Where sheep cannot be adequately protected against severe infection by light stocking, pasture rotation and other measures,

treatment should be repeated in temperate climates under conditions of moderate stocking at intervals of 3 weeks. In warm climates with heavy stocking of pastures, it is necessary to repeat treatment every 2 weeks. It is usually necessary to continue treatment throughout the warmer months of the year and in warmer climates it may be of value to continue dosing during the winter. The repeated administration of copper sulphate is apparently not detrimental to sheep and in fact there is some evidence to indicate that animals so treated make better gains than non-treated animals. Wright and Bozirevich (1931) found that the 1 percent solution of copper sulphate may be administered in the usual doses as often as once a week without harm to sheep. When so administered, there is a marked increase in the copper content of the liver after a period of time with no appreciable pathological changes. In view of the relationship of copper salts and liver to anemia, the increased amount of copper in the livers of sheep treated frequently with copper sulphate solution may be beneficial rather than detrimental to the health of the animals.

The 1 percent solution may be made up on the basis of 1 gram of copper sulphate to 59 cc. of water, or by dissolving  $\frac{1}{4}$  pound of copper sulphate in 1 pint of boiling water and adding cold water to make 3 gallons of the solution. This latter will make a quantity sufficient to dose 100 sheep, allowing 10 percent for waste. Only clear blue crystals should be used in preparing the solution. Porcelain, enamelware or wooden vessels should be employed, as copper sulphate solution will corrode metal.

Various writers have advocated the use of a 1.5 percent solution or stronger solutions of copper sulphate. As shown by Clunies Ross (1934) and Mönning and Quin (1935) solutions of this salt stimulate the closure of the oesophageal groove in sheep with a resultant delivery of the solution directly into the abomasum in a considerable proportion of the cases. No doubt this quality of copper sulphate is responsible to a considerable extent for its efficacy against *H. contortus*. Gordon (1939) has shown recently that the usually prescribed dose in Australia, i.e., 1 fluid ounce of a 4 percent solution of copper sulphate, is not an efficient treatment against *H. contortus* in adult sheep. His investigations indicated that a dose of 2 fluid ounces of a solution of this strength was very effective in adult sheep. Gordon (1939) determined also that the copper sulphate treatment is relatively ineffective against immature *Haemonchus* and stated that apparent failures of treatment in severe outbreaks of haemonchosis in the field could be explained on this basis. As a result of the findings, Gordon suggested that in flocks heavily infected with the parasite, treatment with copper sulphate should be applied every 10 to 14 days.

**COPPER SULPHATE AND NICOTINE SULPHATE.** As previously stated, the copper sulphate and tobacco solution of Lewis and Guberlet and the nicotine sulphate solution of Lamson have been replaced largely by the "Cu-Nic" solution developed by Curtice. This latter solution is made up by adding 1 ounce of 40 percent nicotine sulphate to each gallon of a 1 percent solution of copper sulphate. The dose of the combined solution is 3.5 ounces (100 cc.) for adult animals and 1.5 ounces (50 cc.) for weanling lambs. Experience has shown that this solution is occasionally toxic for weak animals or for very young lambs. Furthermore, the operator should be certain that the precipitate, which forms in this mixture, is not allowed to settle in the container from which the sheep are being dosed. If there is any reason to believe that animals will not tolerate the treatment, the dose should be reduced or trial treatment should be made on a few animals to establish tolerance. For cattle, the "Cu-Nic" solution may be used in the same doses as for the 1 percent solution of copper sulphate. For sheep, the solution is effective against immature as well as mature *Haemonchus* and in addition is a fairly satisfactory treatment for the removal of hookworms, small trichostrongyles and *Moniezia expansa*. In uncomplicated stomach worm infections, it is probably of no great advantage over copper sulphate solution because of its greater toxicity.

**CARBON TETRACHLORIDE.** While the use of this drug in uncomplicated stomach worm infections has been largely discontinued in the United States, it is still popular in Australia, especially in concomitant liver fluke infections. In the United States, it is usually given in gelatin capsules in a dose of 5 cc. for adult sheep and 2.5 cc. for weanling lambs. In Australia, the doses employed are 2 cc. for adult sheep, and 1 cc. for lambs under 6 months of age, the drug being mixed with 4 parts of liquid paraffin. Not infrequently losses follow the use of carbon tetrachloride in sheep and for this reason it should be used with caution. Where the drug is used, precaution should be taken to place the sheep on a diet rich in available calcium for 2 to 3 weeks prior to treatment. Changes in feed other than to provide calcium should not be made for several weeks prior to treatment; animals appear to suffer

fewer reactions when maintained for several weeks on pasture prior to and subsequent to treatment with carbon tetrachloride.

**TETRACHLORETHYLENE.** This treatment, first tested by Hall and Shillinger (unpublished data), has been employed extensively. In the United States, the drug is administered in gelatin capsules in a dose of 5 cc. for adult sheep and 2.5 cc. for lambs. In Australia, it is combined with equal parts of liquid paraffin. While several investigators have reported it to be very effective against the common stomach worm, there is some divergence of opinion on this point with the probability that its efficacy does not approach that of carbon tetrachloride. However, tetrachlorethylene is a much safer treatment. In repeated treatments over a period of time, it is of some value in mixed infections involving stomach worms, hookworms and small trichostrongyles.

**COPPER SULPHATE AND SODIUM ARSENITE.** This mixture in powder form has long been employed in South Africa, where it is known as the "Government Wireworm Remedy." It is composed of 4 parts of copper sulphate, partly dehydrated, and 1 part of sodium arsenite. Special measuring spoons are employed to insure correct dosage, which is as follows: For lambs 2 to 4 months old, 0.2 gram of the mixture; 4 to 6 months old, 0.25 gram; 6 months to 2-tooth animals, 0.375 gram; 2-tooth sheep, 0.5 gram; 4-tooth sheep and over, 0.625 gram. The remedy is said to be contraindicated in the presence of heavy *Trichostrongylus* infections; smaller doses should be used when the sheep are in poor condition or when they are grazing on young grass in the spring. All animals should be kept from water for at least 7 hours before and after treatment; otherwise rapid absorption of the drug may occur and lead to arsenical poisoning.

**PHENOTHIAZINE.** Evidence presented under the discussion of the treatment against nodular worms indicates that this drug represents an effective treatment against *H. contortus*. While its use on more animals may disclose some limitations or contraindications, it appears to be of great value, particularly because of its efficacy in concomitant infections with many of the other gastro-intestinal parasites which are commonly found in sheep. If only the common stomach worm is present, doses as small as 10 grams per adult sheep may be employed; however, if other species are present, it is probably advisable to use a dose of 25 grams.

#### TREATMENT FOR INFECTIONS WITH SMALL TRICHOSTRONGYLES, TRICHOSTRONGYLES SPP., OSTERTAGIA SPP., COOPERIA SPP., AND NEMATODIRUS SPP.

On the basis of present evidence, phenothiazine is the treatment of choice for the removal of worms of these genera from sheep. From tests carried out to date it would appear that in sheep phenothiazine is probably less effective against *Cooperia* spp. and *Nematodirus* spp. than against these other genera. However, the general utilitarian value of the drug against most nematode parasites of the gastro-intestinal tract of sheep ranks it above all others at present. English investigators (Taylor and Sanderson, 1940; Lapage, 1940) have reported that sheep treated with doses of phenothiazine varying from 5 to 30 grams gained more rapidly than untreated controls; *Ostertagia* spp. were the principal nematodes encountered in the test animals.

The "Cu Nic" solution will frequently remove satisfactory percentages of these various worms. In Australia, Gordon and Clunies Ross (1936) found that sheep exposed to continual heavy infection with *Trichostrongylus* spp. were adequately protected by routine treatment at intervals of 3 weeks with a 2 percent solution of copper sulphate and commercial nicotine sulphate. Similar protection was obtained by the use of 15 cc. of a 2 percent solution of copper sulphate followed immediately by 2.5 cc. of tetrachlorethylene repeated at the same intervals. The recommended dose of the 2 percent copper sulphate and nicotine sulphate solution is as follows:

|                       |      |                 |
|-----------------------|------|-----------------|
| Adult sheep           | 2    | ounces (60 cc.) |
| Sheep 12 to 18 months | 1.5  | ounces (45 cc.) |
| Lambs 8 to 12 month   | 1.0  | ounce (30 cc.)  |
| Lambs 4 to 8 months   | 0.75 | ounce (22 cc.)  |
| Lambs under 4 months  | 0.5  | ounce (15 cc.)  |

Treatment for the removal of worms of the above-mentioned genera from cattle is not well established. In limited experiments, Swanson, Porter and Connelly (1940) found that unconditioned phenothiazine administered to calves in doses of 50 to 80 grams (0.44 to 1.1 grams per kilogram of body weight) removed all *T. axei* present. The treatment was approximately 84 percent effective against *O. ostertagi* but only slightly effective against *Cooperia* spp.

#### TREATMENT FOR LUNGWORM INFECTION

*Protostrongylus* and *Muellerius* infections are not susceptible to treatment but numerous drugs have been recommended

against *Dictyocaulus filaria*, chiefly for administration by insufflation or by intratracheal injection. Evidence for the use of these preparations is not convincing. Orloff (1935) recommended injections into the trachea on 2 successive days of 10 cc. of a mixture of 1 cc. of a 10 percent tincture of iodine, 50 cc. of glycerin and 150 cc. of distilled water. The sheep are placed on the back and after injection are held in a sitting position for half a minute. McGrath (1931) found Lugol's solution of no value and responsible for the causation of pneumonic lesions. McGrath believed that the intratracheal injection of the mixture recommended by the New South Wales Department of Agriculture provided good results. This mixture consisted of chloroform 0.5 cc., oleum terebinthinae I cc., and olive oil 2 cc. However, Kausal (1932) was not successful in removing all worms with this mixture. Velu and Zottner (1937) used a dose of 10 cc. of an aqueous solution of 1 mgm. of pyrethrin per dose; this was repeated three times. These treatments are for sheep but could probably be used for calves also.

Until more substantial evidence is obtained for the value of medicinal treatment, the most logical procedure is to give infected animals good nursing treatment, remove them from infected pastures and provide feed which will satisfy all nutritional requirements.

## Anthelmintic Medication for Nematodes of Poultry

### TREATMENT FOR STRONGYLOIDES INFECTION

Gentian violet exerts a specific action against *Strongyloides avium* and, in birds in which treatment is indicated, this would be the drug of choice. Wright and Van Volkenberg (1937) found that a dose of 1 grain (64 mgm.) three times a day for 10 days for birds weighing 3 to 4 pounds removed all of these worms from the small intestine and the ceca. However, this course of treatment resulted in inflammation of the digestive tract. Single doses up to 10 grains were not effective.

### TREATMENT FOR CAPILLARIA COLUMBAE AND C. RETUSA INFECTIONS

There is no established treatment for the removal of capillarids from the lumen of the digestive tract. Carbon tetrachloride has been reported to be of value in chickens when given in a dose of 1 cc. and repeated in 1 week. However, the evidence for this is contradictory as other investigators have not obtained promising results with this drug either in chickens or pigeons. Thymol has been recommended for pigeons in a dose of 5 gm., repeated on alternate days until 3 doses have been given. The last dose is followed by castor oil. Perhaps halogenated hydrocarbons other than carbon tetrachloride might be of benefit, although to be of value any treatment would probably have to be repeated several times. In any case, worms in the ceca would be particularly difficult to remove.

### TREATMENT FOR ORNITHOSTRONGYLUS QUADRIRADIATUS INFECTION

Thymol has been reported to be of value though such reports have not been confirmed. Tetrachlorethylene in doses of 0.5 to 1 cc. will sometimes remove some of the worms, although it does not constitute a dependably effective treatment. In fact, Cuvillier (1937) stated that the lack of any anthelmintic of demonstrated efficacy against the parasite indicated the importance of applying preventive measures.

### TREATMENT FOR AMIDOSTOMUM ANSERIS INFECTION

Schmid (1930) treated geese with carbon tetrachloride in doses of 1 to 1.5 cc. in 8 cc. of flour paste injected into the crop and reported excellent results, the birds improving in condition and the losses in the flock being checked. Schumann (1930) had good results following doses of 1 cc. of carbon tetrachloride administered in gelatin capsules. Jerstad (1936) removed all of 11 *Amidostomum* from a goose with a single dose of 2 cc. of carbon tetrachloride.

### TREATMENT FOR SYNGAMUS TRACHEA INFECTION

Mechanical removal of the worms may be accomplished by means of a fine wire, a barbed feather or other similar devices commonly used by poultrymen. However, the method is tedious and time consuming and not adapted for flock treatment. Several workers have recommended the intratracheal injection of several drops of a 5 percent solution of Aniodol (trioxy-methylene).

Wehr, Harwood and Schaffer (1938) obtained an indicated efficacy of 98 percent for the removal of these worms by insufflation with barium antimonyl tartrate. The birds are placed in a tightly closed container and the finely powdered drug is dispersed throughout the air several times by means of a blower.

Single doses of a number of drugs administered orally will remove some heterakids but few of these drugs exhibit a dependable efficacy. It is probable that in most cases the drug does not actually penetrate into the ceca to any great extent. The flock treatment for *Ascaridia* with tobacco dust mixed with mash will remove some worms over a period of time but results are variable.

Recently McCulloch and Nicholson (1940) reported that phenothiazine in doses varying from 0.05 to 1 gram per bird removed 2,056 *Heterakis* from 12 chickens and failed to remove 889, but of the worms not removed all except 121 had been killed by the action of the drug by the time the birds came to necropsy. Also in repeated doses varying from 0.05 to 0.5 grams per dose, phenothiazine removed 4,663 worms from 15 chickens. All of the 277 *Heterakis* not removed by the time the birds came to necropsy had been killed by the action of the drug.

Rectal injections by means of a hard rubber enema syringe of a mixture of 0.1 cc. of oil of chenopodium in 5 cc. of cottonseed oil for a 1.5 pound (680 gram) bird were found by Hall and Shillinger (1923) to remove 90 percent of the heterakids from chickens. Probably double this dose would be effective for birds weighing 3 pounds (1.36 kgm.) or more. This mixture may be made up at the rate of 1 teaspoonful (4 cc.) of oil of chenopodium in 6 fluid ounces (180 cc.) of cottonseed oil, and given at the rate of  $\frac{1}{3}$  fluid ounce (10 cc.) for birds weighing 3 pounds or more, using a proportionately small dose for smaller birds. The two ingredients should be thoroughly mixed before administration. The tip of the syringe should be passed along the floor of the cloaca and the mixture injected slowly.

## TREATMENT FOR ASCARIDIA COLUMBAE INFECTION

Carbon tetrachloride in repeated doses of 0.5 to 2 cc. administered in liquid paraffin on several consecutive days has been found entirely effective in removing these worms from pigeons. Tetrachlorethylene in a dose of 0.6 cc., preceded and followed by sodium sulphate, is said to constitute an effective treatment.

## TREATMENT FOR ASCARIDIA GALLI INFECTION

Various treatments are available in single doses for the removal of these worms. Tetrachlorethylene in a dose of 1 cc. for average sized birds is very effective. For young chickens, the dose should be reduced in accordance with the weight of the bird. Carbon tetrachloride is not as effective as tetrachlorethylene but n-butylidene chloride in doses of 2 cc. for adult birds removed all ascarids from test birds. These drugs may be administered in gelatin capsules but care should be taken that the drugs do not enter the lungs.

Nicotine sulphate combined with Lloyd's alkaloidal reagent, a selected diatomaceous fuller's earth, is a very effective single dose treatment. Recommendations call for the administration to each bird of a No. 2 capsule containing 35 cgm. (5.45 grains) of a mixture of 6.6 cc. of 40 percent nicotine sulphate solution and 16 grams (4 drams) of Lloyd's reagent.

Certain flock treatments are effective for the removal of *Ascaridia* and obviate the individual treatment of birds. The one recommended first by the California experiment station calls for the addition to the mash of 2 percent by weight of tobacco dust containing at least 1.5 percent of nicotine; this mixture is fed for a period of 3 to 4 weeks and repeated at 3-week intervals. For a single flock treatment, the California station recommended the use of 1 teaspoonful of oil of chenopodium, thoroughly mixed with moist mash, for each lot of 12 chickens.

## TREATMENT FOR ONYSPHIRA MANSONI INFECTION

A treatment which has been found of value consists in the administration of 1 or 2 drops of a 5 percent solution of ereolin under the nictitating membrane of the eye which is first anaesthetized with cocaine or a 5 percent solution of butyn. The ereolin should be washed out promptly with water. The worms killed by the treatment are usually carried down the lacrimal duct. The use of a 10 percent solution of argyrol as a supplementary treatment is of value in relieving the irritation and in helping to control concomitant bacterial infection.

## TREATMENT FOR DISPHARYNX SPIRALIS INFECTION

Whitney (1925) tried carbon tetrachloride and turpentine in pigeons and believed the latter to be more effective. He gave a No. 0 gelatin capsule filled with dry magnesium sulphate 12 to 24 hours prior to treatment; one No. 0 capsule of turpentine was then given night and morning for 4 days, the last dose being followed by 2 cc. of castor oil. Pigeons treated by this method showed marked clinical improvement.

## Bibliography

- ASENJO, C. F. 1940. A preliminary study of the anthelmintic activity *in vitro* of fresh pineapple juice. *J. Am. Pharm. Assoc.*, v. 29(1):8-10.
- BOZICEVICI, J. and WRIGHT, W. H. 1935.—Carbon disulphide for the removal of stomach worms from swine. *Vet. Med.*, v. 30(9):390-393.
- BROWN, H. P. and AUSTIN, J. A. 1939. Treatment of heart worms in dogs with stibsol—a new drug. *J. Am. Vet. Med. Assoc.*, v. 95(752):566-569; correction v. 95(753):738.
- BROWN, H. W. 1932.—Treatment of pin worm (*Enterobius vermicularis*) infestation with hexylresorcinol. *Proc. Soc. Exper. Biol. & Med.*, v. 30(2):221-224.
- 1937.—Observations on the mode of action of several anthelmintics upon *Ascaris lumbricoides* (pig strain). *Am. J. Hyg.*, v. 26(1):72-83.
- CAIUS, J. F. and MHASKAR, K. S. 1923.—The correlation between the chemical composition of anthelmintics and their therapeutic values in connection with the hookworm inquiry in the Madras Presidency. XXII. Summary and conclusions. *Indian J. Med. Res.* v. 11(2):371-375.
- CHOPRA, R. N. and CHANDLER, A. C. 1928.—Anthelmintics and their uses in medical and veterinary practice. 291 pp., pls. 1-5, figs. 1-65. Baltimore.
- CHOPRA, R. N. and SUNDAR RAO, S. 1939.—Chemotherapy of filarial infection. *Indian J. Med. Res.*, v. 27(2):549-562.
- CLUNIES ROSS, I. 1934.—The passage of fluids through the ruminant stomach. II. With observations on the effect of long starvation on anthelmintic efficiency. *Austral. Vet. J.*, v. 10(1):11-23.
- CLUNIES ROSS, I. and GORDON, H. McL. 1934.—The influence of starvation on anthelmintic efficiency. *Austral. Vet. J.*, v. 10(4):135-142.
- 1935.—The effect of starvation on the anthelmintic efficiency of sodium arsenite and tetrachlorethylene. *Ibid.*, v. 11(3):106-109.
- CUVILLIER, E. 1937.—The nematode, *Ornithostrongylus quadriciliatus*, a parasite of the domesticated pigeon, U. S. Dept. Agric., Tech. Bull. No. 569. 36 pp.
- DEEDS, F., STOCKTON, A. B. and THOMAS, J. O. 1939.—Studies on phenothiazine. VIII. Antiseptic value of phenothiazine in urinary tract infections. *J. Pharmacol. & Exper. Therap.*, v. 65(4):353-371.
- DE RIVAS, D. 1926.—The effect of temperature on protozoan and metazoan parasites, and the application of intra-intestinal thermal therapy in parasitic and other affections of the intestine. *Am. J. Trop. Med.*, v. 6(1):47-73.
- 1927.—A further contribution on the treatment of parasitic and other affections of the intestine, by the "intra-intestinal thermal method." *Ibid.*, v. 7(6):389-403.
- 1936.—Diagnosis and treatment of intestinal parasitism by the intra-intestinal thermal method. *J. Parasit.*, v. 22(6):524, 531.
- EHLERS, G. H. 1931.—The anthelmintic treatment of infestations of the badger with spirurids (*Physaloptera* sp.), *J. Am. Vet. Med. Assoc.*, v. 78, n. s. v. 31(1):79-87.
- [FAUST, E. C.] 1930.—Gentian violet therapy for *Strongyloides* infection. [Editorial] *Internat. Med. Digest.*, v. 17(1):57-58.
- FERNÁN-NÚÑEZ, M. 1927.—A contribution to helminthic therapy. *J. Am. Med. Assoc.*, v. 88(12):903-905.
- FREEBORN, S. B. 1916.—Lung parasites. *Country Gentleman*, v. 81(17):889.
- GARIN, C. 1913.—Recherches physiologiques sur la fixation et le mode de nutrition de quelques nématodes parasites du tube digestif de l'homme et des animaux. *Ann. Univ. Lyon*, n. s., I: Sc. Méd., fasc. 34, 160 pp., figs. 1-55.
- GOLDEN, R. and O'CONNOR, F. W. 1934.—The roentgen treatment of filariasis. I. Chyluria. II. Filarial lymphangitis. *Tr. Roy. Soc. Trop. Med. & Hyg.*, v. 27(4):385-398.
- GORDON, H. McL. 1939a.—Anthelmintic efficiency against immature *Haemonchus contortus*. *Austral. Vet. J.*, v. 15(2):57-66.
- 1939b.—The efficiency of various concentrations of solutions of copper sulphate against *Haemonchus contortus*. *Ibid.*, v. 15(5):216-218.
- 1939c.—The anthelmintic efficiency of phenothiazine. *Ibid.*, v. 15(6):245-252.
- GORDON, H. McL. and CLUNIES ROSS, I. 1936.—Medicinal treatment of trichostrongylosis. Efficiency in lambs ex-

- posed to continuous infection. Austral. Vet. J., v. 12(3): 111-113.
- GRAHAME, T., MORGAN, D. O. and SLOANE, J. E. N. 1940.—Report on phenothiazine as an anthelmintic in horses. Vet. Rec., v. 52(36):660-663.
- HABERMANN, R. T. and HARWOOD, P. D. [1939].—Efficacy of recrystallized phenothiazine for the removal of nematodes from the gastrointestinal tract of sheep. Vet. Med., v. 35(1), Jan. 1940:24-29.
- HABERMANN, R. T., HARWOOD, P. D. and HUNT, W. H. 1940.—The efficacy of crude unconditioned phenothiazine for the removal of gastrointestinal parasites from sheep. Proc. Helm. Soc., Wash., v. 7(1):16-18.  
1941.—Critical tests with phenothiazine as an anthelmintic in horses. North Am. Vet., v. 22(2):85-92.
- HALL, M. C. and SHILLINGER, J. E. 1923.—The removal of heterakids from the ceca of chickens by rectal injections of anthelmintics. J. Am. Vet. Med. Assoc., v. 62, n. s. v. 15(5):623-630.  
[1925].—Critical tests of miscellaneous anthelmintics. J. Agric. Res., v. 29(7), Oct. 1, 1924:313-332.  
1926a.—Gastro-intestinal lavage with water at comparatively high temperatures for removing worms from dogs. Am. J. Trop. Med., v. 6(5):341-346.  
1926b.—Eine neue Methode zur Untersuchung der Wirkung der Antihelminthika mit besonderer Berücksichtigung des Merkurochroms. Arch. Schiffs- u. Tropen-Hyg., v. 30(9):391-396.
- HALL, M. C. and WIGDOR, M. 1926.—Miscellaneous anthelmintic investigations. J. Am. Vet. Med., Assoc., v. 69, n. s. v. 22(2):195-217.
- HANSON, K. B. 1933.—Tests of the efficacy of single treatments with tracheal brushes in the mechanical removal of lungworms from foxes. J. Am. Vet. Med. Assoc., v. 82, n. s. v. 35(1):12-33.
- HARWOOD, P. D., HABERMANN, R. T. and JERSTAD, A. C. 1939.—Efficacy of commercial phenothiazine in the removal of roundworms from sheep. Vet. Med., v. 34(7):440-443.
- HARWOOD, P. D., HABERMANN, R. T., ROBERTS, E. H. and HUNT, W. H. 1940.—Preliminary observations on the effectiveness of crude, unconditioned phenothiazine for the removal of worms from horses. Proc. Helm. Soc. Wash., v. 7(1):18-20.
- HARWOOD, P. D., JERSTAD, A. C. and SWANSON, L. E. 1938.—The efficacy of phenothiazine for the removal of ascarids and nodular worms from swine. J. Parasit., v. 24(6), Suppl.:16-17.
- HARWOOD, P. D., JERSTAD, A. C., UNDERWOOD, P. C. and SCHAFER, J. M. 1940.—The efficacy of n-butyl chloride for the removal of intestinal nematodes, especially whipworms, from dogs. North Am. Vet., v. 21(1):35-41.
- HARWOOD, P. D., UNDERWOOD, P. C. and SCHAFER, J. M. 1938.—Treatment of equine strongyloidosis with n-butyl chloride (preliminary note). North Am. Vet., v. 19(7):44-46.
- HATCHER, W. L. 1941.—Phenothiazine poisoning in horses. North Am. Vet., v. 22(3):159-160.
- HAYES, I. M. 1933.—Filariasis or heartworms in dogs. (Notes from discussion at 9. ann. short course for graduate veterinarians, Alabama Polytech. Inst., Feb. 6-11.) Vet. Med., v. 28(4):140-143.
- HUTCHESON, D. 1891.—Wire worms in sheep and goats, and their treatment with sulphate of copper. Agric. J. Cape Town, v. 3(19):179-181.
- JERSTAD, A. C. 1936.—The gizzard worm, *Amidostomum anseris*, of geese in western Washington. Reports of cases. J. Am. Vet. Med. Assoc., v. 89, n. s. v. 42(3):318-320.
- KAUZAL, G. 1932a.—Note on the treatment of lung worm, *Dictyocaulus filaria* (Rud., 1809), infestation of sheep. Austral. Vet. J., v. 8(1):25-28.  
1932b.—The use of carbon tetrachloride in the treatment of kidney worm infestation in the pig. Ibid., v. 8(2):68-69.
- KNOTT, J. 1938.—The treatment of filarial elephantiasis of the leg by bandaging. Tr. Roy. Soc. Trop. Med. & Hyg., v. 32(2):243-252.
- KULIKOV, N. S. and TAMARIN, I. B. 1937.—[A study of treatment of the dictyocaulosis of horses.] [Russian text.] Papers on Helminthology, 30 Year Jubilee, K. J. Skrjabin, p. 302-304.
- KWA TJAON SIOE. [1928].—Strongyloidosis and its treatment with gentian violet. Far East. Assoc. Trop. Med. Tr. 7 Cong. Calcutta (British India [Calcutta], Dec., 1927), v. 3:200-204.
- LAMBERT, S. M. 1923.—Methods of administering anthelmintics to remove whipworms, with a note on a new method. Am. J. Trop. Med., v. 3(4):297-305.
- LAMSON, P. D. and BROWN, H. W. 1935.—Anthelmintic studies on alkylhydroxybenzenes. III. 6-n-alkyl-meta cresols. J. Pharmacol. & Exper. Therap., v. 53(2):227-233.
- LAMSON, P. D., BROWN, H. W. and HARWOOD, P. D. 1934.—The anthelmintic properties of certain alkyl phenols. Am. J. Trop. Med., v. 14(5):467-478.
- LAMSON, P. D., BROWN, H. W., STUGHTON, R. W., HARWOOD, P. D., BALTZLY, R. and BASS, A. D. 1935a.—Anthelmintic studies on alkylhydroxybenzenes. II. Ortho- and para-alkylphenols. J. Pharmacol. & Exper. Therap., v. 53(2): 218-226.  
1935b.—Idem. IV. Isomerism in polyalkylphenols. Ibid., v. 53(2):234-238.  
1935c.—Idem. V. Phenols with other than normal alkyl side chains. Ibid., v. 53(2):239-249.
- LAMSON, P. D., BROWN, H. W. and WARD, C. B. [1930.] Hexyl-resorcinol in canine ascariasis. North Am. Vet., v. 12(1), Jan., 1931:17-21.  
1935.—Anthelmintic studies on alkylhydroxybenzenes. I. Alkylpolyhydroxybenzenes. J. Pharmacol. & Exper. Therap., v. 53(2):198-217.
- LAMSON, P. D. and WARD, C. B. 1932.—The chemotherapy of helminth infestations. J. Parasit., v. 18(3):173-199.
- DE LANGEN, C. D. 1928.—Anguillulosis and the syndrome of the "idiopathie hypereosinophilia." Meded. Dienst Volksgezondheid Nederl-Indië, Foreign Ed., v. 17(4):515-529.
- LAPAGE, G. 1940.—Experiments on the anthelmintic action of phenothiazine. Vet. Rec., v. 52(36):648-657.
- LO MONACO. 1896.—Sur l' action vermicide de la santonine et de quelques-uns de ses dérivés. Arch. Ital. Biol., v. 26:216-225.
- LUCAS. 1937.—[Microfilariae from a dog; treatment with emetin hydrochloride.] [Abstract of report before Queensland Vet. Assoc., Sept. 13.] Austral. Vet. J., v. 13(6): 260-261.
- MACCALLUM, W. G. 1921.—Chemotherapy in infestations with *Dirofilaria immitis*. J. Parasit., v. 7(4):189.
- MCCULLOCK, E. C. and NICHOLSON, L. G. 1940.—Phenothiazine for the removal of *Heterakis gallinae* from chickens. Vet. Med., v. 35(7):398-400.
- MCGRATH, T. T. 1931.—Some observations on the treatment of young sheep for "lungworm" (*Dictyocaulus filaria*) infestation by intratracheal injections. Dept. Agric., New South Wales, Vet. Res. Rpt., No. 6, parts 1-2:36-39.
- MALLOY, D. M. 1926.—Work of the Department of Uncinariasis of Nicaragua in 1924 and 1925. Bull. Internat. Health Board, v. 7(2):77-83.
- MANSON-BAHR, P. 1940.—Phenothiazine as an anthelmintic in threadworm and roundworm infections. Lancet, No. 6122, v. 239, v. 2(26):808-809.
- MÖNNIG, H. O. 1935.—The chemotherapy of oesophagostomiasis in sheep—II. Onderstepoort J. Vet. Sci. & Anim. Ind., v. 5(2):419-438.
- MÖNNIG, H. O. and ORTLEPP, R. J. 1939.—A tetrachlorethylene emulsion as an anthelmintic. Onderstepoort J. Vet. Sci. & Anim. Ind., v. 13(1):193-197.
- MÖNNIG, H. O. and QUIN, J. I. 1935.—Studies on the alimentary tract of the Merino sheep in South Africa. II. Investigations on the physiology of deglutition. Onderstepoort J. Vet. Sci. & Anim. Ind., v. 5(2):485-499.
- MORRIS, H. and MARTIN, J. A. 1931.—Santonin as an anthelmintic for swine. J. Am. Vet. Med. Assoc., v. 78, n. s. v. 31(4):531-538.
- MUELLER, J. F. 1929.—Studies on the microscopical anatomy and physiology of *Ascaris lumbricoides* and *Ascaris megalocephala*. Ztschr. Zellforsch. u. Mikr. Anat., v. 8(3):361-403, pls. 9-13, figs. 1-80.
- O'CONNOR, F. W. 1932.—The aetiology of the disease syndrome in *Wuchereria bancrofti* infections. Tr. Roy. Soc. Trop. Med. & Hyg., v. 26(1):13-47.
- ORLOFF, I. V. 1935.—Le traitement de la dictyocaulose des moutons et des veaux par la méthode des injections intratrachéales en U.R.S.S. Bull. Acad. Vét. France, v. 8(7): 390-401.
- ORTLEPP, R. J. 1935.—The sheep hookworm. Its appearance, life cycle, control and remedial measures. Farming in S. Africa, v. 10(106):10-11.
- ORTLEPP, R. J. and MÖNNIG, H. O. 1936.—Anthelmintic tests, chiefly with tetrachlorethylene, for the removal of the



- hookworm, *Gaigeria pachyseelis*, from infested sheep, with observations on the effects of this drug on other parasitic nematodes. Onderstepoort J. Vet. Sci. & Anim. Ind., v. 7(2):399-417.
- ROBBINS, B. H. 1930.—A proteolytic enzyme in ficin, the anthelmintic principle of Læche de Higuerón. J. Biol. Chem., v. 87(2):251-257.
- ROBERTS, F. H. S. 1939.—The value of phenothiazine in the treatment of oesophagostomiasis in sheep. Austral. Vet. J., v. 15(6):237-244.
- SCHMID, F. 1930.—Beitrag für Geflügelparasiten Behandlung. Tierärztl. Rundschau, Wittenberge, v. 36(19):313-316.
- SCHÜMAN, P. 1930.—Die durch *Strongylus nodularis* hervorgerufene Magenwurmseuche der Gänse. [Abstract.] Klin. Wochenschr., v. 9(48), Beilage: 1973.
- SCHWARTZ, B. 1921.—Hemotoxins from parasitic worms. J. Agric. Res., v. 22(8):379-432.
- SHCHERBOVICH, I. A. 1935.—(Emploi de la santonine dans l'ascaridose du porc). [Russian text; French summary.] Trudy Vsesoiuz. Inst. Gel'mint., v. 1: 143-148.
- SIMONELLI, A. 1936.—Sulla filariosi in Umbria. Nota clinica e terapeutica. N. Ereolani, v. 41(4):169-178.
- SKRJABIN, K. I. and SCHUL'TS, R. Ed. S. 1936.—Les helminthoses pulmonaires des animaux. Bull. Office Internat. Epizoot., v. 12:407-456.
- STRONG, S. B. 1918.—*Trichocephalus dispar*. So. Med. J., v. 11(5):345-347.
- SUCHANEK, H. 1932.—Operation eines Spirocerenknötens beim Hunde mit Hilfe des Oesophagoskopes; Heilung. Wien. Tierärztl. Monatschr., v. 19(13):392-395.
- SWALES, W. E. 1939.—Tests of phenothiazine, a highly efficient anthelmintic. Canadian J. Comp. Med., v. 3(7):188-194.
- SWANSON, L. E., HARWOOD, P. D. and CONNELLY, J. W. 1940.—Phenothiazine as an anthelmintic for the removal of intestinal worms from swine. J. Am. Vet. Med. Assoc., v. 96(756):333-338.
- SWANSON, L. E., PORTER, D. A. and CONNELLY, J. W. 1940.—Efficacy of nonconditioned phenothiazine in removing worms from the alimentary canal of cattle. J. Am. Vet. Med. Assoc., v. 96(759):704-707.
- TAYLOR, E. L. and SANDERSON, K. M. 1940.—Phenothiazine, a remarkably efficient anthelmintic. Vet. Rec., v. 52(36):635-647.
- TRENDELENBURG, P. 1915.—Über die Wirkung des Santonins und seiner Derivate auf die Wurmuskulatur, und Bemerkungen zur Wirkung des Oleum Chenopodii. Arch. Exper. Path. & Pharmacol., v. 79(2-3):190-218.
- TRUM, B. F. 1938.—Oil of chenopodium intravenously. Vet. Bull., U. S. Army, v. 32(2):114-117.
- UNDERWOOD, P. C., WRIGHT, W. H. and BOZICEVICH, J. 1931.—Critical experiments with the intraeecal method of injection for the treatment of whipworms in dogs. North Am. Vet., v. 12(10):41-47.
- VELU, H. and ZOTTNER, G. 1937.—Les pryéthrimines et la prophylaxie de la strongylose pulmonaire. Bull. Acad. Vét. France, v. 10(1):53-58.
- WEIR, E. E., HARWOOD, P. D. and SCHAFFER, J. M. 1938.—Barium antimonyl tartarate as a remedy for the removal of gapeworms from chickens. J. Parasit., v. 24(6), Suppl.: 17.
- WELLS, H. S. 1931.—Observations on the blood sucking activities of the hookworm, *Ancylostoma caninum*. J. Parasit., v. 17(4):167-182.
- WHITNEY, L. F. 1939.—Hydrogen peroxide as a general anthelmintic for dogs. Vet. Med., v. 34(9):560-566.
- WORTON, A. G., KEMPF, G. F., BURRIN, P. L. and BIBBINS, F. E. 1938.—A new enteric coating and a laboratory method for its control. J. Am. Pharm. Assoc., v. 27(1):21-28.
- WRIGHT, W. H. and BOZICEVICH, J. [1931].—Control of gastrointestinal parasites of sheep by weekly treatments with various anthelmintics. J. Agric. Res., v. 43 (12):1053-1069.
- WRIGHT, W. H., BOZICEVICH, J. and GORDON, L. S. 1937.—Studies on oxyuriasis. V. Therapy with single doses of tetrachlorethylene. J. Am. Med. Assoc., v. 109(8):570-573.
- WRIGHT, W. H., BOZICEVICH, J. and UNDERWOOD, P. C. 1931.—Critical experiments with carbon disulphide in the treatment of habronemiasis. J. Roy. Army Vet. Corps, v. 2(2):66-70.
- WRIGHT, W. H. and BRADY, F. J. 1940.—Studies on oxyuriasis. XXII. The efficacy of gentian violet in the treatment of pinworm infestation. J. Am. Med. Assoc., v. 114(10):861-866.
- WRIGHT, W. H., BRADY, F. J. and BOZICEVICH, J. 1938.—Studies on oxyuriasis. VIII. A preliminary note on therapy with gentian violet. Proc. Helm. Soc. Wash., v. 5(1):5-7.
- 1939.—Studies on oxyuriasis. XIV. Controlled tests with various methods of therapy. U. S. Pub. Health Rpts., v. 54(45):2005-2016.
- WRIGHT, W. H. and CRAM, E. B. 1937.—Studies on oxyuriasis. IV. Some aspects of the problem of therapy. Am. J. Dis. Children, v. 54(6):1276-1284.
- WRIGHT, W. H. and RAFFENSPERGER, H. B. 1930.—Anthelmintics for the removal of thorn-headed worms from swine. U. S. Dept. Agric. Misc. Pub. (79), 11 pp.
- WRIGHT, W. H. and SCHAFFER, J. M. 1931.—The anthelmintic value of some halogenated hydrocarbons containing iodine and bromine. J. Parasit., v. 18(2):134.
- 1932.—Critical anthelmintic tests of chlorinated alkyl hydrocarbons and a correlation between the anthelmintic efficacy, chemical structure, and physical properties. Am. J. Hyg., v. 16(2):325-428.
- WRIGHT, W. H., SCHAFFER, J. M., BOZICEVICH, J. and UNDERWOOD, P. C. 1937.—Critical anthelmintic tests of some primary monobrom hydrocarbons. Papers on helminthology, 30 Year Jubileum, K. J. Skrjabiu, pp. 769-779.
- WRIGHT, W. H. and UNDERWOOD, P. C. 1934.—Fouadin in the treatment of infestations with the dog heartworm, *Dirofilaria immitis*. Vet. Med., v. 29(6):234-246.
- WRIGHT, W. H. and VAN VOLKENBERT, H. L. 1937.—Critical tests with various dyes as anthelmintics for chickens. Rev. Med. Trop. y Parasit., v. 3(2):125-132.
- YOUNG, D. B. 1930.—Diagnosis of worm parasites in the fox. Am. Fur Breeder, v. 3(3):31.

## CHAPTER X

### FEEDING HABITS OF NEMATODE PARASITES OF VERTEBRATES

J. E. ACKERT and J. H. WHITLOCK, Kansas State College, Manhattan, Kansas

The obscured habitats of the parasitic nematodes preclude ready observations upon their feeding habits. Indications of their nutritive needs have been gained from chemical analyses of the worm bodies. Weinland (1901) found that glycogen made up one-fourth to one-third of the dry substance of the ascarid body. Flury (1912) was led to believe that ascarids had essentially the same chemical constitution as other animals. He found only such minor differences as a lack of uric acid, creatinine and the substitution of a high molecular alcohol (ascaryl alcohol) for glycerol in combination with fatty acids. From these studies it seemed probable that the nutritive needs of parasitic nematodes are fundamentally the same as those of other animals, although Ackert (1930) has shown that there is no evidence to indicate that *Ascaridia galli* needs Vitamin A, Vitamin B (complex), or Vitamin D.

As most of the research on the feeding habits of nematodes has been upon adult forms, they will be discussed first; then the larval forms will be compared with the adults, and the section will close with a brief review of digestion in the parasitic nematodes. Although there are many diverse groups of nematodes, few methods of parasitic feeding have been evolved. The similarity of these feeding habits in nematode groups which are widely separated morphologically would make a discussion of nutrition from a primarily taxonomic standpoint repetitious; hence the subject will be discussed from an ecological and physiological standpoint rather than from that of a morphological classification.

Ecologically, parasitic nematodes may be grouped as to whether they are associated with the physiological interior or exterior of the host body. The physiological exterior of the body, as here considered, is marked by any epithelial membrane lining a cavity which communicates with the exterior of the host body.

Most of the parasitic helminths are associated with the physiological exterior of the body, particularly the mucosae. This group will be subdivided upon the basis of being attached to the mucosae most of the time or usually unattached. Attached nematodes may hold their positions by the buccal capsule grasping the mucosa (*Ancylostoma*, *Necator*, *Strongylus*) or by penetration of the mucosa (*Physaloptera*, *Trichuris*). Nematodes unattached to the mucosa may be closely associated with it (*Haemonchus*, *Metastrongylus*) or not closely associated with it (*Ascaris*, *Ascaridia*, *Heterakis*, *Oxyuris*). Nematodes inhabiting the physiological interior of their hosts are best exemplified by *Dirofilaria*, *Spirocerca* and *Strongyloides*.

#### Nematodes in the Physiological Exterior of the Body

##### NEMATODES ATTACHED TO MUCOSA BY BUCCAL CAPSULE

The best examples of this group are the hookworms *Ancylostoma* and *Necator* which apparently remain attached to the intestinal mucosa much of the time. The sucker-like oral opening and the adjacent teeth or cutting plates afford effective means of attachment and blood letting. Since the earliest recorded observations, blood has been considered as a probable food of hookworms. Grassi, according to Leichtenstern (1886), saw hookworms eject blood both from the mouth and the anus. Leichtenstern thought, as did Grassi, that much more blood is withdrawn by the parasite than is necessary for its food. As the fecally deposited red cells seemed to be practically unchanged, Leichtenstern inferred that the plasma must be the main source of nourishment. In 1888 Ernst noted the emission of blood from the mouth capsule and Whipple (1909) observed the oral and anal emission of blood in both *Necator* and *Ancylostoma*. Whipple believed that there was a rapid ingestion of blood by the parasite. Ackert and Payne (1923) who took *Necator suillus* repeatedly from the intestines of freshly killed swine frequently noted female specimens with bodies colored red from ingested blood. On the other hand some workers, notably Looss (1905) and Ashford and Igaravidez (1911), maintained that blood is not the normal food of hookworms. They observed worms lacking blood even when they were attached to the intestine. They found tissue elements and shreds of mucosa in both the esophagus and intestine and concluded that the parasites fed primarily on the mucous membranes; the blood in the tract was thought to be due to accidental hemorrhage from hookworm bites. Support for the view that portions of the mucosae serve as food for such worms was given by Hoespli (1930), who found that *Ancylostoma duodenale* is more than a blood sucker. The piece of mucous membrane taken in by the mouth capsule is rasped by the teeth.

Blood from surface vessels pours into the buccal capsule where secretions from the esophageal glands partially digest the blood and loosened portions of the mucosae. "After this digestion has taken place, the liquefied masses are swallowed by the worm." Evidence that disintegration had taken place was furnished by staining the tissue at the bottom of the mouth capsule and in the lumen of the esophagus.

Wells (1931) in a series of ingenious experiments was able to observe living *Ancylostoma caninum* in the act of feeding. He was able to observe the attachment of the hookworms to the mucosa, study the details of the blood-sucking process, the passage of the blood through the intestine of the worm, and its ejection through the anal orifice. From the volumes of blood withdrawn by the parasite and the rapid rate at which it passed through the intestine, Wells was of the opinion that the food of the hookworm consists of simple diffusible substances in the host blood.

In studies upon the food of the dog hookworm, *Ancylostoma caninum*, Hsü (1938) made serial sections of hookworms taken from living hosts and found red blood cells in all worms; he also found fragments of host tissue and white blood cells all of which were in stages of disintegration. As further evidence of blood as food for the hookworms, Hsü reported the finding of pigment granules in the cytoplasm of the worms' intestinal cells, which gave positive iron reaction. These granules, which were found in large quantities throughout the whole intestine, the author interpreted as owing their origin to the breaking down of red blood corpuscles. Hsü did not find intestinal contents of the host, worm eggs, or bacteria in the hookworms' intestines. He concluded that the food of *A. caninum* consisted of blood and mucosa cells.

Other nematodes that attach by means of buccal capsules include such forms as *Strongylus*, *Chabertia* and *Camallanus*, all of which are known to draw intestinal epithelium into their mouths. From the disintegrated condition of the tissue so drawn in, it is probable that epithelial tissue and blood form a portion of their food. Whitlock (unpublished), who has worked extensively with living equine strongyles, has noted in the worm intestines material resembling partially digested blood. Wetzel (1931b), studying *Chabertia ovina* (Fab.) in sheep colons, found that the nematodes feeds on the propria mucosae which it draws into its buccal capsule. The tissue fragments which are loosened by the gnawing of the nematode are partially digested, according to Wetzel, by secretions from the dorsal esophageal gland.

Support of the view that *C. ovina* attacks the mucous membrane is afforded by the work of Kauzal (1936) who found numerous small hemorrhages on the mucous membrane of the large intestine of sheep which he attributed to *C. ovina*. He examined 250 of these specimens quantitatively for iron which he assumed to be derived from haemoglobin. The presence of the haemoglobin and the reddish tint of the intestinal contents of the immature *C. ovina* led Kauzal to infer that this nematode ingests considerable quantities of blood. That the attachment to the intestinal mucosae by the buccal capsule is a widespread feeding phenomenon among the Strongyloidea is further shown by Magath (1919) for *Camallanus americanus* in the turtle intestine and by Hoespli and Hsü (1931) for *Kaliocephalus* sp. in the enteric canal of snakes.

##### NEMATODES ATTACHED MUCH OF THE TIME BY PENETRATION OF THE MUCOSA

Typical examples of this group are *Trichuris* and *Physaloptera*. The food of *Trichuris* apparently is secured while the anterior extremity is imbedded in the mucous membrane of the large intestine. Christofferson (1914) who reviewed the literature on *Trichuris* (*Trichocephalus*) up to 1914, observed a peculiar cell transformation about the imbedded anterior portion of these nematodes. Hoespli (1927) found such changes in human and baboon intestines in which the *Trichuris* made tunnels in the mucosa parallel with the surface of the intestinal lining. Surrounding the anterior ends of the trichurids in these tunnels, the epithelial cells, according to Hoespli, were transformed into syncytial-like structures with eosinophilic homogeneous protoplasm and pyknotic nuclei as a result of the action of a liquifying secretion from the worm. Hoespli's studies (1927, 1933) led him to believe that the liquified syncytial material was taken by the trichurids as food.

That *Trichuris* may take blood was indicated by the studies of Guart (1908) who found blood engorged trichurids. Garin, cited by Otto (1935), likewise found *Trichuris* filled with blood and reported that blood was found in the stools of 50 out of

54 trichuris infected patients. In support of the view that these nematodes may feed on blood were the findings of Li (1933c) and Chitwood and Chitwood (1937) that the adult *Trichuris* bears a stylet capable of insertion sufficient for drawing blood. Chitwood and Chitwood (1937) showed that the anterior muscular part of the trichuroid esophagus possesses muscles capable of the dilation necessary for sucking. Moreover, they found by serial sections a large number of red corpuscles in the esophageal lumen of *Trichuris*. While Smirnov (1936), after a comprehensive study of the literature and of serial sections of worms, concluded that there was no convincing evidence that trichurids feed on blood, the fact remains that Whipple (1909) and Garin (1913) reported the occurrence of hemolytic enzymes in *Trichuris*. From the various studies made it appears that trichurids secure their food from the intestinal mucosa and that it may consist of liquified mucosal tissue and blood elements.

Studies on the food of *Trichinella spiralis* were made by Heller (1933) who introduced encysted trichina larvae enclosed in collodion sacs into the small intestines of cats and rats. While the meat around the larvae was digested in 6 to 8 hours, they made no growth in 1 to 3 days. *Trichinella* larvae enclosed in fine silk bags and thus kept away from the intestinal mucosa likewise did not develop. That these nearly adult trichinae do not feed on intestinal contents seems likely also from other tests by Heller who fed india ink along with meat containing encysted larvae, but failed to find any ink in the worms' intestines. Sections of intestinal tissue made after the encysted trichinae were fed showed that the freed larvae penetrated the intestinal mucosa, where the maturing trichinae doubtless secure their food.

Following the work of Heller, McCoy (1934) injected sterile *Trichinella* larvae from digested rat muscle into the amniotic sac of chick embryos 6 to 15 days old. Definite growth of the maturing larvae occurred in only about 1 or 2 percent of the trichinae. A single female developed to sexual maturity. Better success was attained in a second series of experiments in which the sterile larvae were injected into the amniotic sacs of rat embryos on approximately the 14th day of gestation. In 2 to 5 days, practically all worms were developing at nearly the normal rate and on the fifth day, numerous female trichinae were found with embryos developing in their uteri. These results give further evidence that *Trichinella* normally feeds upon host body fluids secured from the mucous membrane. Moreover, van Someren (1939) reported a functional buccal stylet in *T. spiralis* and indicated that it is used to lacerate the host tissue and release tissue fluids. From the examination of living specimens immediately after recovery, van Someren believed that the food, which is in a fluid state when ingested, consists of tissue fluids, cell contents, or perhaps predigested tissue acted on by a tissue lysate from the anterior esophageal glands.

Among other nematodes attached much of the time to the enteric mucosa is *Physaloptera*. Studies by Hoeppli and Feng (1931) showed that the mucosa about the anterior ends of these attached worms was liquified or partially digested, presumably from esophageal secretions from the nematodes. Studies of sectioned mucosa showed definite excavation of tissue immediately around the anterior ends of the worms, presumably from the taking of the liquified tissue as food.

#### UNATTACHED NEMATODES CLOSELY ASSOCIATED WITH THE MUCOSA

Many nematodes belonging to this group, while having poorly developed buccal capsules, are able to puncture the mucous membrane and draw blood. For example, Stadelmann (1891) found blood corpuscles in the nearly mature *Ostertagia ostertagi* (*Strongylus convolutus*) in nodules of the abomasum, and Dikmans and Andrews (1933) found such stages of *Ostertagia circumcincta* in the mucosa and partly free in the abomasal lumen of sheep. Unfortunately, however, much of the evidence is circumstantial. Thus Ransom (1911), writing of *Haemonchus contortus* and *Ostertagia ostertagi*, stated that they evidently suck blood for the heavily infected hosts are anaemic. Other writers simply state that they suck blood. Veglia's (1916) observations on living worms demonstrated that the oral lancet made definite cutting movements. Fallis (1938) placed *Nematodirus* among the blood suckers on the basis of a spectroscopical analysis of the body fluid which showed the absorption bands for oxyhaemoglobin. In the same year, Davey (1938), who studied the food of nematodes of the alimentary tract of sheep, questioned the spectroscopic demonstration of haemoglobin in nematodes as evidence of their being blood suckers. He found that haemoglobin was present in tissues other than the alimentary canal of *Nematodirus spathiger* and that its absorption bands had different positions from those in the blood of their hosts. These facts, together with his finding of haemoglobin in species of Trichostrongylidae long after any haemoglobin from the host would have decomposed, proved

that these nematodes could synthesize haemoglobin. Davey's (1938) culture tests with serum, blood digests, and defibrinated blood as food for *Ostertagia*, *Cooperia*, *Nematodirus* and *Trichostrongylus* were unsuccessful, as were also those on abomasum fluid for *Ostertagia circumcincta*. These negative results led him to the conclusion that these nematodes with rudimentary buccal capsules probably feed on tissue elements at or in the mucosa.

Other evidence of intimate association of trichostrongyles with the mucosa of the alimentary tract is available from rabbit nematodes. Alicata (1932) experimentally infected rabbits with *Obeliscoides cunctuli* by feeding infective larvae. Examination about 2 months later showed nematodes free on the mucous membrane of the stomach or under the membrane and into the submucosa. That such trichostrongyles feed from the enteric wall was the opinion of Wetzel and Enigk (1937) who, on infecting rabbits with *Graphidium strigosum*, found the stomach mucosa bloody. Enigk (1938) examined the intestinal contents of several sexually mature *G. strigosum* and found a colorless viscous mass containing nuclear remnants apparently from white blood cells, granules and bacteria. Other tests such as feeding the rabbits pulverized charcoal, trypan blue and carmine resulted in these substances being ingested by the nematodes. Also, injecting the hosts intravenously with trypan blue for several days resulted in the worms taking up several blue colored particles presumably desquamated mucosa cells. Enigk concluded that *G. strigosum*'s food consists of gastric mucosa, gastric juice and stomach contents.

Other unattached nematodes closely associated with the mucosa include lungworms which inhabit the bronchi and bronchioles. Hung (1926) studying swine lungs infected with adult *Metastrongylus elongatus* frequently found eosinophiles and red blood corpuscles in the worms' intestines. The findings of Porter (1936), who made similar studies, indicated that the material in the worms' intestines consisted of elements identical with those found in the exudate surrounding the nematodes. In cross sections of the worms, Porter recognized large numbers of eosinophilic and neutrophilic polymorphonuclear leucocytes, lymphocytes and desquamated epithelial cells. Erythrocytes were seen in some instances. These and some of the leucocytes and epithelial cells appeared to have been digested in part by the worms.

From the findings of the investigators cited, the food of many of the unattached strongyles appears to consist mainly of substances derived from the mucosa, namely, leucocytes, erythrocytes, lymphocytes, plasma, exudates and desquamated mucosa cells, but also of some extra-mucosal material such as stomach contents.

#### UNATTACHED NEMATODES NOT CLOSELY ASSOCIATED WITH THE MUCOSA

Chief among the parasitic nematodes not closely associated with the intestinal mucosa are members of the Oxyuroidea and Ascaridoidea. Among the early observations of the food of such nematodes were those of Leuckart (1876) who found that the intestine of *Enterobius vermicularis* usually contained yellow fluid which on microscopical examination proved to be identical with the liquid host feces. Similar observations were made by Leuckart on *Oxyuris equi* whose intestinal contents contained small particles of vegetable material identical with the contents of the horse intestine.

Early in the present century, Weinberg (1907) examined the intestines of many *Ascaris* specimens but could not find red blood corpuscles in them and expressed the opinion that the horse *Parascaris* feeds on the contents of the host intestine.

To ascertain whether *Ascaris* feeds upon intestinal contents, Vogel, cited by Hoeppli (1927), fed powdered animal charcoal to a human patient infected with *Ascaris*. The results of the first test were negative, but in a second test carried out similarly, numerous charcoal particles were found in the intestine of the worm. On the other hand, a number of early workers held to the view that the ascarids are blood suckers. This view was derived, in part, from microscopical and chemical examinations which showed evidence of blood in the intestines of *Ascaris* and related forms. For example, Mueller (1929), on studying specimens of *Anisakis simplex* from the sperm whale stated that the intestine, in all cases, contained blood in considerable quantities with occasional fragments of muscle and other tissues. From the quantities of blood corpuscles present, Mueller was of the opinion that the nematode had a blood-sucking habit. Mueller was unable to determine the nature of the intestinal contents of any other genus of the Anisakinae that he studied.

If such ascarid forms are blood suckers some specimens should be found in contact with the mucosa. Hoeppli (1927) reported on the examination of 350 cadavers in which large numbers of ascarids were found. No evidence was available to show that any of these nematodes were attached to the mu-

cosa. Hoeppli further stated that in Fülleborn's laboratory no cases had been found with the ascarid, *Toxocara canis*, attached to the dog intestine. Other workers on examining large numbers of horse intestines at slaughter houses always found *Parascaris equorum* free in the lumen of the gut.

Standard textbooks carry the statement that *Ascaris lumbricoides* feeds on intestinal contents but gnaws at the mucosa. This statement doubtless is due to the occasional finding of reddish spots in the intestinal epithelium in cases of ascarid infection. While such spots occur occasionally, those who have examined hundreds of mammalian and avian intestines which contained numerous ascarids can testify that in the great majority of cases, no evidences of the adult ascarids attaching the mucosa are available.

As to certain Ascaridoidea being attached to the intestinal wall presumably for feeding, Guiart, cited by Hoeppli (1927), found in the stomach of a dolphin the clear imprint of the worms' lips in pit-like depressions of the mucous membrane. Similar observations were made by Hoeppli (1927) on a *Contracaecum* sp. from a seal from northern waters. It is quite possible that instead of being attached, the dying worms pressed their anterior ends deeply into the mucous membrane of the dead host.

As to the food of ascarids, Archer and Peterson (1930), by giving patients infected with *Ascaris lumbricoides* a barium-cereal-meal, found that the enteric canals of the parasites showed string like shadows, indicating that the nematodes in the host intestine had swallowed the barium. These observations indicated that *Ascaris lumbricoides* feeds on the intestinal contents of man.

Following this work, Li (1933a) fed to six dogs, positive for ascarids, liquid chinese ink or powdered charcoal twice a day for several days. While most of the tests were negative, due presumably to a vermifugal action of the charcoal, one dog gave unquestioned positive evidence. The one female worm from the dog's intestine definitely showed charcoal and beef particles in its enteric tract. In a subsequent series of tests, Li (1933b) fed a mixture of powdered charcoal, clotted blood, striated beef muscle and starch granules to experimental animals harboring ascarids as follows: Dog, *Toxocara canis*; cat, *Toxascaris leonina*; and chicken, *Ascaridia galli*. The results from the dogs gave no positive evidence; that from the cat showed that the worm intestine contained charcoal, blood cells, and beef particles. These findings were confirmed by examination of paraffin sections of the worms. The results from four chickens showed charcoal and beef particles in the intestines of all worms including both male and female specimens. From similar experiments, in which starch granules were substituted for powdered charcoal, all worms recovered showed starch granules and some beef particles.

To ascertain the nature of food of the chicken cecal worm *Heterakis gallinae*, Li (1933b) fed infected chickens powdered charcoal and beef as before. On examination, most of the worms showed charcoal in the entire intestine. In further studies, Li opened the intestines of *Ascaris lumbricoides* from man and mounted the intestinal contents on slides for microscopic examination. While most of these contents could not be identified, Li found in one specimen two *Ascaris* eggs and a piece of striated muscle. The results of Li's experiments (1933a, 1933b) indicate that the intestinal contents of the host constitute part of the food of *Ascaris lumbricoides*, *Toxocara canis*, *Toxascaris leonina*, of mammals; and *Ascaridia galli* and *Heterakis gallinae* of fowls.

The findings of Li and of other workers cited, while showing that certain ascaroids take intestinal contents, do not preclude the possibility that these nematodes may also feed upon the intestinal epithelium. In a study of the food of the fowl nematode, *Ascaridia galli* (Schneider), Aekert and Whitlock (1935) deprived chickens infected with *Ascaridia galli* of food by mouth; the experimental chickens were nourished by intramuscular injections of glucose. The results of the first series of experiments on 141 chickens with worms of various ages indicated that little growth occurred in the worms after the host chickens were taken off the regular feed. In the second series in which 96 additional chickens were used, Aekert, Whitlock and Freeman (1940) used worm infections of one week's duration in the tests. Experimental and control chicks under comparison were of the same age and the developing worms were from the same egg cultures. The results of this series of tests were very uniform, namely, that in the chickens given only water *per os* and intramuscular injections of glucose, the young *Ascaridia galli* ceased growing whereas the worms in the regularly fed control chickens made normal growth. These results indicate that the large nematode of chickens whose mouth parts are very similar to those of mammalian ascarids, did not secure nutriment from the intestinal epithelium of the host. These nematodes may have fed to some extent on duodenal mucus from the goblet cells but Aekert, Edgar and Frick (1939) have shown recently that such mucus may contain an inhibitory

growth factor for young *Ascaridia* that have been grown in the culture media developed by Aekert, Todd and Tanner (1938). This last group of workers prepared a salt-dextrose solution in which young *Ascaridia galli* will grow. On the introduction of mucus from growing chickens into the nutrient solution, the cultured *Ascaridia* ceased growing, whereas the control worms in the nutrient solution continued to increase in length. In the glucose-injected chickens, the duodenal mucus, while containing an inhibitory growth factor, may have afforded the *Ascaridia galli* food sufficient for maintaining life, but not for growth.

The literature cites cases in which blood has been found in the digestive tracts of ascaroids. For example, Mueller (1929) found blood in the intestine of *Anisakis simplex* and Guiart, cited by Lievre (1934), saw some *Ascaris* whose digestive tracts were full of blood. On the other hand, Lievre cited Brumpt as having performed numerous autopsies to see if *Ascaris* caused ecchymotic spots on the mucosa. But Brumpt was unable to find such spots, and the intestinal contents of the worms showed only chyme, never blood.

Indirect evidence of blood as a nutrient of ascarids is available from the finding of haemoglobin in the worms' bodies by such tests as the Benzidine blood test and spectroscopic analysis. That the former is an unreliable test for blood has been shown recently by Davey (1938). Using spectroscopic analysis, Lievre (1934) found traces of haemoglobin in the intestine of the dog ascarid, *Toxocara canis*. Even though the spectroscopic examination of blood was positive in 75 percent of the cases, the quantity of haemoglobin noticed was so small that Lievre was led to think that the haemoglobin had come from the flesh colored food of the animal. Lievre, on macerating the intestines of *Ascaris lumbricoides*, *Parascaris equorum* and *Ascaris suum* was unable to find any haemoglobin present in these worms by spectroscopic analysis. He concluded that there is no haemophagia in *Ascaris* and only in exceptional circumstances would there be ingestion of blood. Davey (1938) demonstrated haemoglobin in the dermo-muscular tube of *Toxocara canis* and in tissues other than the alimentary canal of *Ascaris*. He found, further, that the absorption bands of the haemoglobin in the tissues had different positions from those in the blood of their hosts, indicating that these nematodes were able to synthesize haemoglobin. Thus the presence of haemoglobin in the tissues of nematodes is not necessarily evidence that they feed on blood.

Further indicative evidence that ascarids may take blood is available from the work of Schwartz (1921) who found that the body fluid of *Ascaris lumbricoides* inhibited coagulation of rabbit blood to a moderate extent. Extracts of *Parascaris equorum* and of *Toxocara* sp. had a slight effect on the coagulation of sheep's blood. Whether or not this property of the ascarid body is utilized by the living nematodes is unknown. It is conceivable that ascarid nematodes living with hookworms which are known to draw blood in excess would swallow blood from time to time. But as other writers have indicated, this would be exceptional rather than normal.

In the light of our present knowledge, it appears that the oxyuroids and ascaridoids feed normally on the intestinal contents of the host including also any mucus, desquamated mucosa cells, and blood elements that may be free in the lumen of the intestine.

## Nematodes in the Physiological Interior of the Body

The nematodes that live in the physiological interior of the body are exemplified by *Diroflaria*, *Spirocerca* and intra-mucosal *Strongyloides*. In a recent study, Hsü (1938a) was led to believe that *Diroflaria immitis* feeds exclusively on red and white blood cells. In the case of *Diplotriacna tricuspis*, Hsü (1938b), after studying the intestinal contents of this parasite of the crow, concluded that the worms' food consisted of the inflammatory exudate in the thoracic cavity. While there was evidence of blood being ingested, Hsü believed that it is not taken normally. As the adult *Wuchereria bancrofti* normally lives in lymph vessels and nodes, it doubtless normally feeds upon lymphocytes and other constituents of lymph. When such encapsulation as shown by Faust (1939) occurs, the encapsulated worm dies, apparently from lack of food.

In a further study of the food of nematodes, Hsü (1938a) concluded that *Spirocerca lupi* feeds on inflammatory cells that pass through the nodule walls.

Observations on the food of another nematode of this group, *Strongyloides stercoralis*, were recorded by Askanazy (1900) who concluded that the mother worms in the intestinal mucosa fed on chyle; he found no indication that they take blood. Faust (1935), studying *Strongyloides* in the mucosa of the jejunum, found evidence of lytic action by the female worms, particularly around the head of the worm where disintegration of the tissue was observed. Considering the facts that the adult females spend much of their time in the intestinal mucosa and

that they have not been observed to take blood, it is probable that they feed on chyle and the partially disintegrated tissues in their tunnels.

### The Food of Larval Parasitic Nematodes

The nutrition of the larval parasitic nematodes is fundamentally like that of adult parasitic or free-living nematodes subject to the modifications imposed by the environment and the structure of the larvae in question. For example, the researches of McCoy (1929), Lepage (1933, 1937) and Glaser and Stoll (1938) on the free-living stages of *Strongylina* have revealed no essential differences between the mode of nutrition of these immature forms and that of the free-living stages of Rhabdiasidae (see Chu, 1936) and Strongyloididae (see Faust, 1932). The feeding mechanisms (buccal capsule and rhabditoid esophagus) and sources of food (bacteria or fluid organic matter) are essentially the same. The method of feeding of *Rhabditis* as described by Chitwood and Chitwood (1938, p. 76 of this series) is probably typical of this group.

Of the larvae of heteroxenous, parasitic nematodes in their intermediate host, no complete study of the feeding habits is available. But their locations in the intermediate hosts are fundamentally similar to those of various adult forms in primary hosts. Since many of such larvae increase in size without the presence of reserve food stuff, they must secure their nourishment from their host. From the foregoing it would be logical to conclude that larval nematodes in secondary hosts feed as do adult nematodes in analogous positions in primary hosts. Inactive encysted forms are at such low levels of metabolic activity in both types of host that simple diffusion is probably more than adequate to maintain the parasite.

The nutrition of immature nematodes in a primary host is, as far as known, like that of adult nematodes in similar positions except for (a) larval nematodes carrying reserve food-stuff and (b) larval nematodes which may be nourished by diffusion. For the rest it is possible to find a larval nematode feeding habit identical with each major type of adult nematode method of feeding.

Wetzel (1930) has shown fourth stage *Oxyuris equi* to feed like adult *Strongylus* sp. Ortlepp (1937) found the same true of larval *Gaigeria pachyscelis*. According to Aekert (1931), *Ascaridia galli* larvae penetrate the mucosa of the small intestine and feed much as do *Physaloptera* sp. or *Strongyloides* sn.

In a study of *Cooperia curticei*, Andrews (1939) noted the third stage larvae feeding in the lumen of the gut. The fourth stage larvae of this parasite had their anterior ends in the crypts of Lieberkühn and grew while in this position indicating the same type of nutrition as that observed in the adult *Trichostrongylidae*.

Immature *Probstmayria vivipara* are found free in the gut tube like ascarids indicating a similar mode of nutrition. According to Ransom (1911) immature *Oesophagostoma columbianum* feed on the cheesy material in the nodule making their mode of nutrition essentially similar to such forms as *Gnathostoma*. Ascarid larvae in the blood stream ingest and digest blood cells, according to Smirnov (1935), hence resembling adult *Dirofilaria*. Wetzel (1931a) has reported a case of what he considers to be extra-intestinal digestion by the fourth stage larva of *Dermatophys veligera* which attaches to the intestinal mucosa by means of four cephalic hooks, a unique attachment mechanism in nematodes.

The recent development of culturing techniques for parasitic nematodes promises more information regarding their food. However, to date only one parasitic nematode has been cultured throughout its life cycle. This is *Necoplectana glaseri* which is parasitic in the Japanese beetle, *Popillia japonica* (Glaser, 1932). Attempts to grow *Haemonchus contortus* of sheep by Lepage (1933) and Glaser and Stoll (1938), and *Ascaridia galli* of chickens by Aekert, Todd and Tanner (1938) have been only partially successful. Hence, these are included with the discussion of the nutrition of the larval forms. No direct observations of the food of these parasitic nematodes have been made, but the fact that the nematodes have grown and developed in an artificial environment indicates that at least part of the environment is a source of food. Table 1 lists these attempts at culturing parasitic nematodes.

From these considerations it appears that the food of larval parasitic nematodes may include bacteria, enteric contents, vascular fluids and elements, and mucosal cells and tissues.

### Digestion in Parasitic Nematodes

Most of the parasitic nematodes are placed in intimate contact with the host's physiological fluids which carry nutrient materials to its cells. Since these nutrients are in their simplest diffusible form it might be assumed that much of the nourishment of parasitic nematodes is derived from this source and that no true digestion is required. However, a number of

TABLE 1.— Summary of attempts to culture parasitic nematodes.

| Author                         | Nematode                    | Degree of Growth  | Successful Culture Media                                    | Normal host      |
|--------------------------------|-----------------------------|-------------------|---|------------------|
| Glaser (1932)                  | <i>Necoplectana glaseri</i> | Complete          | Dextrose infusion with yeast                                | veal agar beetle |
| McCoy and Glaser (1936)        | <i>Necoplectana glaseri</i> | Complete          | Permented potato medium                                     | Japanese beetle  |
| McCoy and Girth (1938)         | <i>Necoplectana glaseri</i> | Complete          | Veal infusion & preservatives                               | Japanese beetle  |
| Glaser and Stoll (1938)        | <i>Haemonchus contortus</i> | Partial           | Agar, liver extract, sheep fourth larval defibrinated blood | Sheep            |
| Aekert, Todd and Tanner (1938) | <i>Ascaridia galli</i>      | Measurable growth | Incubating hens' eggs, starch, dextrose, commercial agar    | Chicken          |

workers have demonstrated the existence of a true digestion in phylogenetically widely separated parasitic nematodes; hence, it is probable that they all carry on some form of digestion. According to the location of the digestive processes, various workers have distinguished between an intestinal and extra-intestinal digestion. Much of the evidence of extra-intestinal digestion rests upon the occurrence of necrotic or cytolyzed material around the anterior attached end or within the buccal capsule of parasitic worms. That such a condition of the host tissue is so often interpreted as extra-intestinal digestion is somewhat questionable since the effect of parasite excretions, simple trauma, mechanical pressure, and heterophilogenous proteolytic enzymes upon the host tissue would produce many of the conditions described as extra-intestinal digestion. This form of digestion may be possible in some nematodes, however, since Hoeppli (1927) has discovered an epitheliolytic material present in the anterior end but not in the posterior portion of *Strongylus*.

The intestinal digestion in nematodes has been the subject of work by a considerable number of investigators. Most of the studies have been confined to the demonstrations of enzymes within extracts of the parasites. Because of the early workers' limited knowledge of enzyme action, much of their results need confirmation before they can be accepted. Flury (1912), for example, made no attempt to critically evaluate the research of other workers. He simply listed the worker's name and the enzymes which he reported. In little of this early work was the action of bacterial enzymes adequately controlled. The demonstration of a peptolytic enzyme in the gut of *Toxocara canis* by Abderhalden and Heise (1909) is questionable for this reason. Nor was any particular attempt made in the early work to differentiate between intracellular and extracellular enzymes. Most of it was done with extracts of the parasite being studied, and peroxidases and proteases were reported as though the question of their respective origins was of little importance.

Recent researches have been more accurate. Enigk (1938) showed that *Graphidium strigosum* produces an amylase and a protease which are active in the gut tube of the parasite. He was unable to demonstrate a lipase. Chitwood (1938) demonstrated in an extract of the esophagus of *Ascaris lumbricoides*, a proteolytic enzyme which was inactive at its isoelectric point (pH 8.0) and most active in a weak acid solution. The fact that such workers as Wetzel (1928) and Hoeppli and his co-workers have demonstrated digested epithelial cells within the alimentary tracts of certain parasitic nematodes, gives evidence of the existence of proteolytic enzymes in parasitic nematodes. Enigk's (1938) finding of a varying reaction in the gut tube of *Graphidium strigosum* (pH 7.0 at the ends and 4.4-4.8 in the middle), and Van Someren's (1939) report of an acid reaction in the intestine and rectum of *Trichinella spiralis* are additional confirmation of the presence of enzymes because alteration of the reaction of the digestive tract of animals is universally coordinated with the optimum pH for the enzymes present.

Anticoagulants in blood sucking nematodes have been demonstrated by a number of workers. While such products are not primarily digestive, they doubtless prevent blocking of the parasite's alimentary tract with clotted blood; hence, they are an aid to digestion. Such products have been reported by Schwartz (1921) and Hoeppli and Feng (1933).

Careful consideration of the relative values of the researches demonstrating the presence of enzymes leads to the conclusion that at least one and probably more proteolytic enzymes are

present in the intestine of many parasitic nematodes as well as at least one amylolytic enzyme. Demonstrations of lipases have been impossible or questionable. Although little research has been done on digestion in parasitic nematodes, the demonstration of these enzymes lends weight to the hypothesis that digestion in parasitic nematodes is essentially like that of other animals possessing a digestive tract.

### Bibliography

- ABDERHALDEN, E., and HEISE, R. 1909.—Neber das Vorkommen peptolytischen Fermente bei den Wirbellosen. *Ztschr. Physiol. Chem.*, v. 62:136-138.
- ACKERT, J. E. 1930.—Vitamin requirements of intestinal nematodes. *Anat. Rec.*, v. 47(3):363.  
1931.—The morphology of life history of the fowl nematode *Ascaridia lineata* (Schneider). *Parasit.*, v. 23(3):360-379, pls. 13, 14, figs. 1-50.
- ACKERT, J. E., EDGAR, S. A. and FRICK, L. P. 1939.—Goblet cells and age resistance of animals to parasitism. *Tr. Am. Micr. Soc.*, v. 58:81-89.
- ACKERT, J. E. and PAYNE, FLORENCE KING. 1923.—Investigations on the control of hookworm disease. XII. Studies on the occurrence, distribution and morphology of *Necator suillus*, including descriptions of the other species of *Necator*. *Am. J. Hyg.*, v. 3(1):1-25, pls. 1-2, figs. 1-23.
- ACKERT, J. E., TODD, A. C. and TANNER, W. A. 1938.—Growing larval *Ascaridia lineata* (Nematoda) *in vitro*. *Tr. Am. Micr. Soc.*, v. 57:292-296.
- ACKERT, J. E. and WHITLOCK, J. H. 1935.—Studies on ascarid nutrition. *J. Parasit.*, v. 21:428.
- ACKERT, J. E., WHITLOCK, J. H. and FREEMAN, A. E., JR. 1940.—The food of the fowl nematode, *Ascaridia lineata* (Schneider). *J. Parasit.*, v. 26(1):17-32, fig. 1.
- ALICATA, JOSEPH E. 1932.—Life history of the rabbit stomach worm *Obeliscoides cuniculi*. *J. Agr. Res.*, v. 44(5):401-419, figs. 1-12.
- ANDREWS, J. S. 1939.—Life history of the nematode *Cooperia curticei* and development of resistance in sheep. *J. Agr. Res.*, v. 58:771-785, figs. 1-3.
- ARCHER, V. W. and PETERSON, C. H. 1930.—Roentgen diagnosis of ascariasis. *J. Am. Med. Assoc.*, v. 95:1819-1821.
- ASHFORD, B. K. and IGARAVIDEZ, P. G. 1911.—Uncinariasis in Porto Rico. U. S. 61st Cong., 3d Sess., Senate Doc. 808, 335 pp.
- ASKANAZY, M. 1900.—Ueber Art und Zweck der Invasion der *Anguillula intestinalis* in die Darmwand. *Centr. Bakt. [etc.]*, Abt. 1, v. 27:569, figs. 1-4.
- CHITWOOD, B. G. 1938.—Notes on the physiology of *Ascaris lumbricoides*. *Proc. Helm. Soc. Wash.*, v. 5(1):18-19.
- CHITWOOD, B. G. and CHITWOOD, M. B. 1937.—The histology of nemie esophagi. VIII. The esophagus of representatives of the Enoplida. *J. Wash. Acad. Sc.*, v. 27(12):517-531, figs. 1-2.
- CHRISTOFFERSON, N. R. 1914.—*Trichocephalus dispar* im Darmkanal des Menschen. *Zieglers Beitr. Path. Anat. & Allg. Path.*, v. 57:474-515.
- CHU, TSO-CHIH. 1936.—Studies on the life history of *Rhabdias fuscovenosa* var. *catancensis* (Rizzo, 1902). *J. Parasit.*, v. 22(2):140-160, figs. 1-8.
- DAVEY, D. G. 1938.—Studies on the physiology of the nematodes of the alimentary canal of sheep. *Parasit.*, v. 30(3):278-295, fig. 1.
- DIKMANS, G. and ANDREWS, J. S. 1933.—A note on the life history of *Ostertagia circumcincta*. *J. Parasit.*, v. 20(2):107.
- ENIGK, K. 1938.—Ein Beitrag zur Physiologie und zum Wirt-Parasitverhältnis von *Graphidium strigosum* (Trichostromyidae, Nematoda). *Ztschr. Parasitenk.*, v. 10(3):386-414.
- ERNST, J. 1888.—Einige Fälle von Ankylostomiasis, nebst Sectionsbefunden. *Deutsch. Med. Wochenschr.*, v. 14:291-294.
- FALLIS, A. MURRAY. 1938.—A study of the helminth parasites of lambs in Ontario. *Tr. Roy. Canad. Inst.*, v. 22, pt. 1 (47):81-128, figs. 1-30.
- FAUST, ERNEST CARROLL. 1932.—The symptomatology, diagnosis and treatment of *Strongyloides* infection. *J. Am. Med. Assoc.*, v. 98:2276-2277.  
1935.—Experimental studies on human and primate species of *Strongyloides*. IV. The pathology of *Strongyloides* infection. *Archiv. Path.*, v. 19:769-806.  
1939.—Human helminthology Philadelphia. Lea & Febiger. 780 pp.
- FLURY, FERDINAND. 1912.—Sur Chemie und Toxikologie der Ascariden. *Arch. Exper. Path. & Pharmacol.*, v. 67:275-392.
- GARIN, C. 1913.—Recherches physiologiques sur la fixation et le mode de nutrition de quelques nematodes parasites du tube digestif de l'homme et des animaux. *Univ. Lyon. n.s., I. Sc. Med.*, v. 34:160 pp., figs. 1-55.
- GLASER, R. W. 1932.—Studies on *Necoaplectana glaseri*, a nematode parasite of the Japanese beetle (*Popillia japonica*). *N. J. Dept. Agr. Circ. No. 211*. 34 pp. pls. 1-3, figs. 1-17.
- GLASER, R. W. and STOLL, NORMAN R. 1938.—Sterile culture of the free-living stages of the sheep stomach worm, *Haemonchus contortus*. *Parasit.*, v. 30(3):324-332, figs. 1-3.
- GUIART, J. 1908.—Le trichocephale vit aussi dans l'intestin grele et se nourrit de sang. *Lyon Med.*, v. 110(6):325-326.
- HELLER, M. 1933.—Entwickelt sich die *Trichinella spiralis* in der darmlichtung ihres wirtes? *Ztschr. Parasitenk.*, v. 5:370-392.
- HOEPLI, R. 1927.—Ueber Beziehungen zwischen dem biologischen Verhalten parasitischer Nematoden und histologischen Reaktionen des Wirbeltierkoerpers. *Arch. Schiffs-u. Tropen-Hyg.*, v. 31(3):207-290.  
1930.—Parasitic nematodes and the lesions they produce. *Natl. Med. J. China.*, v. 16:103-110.  
1933.—On histolytic changes and extra-intestinal digestion in parasite infections. *Lingnan Sc. J.*, v. 12 (Suppl.):1-11, pl. 1, figs. 1-3.
- HOEPLI, R. and FENG, L. C. 1931.—On the action of esophageal glands of parasitic nematodes. *Chinese Med. J.*, v. 17:589-598, pls. 1-3, figs. 1-6.  
1933.—The presence of an anticoagulin in the esophagus of *Bunostomum trigonocephalum* from the intestine of sheep. *Arch. Schiffs-u. Tropen-Hyg.*, v. 37(4):176-182.
- HOEPLI, R. and HSÜ, H. F. 1931.—Histological changes in the digestive tract of vertebrates due to parasitic worms. *Chinese Med. J.*, v. 17:557-566, pls. 1-2, figs. 1-4.
- HSÜ, H. F. 1938a.—Studies on the food and the digestive system of certain parasites. I. On the food of the dog hookworm *Ancylostoma caninum*. *Bul. Fan Mem. Inst. Biol., Zool., Ser.*, v. 8(2):121-132, pls. 12, 13, figs. 1-6.  
1938b.—Idem. II. On the food of *Schistosoma japonicum*, *Paragonimus ringeri*, *Dioflaria immitis*, *Spirocerca sanguinolenta*, and *Rhabdias* sp. *Ibid.*, v. 8:347-353.  
1938c.—Idem. IV. On the food of *Diplotriacna tricuspis* (Nematoda). *Ibid.*, v. 8:403-406.
- HUNG, SEE-LU. 1926.—The histological changes in lung tissue of swine produced by *Metastrongylus clongatus*. *N. Am. Vet.*, v. 7(1):21-23, figs. 1-3.
- KAUZAL, G. 1936.—Further studies on the pathogenic importance of *Chabertia ovina*. *Austral. Vet. J.*, v. 12:107-110.
- LAPAGE, GEOFFREY. 1933.—Cultivation of parasitic nematodes. *Nature*, v. 131:583-584.  
1937.—Nematodes parasitic in animals (Monograph). London. Methuen and Co., Ltd. 172 pp.
- LEICHTENSTERN, O. 1886.—Weitere Beiträge zur Ankylostomafrage. *Deut. Med. Wochenschr.*, v. 12:173-176.
- LEUCKART, R. 1876.—Die Menschlichen Parasiten. pp. 301, 345.
- LI, H. C. 1933a.—Parasitic nematodes: Studies on their intestinal contents. I. The feeding of dog ascaris, *Toxocara canis* (Werner, 1782). II. The presence of bacteria. *Lingnan Sc. J.*, v. 12 (Suppl., May):33-41, pl. 1, figs. 1-3.  
1933b.—Feeding experiments on representatives of Ascaroidea and Oxyuroidea. *Chinese Med. J.*, v. 47:1336-1342.  
1933c.—On the mouth-spear of *Trichocephalus trichu-*

- rus and of a *Trichocephalus* sp. from monkey, *Macacus rhesus*. *Ibid.*, v. 47:1343-1346.
- LIEVRE, H. 1934.—A propos de l'Hématophagie des *Ascaris*. *Comp. Rend. Soc. Biol., Paris*, v. 116:1079.
- LOOSS, A. 1905.—The anatomy and life history of *Ancylostoma duodenale* Dub. *Rec. Egypt. Govt. Sch. Med.*, v. 3:1-158, pls. 1-10, figs. 1-100, photos 1-6.
- MCCOY, E. E. and GIRTH, H. B. 1938.—The culture of *Neoplectana glaseri* on veal pulp. *N. J. Dept. Agric. Circ.* No. 285. 12 pp.
- MCCOY, E. E. and GLASER, R. W. 1936.—Nematode culture for Japanese beetle control. *N. J. Dept. Agric. Circ.* No. 265. 9 pp. figs. 1-5.
- MCCOY, OLIVER R. 1929.—The suitability of various bacteria as food for hookworm larvae. *Am. J. Hyg.*, v. 10(1):140-156.  
1934.—The development of adult trichinae in chick and rat embryos. *J. Parasit.*, v. 20(6):333.
- MAGATH, THOMAS BYRD. 1919.—*Camallanus americanus*, nov. spec. *Tr. Am. Micr. Soc.*, v. 38(2):49-170, pls. 7-16, figs. 1-133.
- MUELLER, JUSTUS F. 1929.—Studies on the microscopical anatomy and physiology of *Ascaris lumbricoides* and *Ascaris megalocephala*. *Ztschr. Zellforsch.*, v. 8(3):361-403, pls. 1-18, figs. 1-80.
- ORTLEPP, R. J. 1937.—Observations on the morphology and life-history of *Gaigeria pachyseclis* Raill. and Henry, 1910: A hookworm parasite of sheep and goats. *Onderstepoort J. Vet. Sc. & Anim. Indus.*, v. 8(1):183-212, figs. 1-18.
- OTTO, G. F. 1935.—Blood studies on trichurisinfested and worm-free children in Louisiana. *Am. J. Trop. Med.*, v. 15(6):693-704.
- PORTER, DALE A. 1936.—The ingestion of the inflammatory exudate by swine lungworms. *J. Parasit.*, v. 22(4):411-412, fig. 1.
- RANSOM, B. H. 1911.—The nematodes parasitic in the alimentary tract of cattle, sheep, and other ruminants. *U. S. Dept. Agric., Bur. Anim. Indus. Bul. No. 127*, 132 pp., figs. 1-152.
- SCHWARTZ, BENJAMIN. 1921.—Effects of secretions of certain parasitic nematodes on coagulation of blood. *J. Parasit.*, v. 7(3):144-150.
- SMIRNOV, G. G. 1935.—Nutrition of the *Ascaris* larvae in the process of migration. *Parazit., Perenosch. i Iadovit. Zhivotn. Storn. Rabot. . . . Pavlovskii 1909-1935*, pp. 298-306. Russian with English summary.  
1936.—On the question of hematophagia in threadworms and whipworms. *Trudy Sec. Parasit., U.S.S.R. Inst. Exper. Med.*, v. 2:229-239. [Russian with English summary.]
- VAN SOMEREN, VERNON D. 1939.—On the presence of a buccal stylet in adult *Trichinella*, and the mode of feeding of the adults. *J. Helml.*, v. 17(2): 83-92, figs. 1-5.
- STADELMANN, H. 1891.—Ueber den anatomischen Bau des *Strongylus convolutus* Ostertag, nebst einigen Bemerkungen zu seiner Biologie. *Inaug.-Diss., Berlin*.
- VEGLIA, FRANK. 1916.—The anatomy and life history of the *Hacmonchus contortus* (Rud.). *Dept. Agric. Union S. Africa*, 3d & 4th Rpts. Director Vet. Res., pp. 347-500, figs. 1-60.
- WEINBERG, M. 1907.—Du Role des Helminthes. *Ann. Inst. Pasteur*, v. 21: 417-533.
- WEINLAND, ERNST. 1901.—Ueber den Glycogengehalt einiger parasitischer Würmer. *Ztschr. Biol.*, v. 41: 69-74.
- WELLS, HERBERT S. 1931.—Observations on the blood sucking activities of the hookworm, *Ancylostoma caninum*. *J. Parasit.*, v. 17(4): 167-182, fig. 1.
- WETZEL, R. 1928.—Pathogenic effects of *Strongylus equinus*, *dentatus* and *vulgaris* on mucosa of colon of horses. *Deutsch. Tierärzte Wochenschr.*, v. 36: 719-722.  
1930.—The biology of the fourth stage larvae of *Oxyuris equi*. *J. Parasit.*, v. 17: 95-97, fig. 1.  
1931a.—On the biology of the fourth stage larva of *Dermatoyxys veligera* (Rudolphi 1819) Schneider 1866, an oxyurid parasitic of the hare. *Ibid.*, v. 18:40-43.  
1931b.—On the feeding habits and pathogenic action of *Chabertia ovina* (Fabricius, 1788). *N. Am. Vet.*, v. 12(9): 25-28, fig. 1.
- WETZEL, R. and ENIGK, K. 1937.—Zur Biologie von *Graphidium strigosum*, dem Magenwurm der Hasen und Kaninchen. *Deutsch. Tierärzte. Wochenschr.*, v. 45(25): 401-405, figs. 1-3.
- WHIPPLE, G. H. 1909.—The presence of a weak hemolysin in hookworm and its relation to the anemia of uncinariasis. *J. Exper. Med.*, v. 11: 331-343.

## CHAPTER XI

# CHEMICAL COMPOSITION AND METABOLISM OF NEMATODE PARASITES OF VERTEBRATES, AND THE CHEMISTRY OF THEIR ENVIRONMENT

THEODOR VON BRAND, Department of Biology, Catholic University of America and  
THEODORE LOUIS JAHN, Department of Zoology, State University of Iowa

The metabolic processes of nematode parasites comprise a subject which has been under investigation for many years. Progress in the field, however, has been particularly rapid during the last decade, and it is the purpose of the present authors to present a summary of the known facts of metabolism together with the related subjects of the chemistry of the worms and of their environment. Recent reviews which deal with some of the subject matter here presented are those of Slater (1928), McCoy (1935), Lapage (1938) and v. Brand (1934, 1938).

### Peculiarities of Environment Which May Influence Metabolism

The wide differences in the habitats of the various nematode parasites of vertebrates are undoubtedly correlated with wide differences in metabolic processes. The organisms which live in the digestive tract, blood stream, lungs, kidneys, subcutaneous tissue, etc., are subject to quite a variety of environmental conditions. In those cases where open contact with the blood stream or lymph is maintained the parasites are, of course, subjected to an environment very similar to that of the cells of the host body. Whenever a nematode is surrounded by a cyst wall which reduces the availability of oxygen, or is located in a region deprived of free blood circulation, metabolic processes are probably different from the processes in those species which live in the blood stream. Species that live in the digestive tract have an environment which is peculiar in many respects. The chemistry of blood is adequately described elsewhere, and the chemical environment within cysts and in chemically isolated tissues is practically unknown (except for cestode cysts, Schopper, 1932). Therefore, the present discussion of environment is limited to the chemistry of the digestive tract.

From the viewpoint of nematology the chemistry of the intestinal contents is interesting for several reasons. A thorough knowledge of the chemistry of the environment may allow a better understanding of the physiology of the intestinal parasites, it may aid in the formulation of culture media suitable for growth *in vitro* (cf. Glaser and Stoll, 1938), and it may shed light on the problems of host specificity and on the possibility that experimental modifications of intestinal contents may be of use in controlling the activities of the nematodes. The effect on nematodes of many of the substances found in the intestine has not been studied. In the hope that the present discussion might serve as a partial outline of substances to be investigated, the authors have included a general discussion of the chemical compounds present.

### THE SEQUENCE OF CHEMICAL EVENTS IN THE DIGESTIVE TRACT

In any discussion of the chemical composition of the contents of the digestive tract it is necessary to keep in mind the sequence of events which occurs as the ingesta pass through the alimentary canal. The chemical composition of the contents of the gut varies with diet, with species, and with the state of health. However, in any healthy animal on a constant diet there is a definite sequence to the chemical changes which occur.

In man, the stomach receives the mixture of food and saliva. To this is added mucus, pepsin, and hydrochloric acid. The material present in the duodenum is derived from four sources: chyme from the stomach, bile, pancreatic juice, and succus entericus. The stomach contents when emptied into the duodenum consist, among other things, of proteoses and peptones, starch, sugars, fat droplets, some fatty acids and glycerol, hydrochloric acid, plant fragments containing cellulose and undigested plant tissue, and water.

The bile contains mucin, the pigments biliverdin and bilirubin, the bile salts Na-taurocholate and Na-glycocholate, cholesterol, lecithin, fats, soaps, inorganic salts and water. The relative amounts of taurocholate and glycocholate vary with the species; the dog, for example, is entirely lacking in glycocholate. The pancreatic secretion contains  $\text{Na}_2\text{CO}_3$  and the enzymes trypsin, lipase, and amylase. The succus entericus contributes the enzymes erepsin, lipase, maltase, invertase, lactase, and rennin, and a large amount of mucus and desquamated epithelial cells. Due to partial sterilization of food, or to the action of hydrochloric acid and bile salts, living bacteria are present only in small numbers in the duodenum and in

normal men may sometimes be absent (Kellogg, 1933). As these materials pass through the duodenum and jejunum digestion is completed, and the products of digestion and most of the bile salts are absorbed. The bacteria increase in numbers, utilize some of the products of digestion and decompose others. As the material passes through the large intestine water is absorbed, and calcium, magnesium, iron, and phosphates are secreted by the intestinal wall.

The feces of an animal on a carnivorous diet are composed mostly of the intestinal secretions and bacteria. If vegetables make up a considerable part of the diet, the bulk of the feces is increased, and plant fragments appear in the feces, sometimes with the contained plant protoplasm only partially digested. The large bulk of undigested cellulose stimulates peristalsis, and consequently causes a more rapid passage of ingesta through the intestine, which results in the absorption of less water by the colon and a more liquid feces.

The materials which are present in the digestive tract and which may affect the metabolism of nemas are for convenience discussed under the following headings: (1) Composition of the intestinal gases, (2) Hydrogen ion concentration, (3) Dissolved materials (exclusive of gases), (4) Antienzymes. Nematodes, especially those which live in tissues, are known to secrete digestive enzymes, but these are more properly discussed under the subject of nutrition of the worms.

### COMPOSITION OF THE INTESTINAL GASES

The composition of the gases in or in contact with the ingesta varies greatly in different parts of the digestive tract. The gas tension of the stomach contents varies at different periods following a meal and depends on the amount of air ingested with food. The action of HCl causes a release of bound  $\text{CO}_2$ , most of which is probably absorbed either in the stomach or upper intestine. The oxygen ingested with the food apparently undergoes a rapid decrease so that it is almost absent from the intestine below the duodenum. The analyses of von Brand and Weise (1932) show that very little oxygen is introduced into the intestine by the bile. These investigators also studied the oxygen content of fluid intestinal matter and of intestinal gases. They found that the oxygen content of the fluid of both the large and small intestines of almost all animals examined was practically nil. The only exception was one pig which contained quite appreciable amounts of oxygen. This might have been caused by the swallowing of large amounts of air, perhaps at the time of slaughtering. The values for all animals except the pig correspond to about 5 percent saturation. The data of several investigators on the oxygen content of intestinal fluids and gases are summarized in Tables 13 and 14. The data in Table 14 demonstrate the absence of oxygen in the gaseous content of the intestine of all animals except the pig. Long and Fenger (1917) found that oxygen was present in appreciable quantities in the intestinal gases of the pig, and this was confirmed by v. Brand and Weise. It has been assumed by Slater (1925) that the intestinal walls give off oxygen to the intestinal contents during digestion. This has not been proved experimentally, and Long and Fenger (1917) found that the oxygen content was lowest during active digestion. It seems probable (as indicated by the data of Melver, Redfield, and Benedict, 1926) that oxygen may diffuse inward from the intestinal wall, but it is also very likely that the bacteria present near the wall would consume this immediately so that very little oxygen from this source would ever reach the central portion of the lumen. The available evidence indicates that the environment of intestinal helminths is not devoid of oxygen but contains oxygen in only small quantities. Worms which live close to the intestinal wall may have access to larger amounts. In the case of the hookworm it is apparent from the observations of Wells (1931) that the blood sucking activities represent largely a respiratory function.

Analyses of intestinal gases other than  $\text{O}_2$  are not numerous. The intestinal gases of man vary with diet. Ruge (1861) gives the following data for percentage composition:

| Diet       | $\text{CO}_2$ | $\text{H}_2$ | $\text{CH}_4$ | $\text{N}_2$ |
|------------|---------------|--------------|---------------|--------------|
| Vegetables | 21.34         | 1.5-4.0      | 44.55         | 10.19        |
| Meat       | 8.13          | 0.7-3.0      | 26.37         | 45.64        |
| Milk       | 9.16          | 43-54        | 0.9           | 36.38        |



The data of Fries (1906) show that the gases of man on a mixed diet are similar to those given above for a meat diet. Further analyses are given by Basch (1908). The absorption of intestinal gases is discussed by Meyer, Redfield, and Benedict (1926), and the subject of human gastro-intestinal gases is reviewed by Ziegler and Hirsch (1925) and Lloyd Jones and Liljedahl (1934).

The intestinal gases of the dog were analyzed by Planer (1860). His analyses demonstrated large amounts of CO<sub>2</sub> and N<sub>2</sub>, and a smaller amount of H<sub>2</sub> throughout the digestive tract, a small amount of O<sub>2</sub> in the small intestine, and a small amount of H<sub>2</sub>S in the large intestine when the dog was on bread or meat diets. On a vegetable diet H<sub>2</sub> largely replaced the N<sub>2</sub>, while O<sub>2</sub> and H<sub>2</sub>S were absent. In these analyses methane is conspicuously absent.

The intestinal gases of various herbivores have been analyzed by Tappeiner (1883), and the literature is summarized by Scheumert and Schieblich (1927). The data of Tappeiner (1883) on the percentage composition of the gases in cattle, sheep, and goats (all of which were quite similar) are as follows:

|                       | Rumen   | Small Intestine | Caecum and Colon |
|-----------------------|---------|-----------------|------------------|
| CO <sub>2</sub> ..... | 65      | 62.92           | about 30         |
| O <sub>2</sub> .....  | .5      | 0               | 0                |
| CH <sub>4</sub> ..... | 30      | .04-6.6         | 38.53            |
| H <sub>2</sub> .....  | 0.6-4.7 | 0.37            | 2.6              |
| N <sub>2</sub> .....  | 1-40    | 1               | 23.34            |

Data for the horse (Tappeiner, 1883) are:

|                       | Stomach | Small Intestine | Caecum and Colon | Rectum |
|-----------------------|---------|-----------------|------------------|--------|
| CO <sub>2</sub> ..... | 75      | 15-43           | 55-85            | 29     |
| O <sub>2</sub> .....  | 0       | 0.57-7.6        | 0-14             | 0      |
| CH <sub>4</sub> ..... | 0       | 0               | 11-33            | 57     |
| H <sub>2</sub> .....  | 14      | 20-24           | 1.7-2.2          | 0.8    |
| N <sub>2</sub> .....  | 10      | 37-60           | .9-10.0          | 13     |

Data for the rabbit (Tappeiner, 1883) are:

|                       | Stomach | Small Intestine | Caecum and Colon |
|-----------------------|---------|-----------------|------------------|
| CO <sub>2</sub> ..... | 32      | 75              | 6                |
| O <sub>2</sub> .....  | 0       | 0               | 0.6              |
| CH <sub>4</sub> ..... | 0       | 2               | 21.0             |
| H <sub>2</sub> .....  | 0       | 18              | 0.6              |
| N <sub>2</sub> .....  | 68      | 6               | 72               |

Long and Feuger (1917) found a large amount of N<sub>2</sub> (74—92%) somewhat less CO<sub>2</sub> (5—28%), and about 5 percent O<sub>2</sub>, but no methane or H<sub>2</sub> present in the small intestine of hogs.

The production of methane is probably caused mostly by bacterial decomposition of cellulose, although the data of Ruge indicate that it can also be produced by bacterial action when the animal is on a meat diet. The analyses of Tappeiner and others also indicate the presence of H<sub>2</sub>S in some cases. H<sub>2</sub>S and N<sub>2</sub> must be formed by the action of bacteria on protein. Ammonia is also formed by bacterial decomposition of protein, but it is usually bound by the acids of the intestine. Most of the CO<sub>2</sub> is probably of bacterial origin, although in the duodenum it may also be formed by the Na<sub>2</sub>CO<sub>3</sub> of the pancreatic juice and the acid of the chyme. The NH<sub>3</sub> and the CO<sub>2</sub> of the succus entericus are discussed by Herrin (1937). Most of the intestinal gases are eliminated from the body by the lungs. Taeke (1884) found that 10 to 20 times as much of the intestinal gases of rabbits escape by means of the blood and lungs as by way of the anus.

The effect of the gases other than oxygen on intestinal nematodes is entirely unknown. Methane, H<sub>2</sub>, and N<sub>2</sub> are probably without either beneficial or harmful effects. The utilization of oxygen will be discussed in the part dealing with metabolism of adult nematodes. The effect of CO<sub>2</sub> is unknown. Since it is incapable of further oxidation and since there is no evidence of chemosynthesis in the nematodes, the only apparent effect it could have would be the adjustment of intracellular pH. Since intestinal nematodes live in a medium usually saturated with CO<sub>2</sub>, it is conceivable that they may depend on this substance as an intracellular buffer. Therefore, it may become important to maintain a high CO<sub>2</sub> content in *in vitro* cultures (Cf. possible role in growth of intestinal protozoa, Jahn, 1934, 1936). It should be noted that Weinland (1901) found that *Ascaris* survived longer *in vitro* when the medium was saturated with CO<sub>2</sub>.

TABLE 13.—Oxygen Content of Fluid Intestinal Masses

| Animal | Part of intestine | Oxygen in volume per cent mean and ( ) extremes | Number of determinations | Investigator            |
|--------|-------------------|---|--------------------------|-------------------------|
| Horse  | Sm.intestine      | 0.024<br>(0.016-0.031)                          | 2                        | Toryu (1934)            |
| Dog    | Sm.intestine      | 0.028   | 1                        | v. Brand & Weise (1932) |
| Cattle | Sm.intestine      | 0.013<br>(0.00-0.025)                           | 2                        | v. Brand & Weise (1932) |
| Sheep  | Sm.intestine      | 0.012<br>(0.00-0.025)                           | 4                        | v. Brand & Weise (1932) |
| Pig    | Sm.intestine      | 0.083<br>(0.00-0.358)                           | 6                        | v. Brand & Weise (1932) |
| Cattle | Lg.intestine      | 0.010<br>(0.00-0.023)                           | 3                        | v. Brand & Weise (1932) |
| Pig    | Lg.intestine      | 0.00  | 1                        | v. Brand & Weise (1932) |

TABLE 14.—Oxygen Content of Intestinal Gases

| Animal | Part of intestine | Oxygen in volume per cent mean and ( ) extremes | Number of determinations | Investigator            |
|--------|-------------------|---|--------------------------|-------------------------|
| Horse  | Sm.intestine      | 0.67<br>(0.57-0.76)                             | 3                        | Tappeiner (1883)        |
| Cattle | Sm.intestine      | 0.00  | 1                        | Tappeiner (1883)        |
| Cattle | Sm.intestine      | *   | *                        | v. Brand & Weise (1932) |
| Goat   | Sm.intestine      | *   | *                        | Tappeiner (1883)        |
| Sheep  | Sm.intestine      | *   | *                        | v. Brand & Weise (1932) |
| Pig    | Sm.intestine      | 5.5<br>(1.2-14.2)                               | 9                        | Long & Feuger (1917)    |
| Pig    | Sm.intestine      | 4.2<br>(0.4-8.2)                                | 6                        | v. Brand & Weise (1932) |
| Dog    | Sm.intestine      | 0.2<br>(0.0-0.7)                                | 8                        | Planer (1860)           |
| Horse  | Lg.intestine      | 0.07<br>(0.00-0.14)                             | 4                        | Tappeiner (1883)        |
| Cattle | Lg.intestine      | 0.00  | 1                        | Tappeiner (1883)        |
| Goat   | Lg.intestine      | 0.03<br>(0.00-0.07)                             | 3                        | Tappeiner (1883)        |
| Sheep  | Lg.intestine      | 0.00  | 1                        | Tappeiner (1883)        |
| Rabbit | Lg.intestine      | 0.62  | 1                        | Tappeiner (1883)        |
| Dog    | Lg.intestine      | 0.00  | 6                        | Planer (1860)           |

\*Not enough gas for analysis.

## HYDROGEN ION CONCENTRATION

The pH of the stomach and intestine has been measured for a large number of animals, and some of the representative data are listed in Tables 15 and 16. Contents of the stomach of carnivores, omnivores, and herbivores with a simple stomach, and of the abomasum of ruminants are distinctly acid in character due to the secretion of hydrochloric acid. The rumen and omasum vary from neutral to distinctly alkaline. The pH of the duodenum is extremely variable but is usually acid because of the introduction of HCl from the stomach. The pH of the remainder of the small intestine is less acid than the duodenum, and there is usually a progressive rise toward neutrality or to a slight alkalinity; the pH seldom reaches a value higher than 8.0 or 8.2. The colon and caecum of most animals are neutral, slightly acid, or slightly alkaline. Some of the recent literature is reviewed by Lenkeit (1933).

The pH of the intestine may be lowered by the administration of large quantities of lactose, especially if the diet is low in protein. Robinson and Duncan (1931) found that the pH of the rat intestine could be lowered about one pH unit by the administration of 25 percent lactose with a low protein diet (other literature is cited by these authors). In man it is known that the acidity of the intestine may be considerably decreased if large amounts of lactose accompanied by *Lactobacillus acidophilus* are ingested (literature cited by Kopeloff, 1926, and Frost and Hankinson, 1931). Comparable results have been obtained with the domestic fowl (Ashcraft, 1933). The direct addition of mineral salts such as NaCl, MgSO<sub>4</sub>, CaCl<sub>2</sub>, Ca(OH)<sub>2</sub>, CaSO<sub>4</sub>, NaHCO<sub>3</sub>, and NH<sub>4</sub>Cl to the diet may have no effect on pH in experimental animals (McClendon et al, 1919; Heller, Owens, and Portwood, 1935; Musschl, Blish, and Ackerson, 1933). However, positive results with mineral salts have been obtained by Robinson (1922), Shohl and Bing (1928) and others. A deficiency of vitamin D is also known to cause the intestinal contents to become alkaline due to lack of Ca absorption (Zucker and Matzner, 1923; Jephcott and Bacharach,

TABLE 15.—The pH of Stomach Contents

| Animal                    | pH                                       | Author   |
|---------------------------|--|--|
| Man                       | minimum pH<br>1.0 to 2.5                 | McClendon and Medes (1925)<br>Kahn and Stokes (1926) |
| Rat                       | 3.2-4.6                                  | Sun, Blumenthal, Slifer, Herber and Wang (1932)      |
| Rat                       | 1.8-5.6 (av. 3.6)                        | Eastman and Miller (1935)                            |
| Rat                       | 3.3-3.9                                  | Kofoid, McNeil and Cailleau (1932)                   |
| Horse                     | 1.13-6.8 (50%<br>between 1.1<br>and 3.3) | Schwarz, Steinmetzer, and<br>Caitham (1926)          |
| Rabbit                    | 1.8                                      | McLaughlin (1931)                                    |
| Cat                       | 3.34                                     | McLaughlin (1931)                                    |
| Dog                       | 1.5-2.0                                  | Mann and Bollman (1930)                              |
| Dog                       | 2.0-6.0                                  | Schwarz and Danziger (1924)                          |
| Dog                       | 1.37-5.7<br>(av. 3.47)                   | Nagl (1928)  |
| Chick, gizzard            | 3.39                                     | McLaughlin (1931)                                    |
| Chick, gizzard            | 2.9-3.2                                  | Ashcraft (1933)                                      |
| Chicken<br>proventriculus | 5.9                                      | McLaughlin (1931)                                    |
| Chicken<br>proventriculus | 4.8-5.7                                  | Ashcraft (1933)                                      |
| Cattle<br>abomasum        | 2.0-4.1                                  | Schwarz and Kaplan (1926)                            |
| Cattle<br>abomasum        | 3.8                                      | Mangold (1925)                                       |
| Sheep<br>abomasum         | 3.15-5.25<br>(av. 4.0)                   | Davey (1938)   |
| Cattle rumen              | 8.89 (8.61-9.68)                         | Schwarz and Gabriel (1926)                           |
| Cattle rumen              | 7.5-8.0                                  | Kreipe (1927)  |
| Sheep rumen               | 7.0-7.6                                  | Ferber (1928)  |

1926; Redman, Willimott, and Wokes, 1927). The pH may be appreciably lowered by addition of cod liver oil to a rachitogenic diet. The effect of varying the proportions of protein, fat, and carbohydrate has been reported to cause no marked change in the pH of the intestine of rats (Redman, Willimott and Wokes, 1927), dogs (Grayzel and Miller, 1928; Graham and Emery, 1928), or man (Hume, Denis, Silverman, and Irwin, 1924). However, the data of Robinson and Duncan (1931) show consistently higher pH values for rats fed on grain and alfalfa than for rats on a high protein diet (Table 16). Eastman and Miller (1935) studied the effect of a number of diets on gastro-intestinal pH in rats.

It has been suspected for some time that the pH of the central portion of the lumen is not the same as that close to the intestinal wall. Evidence for this is found in the feces in that

the surface of stools is more alkaline, apparently because of secretion of alkaline salts by the intestinal wall. Kofoid, McNeil, and Cailleau (1932) reported differences in the pH of contents and wall throughout the digestive tract of the rat. (Table 16). Robinson (1935) studied the effect of placing various salt solutions in the small intestine of dogs on the pH of the solution and decided that each portion of the digestive tract tended to produce a characteristic pH value in the solution, regardless of the initial pH. He concluded that the pH of the region close to the wall increases regularly from pH 6.5 to 7.5 or 8.0 throughout the length of the small intestine and pointed out that the pH close to the wall is probably largely independent of changes produced in the lumen by the action of bacteria. Ball (1939), by means of a capillary glass electrode, has measured the pH of the wall (data given in Table 16).

The possibility that pH may be a limiting factor in the distribution of sheep nematodes was investigated by Davey (1938). He found that *Ostertagia circumcincta* was able to live between pH 3.2 and pH 9.0. This range allows it to live in the abomasum of sheep (pH 3.2-5.25) but apparently may be one reason why it does not infest the stomach of the dog (pH 2 or less) or horse (pH 1.1-6.8) or the abomasum of cattle (pH 2.0 to 4.1). Two duodenal species from sheep, *Trichostrongylus colubriformis* and *T. vitrus*, were able to stand a continuous acidity as low as pH 3.6, but five other species (*Nematodirus filicollis*, *N. spathiger*, *Cooperia oncophora*, *Cooperia curticei*, *Strongyloides papillosus*) from the middle and lower small intestine were killed at acidities of pH 3.9 to 4.6. Since the duodenum is more acid than the ileum, the low resistance to acidity may be an important factor in preventing the five species from the middle and lower intestine from infesting the duodenum. It has been suggested by Lapage (1935a, 1938) that pH has an influence on the second ecdysis of trichostrongylid larvae (outside of the host) and that this may be of importance in allowing development of the parasite. The third ecdysis (in the intestine) might be similarly affected.

It seems possible that the presence of nemas in the digestive tract might cause a change in gastro-intestinal pH, either directly (perhaps because of lesions in the epithelium) or indirectly through the systemic reactions of the host. In cases of ancylostomiasis and intestinal schistosomiasis Eldin and Hassan (1933) found evidence of gastric disturbance which disappeared after removal of the worms. Fernandez (1934), however, found no correlation between gastric acidity and helminth parasites.

DISSOLVED SUBSTANCES (EXCLUSIVE OF GASES)

The dissolved materials of the digestive tract consist of the ingesta and various secretions listed above, the products of digestion, and the products of bacterial decomposition. Many of these, especially the carbohydrates, may serve as food for nematodes; many others may be toxic and may be effective in

TABLE 16.—The pH of the digestive tract contents.

| Animal and Diet     | Duodenum                  | Jejunum          | Ileum             | Caecum           | Colon     | Investigator                                     |
|---------------------|---------------------------|------------------|-------------------|------------------|-----------|--|
| Man                 | 2.27-7.8                  | ---              | ---               | ---              | ---       | Long and Fenger (1917)                           |
| Man                 | 4.7-6.5                   | 7.0              | 6.1-7.3           | ---              | ---       | Karr and Abbott (1935)                           |
| Man                 | 4.5-5.1                   | ---              | 5.9-6.5           | ---              | ---       | McClendon (1920)                                 |
| Dog                 | 2.0-7.6                   | 7.0-7.6          | 6.0-8.0           | ---              | 7.4       | Mann and Bollman (1930)                          |
| Dog                 | 6.2-6.5                   | 6.0-7.0          | 6.0-7.0           | 6.0-6.5          | ---       | Graham and Emery (1927-28)                       |
| Dog                 | 5.9                       | 6.0-6.27         | 6.36              | 6.57             | 6.84      | Grayzel and Miller (1928)                        |
| Dog                 | ---                       | ---              | ---               | ---              | 7.6-8.4   | Heupke (1931)                                    |
| Cat                 | 6.5                       | 6.9              | 6.8               | ---              | 5.25      | McLaughlin (1931)                                |
| Rat                 | 6.5                       | to               | 7.2               | 6.5-7.2          | 6.4-6.6   | Sun, Blumenthal, Slifer, Herber, and Wang (1932) |
| Rat—grain & alfalfa | 6.75 <sup>1</sup>         | 7.7 <sup>1</sup> | 8.2 <sup>1</sup>  | 7.0              | 7.2       | Robinson and Duncan (1931)                       |
| Rat—high protein    | 6.4 <sup>1</sup>          | 6.8 <sup>1</sup> | 7.3 <sup>1</sup>  | 7.3              | 7.2       | Robinson and Duncan (1931)                       |
| Rat—high base       | 6.4 <sup>1</sup>          | 6.8 <sup>1</sup> | 7.25 <sup>1</sup> | 7.0              | 7.2       | Robinson and Duncan (1931)                       |
| Rat                 | 5.9 <sup>1</sup>          | 6.6 <sup>1</sup> | 6.9 <sup>1</sup>  | 6.4              | 6.6       | Eastman and Miller (1935)                        |
|                     | (4.2-6.9)                 | (5.0-7.3)        | (5.6-7.7)         | (5.1-7.4)        | (5.4-7.5) |  |
| Rat—lumen of gut    | ---                       | ---              | 7.13              | 7.13             | 7.33      | Kofoid, McNeil, and Cailleau (1932)              |
| Rat—wall of gut     | 6.93                      | ---              | 7.34              | 7.34             | 6.95      | Kofoid, McNeil, and Cailleau (1932)              |
| Rat—wall of gut     | 6.34                      | ---              | 6.89              | 7.06             | 6.91      | Ball (1939)                                      |
| Rabbit              | 7.35                      | ---              | 8.0               | 6.26             | ---       | McLaughlin (1931)                                |
| Cattle              | 6.68                      | 8.42             | 8.2               | 8.2              | ---       | Danniger, Pfragner, and Schultes (1928)          |
| Cattle              | ---                       | ---              | ---               | ---              | 7.4-8.4   | Heupke (1931)                                    |
| Horse               | 6.72                      | ---              | 7.09              | 8.12             | ---       | Danniger, Pfragner, and Schultes (1928)          |
| Hogs, calves, lambs | Indefinitely variable     | 6.48 to 7.76.    | ---               | ---              | ---       | Long and Fenger (1917)                           |
|                     | More often acid than alk. |                  |                   |                  |           |  |
| Fowl                | 6.3                       | ---              | 6.22              | 1.9 <sup>2</sup> | ---       | McLaughlin (1931)                                |
| Fowl—meat scrap     | 5.96                      | ---              | 7.1               | 7.0              | 7.2       | Ashcraft (1933)                                  |
| Fowl—+ 20% laetose  | 6.51                      | ---              | 7.16              | 5.1              | 6.3       | Ashcraft (1933)                                  |

<sup>1</sup>Intestine divided into three approx. equal portions so that the measurements given may not correspond exactly to those of the duodenum, jejunum, and ileum.

<sup>2</sup>Possibly a misprint in the original paper.

causing the localization of nematodes in certain portions of the digestive tract.

The possibility that bile salts may affect the growth of intestinal parasites has been recognized for some time. According to Moorthy (1935) fresh bile from certain species of *Barbus* and from sheep and man is capable of killing *Cyclops* and of activating the enclosed larvae of *Draconculus medincensis* to escape. De Waele (1934) claimed that the cestode, *Taenia hydatigena* (*Cysticercus pisiformis*), is able to infest dogs because of the absence of Na-glycocholate in dog bile and that since Na glycocholate is toxic to the organism it can not develop in animals which secrete this substance.

Davey (1938) has investigated the effect of bile salts on sheep nematodes. He found that the species which infest the duodenum (*Trichostrongylus colubriformis* and *T. vitrinus*) of sheep were much more resistant to Na-taurocholate and Na-glycocholate than other species (*Nematodirus fillicollis*, *N. spathiger*, *Cooperia oncophora*, *Cooperia curticei*, and *Ostertagia circumcincta*) from the lower small intestine and abomasum. *Cooperia curticei*, which lives closer to the opening of the bile duct than the other species except *Trichostrongylus colubriformis* and *T. vitrinus*, has a resistance second only to *Trichostrongylus*. Since the bile salts are introduced by the bile duct and are largely reabsorbed in the small intestine, the concentration of bile salts decreases along the intestine. The high concentration in the upper small intestine probably prevents species other than *Trichostrongylus* from living in that region. In these experiments glycocholate seemed to be somewhat more toxic to *Trichostrongylus* than taurocholate. Davey mentioned the possibility that differential susceptibility to the two bile salts might be a factor in the determination of host specificity.

The products of bacterial decomposition are of several types:

1. Products of carbohydrate decomposition from:
  - a. Hydrolysis of cellulose to glucose in the rumen and large intestine of herbivores.
  - b. Fermentation of simple sugars to lower fatty acids in the small and large intestine of all vertebrates and in the rumen of ruminants.
2. Products of protein decomposition from:
  - a. Hydrolysis of proteins to amino acids in the upper small intestine.
  - b. Fermentation of amino acids to aporrhegmas and to lower products in the lower small intestine and large intestine of animals with simple stomachs and in the rumen of ruminants. Some of the products of fermentation are indol, skatol, paraeresol, phenol, volatile fatty acids, H<sub>2</sub>S, histamine, and tyramine. The relative amounts of these products depend on the type of protein and on the species of bacteria present.

At present there is little evidence that these substances are useful or harmful to intestinal nemas. Glucose is probably absorbed by nemas, and on this assumption changes in the diet or in the bacterial flora which would affect the distribution of glucose should affect the parasites. From the studies of Grove, Olmstead, and Koenig (1929) on the lower fatty acids in feces it seems as if the quantity and perhaps the distribution of these materials along the digestive tract is greatly affected by diet. It is also probable that the products of protein putrefaction may exert beneficial or harmful effects on the parasites. If so, then experiments in which the amount of protein putrefaction is controlled are in order. Such control is possible by the administration of large amounts of lactose and bacteria which ferment glucose to acid (review, Arnold, 1933). This treatment results in the replacement of the protein putrefying organisms of the coli-aerogenes group by those which ferment carbohydrate. The change in type of fermentation products is probably due to both the protein sparing action of carbohydrate and the change in flora produced by increased acidity of the intestine. Putrefaction could also be decreased by increasing the rate of passage of ingesta. It is possible to increase protein putrefaction at least in the large intestine by feeding such large quantities of protein that some of it escapes complete digestion and absorption in the small intestine. The putrefying organisms also increase under conditions of achlorhydria which result in an alkalization of the intestine, and if the achlorhydria is severe they may even become implanted in the stomach. It seems probable that experimental modification of the intestinal contents through modification of the intestinal flora may bring about changes in the distribution of nemas along the intestine, and perhaps such experiments may result in methods of controlling or eliminating certain species. Any changes which may prevent ecdysis of larval nematodes might be extremely useful (Lapage, 1938).

It is known that H<sub>2</sub>S is highly toxic to vertebrates and that it easily passes through most animal membranes. The studies of Enigk (1936) on the lethal effects of H<sub>2</sub>S on the eggs of *Ascaris lumbricoides* and the studies of Lapage (1935) on the

infective larvae of *Trichostrongylus* suggest that the outer covering of eggs and larvae may be permeable to H<sub>2</sub>S and other sulfur compounds. Lapage (1935b) obtained considerable evidence that the permeability of the sheaths of larvae is changed by sulfur compounds. In these experiments the effect of pH was not carefully controlled, but the effect of 1 percent Na<sub>2</sub>S on the ecdysis of infective larvae was more pronounced than that of 1 or 2 per cent NaOH. The sheaths became greatly distended due to intake of water. If this effect is really due to the sulfur compounds, this type of effect may give a chemical basis for the statements of Mudie (1934) and Johnston (1934) that the eating of garlic will cause the disappearance of threadworms from the human digestive tract. Lapage (1938) suggested that compounds which yield H<sub>2</sub>S when subjected to the action of intestinal bacteria might eventually be used as anthelmintics.

Some of the products of protein putrefaction, especially H<sub>2</sub>S, rapidly combine with molecular oxygen and when in solution produce very low oxidation-reduction potentials. Bergeim (1924) devised a chemical method of obtaining an index of the reducing power of intestinal contents, and he found that the amount of reduction varied with diet. Preliminary electrical measurements of the oxidation-reduction potential of the rat digestive tract (Jahn, 1933) have shown that the Eh value may be as low as -200 mv. in the caecum and somewhat higher in the lower small intestine. These measurements are well within the "anaerobic" range and support the conclusions mentioned above that oxygen is very scarce in the small intestine and absent in the caecum.

The osmotic pressure of the digestive tract is usually somewhat higher than that of the serum and tissues. Schopfer (1932) gives the following freezing point depressions for various animals: sheep, 0.70-0.83° C.; cow, 0.80° C.; horse, 0.74-0.77° C.; hog, 0.9-1.0° C.; and the elasmobranch *Scylliorhinus*, 2.4° C. With the exception of the elasmobranch the serum of the above animals has a molecular depression of about 0.55 to 0.65° C. Davey (1936b) gave a value of 0.55-0.63° C. for the abomasal contents of sheep. The osmotic pressure of the intestinal contents probably varies considerably with salt intake, but absorption and excretion are apparently rapid enough to prevent the osmotic pressure from ever becoming more than twice that of the blood. As will be discussed below (General Chemical Composition) the osmotic pressure of the medium determines that of the worms. However, the effect of this change in osmotic pressure on worm metabolism is unknown. Davey (1938) has shown that *Ostertagia circumcincta* is capable of living in NaCl which varied from .4 percent to 1.3 percent (0.9 percent is equivalent to a freezing point depression of 0.6° C.). In balanced salt solutions the range would probably be greater.

## ANTIENZYMES

Since the nematodes of the vertebrate digestive tract live in a medium high in the concentration of proteolytic enzymes, the question of how they are able to resist digestion has often been mentioned in the literature. The mechanism seems to be at least dual: (1) the cuticle is relatively indigestible, and (2) the worms contain or secrete antienzymes, i.e., substances which inactivate the digestive enzymes. Evidence for this latter mechanism was first described by Weinland (1903) who described a substance with antitryptic powers in aqueous extracts of *Ascaris*. Dastre and Stassano (1904) believed that the action was antikinase, but the experiments of Hamill (1906) confirmed the original conclusions of Weinland (1903). Hamill (1906) ascribed the following properties to the antienzyme: highly soluble in water and weak alcohol; insoluble in 85 percent alcohol; thermostable in neutral or acid solutions; thermolabile in weakly or strongly alkaline solutions; readily diffusible through membranes which retain colloids. Harned and Nash (1932) described an improved method for preparing high concentrations of antitrypsin by fractional precipitation with alcohol. They claimed that by varying the concentration of alcohol a preparation of antitrypsin could be obtained almost free of *Ascaris* protease. These investigators were able to demonstrate that their antitrypsin preparation also contained a weak antipepsin. A powerful trypsin inhibiting fraction was also recently isolated by Collier (1941) from *Ascaris*. An antitrypsin with chemical properties similar to those of *Ascaris* antitrypsin has been prepared from egg white by Balls and Swenson (1934).

Sang (1938) investigated the mechanism of the action of *Ascaris* antienzyme and confirmed the conclusion that the substance exerted both an antitryptic and an antipeptic activity. However, he could not confirm the result of Harned and Nash (1932) that the ratio of protease to antienzyme could be varied. Sang concluded that *Ascaris* protease and *Ascaris* antitrypsin and antipepsin are all one and the same substance, and he pro-

posed that this substance be called "ascarase." His investigations showed that ascarase was readily diffusible and that it either is or is associated with a substance of the order of a primary albumose. It was precipitated by ammonium sulphate and 70 per cent alcohol, and was not destroyed by trypsin. Ascarase did not inhibit the action of papain. Von Bonsdorff (1939) was unable to confirm the existence of antitrypsin or antipepsin in *Ascaris* extracts, but he did find that the extracts inhibited proteolysis of casein by depepsinized gastric juice at pH 7.4.

Stewart and Shearer (1933) studied the digestion of protein by infected and noninfected sheep and concluded that the nematodes of the stomach inhibited the normal digestive processes. They then obtained an extract from the worms which was capable of producing a 40 to 75 per cent inhibition of the peptic digestion of casein. For this substance and for similar antienzymes of nematodes they suggested the term "nezyme." Andrews (1938) could not repeat the results of Stewart and Shearer on the lowered digestive action of infected sheep. He found that the digestibility coefficients were the same in infected and noninfected animals. Infected sheep did not gain weight as rapidly as controls, but Andrews concluded that this was probably caused by intestinal irritation.

The existence of antienzymes has also been reported for cestodes. However, de Waele (1933), on the basis of experiments on *Taenia saginata*, has questioned the existence of antienzymes and has assumed that protection of the worms from enzyme action is due entirely to the resistance of the cuticle. One basis for this assumption is found in the fact that pieces of worms but not whole worms may be digested by trypsin. This conclusion is subject to criticism in that when worm fragments are placed in an enzyme solution considerable dilution of any antienzyme may occur by diffusion and the antienzyme may thereby be rendered ineffective. In view of the chemical isolation of the antienzyme mentioned above (Hamill, 1906; Nash and Harned, 1932; Collier, 1941) de Waele's conclusion certainly can not be extended to the nematodes.

## General Chemical Composition

### DRY WEIGHT

There have been only a few determinations of the dry weight of parasitic nematodes, and the values recorded are fairly high. The average figures reported for *Ascaris lumbricoides* are 20.7 percent (Weinland, 1901) and 15 percent (Flury, 1912), for *Parascaris*, 21 percent (Schimmelpfennig, 1903) and 14.8 percent (Flury, 1912), and for a larval *Eustrongylides*, 25 percent (V. Brand, 1938). Flury (1912) measured the dry weight of various parts of the body and obtained the following results:

|                           | Dry weight in percent of fresh weight |                           |
|---------------------------|---------------------------------------|---------------------------|
|                           | <i>Ascaris lumbricoides</i>           | <i>Parascaris equorum</i> |
| Body wall .....           | 23.5-25.0                             | 25.0                      |
| Alimentary tract .....    | 27.5                                  | 24.9                      |
| Body fluid .....          | 4.0-6.7                               | 5.0                       |
| Reproductive organs ..... | 25.0-33.3                             | 24.0-27.4                 |

It can be calculated from Flury's figures that these values represent the following fractions of the total dry weight: body wall 65 percent, alimentary tract 3 percent, body fluid 10 percent, and reproductive organs 20 percent.

### CARBOHYDRATES

Storage of carbohydrates in the form of polysaccharides seems to be quite common among the parasitic nematodes. Although chemical analyses have been made only for *Ascaris*, it seems likely that in this respect other species are very similar. Weinland (1901) and Flury (1912) found an optical rotation of +183° to +193° for the polysaccharide of *Ascaris*. Since these workers and Campbell (1936) identified the sugar resulting from hydrolysis as glucose, and since the solubility of the polysaccharide and its color reaction with iodine are typical of glycogen, it seems probable that the substance is true glycogen. Campbell (1936), however, observed antigenic properties of a polysaccharide fraction isolated from *Ascaris*. It does not seem likely that pure glycogen would be capable of inducing the formation of specific anti-bodies. One should therefore expect that another polysaccharide is associated, perhaps in very small amounts only, with the glycogen. However, in so far as metabolic processes are concerned, it is justifiable to speak of glycogen alone.

The occurrence of large amounts of glycogen in ascarids was established in a qualitative or semi-quantitative way by Claude Bernard (1859) and Foster (1865), but Weinland (1901) was the first to undertake a large series of quantitative determinations. The more recent data on the glycogen content are summarized in the following table:

| Species                          | Sex   | Glycogen in % of fresh substance | Country | Investigator                  |
|----------------------------------|-------|----------------------------------|---------|-------------------------------|
| <i>Ascaris lumbricoides</i> ...  | ?     | 5.4                              | Germany | Weinland, 1901                |
| <i>Ascaris lumbricoides</i> ...  | ?     | 6.6                              | Germany | Schulte, 1917                 |
| <i>Ascaris lumbricoides</i> ...  | ♀     | 7.2                              | Denmark | v. Brand, 1934                |
| <i>Ascaris lumbricoides</i> ...  | ♀     | 8.7                              | Russia  | Smorodincev and Bebesin, 1936 |
| <i>Ascaris lumbricoides</i> ...  | ♂     | 6.1                              | Russia  | Bebesin, 1936                 |
| <i>Ascaris lumbricoides</i> ...  | ♀     | 5.3                              | USA     | v. Brand, 1937                |
| <i>Ascaris lumbricoides</i> ...  | ♂     | 5.8                              | USA     | v. Brand, 1937                |
| Dog <i>Ascaris</i> .....         | ?     | 4.5                              | Germany | Weinland, 1901                |
| <i>Parascaris equorum</i> ...    | ♀     | 2.1                              | Germany | Schimmelpfennig, 1903         |
| <i>Parascaris equorum</i> ...    | ♀     | 3.8                              | Japan   | Toryu, 1933                   |
| <i>Parascaris equorum</i> ...    | ♂     | 2.9                              | Japan   | Toryu, 1933                   |
| <i>Ancylostoma caninum</i> ...   | mixed | 1.6                              | USA     | v. Brand and Otto, 1938       |
| <i>Strongylus vulgaris</i> ...   | ?     | 3.5                              | Japan   | Toryu, 1933                   |
| <i>Filaria equina</i> .....      | ?     | 2.2                              | Japan   | Toryu, 1933                   |
| Larval <i>Eustrongylides</i> ... | ..    | 6.9                              | USA     | v. Brand, 1938                |

Apparently the glycogen content of parasitic nematodes is always high. The lowest value amongst the intestinal nematodes was found in *Ancylostoma*. This may be related to the fact that the hookworms have access to larger amounts of oxygen than the other intestinal helminths. It is curious that *Ascaris lumbricoides* analyzed in Denmark and Russia yielded higher average glycogen values than those in USA and Germany. It is unknown whether this is caused by a different diet of the host and therefore of the parasite in various countries, or merely to different handling of the pigs before slaughtering.

Sexual differences in glycogen content of parasitic nematodes do not seem to be pronounced. Smorodincev and Bebesin (1936) and Toryu (1933) found more glycogen in females than in males of *Ascaris* and *Parascaris*. Von Brand (1937), on the other hand, found slightly more polysaccharide in male ascarids.

So far, only adult nematodes of warm-blooded hosts have been analyzed, and contrary to what is known about many free-living invertebrates, no evidence of seasonal variation in the amount of stored glycogen has been found. The obvious explanation of this difference lies in the uniform conditions under which the parasitic organisms live throughout the year. From this viewpoint, it should prove interesting to survey parasites from poikilothermic and heterothermic hosts, in which such variations are more likely to occur.

The glycogen distribution in various organs and tissues has been investigated both by quantitative chemical methods and by differential staining. Toryu's (1933) analyses of various organs of *Parascaris equorum* are summarized in the following table:

| Organ   | —Glycogen in percent of— |     |                |     |
|---|--------------------------|-----|----------------|-----|
|   | fresh substance          |     | total glycogen |     |
|   | ♀                        | ♂   | ♀              | ♂   |
| Body wall (cuticle + sub-cuticle + muscles) ..... | 5.8                      | 4.9 | 66             | 96  |
| Intestine .....                                   | 0.6                      | 0.6 | 2              | 2   |
| Ovary .....                                       | 6.5                      | ... | 23             | ... |
| Uterus .....                                      | 1.6                      | ... | 9              | ... |
| Male reproductive system .....                    | ...                      | 0.5 | ...            | 2   |

The body wall is obviously the most important storage place for glycogen in worms of both sexes.

Differential glycogen staining has been used chiefly by v. Kemnitz (1912) and Martini (1916) working with *Ascaris* and *Oxyuris*, respectively. These workers extended the earlier investigations of Brault and Loeper (1904) and Busch (1905). It seems that in both cases the most intensive glycogen reactions are found in the plasmatic bulbs of the muscle cells of the body wall and in the hypodermis, especially in the region of the lateral chords, but it was also found in other organs, for example, the intestine (compare also Hirsch and Bretschneider, 1937) and the reproductive organs. Glycogen, however, was never found in the cuticle, the phagocytic organs and the nervous system. Additional data on the glycogen morphology of other parasitic nematodes (*Parascaris*, *Sclerostomum*, *Heterakis* and *Ancylostoma*) are found in the papers of Busch (1905), v. Kemnitz (1912), Fauré-Fremiet (1913), Toryu (1933) and Giovannola (1935). In these cases, the general

pattern of glycogen storage seems to be similar to that of *Ascaris*. In accordance with the quantitative chemical observations much less glycogen was found by morphological methods in hookworms than in ascarids. In the former, however, the rays of the bursa are an important storage place, and probably represent an energy reserve for the male during the periods of copulation when it is detached from the intestinal wall (Giovannola, 1935).

Not much is known about the occurrence of carbohydrates of lower molecular weight in parasitic nematodes. Weinland (1901) found 1.6 percent, and Schulte (1917) found 0.9 percent glucose in *Ascaris lumbricoides*. It is, however, questionable whether these figures are not too high, due to a partial breakdown of glycogen during the analyses. According to Foster (1865) and v. Brand (1934) only very small amounts of reducing sugar occur in *Ascaris*. Fauré-Fremiet (1913) found 0.15 percent glucose in the body fluid of *Parascaris*.

#### ETHER EXTRACTABLE MATERIAL

The parasitic nematodes seem to contain only small amounts of material extractable with ether or petrol ether. The mean values for *Ascaris lumbricoides* vary from 1.2 to 1.6 percent (Weinland, 1901; Flury, 1913; Schulte, 1917; v. Brand, 1934; Smorodincev and Bobesin, 1936), and the value for a larval *Eustrongylides* is 1.1 percent (v. Brand, 1938).

The chemical compounds comprising the ether extract seem to be quite similar in *Ascaris* and *Parascaris* (Flury, 1912; Fauré-Fremiet, 1913; Schulz and Becker, 1933). According to Flury (1912) 100 gm of ether extractable material from *Ascaris* contains the following:

|                               |          |
|-------------------------------|----------|
| Volatile fatty acids .....    | 31.07 gm |
| Saturated fatty acids .....   | 30.89 gm |
| Unsaturated fatty acids ..... | 34.14 gm |
| Unsaponifiable matter .....   | 24.72 gm |
| Glycerol .....                | 2.40 gm  |
| Lecithin .....                | 6.61 gm  |

The volatile fatty acids were represented chiefly by valeric and butyric acids, with small amounts of formic, propionic and acrylic acid. In *Parascaris* the whole series of volatile fatty acids has been reported (Schimmelpfennig, 1903). The saturated fatty acids of higher molecular weight were recognized as stearic acid with a small admixture of palmitic acid. Oleic acid was the chief representative of the unsaturated fatty acids. Flury's value for glycerol is probably too low. Schulz and Becker (1933), using newer methods, found glycerol values ranging up to 8.8 percent. It is, therefore, unnecessary to assume as seemed necessary to Flury (1912) that there is a combination of part of the fatty acids with the unsaponifiable matter. It is probable that all the fatty acids are present in form of glyceryl esters. The unsaponifiable material is of special interest because it contains a compound which so far has been found in no other animal. This substance was found independently by Flury (1912) and Fauré-Fremiet (1913), and it is known as ascaryl alcohol. It was recently reinvestigated by Schulz and Becker (1933), who assigned it the formula  $C_{25}H_{46}O_4$ . They state that its configuration is not yet sufficiently known, but that it may be an ethereal combination of glycerol with some higher alcohol. According to Fauré-Fremiet (1913) ascaryl alcohol occurs in the female reproductive cells only. Under these circumstances one wonders why neither Flury (1912) nor Schulz and Becker (1933) mention any other unsaponifiable substance, which should be expected in other parts of the body. Fauré-Fremiet (1913) found small amounts of cholesterol in the body fluid, the eggs, and the testes of *Ascaris*, but Bondouy (1910) found no cholesterol in *Strongylus equinus*. The ether extract of the latter species seems to be characterized by the presence of soaps.

Little is known about the distribution of the ether extractable material in different organs. Flury (1912) found it to comprise 1.00 percent of the body wall of *Ascaris* and 4.0 to 6.25 percent of the reproductive organs. The latter figure agrees with that given by Fauré-Fremiet (1913) for the testes. If allowances are made for the relative weights of body wall and reproductive systems, it seems probable that roughly the same amount of ether extractable material is stored in both these places. This is in marked contrast to the distribution of glycogen.

Microscopical examinations (v. Kemnitz, 1912; Fauré-Fremiet, 1913; Mueller, 1928/29; Hirsch and Bretschneider, 1937) have shown that fat droplets are deposited in the plasma bulbs of the muscles of *Ascaris*, in which the nuclei are usually surrounded by an accumulation of fat, in the four chords, and

especially in the subcuticula. Stainable fat was also found in ganglion cells, the intestinal cells, and the reproductive organs. According to Mueller (1928/29) considerably more fat can be demonstrated with osmic acid in *Parascaris* than in *Ascaris*, although the pattern of fat deposition is the same in both species.

#### NITROGEN CONTAINING SUBSTANCES

Flury (1912) found 8.1 percent proteins in *Ascaris*. This is somewhat less than should be expected from Weinland's (1901) N figure of 1.80 percent. Flury (1912) ascertained the presence of albumin, globulin, albumoses and peptones, purinebases, amines and ammonia, and he identified a series of amino acids as degradation products of the worm protein. Recently Yoshimura (1930) performed a quantitative analysis of the amino acids resulting from the hydrolysis of ascarids with sulfuric and hydrochloric acid. His results are summarized in the following table:

| Amino acids in percent of dry substance upon hydrolysis with |                   |                 |               |
|--|-------------------|-----------------|---------------|
|  | hydrochloric acid |                 | sulfuric acid |
| Leucine .....  | 3.70              | Leucine .....   | 15.54         |
| Alanine .....  | 1.45              | Histidine ..... | 0.45          |
| Valine .....   | 0.79              | Arginine .....  | 1.28          |
| Proline .....  | 3.41              | Lysine .....    | 2.58          |
| Isoleucine .....   | 1.45              | Tyrosine .....  | 2.09          |
| Serine .....   | 0.72              |                 |               |
| Glutamic acid .....  | 3.93              |                 |               |
| Aspartic acid .....  | 0.36              |                 |               |
| Glycocoll .....  | 0.29              |                 |               |
| Phenylalanine .....  | 0.02              |                 |               |

The N containing substances constituting the cuticle have already been discussed in another chapter (see page 32), and that characteristic of the eggs (chitin) is mentioned on page 177.

Fauré-Fremiet (1913) described under the name of ascardinine an intracellular protein of the spermatozoa of *Ascaris*. It contains 17.5 percent N, but no phosphorus or sulfur. The chemical constitution of this interesting compound is not yet sufficiently known. It is insoluble in cold distilled water, but dissolves rapidly in water of 50 to 51 °C. This critical temperature varies greatly if the substance is dissolved in various salt solutions (Fauré-Fremiet and Filhol, 1937). According to Champetier and Fauré-Fremiet's (1937) roentgenographic studies ascardinine seems to be a semi-crystalline substance, but it can be changed experimentally into an amorphous state.

In recent years an increasing amount of attention has been given to the occurrence of respiratory pigments in parasitic nematodes. Haemoglobin seems to be widely distributed. It has been found in *Dioctophyma*, *Ascaris*, *Parascaris*, *Toxocara*, *Nematodirus*, species of *Trichostrongylus*, *Camallanus*, *Spirocerca*, a larval *Eustrongylides* and larvae of *Trichinella* (Aducci, 1889; Flury, 1912; Fauré-Fremiet, 1913; Keilin, 1925; Kruger, 1936; v. Brand, 1937; Davey, 1938; Stannard, McCoy and Latchford, 1938; Wharton, 1938, 1941; Hsü, 1938; Janicki, 1939). The best known case is that of *Ascaris* where it is found both in the body fluid and the body wall. The absorption bands of the haemoglobins occurring at these two places are slightly different, and this indicates the presence of two kinds of haemoglobin (Keilin, 1925). In all the above cases, where haemoglobin has been found beyond the intestinal wall, one can safely assume that it has been synthesized by the worm. Parts of the host haemoglobin molecule may, of course, be used in this process, but no definite data on this possibility have been obtained. Obviously, haemoglobin found in the intestinal tract of a worm will not fall in the same category, though in some instances it may play a physiologically similar role (hookworm, for example).

The only other respiratory pigments found so far are cytochrome, which is known to occur in *Ascaris*, *Parascaris* and *Camallanus* where the highest concentration is found in the eggs and sperm (Keilin, 1925; Wharton, 1941) and flavine found by Gorrévitch (1937) in *Parascaris*.

## INORGANIC SUBSTANCES

*Ascaris lumbricoides* according to Flury (1912) contains 0.76 percent inorganic substances, and a larval *Eustrongylides* according to v. Brand (1938) contains 1.1 percent.

A quantitative analysis of the inorganic substances of *Ascaris* by Flury (1912) gave the following results:

|                        |                          |
|------------------------|--------------------------|
| Na .....               | 1.104% of the dry weight |
| K .....                | 0.607                    |
| Ca .....               | 0.404                    |
| Mg .....               | 0.058                    |
| Al .....               | 0.131                    |
| Fe .....               | 0.019                    |
| Cl .....               | 1.272                    |
| PO <sub>4</sub> .....  | 1.315                    |
| SO <sub>4</sub> .....  | 0.114                    |
| SiO <sub>2</sub> ..... | 0.029                    |

Neither copper nor manganese was found, and it can be said that on the whole the composition of the ash of *Ascaris* seems to be quite similar to that of free living organisms.

The osmotic pressure of the tissues of several *Ascaris* species and that of the body fluid of *Parascaris* (Vialli, 1923, Schopfer, 1926, 1932) is similar, but not identical to that found in the host intestine. The osmotic pressure of the worms always seems to be a little lower, so that they live in a slightly hypertonic environment. It is noteworthy that chlorides seem to play only a minor role in producing the normal osmotic pressure of the body fluid of *Parascaris* (Marec, 1865, Schopfer, 1932). The total osmotic pressure corresponds to a freezing point depression ( $\Delta$ ) of  $-0.62^{\circ}\text{C}$ , whereas the osmotic pressure due to the chlorides is equivalent to a  $\Delta$  value of  $-0.12^{\circ}\text{C}$ . The osmotic pressure varies directly with that of the environment.<sup>1</sup> The osmotic pressure of *Proleptus obtusus* living in the marine elasmobranch *Scylliorhynchus* is considerably higher than that of the other parasites mentioned and is slightly higher than that of *Scylliorhynchus* blood ( $\Delta = -2.40^{\circ}$ , Schopfer, 1932).

<sup>1</sup>Panikkar and Sproston (1941) give data for *Augustineacum* sp. from the intestine of the tortoise. It is of interest that according to Stoll (1940) the first parasitic ecdysis of *Haemonchus contortus* is favored by hypotonic solutions.

## Metabolism of Adult Nematodes

### METABOLISM UNDER ANAEROBIC CONDITIONS

Most of the experiments on nematodes under anaerobic conditions have been performed with *Ascaris lumbricoides*. Bunge (1889) found that this species can be kept for several days in the absence of oxygen and that it produces during this time carbon dioxide and a volatile acid. Considerable progress was made by Weinland (1901) who performed quantitative determinations of the amounts of various substances consumed and produced and who recognized that carbohydrates were predominantly used. In starvation experiments of several days' duration he found that 100 gm of worms consumed 0.7 gm glycogen and 0.1 gm glucose in 24 hours. He found among the end products 0.4 gm carbon dioxide and 0.3 gm of a volatile fatty acid which he identified as valeric acid. Later Weinland (1904) found that caproic acid was also present in the ether soluble excreta of *Ascaris*. A quantitative study of fat and nitrogen in similar starvation experiments led Weinland (1901) to the conviction that both carbon dioxide and fatty acids were derived from the breakdown of glycogen, and he compared this process to the fermentations produced by microorganisms. This view concerning the anaerobic processes of *Ascaris* is still valid, although subsequent investigations necessitated certain changes in Weinland's conclusions. In the first place it was found that in addition to valeric and caproic acids, some formic, butyric (Flury, 1912) and lactic acid (v. Brand, 1934a) were also present in the excreta. At present it is certain that valeric acid is the chief end product, but there is some uncertainty as to the type of valeric acid excreted. It seems probable that it is normal valeric acid (Waechter, 1934), although Flury (1912) believed that he had identified iso-valeric acid. Krüger (1936) suggested the presence of methyl-ethyl-acetic acid, but Oesterlin (1937) pointed out that this identification was insufficiently supported by Krüger's data.

The second necessary modification of Weinland's conclusions concerns the intensity of the fermentation process. It was found that with increasing length of starvation a decreasing daily amount of glycogen was used and that less carbon dioxide was produced (Weinland, 1901; Schulte, 1917; v. Brand, 1934a, 1937; Krüger, 1936). In experiments conducted for only 24 hours with fresh worms about 1.4 gm of glycogen was used. This is twice as much as Weinland (1901) found for the aver-

age daily glycogen consumption (0.7 gm) in experiments which lasted as long as 6 days. It is, however, curious and not yet sufficiently understood, that despite the different lengths of their experimental periods, most of the above mentioned investigators found that between 0.2 and 0.3 gm of valeric acid was produced per day. Krüger (1936), however, found that about 0.5 gm fatty acid was excreted during the first 24 hours.

The last complete biochemical balance under anaerobic conditions was given by v. Brand (1934a) for females of *Ascaris lumbricoides*. He found that 100 gm of worms consumed, during 24 hours at  $37^{\circ}\text{C}$ , 1.39 gm glycogen and produced 0.71 gm carbon dioxide, 0.22 gm valeric acid, and 0.02 gm of lactic acid. No complete data are available for males. It has been found, however, that the glycogen consumption is identical in both sexes during the first 24 hours and that the more active males later consume more glycogen than the females (v. Brand, 1937a).

*Parascaris equorum* seems to have a quite similar carbohydrate metabolism. Fischer (1924) ascertained the production of small amounts of lactic acid. Toryu (1936a) found a small amount of lactic and propionic acid and a large amount of valeric acid, but no formic, acetic, butyric, caproic, malic, citric or succinic acids. His glycogen/acid balance for the first 24 hours of anaerobiosis for 100 gm of worms was as follows: Consumed: 1.39 gm glycogen. Produced: 0.65 gm valeric acid and 0.02 gm lactic acid. In addition carbon dioxide was produced and the amount of carbon dioxide differed markedly for females and males (Toryu, 1936b). It is not clear what animals were used for the glycogen/acid experiments, and therefore it is impossible to introduce reliable carbon dioxide values into the above balance.

The above data indicate that the end products of the anaerobic carbohydrate metabolism are chiefly lower fatty acids and therefore noticeably different from that of a vertebrate muscle. This concept has been criticized chiefly by Fischer (1924) and Slater (1925). The former investigator concedes that living *Parascaris* excrete only a small amount of lactic acid, and a larger amount of an unidentified acid. He found, however, that in minced material the production of lactic and the liberation of phosphoric acid was sufficient to account for the whole acidity observed in aerobically conducted experiments. Therefore, he concluded that there was no great difference between the glycogen breakdown in *Parascaris* and in vertebrates. In the opinion of the present writers, however, his observation indicates merely that through changes in the experimental conditions the course of the chemical reactions can be changed—a phenomenon well known in experiments with yeast and other lower plants. It should be remembered that Weinland (1902) found the same end products with extracts of *Ascaris* under anaerobic conditions as he had found in experiments with whole worms.

Slater (1925) demonstrated that bacteria capable of transforming sugar into volatile fatty acids could be isolated from a saline solution in which ascarids had been immersed. He failed, however, to show that they were present in sufficient numbers to account for all the organic acids produced in experiments with worms, and, furthermore, he did not demonstrate any substance which could have served as a substrate for such bacterial fermentation.

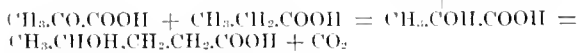
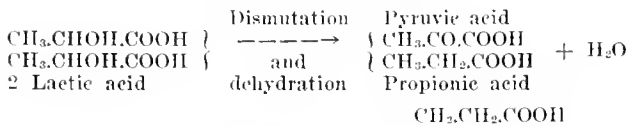
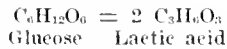
Several lines of evidence have been brought forward which seem to indicate a direct connection between nematodes and the production of lower fatty acids. The following two may be mentioned. The volatile acids are found not only in saline in which worms have been kept, but also in distillates of minced worms (Weinland, 1901) and in the ether extract of whole worms (Flury, 1912; Schimmelpfennig, 1903). Valeric acid has, furthermore, been found under both aerobic and anaerobic conditions, although one should expect that such a difference in the external conditions should have a deep influence on the development of a bacterial flora in the surroundings. For further information on this controversy compare the discussion of Slater (1928) with those of Weinland (1901) and v. Brand (1934b).

Several methods have been discussed in which valeric acid may originate from carbohydrate. Weinland (1901) favored the following equation:  $4\text{C}_6\text{H}_{12}\text{O}_6 = 9\text{CO}_2 + 3\text{C}_5\text{H}_{10}\text{O}_2 + 9\text{H}_2$ . It must, however, be emphasized, that the postulated hydrogen could not be found. Weinland (1901) had to assume that it was used at once in other reactions. He also discussed an equation proposed by Koenigs:

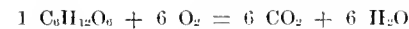
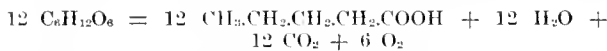
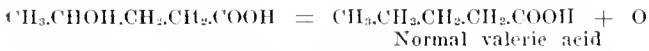


Weinland rejected this equation because it did not predict nearly as much carbon dioxide as he found to be present. However, the excess might have originated either from bicarbonate or from protein decomposition. Jost (1928) has given the following chain of reactions which leads to Koenigs' equa-

tion. These equations are purely theoretical, but the series is interesting in that it shows a possible link between the production of lactic and valeric acids.



γ Hydroxy-valeric acid



In effect, then, 12 molecules of sugar would be transformed into 12 molecules of valeric acid, carbon dioxide and water, and the oxygen liberated during this process would be sufficient to oxidize completely a thirteenth molecule of sugar.

Toryu (1936a) proposed the equation:  $4\text{C}_6\text{H}_{12}\text{O}_6 = 4\text{CO}_2 + 4\text{C}_5\text{H}_{10}\text{O}_2 + \text{H}_2\text{O}$ . This equation needs no further consideration, since the O and H atoms on the two sides do not balance. Correctly written it would read:  $4\text{C}_6\text{H}_{12}\text{O}_6 = 4\text{CO}_2 + 4\text{C}_5\text{H}_{10}\text{O}_2 + 4\text{H}_2\text{O} + 2\text{O}_2$ . This obviously corresponds closely to an intermediate step of Koenigs' equation as formulated by Jost.

The amount of heat produced during the metabolism of *Ascaris lumbricoides* was first determined directly by Krummacher (1919). His experiments, however, were performed at a time at which oxygen was regarded as an inert gas for these worms. Krummacher's experiments were neither clearly aerobic nor anaerobic, and the data obtained are therefore difficult to interpret. Meier (1931), on Krummacher's suggestion, performed similar experiments under anaerobic conditions. He found a heat production of 0.300 gm cal per gm of worm per hour. On the basis of Weinland's chemical data and his own heat determinations he calculated that the fermentation process yields 22 percent of the energy obtainable by total oxidation of the carbohydrate. This is considerably more than usually found in bacterial fermentations. Undoubtedly, however, Meier's figure of 22 percent is far too high. His experimental periods lasted only from 4 to 12 hours, and he used presumably fresh worms. Therefore, the carbohydrate consumption must have been much higher than Weinland's figure. Furthermore, Schulte (1917) has demonstrated by direct comparisons of the heat of combustion with the glycogen content of fresh and starving ascarids that the carbohydrate metabolism accounts for only 80 percent of the total loss of calories from the body. Meier, however, assumed that the total heat production was due to carbohydrate fermentation. At present the data necessary for an exact balance sheet of the energies involved seems to be unavailable. A fair guess would place the energy yield of the fermentation between 6 and 12 percent. This is still more than that usually found in bacterial fermentations. Lactic acid fermentation, for example, yields only about 2.6 percent, and alcoholic fermentation yields 4 percent.

Changes under anaerobic conditions in the material extractable with ether have been studied less thoroughly than the changes in glycogen content. Weinland (1901) found that there was no change in the fat content of ascarids during starvation, and v. Brand (1934a) reached the same conclusion. Schulte (1917), on the other hand, observed a fat increase of 0.08 gm per 100 gm animals per day. He considered this fat to be a product of carbohydrate fermentation. It seems certain, at least, that no fat is consumed under anaerobic conditions. This is not astonishing, because it seems hardly possible that an anaerobic process could yield energy from an oxygen poor substance like fat (Weinland, 1901).

The nitrogen metabolism of *Ascaris* is not very great. For 100 gm of worms the amount of nitrogen excreted in 24 hours was found by Weinland (1904b) to be 15 to 20 mgm and by v. Brand (1934a) to be 29 mgm. One third of the excreted N is ammonia, and the greater part of the remainder can be precipitated by phosphotungstic acid (Weinland, 1904b). Flury (1912) found that the worms excreted not only ammonia but small amounts of amine bases, substances which gave the

biuret reaction, hydrogen sulfide (also Krüger, 1936), and mercaptan. According to v. Brand (1934a) about one fourth of the total excreted N is contained in discharged eggs. Chitwood (1938) found urea in a concentration of about 0.02 percent in the fluid from the excretory pore of freshly collected worms. After 24 hours of starvation the tests for urea were negative, and Chitwood doubts that the urea was formed by the worm. It may have been obtained from the host.

#### METABOLISM UNDER AEROBIC CONDITIONS

Weinland (1901) believed that *Ascaris* did not consume oxygen. However, he did observe that more carbon dioxide was evolved under aerobic than under anaerobic conditions. He explained this on the assumption that the extra carbon dioxide was due either to the metabolism of aerobically developing eggs or to that of an aerobic bacterial flora. His view was generally accepted until Adam (1932) proved that *Ascaris* was able to consume oxygen. The observations of Adam were soon confirmed and extended to other forms. The following table summarizes some of these data on oxygen consumption.

| Species                     | Sex | O <sub>2</sub> consumption in gm per 100 gm worms in 24 hrs. at body temp. | Investigator            |
|-----------------------------|-----|--|-------------------------|
| <i>Ascaris lumbricoides</i> | ♂   | 0.38   | Adam, 1932              |
| <i>Ascaris lumbricoides</i> | ♀   | 0.21   | Adam, 1932              |
| <i>Ascaris lumbricoides</i> | ♀   | 0.21   | v. Brand, 1934          |
| <i>Ascaris lumbricoides</i> | ♀   | 0.13   | { Harwood and           |
| <i>Ascaris lumbricoides</i> | ♂   | 0.21   | { Brown, 1934           |
| <i>Ascaris lumbricoides</i> | ?   | 0.55*  | Krüger, 1936            |
| <i>Ascaris lumbricoides</i> | ?   | 0.27   | Krüger, 1936            |
| <i>Parascaris equorum</i>   | ♀   | 0.08   | Toryu, 1936             |
| <i>Parascaris equorum</i>   | ♂   | 0.35   | Toryu, 1936             |
| <i>Setaria equinum</i>      | ?   | 0.89   | Toryu, 1936             |
| <i>Ancylostoma caninum</i>  | ?   | more than ten times as much as female <i>Ascaris</i>                       | Harwood and Brown, 1934 |

The oxygen consumption of both *Setaria* and *Ancylostoma* is considerably higher than that of *Ascaris* or *Parascaris*. The former undoubtedly have easier access to oxygen and may therefore be better adapted to aerobic metabolism.

The amount of oxygen consumed by *Ascaris* is influenced by several factors. One factor is size, and small animals consume relatively more than large ones. However, it is doubtful if the difference in oxygen consumption of males and females can be explained merely on the basis of size. Krüger (1936) gave a formula which allows one to calculate approximately the increase of oxygen consumption with increasing weight. The formula is applicable only to worms which weigh over 1.4 gm. In smaller worms the increase is more rapid. Krüger stated nothing about the sex of his worms, but the deviation of his data from the formula begins near the average weight of males. In a recent paper Krüger (1940) shows that the O<sub>2</sub> consumption of ascarids of various sizes is fairly constant if referred to surface rather than weight.

The oxygen consumption of starving ascarids kept for long periods of time at the oxygen tension of air show a general tendency to increase (v. Brand, 1934a; Krüger, 1937). This might be an indication of adaptation to the abnormally high oxygen tension.

The oxygen consumption of *Ascaris* varies directly with the oxygen tension, regardless of whether whole worms, parts of worms or even minced material is used (Harnisch, 1933; Krüger, 1936). This is a striking contrast to what is known from massively built free-living organisms, like actinians. In these a similar dependence is observed in whole animals, but it disappears if minced material is used. The diffusion rate of oxygen is the limiting factor, and if the path through which oxygen has to diffuse is shortened by using minced animals, the oxygen consumption remains virtually unchanged over a wide range of tensions. This explanation can not hold for *Ascaris*. Harnisch, however, has found that the oxygen consumption of planarians and *Chironomus* larvae, which is normally independent of oxygen tension, may become dependent if the animals are subjected to anaerobic conditions prior to the experiments. In his opinion two kinds of aerobic processes must be distinguished: (1) a primary aerobic process which is considered to be independent of the oxygen tension, and (2) a secondary process which is considered to be dependent. In *Ascaris* only

\* Krüger (1936) gives data of various sized worms. Those for worms of about the average size of males and females have been introduced in the table, the higher figure being for worms of 1.5 gm, the lower for worms of 4.5 gm.

the secondary aerobic process is present. Harnisch (1937) offered support of this view in the observation that washed minced *Ascaris* material has only a negligible oxygen consumption. The same material, suspended in *Ascaris* body fluid, has a very high oxygen consumption and surpasses even that of non-minced material. According to Harnisch this indicates the presence of a powerful oxidizing mechanism outside of the cells which may govern the entire aerobic processes of *Ascaris*. This, he claims, is in accordance with his explanation of experiments with artificially induced secondary aerobic processes in *Chironomus*. The cellular agents which govern the primary aerobic processes in *Chironomus*, however, could not be removed from the cells by washing (Harnisch, 1936).

The data of Kempner (1937) show that in a variety of biological materials the effect of oxygen tension on oxygen consumption varies with pH, CO<sub>2</sub> tension, salt content, and temperature. It is apparent that certain tissues heretofore considered to have a respiratory mechanism unaffected by oxygen tension really show an independence only in alkaline CO<sub>2</sub>-free media in a certain temperature range. These observations of Kempner indicate that the whole question of oxygen tension versus oxygen consumption should be reexamined, and that the respiration of no material can be said to be completely dependent or independent of O<sub>2</sub> tension unless the effects of the above factors have been investigated. It is possible that these factors may have some effect on the nematode data discussed above. A discussion of the theoretical relationship between oxygen tension and oxygen consumption is given by Marsh (1935).

It seems that all the different organs of *Ascaris* are able to consume oxygen. This has been shown for the body wall, intestine, ovaries, uterus and even the body fluid (Harnisch, 1935, 1937; Krüger, 1936). The largest absolute amount is consumed by the body wall, although the intestine shows the highest rate of oxygen consumption.

It is now generally believed that ascarids evolve larger amounts of carbon dioxide under aerobic than under anaerobic conditions (Weinland, 1901; v. Brand, 1934a; Krüger, 1936), and Harnisch (1937) has abandoned his previous contention to the contrary. The respiratory quotient in air is consistently very high. In fresh worms it may be about 4 or even higher, and in worms kept for several days in saline it is between 1.27 and 1.88 (Krüger, 1937). This indicates that the oxidation of metabolites is not complete and that even in the presence of oxygen the metabolism consists in part of anaerobic fermentations.

The excretion of organic acids under aerobic conditions, first seen by Weinland (1901), is definite proof of the presence of fermentations. The acids have been identified as small amounts of lactic acid (v. Brand, 1934a), formic, acetic, and probably butyric acid, a large amount of valeric acid and some unidentified higher acids (Oesterlin, 1937). Since these products are similar to those formed under anaerobic conditions (see above), it seems likely that the fermentations going on under aerobic and anaerobic conditions are identical. The amounts of acids excreted at the oxygen tension of air are definitely lower than under strictly anaerobic conditions (v. Brand, 1934a; Krüger, 1936, 1937), but at low oxygen tensions even more acids are excreted (Krüger, 1936).

It is customary in the nematode literature to refer to the oxidations which involve oxygen consumption and which lead to the production of carbon dioxide and water as oxidative metabolism and to refer to the molecular rearrangements and oxido-reductions which lead to the production of carbonic, lactic, valeric, and other acids and in which oxygen is not consumed as fermentative metabolism. Von Brand (1934a) and Krüger (1937), by basing calculations on the ratio of anaerobically evolved carbon dioxide to anaerobically excreted acids or similar data at low oxygen tensions, calculated the amounts of aerobically evolved carbon dioxide which originated in fermentative and in oxidative metabolism. This latter figure was used, in connection with the oxygen consumption, to calculate the true respiratory quotient which was found to be about 0.9 or 1.0. In some cases very low quotients were found, and these data are difficult to explain at the present time. The opinion of Harnisch (1933) that the aerobic processes do not lead to the production of CO<sub>2</sub> and that the respiratory quotient is zero has been generally abandoned.

Krüger (1936) found that the uncorrected respiratory quotient of ascarids kept in air instead of saline fell rapidly to about 1.0 and remained at this level for some time. This would indicate (Krüger, 1937) either that the fermentations cease altogether, or that the fermentations present do not lead to carbon dioxide production (e.g., lactic acid formation).

The question of what substances are oxidized has received some attention by v. Brand (1934a). He found that under aerobic conditions somewhat less glycogen is consumed than

under anaerobic ones. On an assumption similar to that made above for the carbon dioxide, he calculated the amounts of the consumed glycogen which had apparently been decomposed by fermentative and by oxidative metabolism. He arrived at the following balances:

Uncorrected balance for 100 gm worms starving at 37° C. under aerobic conditions:

Decomposed: 1.18 gm glycogen. Consumed: 0.21 gm oxygen. End products: 0.84 gm carbon dioxide + 0.16 gm valeric acid + 0.01 gm lactic acid.

Oxidative part of the metabolism:

Decomposed: 0.37 gm glycogen. Consumed: 0.21 gm oxygen. End products: 0.34 gm carbon dioxide + ?.

Fermentative part of the metabolism:

Decomposed: 0.86 gm glycogen. End products: 0.48 gm carbon dioxide + 0.16 gm valeric acid + 0.01 gm lactic acid.

The amount of glycogen which disappeared was so great that complete oxidation to carbon dioxide and water could not be assumed for all of that which was calculated to undergo oxidative metabolism. Probably only a partial oxidation takes place (formation of aldehydes?).

Harnisch (1935) thought that possibly iso-valeric acid would be oxidized to acetoacetic acid or  $\beta$  hydroxy-butyric acid which in turn would be decomposed to acetone and carbon dioxide. However, chemical determinations on the excreta do not favor this view. This statement applies also to v. Brand's (1934a, b) original theory that fats may be changed into carbohydrate.

It seems as if *Ascaris*, in contrast to many free-living animals, does not contract a noticeable oxygen debt during a period of anaerobiosis (Adam, 1932; Harnisch, 1933). It was found (v. Brand, 1937b), however, that ascarids subjected to 20 hours anaerobiosis and then brought for 2 to 6 hours into aerobic conditions, resynthesized 1/20 to 1/10 of the glycogen consumed during the anaerobic period. This resynthesis is clearly an aerobic process, and it is apparently much less pronounced in *Ascaris* than in similarly treated vertebrate muscles. This may be due to the fact that in vertebrate muscle the end products accumulate, whereas in *Ascaris* they are excreted, and only those present in the body at the beginning of the aerobic period are available for resynthesis. It is unknown whether lactic acid or the lower fatty acids are resynthesized to glycogen.

There is still some controversy concerning the significance of the aerobic processes of *Ascaris*. Harnisch (1933) assumed that the aerobic processes would yield no energy, and he still thinks (Harnisch, 1935) that they play no role in the normal energy supply of the organism. This view is similar to that of Krüger (1937) who states that they are probably not linked to any specific organ function and that any derived energy is probably wasted. The present writers are of the opinion that at this time no definite statements regarding the possible utilization of this energy can be made.

The fact that the rate of the fermentative processes is reduced at the oxygen pressure of air, seems to indicate rather clearly that fermentations and oxidations are not entirely independent as Harnisch (1933) originally assumed. Whether Krüger's (1937) view is correct that the oxidations follow essentially the same course as in truly aerobic organisms, or whether Harnisch (1937) is right in assuming that they correspond only to the secondary aerobic processes occurring in free living animals only under special conditions, must be decided by future investigations.

The aerobic metabolism of *Parascaris equorum* has been studied by Toryu (1934 to 1936b). He found an almost identical glycogen consumption under aerobic and anaerobic conditions, but since the worms excreted slightly less organic acids under aerobic conditions, he concluded that a small amount of glycogen was oxidized. Apparently the aerobic metabolism of *Parascaris* follows the same pattern as that of *Ascaris*.

The question of whether or not parasitic nematodes use fat under aerobic conditions is difficult to answer satisfactorily at the present time. In v. Brand (1934a) aerobic experiments no fat was used. In view, however, that his experiments lasted only 24 hours and that in general carbohydrate is consumed before the fat reserves are attacked, these experiments can not be accepted as conclusive evidence that no fat may be used during longer periods of starvation. Mueller (1928/29) observed that in explanted pieces of *Ascaris* a loss of morphologically demonstrable fat occurred after several days, and Hirsch and Bretschneider (1937) have shown that in starving ascarids much of the stainable fat disappeared from the intestinal cells after 6 days. These observations are suggestive that fat may be used, but they should be confirmed by quantitative chemical methods.<sup>1</sup> Bondouy (1910) detected a lipase in *Strongylus equinus*, and the possible significance of its presence warrants further study.

<sup>1</sup>In a recent paper v. Brand (1914) showed that *Ascaris* uses no fat for production of energy during an aerobic starvation period of 5 days.



The aerobic and anaerobic nitrogen metabolism of *Ascaris* has been compared by v. Brand (1934a). The amounts of nitrogen excreted both in soluble excreta and in eggs were very nearly identical in both cases. He assumed that at least a large part of the N metabolism was involved in the transformation of the protoplasm of the body into that of eggs. He also considered it likely that at least a large part of the nitrogen metabolism was always anaerobic. This view is supported by the fact that free-living animals, like the leeches, show, in contrast to *Ascaris*, a marked difference in the amount of nitrogen excreted under aerobic and under anaerobic conditions.

#### DEDUCTIONS CONCERNING THE METABOLISM IN VIVO

Deductions concerning the nature of the metabolism of internal parasites can be drawn only from the chemical composition of their surroundings and their metabolism *in vitro*. Of special interest is the question of whether the nematodes parasitizing the intestine lead an anaerobic or an aerobic life. On the basis of the investigations of Bunge (1889) and Weinland (1901) the first possibility was accepted for many years as an undisputed fact. More recently certain investigators (Slater, 1925; Mueller, 1928/29; Adam, 1932; Davey, 1938a and b) have held the opposite view, i.e., that the worms can get enough oxygen in the intestine to allow an oxidative metabolism. Recently v. Brand (1938) has reviewed the question, and he believes that a general answer can not be given. Apparently the size or relative surface and the presence of respiratory pigments will have a great influence on whether a worm can or can not obtain sufficient oxygen at the low tensions prevailing in the intestine. Large parasites, like *Ascaris* or *Parascaris*, must be regarded as predominantly anaerobic organisms. As mentioned above, they show a marked fermentative metabolism even in air. Since their oxygen consumption is dependent on the oxygen pressure, one can be reasonably sure that fermentative metabolism will be relatively much greater in the intestine. Further signs of their adaptation to an anaerobic life are that they are remarkably resistant to the lack of oxygen *in vitro* and that they are able to excrete the end products of anaerobic metabolism. It seems, however, quite possible that the small amounts of oxygen available in the intestine are not entirely without significance. This may be indicated by the observations that the worms contain some haemoglobin, that stimulated *Ascaris* die much more rapidly in absence than in presence of oxygen, and finally that they are able to perform under suitable conditions such a clearly aerobic process as the resynthesis of glycogen.

Small nematodes, on the other hand, offer better opportunities for the diffusion of oxygen because of their relatively larger surface. This may explain why the sheep nematodes do not show (Davey, 1937, 1938a and b) the same resistance against lack of oxygen as *Ascaris*. The conclusion of Davey that these worms lead an aerobic life under natural conditions is, therefore, probably only in apparent contradiction with the statement made above in regard to large helminths.

An entirely different way of getting oxygen may be realized in worms sucking larger amounts of blood from their hosts. According to Wells (1931) the blood sucking activities of hookworms seem to serve largely as a respiratory function. His data allow the calculation that under optimal conditions 100 gm of worms could obtain 20 gm of oxygen from this source in 24 hours. This would be about ten times as much as Harwood and Brown (1934) found to be the actual oxygen consumption.

No data are known about the metabolism of adult parasitic nematodes which normally live outside the intestine. It is therefore unnecessary to enter into a similar discussion concerning their metabolism. On the whole one may assume that they will have frequently, though probably not in every case, better opportunities to get larger amounts of oxygen than the intestinal helminths.

#### SYNTHESIS OF RESERVE SUBSTANCES

There are only a few investigations which concern the question of the synthesis of reserve substances in parasitic nematodes. Hoffman (1934) and Krüger (1936) have shown that the heat production and the oxygen uptake of ascarids under both anaerobic and aerobic conditions are increased if sugar is present in the surrounding medium. Hirsch and Bretschneider (1937) fed ascarids iron saccharate and concluded from their histological investigation that it was absorbed as colloid and broken down only in a certain part of the intestinal cells into iron and sugar.

Quantitative determinations of the glycogen content of carbohydrate-fed ascarids have been performed by Weinland and Ritter (1902). They found no increase in the glycogen content of animals kept in solutions containing various carbohy-

drates, although glucose caused a lowering of the rate of utilization of body glycogen. More positive results were achieved by injecting the sugar solutions into the animals. In these experiments new glycogen was formed after injection of glucose and probably levulose. The consumption of body glycogen was decreased by injections of maltose and perhaps galactose, but not by injections of lactose.

Von Brand and Otto (1938) compared the glycogen content of hookworms from dogs which had been starved for 48 to 72 hours before death with those from dogs which had been given so much sugar during a similar period that the liver glycogen rose from 0.06 percent to 5.04 percent. No difference whatever in glycogen content of the worms was found. This may be related to the fact that hookworms obtain their food from the tissues rather than from the lumen of the intestine and therefore can gain their maximal food requirements even from a starving host.

So far no experiments have been performed on the deposition of fat in parasitic nematodes except the above mentioned doubtful results of Schulte (1917) concerning the fat increase in ascarids under anaerobic conditions. The whole question of synthesis should prove interesting for future investigations.

#### Metabolism of Eggs and Larvae

The eggs of many parasitic nematodes show, like the adults, a surprising degree of resistance to lack of oxygen. The eggs of such forms as *Ancylostoma*, *Parascaris*, *Trichocephalus* or *Nematodirus* can be kept for days or even weeks in the absence of oxygen, but they do not complete their development (Looss, 1911; Bataillon, 1910; Zawadowski, 1916; Fauré-Fremiet, 1913; Zawadowski and Orlov, 1927; Zviaginzev, 1934; Dinnik and Dinnik, 1937). In *Parascaris* oxygen is unnecessary only during the early stages, i.e., maturation, fertilization and perhaps the first cleavage stages; for further development oxygen is indispensable (Fauré-Fremiet, 1913; Szweikowska, 1929; Dyrkowska, 1931). The need of oxygen for completion of development seems to be a general requirement, although the stage of development at which oxygen becomes necessary seems to vary somewhat with different species. Zawadowski and Schalimow (1929), Schalimow (1931), and Wendt (1936) conclude that the necessity for oxygen begins in *Enterobius vermicularis* with the tadpole stage, and in *Oxyuris equi* with the gastrula stage. Relatively low oxygen pressures, however, are sufficient to insure normal development in *Ascaris* and *Ancylostoma* (Brown, 1928; McCoy, 1930).

The amount of oxygen consumed by one *Ascaris* egg in developing from the one-cell stage to the motile embryo is about 0.0025 cmm with only slight variations whether the development is completed in 21 days at 23°C or in 11 days at 30°C (Brown, 1928). Huff (1936) obtained a value of 0.0041 cmm for *Ascaris*, and Nolf (1932) obtained a value of 0.0027 for the eggs of *Trichuris*. It is surprising that an *Ancylostoma* egg requires for its development from the morula stage to the fully developed larva almost exactly the same amount of oxygen (0.0028 cmm at 23° C. according to McCoy, 1930) as an *Ascaris* egg, although development of *Ancylostoma* is completed in about 24 hours. Since these eggs are about the same size, it seems as if the difference in the rate of oxygen consumption mentioned above for the adults of these species is also present in the embryonic stages.

Huff (1936) observed that the oxygen consumption of *Ascaris* eggs increased more than five times after removal of the albuminous coating by antiformin. Friedheim (1933) found that the oxygen consumption of *Ascaris* eggs is considerably increased if they are immersed in a dilute solution of hallochrome (a pigment which is a reversible oxidation-reduction system isolated from the polychaete worm *Halla parthenopea* and which has an accelerative effect on respiration). The mechanism of the increase in respiration by either of these two methods is not known. Friedheim (1933) apparently used mixed stages of fertilized eggs, and there seems to be no reason for assuming that hallochrome could penetrate the egg shell. Therefore, one might expect the acceleration obtained to be due to an increase in the effective oxygen tension or to an increase of respiration in only those eggs on which an impermeable shell had not yet been formed. The experiments of Huff might also be explained as being caused by an increase in effective oxygen tension because of slow diffusion of oxygen through the albuminous coat, but no data concerning these possibilities are available. Since the R. Q. is always less than 1.0 (see below) the possible effect of oxygen tension could not be merely to change the ratio of oxidative and fermentative metabolism. The accelerations produced by Friedheim (1933) and Huff (1936) must, for the present, be accredited to changes in the rate of oxidative metabolism, and the reasons for the changes remain obscure.

The oxygen consumption of *Ascaris* or *Parascaris* eggs has also been reduced experimentally by ultracentrifuging and by exposure to cyanide (Zawadowsky, 1926; Huff and Boell, 1936). About 90 percent of the respiration was sensitive to cyanide, and it seemed that ultracentrifuging affected only the cyanide sensitive respiratory mechanism.

The respiratory quotient of *Parascaris* and *Ascaris* eggs has been found to be below 1, and this indicates that, in contrast to results on tissues of the adult worm, no fermentative processes are present in the eggs. The respiratory quotient determined at the beginning of development was about .80, and, with some variations in the case of *Parascaris*, it increased during the later stages to .92-.98 (Fauré-Fremiet, 1913a, 1913; Huff, 1936). The total energy liberated by one *Parascaris* during its development was  $50 \times 10^{-7}$  cal. (Fauré-Fremiet, 1913). Nolf (1932) found that the R. Q. of *Trichuris* decreased from a value of 1.0 for the first 5 days of development to a value of 0.73 for the 8th to 15th days.

In considering the chemical changes which occur in the eggs of parasitic nematodes during their development, one must distinguish clearly between processes which lead to the formation of the egg shells and processes which liberate energy. The shells, as far as they are formed from the ovum, consist essentially of the shell proper and the vitelline membrane. The shell is composed of chitin in such species as *Parascaris*, *Ascaris*, *Diectophyma* and *Enterobius* (Fauré-Fremiet, 1913; Szejnkowska, 1929; Schmidt, 1936; Wottge, 1937; Chitwood, 1938; Jacobs and Jones, 1939). The investigations of Fauré-Fremiet (1913) and Szejnkowska (1929) have demonstrated that in *Ascaris* about half the glycogen stored in the oöcytes was used to form the glucosamine incorporated in the chitin. The latter has shown in addition that 26 percent of the total nitrogen of the egg was used during the chitin formation.

The vitelline membrane of the eggs of these and other species is of a lipid nature (Fauré-Fremiet, 1913; Zawadowsky, 1928). Fauré-Fremiet considered it to be mainly ascaryl alcohol. Wottge (1937) obtained a positive reaction for cholesterol, and Chitwood (1938) and Jacobs and Jones (1939) demonstrated that it gave sterol reactions. During the secretion of this layer certain changes in the chemical nature of the ether soluble substances, perhaps a saponification, seemed to occur. The necessity for further studies is indicated.

Chemical analyses of the egg indicate that both glycogen and fat are oxidized, and these data are in accordance with the above data on the respiratory quotient. Szejnkowska (1929) found in *Parascaris* eggs just after fertilization about 0.46 percent volatile fatty acid and 0.53 percent higher fatty acids. After formation of the second polar body these substances had diminished to 0.34 and 0.36 percent respectively. For the same period it was calculated that in addition to the glycogen used in the formation of chitin an amount of glycogen corresponding to about 2.7 percent of the egg weight had disappeared. From Fauré-Fremiet's (1912, 1913) experiments it would appear that both fat and glycogen were used during the later developmental stages. All of these experiments were conducted under aerobic conditions. Dyrkowska (1931) found by the use of staining methods that the glycogen content of *Parascaris* eggs kept under anaerobic conditions underwent a slight diminution and that there was a marked decrease in the fat content. It seems desirable that this decrease in fat content should be verified with quantitative chemical methods since, as already stated above, it is difficult to understand how processes which liberate energy from fat could occur in the absence of oxygen. It should, furthermore, be remembered that Fauré-Fremiet (1913) gained the impression that the amount of fat in anaerobically kept eggs tended to increase.

With the exception of the above mentioned shifting of nitrogen from the ovum to the chitin shell, nothing is known about the nitrogen metabolism of eggs. Szejnkowska (1929) found no change in the total nitrogen content during the time of maturation, and Kosmin (1928) found the same nitrogen content (1.78 percent) in undeveloped and developed eggs. She points out that this may be caused by the impermeability of the vitelline membrane for protein degradation products which consequently might accumulate in the interior of the egg shells.

The fully developed embryo of *Ascaris* contains glycogen, even in eggs which have been stored for 6 months (Stepanow-Grigoriew and Hoeppli, 1926). This observation has a bearing on Pintner's theory (1922) concerning the physiological reason for the migration of parasitic worms through the host body prior to life in the intestine. Pintner was of the opinion that the chief function of the migration was to allow the worms to live for a time under aerobic conditions. This would allow them to accumulate a glycogen reserve which later on would enable them to begin life in the anaerobic intestine. The above mentioned observation of Stepanow-Grigoriew and Hoeppli (1926)

is not what one might expect on the basis of this theory. However, Stepanow-Grigoriew and Hoeppli (1926) and Giovannola (1936) found a definite accumulation of glycogen during the migration.

The fact that glycogen is still present in old embryos also indicates that the rate of metabolism in fully developed eggs is probably very much lower than in the developing eggs, and this problem seems worthy of quantitative consideration.

The young larvae of *Ascaris*, on the other hand, have a high level of metabolism, as evidenced by the investigation of Fenwick (1938). He found a preliminary phase of about half an hour during which the newly hatched larvae showed a low oxygen consumption. This he explained on the assumption that they had not yet become sufficiently adjusted to the new environment. Then followed an intermediate phase, lasting about an hour, in which 1,000 larvae consumed per hour 9.3 cmm oxygen at 37° C. After this the oxygen consumption decreased to a third level (0.928 cmm per 1,000) which was about 1/10 that of the second level. This new rate of oxygen consumption was maintained throughout the rest of the experiments. Fenwick explained the high rate of the intermediate stage on the assumption that it was caused by the removal of an oxygen debt which the larvae had contracted while living within the egg shells. An investigation of the respiratory quotient of eggs containing infective embryos should prove helpful in answering this question.

The rate of metabolism of *Trichinella* larvae, according to the data of Stannard, McCoy and Latchford (1938), was about as high as that of *Ascaris* larvae in the third of Fenwick's stages. At body temperature in Tyrode solution the *Trichinella* larvae consumed 2.24 cmm oxygen per mgm dry weight per hour. In saline the value was 1.70, and in Tyrode without bicarbonate it was 1.78. The figures for 1,000 larvae in these solutions can be calculated to be about 1.12, 0.85 and 0.88 cmm oxygen per hour, respectively. The respiration was independent of the oxygen tension in the range of 1 to 100 percent oxygen. It was very sensitive to cyanide, but was stimulated by carbon monoxide and paraffinylene diamine. The respiratory quotient of the *Trichinella* larvae was always above 1, and the averages were from 1.13 to 1.17. It seems probable that under aerobic conditions some fermentations may take place, but most of the oxidative processes apparently proceed to completion. Fermentation alone was sufficient to keep the worms alive under anaerobic conditions, but apparently oxygen was necessary for enabling them to move.

The fermentation processes of the *Trichinella* larvae are very interesting, since they lead not only to the formation of carbon dioxide but to the formation of other as yet unidentified substances which are known to be non-acidic. In this respect they differ from all the other helminths. It is remarkable, furthermore, that substances like iodoacetate and others, which rapidly inhibit alcoholic fermentation or muscle glycolysis, were quite slow in their action on the anaerobic carbon dioxide production of these larvae (Stannard, McCoy and Latchford, 1938). McCoy, Downing and Van Voorhis (1941) showed that radio-active phosphorus fed to the host penetrates rapidly into the larvae. This observation indicates that the larvae may have an active metabolism inside the cyst.

The *Trichinella* larvae are clearly aerobic rather than anaerobic organisms. This is also true for the larvae of *Eustrongylides*, investigated by v. Brand (1938). He found that these worms survived much longer under aerobic than under anaerobic conditions. One hundred grams of worms in the presence of oxygen consumed 0.3 gm of glycogen in 24 hours at 37° C., and no organic acids could be found. Under anaerobic conditions 0.9 gm glycogen was consumed and organic acids equivalent to 30 cc n/10 acid were produced. The ratio between aerobically and anaerobically consumed glycogen was 1:3, a ratio which places these worms intermediate between most free-living forms which have ratios of about 1:5 and *Ascaris* with one of 1.0:1.3.

The experiments mentioned so far were performed with larvae which had been living under natural conditions in a host. From free-living stages of parasitic nematodes data are only available for *Ancylostoma caninum*. McCoy (1930) found that the oxygen consumption of infective larvae varied greatly with the temperature. At 7° C. it was imperceptible, but in the range of 17° C. to 42° C. the oxygen consumption increased about 9 percent for every degree rise in temperature, and followed an exponential curve, the  $b$  constant, of which was 1.9879. The actual oxygen consumption at 37° C. corresponded to 0.47 cmm per 1,000 larvae per hour, a figure somewhat lower, but of the same order of magnitude as those mentioned above for *Ascaris* and *Trichinella* larvae.

The free-living larvae of *Necator americanus*, and *Ancylostoma caninum* seem to derive their energy primarily from fatty substances stored in their body (Payne, 1923; Rogers, 1939), and the amount of fat demonstrable seems to be

characteristic of the physiological age of the larvae (Payne, 1923; Cort, 1925). A decrease in the amount of fat granules was also observed by Giovannola (1936) in the filariform larvae of several species, especially if they were kept at 37° C.

It seems, however, that these larvae also consume glycogen. Giovannola (1936) found small amounts of glycogen in young rhabditiform larvae of *Necator*, *Ancylostoma* and *Nippostrongylus*, but none in the filariform stages. A comparable observation was made by Stepanow-Grigoriev and Hoeppli (1936) who found glycogen in one- or two-day old filariform larvae of *Strongyloides*, but never in three- to nine-day old larvae.

## Bibliography

### ENVIRONMENT AND ITS INFLUENCE ON METABOLISM

- ANDREWS, J. S. 1938.—Effect of infestation with the nematode *Cooperia curticei* on the nutrition of lambs. *J. Agric. Res.*, v. 57:349-360.
- ARNOLD, L. 1933.—The bacterial flora within the stomach and small intestine. The effect of experimental alterations of acid base balance and of the age of the subject. *Am. J. Med. Sc.*, v. 186:471-480.
- ASHCRAFT, D. W. 1933.—Effect of milk products on pH of the intestinal contents of domestic fowl. *Poultry Sc.*, v. 12:292-298.
- BALL, B. H. 1939.—The pH of the digestive tract in the living albino rat as determined by the capillary glass electrode. *Am. J. Physiol.*, v. 128:175-178.
- BALLS, A. K. and SWENSON, T. L. 1934.—The antitrypsin of egg white. *J. Biol. Chem.*, v. 106:409-419.
- BASCH, S. 1908.—The stomach and intestinal gases. *N. Y. Med. J.*, v. 88:684-689, 738-741.
- BERGEIM, O. 1924.—Intestinal chemistry. I. The estimation of intestinal reductions. II. Intestinal reductions as measures of intestinal putrefaction, with some observations on the influence of diet. *J. Biol. Chem.*, v. 62:45-60.
- VON BONSDORFF, B. 1939.—Influence of intestinal worms on proteolytic activity in vitro of trypsin, papain, and pepsin and human gastric juice at neutral reaction. *Acta. Med. Scand.*, v. 100:459-482.
- VON BRAND, T. 1934.—Das Leben ohne Sauerstoff bei wirbelloser Tieren. *Ergeb. Biol.*, v. 10:37-100.
- 1938.—The nature of the metabolic activities of intestinal helminths in their natural habitat: aerobiosis or anaerobiosis? *Biodynamica* (41), 13 pp.
- VON BRAND, T. and WEISE, W. 1932.—Beobachtungen über den Sauerstoffgehalt der Umwelt einiger Entoparasiten. *Ztschr. Vergleich. Physiol.*, v. 18:339-346.
- COLLIER, H. B. 1941.—A trypsin inhibiting fraction of *Ascaris*. *Canad. J. Res.*, v. 19B: 90-98.
- DANNIGER, R., PFRAGNER, K. and SCHULTES, H. 1928.—Über die absolute Reaktion in dem Inhalt der einzelnen Darmabschnitte von Pferd und Rind. *Pflüger's Arch.*, v. 220: 430-433.
- DASTRE, A. and STASSANO, H. 1903.—Existence d'une Antikinasé chez les parasites intestinaux. *Comp. Rend. Soc. Biol. Paris*, v. 55:131-132.
- DAVEY, D. G. 1936.—Notes on the osmotic pressure of the contents of the stomach compartments of the sheep. *J. Agric. Sc.*, v. 26:328-330.
- 1938.—Studies on the physiology of the nematodes of the alimentary canal of sheep. *Parasit.*, v. 30:278-295.
- EASTMAN, I. M. and MILLER, E. G., JR. 1935.—Gastrointestinal pH in rats as determined by the glass electrode. *J. Biol. Chem.*, v. 110:255-262.
- ELDIN, M. S. and HASSAN, A. 1933.—Gastric functions in helminth infections. *J. Egypt. Med. Assoc.*, v. 16:735-752. [Abstract] *Trop. Dis. Bull.*, 1934, v. 31:767.
- ENIGK, C. 1936.—Untersuchungen über die Abtötung der Spulwurmeier und Coccidienoocyten durch Chemikalien. *Arch. Wiss. Prakt. Tierheilk.* v. 70:439-448.
- FERBER, K. E. 1928.—Die Zahl und Masse der Infusorien im Pansen und ihre Bedeutung für den Eiweissaufbau beim Wiederkäuer. *Ztschr. Tierzücht. & Züchtungsbiol.*, v. 12: 31-63.
- FERNANDEZ, F. 1934.—Parasitismo intestinal y jugo gastrico. *Med. Paises Cálidos, Madrid*, v. 7:336-338. [Abstract] *Trop. Dis. Bull.*, 1934, v. 31:767.
- FRIES, J. A. 1906.—Intestinal gases of man. *Am. J. Physiol.*, v. 16:468-474.
- FROST, W. D. and HANKINSON, H. 1931.—*Lactobacillus acidophilus*, an annotated bibliography to 1931. Milton, Wis.
- GLASER, R. W. and STOLL, N. R. 1938.—Development under sterile conditions of the sheep stomach worm *Hacmonchus contortus* (Nematode). *Science*, v. 87:259-260.
- GRAHAM, W. R. and EMERY, E. S. 1927-28.—The reaction of the intestinal contents of dogs fed on different diets. *J. Lab. & Clin. Med.*, v. 13:1097-1108.
- GRAYZEL, D. M. and MILLER, E. G., JR. 1928.—The pH of the contents of the gastrointestinal tract in dogs, in relation to diet and rickets. *J. Biol. Chem.*, v. 76:423-436.
- GROVE, E. W., OLMSTED, W. H. and KOENIG, K. 1929.—The effect of diet and catharsis on the lower volatile fatty acids in the stools of normal men. *J. Biol. Chem.*, v. 85: 127-136.
- HAMILL, J. M. 1906.—On the mechanism of protection of intestinal worms, and its bearing on the relation of enterokinase to trypsin. *J. Physiol.*, v. 33:479-492.
- HARNED, B. K. and NASH, T. P., JR. 1932.—The protection of insulin by antiproteases, and its absorption from the intestine. *J. Biol. Chem.*, v. 97:443-456.
- HELLER, V. G., OWENS, J. R. and PORTWOOD, L. 1935.—The effect of the ingestion of saline waters upon the pH of the intestinal tract, the nitrogen balance and the coefficient of digestibility. *J. Nutrition*, v. 10:645-651.
- HERRIN, R. C. 1937.—Ammonia content, pH, and carbon dioxide tension in the intestine of dogs. *J. Biol. Chem.*, v. 118:459-470.
- HEUPKE, W. 1931.—Über die Sekretion und Excretion des Dickdarms. *Ztschr. Gesam. Exper. Med.*, v. 75:83-125.
- HUME, H. V., DENIS, W., SILVERMAN, D. N. and IRWIN, E. L. 1924.—Hydrogen ion concentration in the human duodenum. *J. Biol. Chem.*, v. 60:633-645.
- JAHN, T. L. 1933.—Oxidation-reduction potential as a possible factor in the growth of intestinal parasites in vitro. *J. Parasit.*, v. 20:129.
- 1934.—Problems of population growth in the protozoa. *Symposia in Quant. Biol. (Cold Spring Harbor)*, v. 2:167-180.
- 1936.—Effect of aeration and lack of CO<sub>2</sub> on growth of bacteria-free cultures of protozoa. *Proc. Soc. Exp. Biol. & Med.*, v. 33:494-498.
- JEPHCOTT, H. and BACHARACH, A. L. 1926.—A rapid and reliable test for vitamin D. *Biochem. J.*, v. 20:1351-1355.
- JOHNSTON, F. 1934.—Threadworms. *Brit. Med. J.*, v. 1:224.
- KAHN, G. and STOKES, J. 1926.—The comparison of the electrometric and colorimetric methods for determination of the pH of gastric contents. *J. Biol. Chem.*, v. 69:75-84.
- KARR, W. G. and ABBOTT, W. O. 1935.—Intubation studies of the human small intestine. IV. Chemical characteristics of the intestinal contents in the fasting state and as influenced by the administration of acids, of alkalies, and of water. *J. Clin. Invest.*, v. 14:893-900.
- KELLOGG, E. L. 1933.—The duodenum. P. B. Hoeber, Inc., N. Y.
- KOFOID, C. A., McNEIL, E. and CAILLEAU, R. 1932.—Electrometric pH determinations of the walls and contents of the gastro-intestinal tracts of normal albino rats. *Univ. Calif. Pub. Zool.*, v. 36:347-355.
- KOPELOFF, N. 1926.—*Lactobacillus acidophilus*. Baltimore, 1926.
- KREIPE, H. 1927.—Dissertation. Kiel. Cited by Lenkeit (1933).

- LAPAGE, G. 1935a.—The second ecdysis of infective nematode larvae. *Parasit.*, v. 27:186-206.  
1935b.—The second ecdysis of the infective larvae of certain Trichostrongylidae in solutions of sodium sulphide and of organic compounds containing sulphur. *J. Helm.*, v. 13:103-114.  
1938.—Nematodes parasitic in animals. Chem. Pub. Co., N. Y.
- LENKEIT, W. 1933.—Neuere Ergebnisse der vergleichenden Physiologie der Verdauung der Säugetiere. *Ergeb. Physiol.*, v. 35:373-631.
- LLOYD-JONES, O. and LILJEDAHL, E. M. 1934.—Alimentary Gas. *Med. Rec.*, v. 139:320-323.
- LONG, J. H. and FENGER, F. 1917.—On the normal reaction of the intestinal tract. *J. Am. Chem. Soc.*, v. 39:1278-1286.
- MANGOLD, E. 1929.—Handbuch der Ernährung und des Stoffwechsels der landwirtschaftlichen Nutztiere, v. 2:202.
- MANN, F. C. and BOLLMAN, J. L. 1930.—The reaction of the content of the gastro-intestinal tract. *J. Am. Med. Assoc.*, v. 95:1722-1724.
- McCLENDON, J. F. 1920.—Hydrogen-ion concentration of the contents of the small intestine. *Proc. Nat. Acad. Sci.*, v. 6:690-691.
- McCLENDON, J. F. and MEDES, GRACE. 1925.—Physical chemistry in biology and medicine. Saunders Co.
- McCLENDON, J. F., MYERS, F. J., CULLIGAN, L. C., and GYDESEN, C. S. 1919.—Factors influencing the hydrogen ion concentration of the ileum. *J. Biol. Chem.*, v. 38:535-538.
- McCoy, O. R. 1935.—The physiology of the helminth parasites. *Physiol. Rev.*, v. 15:221-240.
- McIVER, M. A., REDFIELD, A. C., and BENEDICT, E. B. 1926.—Gaseous exchange between blood and lumen of stomach and intestines. *Am. J. Physiol.*, v. 76:92-111.
- McLAUGHLIN, A. R. 1931.—Hydrogen ion concentration of the alimentary tracts of fowl, cat, and rabbit. *Science*, v. 73:191-192.
- MOORTHY, V. N. 1935.—The influence of fresh bile on guinea-worm larvae encysted in *Cyclops*. *Indian Med. Gaz.*, v. 70:21-23. [Abstract] *Trop. Dis. Bull.*, 1935, v. 32:654.
- MUDIE, E. C. 1934.—Threadworms. *Brit. Med. J.*, v. 1:224.
- MUSSEHL, F. E., BLISH, M. J. and ACKERSON, C. W. 1933.—Effect of dietary and environmental factors on the pH of the intestinal tract. *Poultry Sci.*, v. 12:120-123.
- NAGL, F. 1928.—Über die Titrations- und Ionenacidität im Mageninhalt des Hundes nach verschiedener Fütterung. *Arch. Wiss. & Prak. Tierheilk.*, v. 58:198-203.
- PLANER. 1860.—Die Gase des Verdauungsschlauches und ihre Beziehungen zum Blute. *Sitzungsb. Akad. Wiss. Wien, Math.-Naturw. Kl.*, v. 42:307-354.
- REDMAN, T., WILLIMOTT, S. G. and WOKES, F. 1927.—The pH of the gastrointestinal tract of certain rodents used in feeding experiments, and its possible significance in rickets. *Biochem. J.*, v. 21:589-605.
- ROBINSON, C. S. 1922.—Hydrogen ion concentration of the human feces. *J. Biol. Chem.*, v. 52:445.  
1935.—The hydrogen ion concentration of the contents of the small intestine. *J. Biol. Chem.*, v. 108:403-408.
- ROBINSON, C. S. and DUNCAN, C. W. 1931.—The effect of lactase and the acid-base value of the diet on the hydrogen ion concentration of the intestinal contents of the rat and their possible influence on calcium absorption. *J. Biol. Chem.*, v. 92:435-447.
- RUGE, E. 1861.—Beiträge zur Kenntniss der Darmgase. *Sitzungsb. Akad. Wiss. Wien, Math.-Naturw. Kl.*, v. 44:739-762.
- SANG, J. H. 1938.—The antiproteolytic enzyme of *Ascaris lumbricoides* var. *suus*. *Parasit.*, v. 30:141-155.
- SCHUEBERT, A. and SCHIEBLICH, M. 1927.—Einfluss der Mikroorganismen auf die Vorgänge im Verdauungstraktus bei Herbivoren. *Handb. Norm. & Path. Physiol.*, v. 3:967-1000.
- SCHOPFER, W. H. 1932.—Recherches physico-chimiques sur le milieu interieur de quelques parasites. *Rev. Suisse Zool.*, v. 39:59-194.
- SCHWARZ, C. and DANZIGER, H. 1924.—Beiträge zur Physiologie der Verdauung. IV. Die H-Ionenkonzentrationen des ans dem Magen austretenden Mageninhaltes zugleich ein Beitrag zur Kenntnis der Magenentleerung. *Pflüger's Arch.*, v. 202:478-487.
- SCHWARZ, C. and KAPLAN, H. 1926.—Die H-Ionenkonzentration im Labmageninhalt des Rindes. *Pflüger's Arch.*, v. 213:562-594.
- SCHWARZ, C. and GABRIEL, F. 1926.—Die H-Ionenkonzentrationen im Panseninhalt des Rindes. *Pflüger's Arch.*, v. 213:814-815.
- SCHWARTZ, C., STEINMETZER, K. and CAITHAML, K. 1926.—Beiträge zur Physiologie der Verdauung. XVII. Die H-Ionenkonzentrationen im Mageninhalt des Pferdes. *Pflüger's Arch.*, v. 213:595-601.
- SHOHL, A. T. and BING, F. C. 1928.—Rickets in rats. IX. pH of the feces. *J. Biol. Chem.*, v. 79:269-274.
- SLATER, W. K. 1925.—The nature of the metabolic processes in *Ascaris lumbricoides*. *Biochem. J.*, v. 19:604-610.  
1928.—Anaerobic life in animals. *Biol. Rev.*, v. 3:303-328.
- STEWART, J. and SHEARER, G. D. 1933.—The effects of nematode infestations on the metabolism of the host. Third Report Univ. Cambridge, Inst. Anim. Path., pp. 58-129.
- SUN, T., BLUMENTHAL, P. R., SLIFER, E. H., HERBER, E. C. and WANG, C. C. 1932.—The hydrogen-ion concentration of the alimentary tract of normal albino rats. *Physiol. Zool.*, v. 5:191-197.
- TACKE, B. 1884.—Über die Bedeutung der brennbaren Gase im thierischen Organismus. *Inaug. Diss. Berlin*.
- TAPPEINER. 1883.—Die Gase des Verdauungsschlauches der Pflanzenfresser. *Ztschr. Biol.*, v. 19:228-279.
- TORYU, Y. 1934.—Contributions to the physiology of the *Ascaris*. II. The respiratory exchange in the *Ascaris*, *Ascaris megalocephala* Cloq. *Sc. Rpt. Tohoku Imp. Univ.*, 4th Ser., v. 9:61-70.
- DE WAELE, A. 1933.—Sur la Migration des Cestodes. *Bull. Cl. Sc. Acad. Roy. Belg.*, Ser. 5, v. 19:649-660.  
1934.—Etude de la fonction biliare de l'invagination chez le cysticerque des cestodes. *Ann. Parasit.*, v. 12:452-510.
- WEINLAND, E. 1901.—Über Kohlehydratzersetzung ohne Sauerstoffaufnahme, einen tierischen Gärungsprozess. *Ztschr. Biol.*, v. 42:55-90.  
1903.—Über Antifermente. I and II. *Ibid.*, v. 44:1-15, 45-60.
- WELLS, H. S. 1931.—Observations on the blood sucking activities of the hookworm, *Ancylostoma caninum*. *J. Parasit.*, v. 17:167-182.
- ZIEGLER, J. and HIRSCH, W. 1925.—Über den Gasgehalt des Magendarmkanals und die Mittel zu seiner Beseitigung in Hinsicht auf die Röntgenuntersuchung. *Fortschr. Geb. Röntgenstrahlen*, v. 33:698-708.
- ZUCKER, T. F. and MATZNER, M. J. 1924.—On the pharmacological action of the antirachitic active principle of cod liver oil. *Proc. Soc. Exp. Biol. & Med.*, v. 21:186-187.

#### GENERAL CHEMICAL COMPOSITION

- ADUCCO, V. 1889.—La substance colorante rouge de *Eustrongylus gigas*. *Arch. Ital. Biol.*, v. 11:52-69.
- BERNARD, CLAUDE. 1859.—De la matière glycogène chez les animaux dépourvus de foie. *Compt. Rend. Soc. Biol. Paris*, Ser. 3, v. 1:53-55.
- BONDROY, T. 1910.—Chimie biologique du *Sclerostomum equinum*. Thèse, Paris, p. 58.
- v. BRAND, TH. 1934.—Der Stoffwechsel von *Ascaris lumbricoides* bei Oxybiose und Anoxybiose. *Ztschr. Vergleich. Physiol.*, v. 21:220-235.  
1937a.—The anaerobic glycogen consumption in *Ascaris* females and males. *J. Parasit.*, v. 23:68-72.

- 1937b.—Haemoglobin in a larval *Eustrongylides*. J. Parasit., v. 23:316-317.
- 1938.—Physiological observations on a larval *Eustrongylides*. (Nematoda). J. Parasit., v. 24:445-451.
- v. BRAND, TH. and OTTO, G. F. 1938.—Some aspects of the carbohydrate metabolism of the hookworm, *Ancylostomum caninum*, and its host. Am. J. Hyg., v. 27:683-689.
- BRAULT, A. and LOEPER, M. 1904.—Le glycogène dans le développement de certains parasites (Cestodes et Nématodes). J. Physiol. & Path. Gen., v. 6:503-512.
- BUSCH, P. W. C. M. 1905.—Over de localisatie van het glycogeen bij enkele Darmparasieten. Diss. Utrecht. 109 pp.
- CAMPBELL, D. H. 1936.—An antigenic polysaccharide fraction of *Ascaris lumbricoides* (from hog). J. Infect. Dis., v. 59:266-280.
- CHAMPÉTIER, G. and FAURÉ-FREMIET, E. 1937.—Étude roentgenographique d'une protéine intracellulaire. Compt. Rend. Acad. Sc. Paris, v. 204:1901-1903.
- DAVEY, D. G. 1938.—Studies on the physiology of the nematodes of the alimentary canal of sheep. Parasit., v. 30:278-295.
- FAURÉ-FREMIET, E. 1913.—Le cycle germinatif chez l'*Ascaris megalocéphala*. Arch. Anat. Micr., v. 15:435-757.
- FAURÉ-FREMIET, E. and FILHOL, J. 1937.—La température de dispersion d'une protéine intracellulaire, l'ascaridine. J. Chim. Physique, v. 34:444-451.
- FLURY, F. 1912.—Zur Chemie und Toxikologie der Ascariden. Arch. Exper. Path. & Pharmacol., v. 67:275-392.
- FOSTER, M. 1865.—On the existence of glycogen in the tissues of certain Entozoa. Proc. Roy. Soc., v. 14:543-546.
- GIOVANNOLA, A. 1935.—Osservazioni sulla nature delle coste nella borsa caudale degli Ancylostomi. Arch. Ital. Sc. Med., Colon., v. 16, Fase. 6:1-7.
- GOURÉVITCH, M. A. 1937.—Sur le dosage de la flavine; la flavine chez les invertébrés. Bull. Soc. Chim. Biol., v. 19:125-129.
- HIRSCH, G. C. and BRETSCHNEIDER, L. H. 1937.—Die Arbeitsräume in den Darmzellen von *Ascaris*, die Einwirkung des Hungerns, die Sekretbildung. Cytologia, Tokyo, Fujii Jub., v. 7:424-436.
- Hsü, H. F. 1938.—Studies on the food and the digestive system of certain parasites. II. On the food of *Schistosoma japonicum*, *Paragonimus ringeri*, *Dirofilaria immitis*, *Spirocerca sanguinolenta* and *Rhabdias* sp. Bull. Fan Mem. Inst., Biol., Zool. Ser., v. 8:347-366.
- JANICKI, M. J. 1939.—Untersuchungen zur Ernährungsfrage von *Dioctophyme renale* (Goeze, 1782). Zool. Poloniae, v. 3:189-223.
- KEILIN, D. 1925.—On cytochrome, a respiratory pigment common to animals, yeasts and higher plants. Proc. Roy. Soc. Lond., Ser. B, v. 98:312-339.
- v. KEMNITZ, G. 1912.—Die Morphologie des Stoffwechsels bei *Ascaris lumbricoides*. Arch. Zellforsch., v. 7:463-603.
- KRÜGER, F. 1936.—Untersuchungen zur Kenntnis des aeroben und anaeroben Stoffwechsels des Schweinepulwurm (*Ascaris suilla*). Zool. Jahrb. Abt. Allg. Zool. & Physiol., v. 57:1-56.
- MARCEP, W. H. 1865.—Chemical examination of the fluid from the peritoneal cavity of the Nematoda Entozoa. Proc. Roy. Soc. Lond., v. 14:69-70.
- MARTINI, E. 1916.—Die Anatomie der *Oxyuris curvula*. Ztschr. Wiss. Zool., v. 116:142-543.
- MUELLER, J. F. 1928-29.—Studies on the microscopical anatomy and physiology of *Ascaris lumbricoides* and *Ascaris megalocéphala*. Ztschr. Zellforsch., v. 8:361-403.
- PANIKKAR, N. K. and SPROSTON, N. G. 1941.—Osmotic relations of some metazoan parasites. Parasit., v. 33:214-223.
- SCHIMMELPFENNIG, G. 1903.—Über *Ascaris megalocéphala*. Beiträge zur Biologie und physiologischen Chemie derselben. Arch. Wiss. & Prakt. Tierheilk., v. 29:332-376.
- SCHULTE, H. 1917.—Versuche über Stoffwechselforgänge bei *Ascaris lumbricoides*. Pflügers Archiv., v. 166:1-44.
- SCHOPFER, W. H. 1926.—Recherches physico chimiques sur les liquides de parasites (*Ascaris*). II. Parasit., v. 18:277-282.
- 1932.—Recherches physico-chimiques sur le milieu intérieur de quelques parasites. Rev. Suisse Zool., v. 39:59-194.
- SCHULZ, FR. N. and BECKER, M. 1933.—Über Ascarylalkohol. Bioch. Ztschr., v. 265:253-259.
- SMORODINCEV, I. and BEBESIN, K. 1936.—La teneur en glycogène des Ascarides. Compt. Rend. Acad. Sc. U. R. S. S., n. s., v. 2:189-191.
- STANNARD, J. W., MCCOY, O. R. and LATCHFORD, W. B. 1938.—Studies on the metabolism of *Trichinella spiralis* larvae. Am. J. Hyg., v. 27:666-682.
- STOLL, N. R. 1940.—In vitro conditions favoring ecdysis at the end of the first parasitic stage of *Hacmonchus contortus* (Nematoda). Growth, v. 4:383-406.
- TORYU, Y. 1933.—Contributions to the physiology of the *Ascaris*. I. Glycogen content of the *Ascaris*, *Ascaris megalocéphala* Clog. Sc. Rpt. Tohoku Imp. Univ., 4th Ser., v. 8:65-74.
- VIALLI, M. 1923.—Ricerca sulla pressione osmotica. II. nei Verm. Rendic. Ist. Lombardo Sc. & Lett., v. 56: Rpts. 1-4.
- WEINLAND, E. 1901a.—Über den Glykogengehalt einiger parasitischer Würmer. Ztschr. Biol., v. 41:69-74.
- 1901b.—Über Kohlehydratzeretzung ohne Sauerstoffaufnahme, einen tierischen Gärungsprozess. Ztschr. Biol., v. 42:55-90.
- WHARTON, G. W. 1938.—Hemoglobin in turtle parasites. J. Parasit., v. 24. Suppl.: 21.
- 1941.—The function of respiratory pigments of certain turtle parasites. J. Parasit., v. 27:81-87.
- YOSHIMURA, SH. 1930.—Beiträge zur Chemie der Ascaris. J. Biochem., v. 12:27-34.

#### METABOLISM OF ADULT NEMATODES

- ADAM, W. 1932.—Über die Stoffwechselprozesse von *Ascaris suilla* Duj. I. Teil. Die Aufnahme von Sauerstoff aus der Umgebung. Ztschr. Vergleich. Physiol., v. 16:229-251.
- BONDROY, T. 1910.—Chimie biologique du *Sclecrostomum equinum*. Thèse, Paris. 58 pp.
- v. BRAND, TH. 1934a.—Der Stoffwechsel von *Ascaris lumbricoides* bei Oxybiose und Anoxybiose. Ztschr. Vergleich. Physiol., v. 21: 220-235.
- 1934b.—Das Leben ohne Sauerstoff bei wirbellosen Tieren. Ergeb. Biol., v. 10:37-100.
- 1937a.—The anaerobic glycogen consumption in *Ascaris* females and males. J. Parasit., v. 23:68-72.
- 1937b.—The aerobic resynthesis of glycogen in *Ascaris*. J. Parasit., v. 23:316-317.
- 1938.—The nature of the metabolic activities of intestinal helminths in their natural habitat: aerobiosis or anaerobiosis? Biodynamica, No. 41:1-13.
- 1941.—Aerobic fat metabolism of *Ascaris lumbricoides*. Proc. Soc. Exp. Biol. Med., v. 46:417-418.
- v. BRAND, TH. and OTTO, G. F. 1938.—Some aspects of the carbohydrate metabolism of the hookworm, *Ancylostomum caninum*, and its host. Am. J. Hyg., v. 27:683-689.
- BUNGE, G. 1889.—Weitere Untersuchungen über die Athmung der Würmer. Ztschr. Physiol. Chem., v. 14:318-324.
- CHITWOOD, B. G. 1938.—Notes on the physiology of *Ascaris lumbricoides*. Proc. Helm. Soc. Wash., v. 5:18-19.
- DAVEY, D. G. 1937.—Physiology of nematodes. Nature, v. 140:645.
- 1938a.—The respiration of nematodes of the alimentary tract. J. Exper. Biol., v. 15:217-224.
- 1938b.—Studies on the physiology of the nematodes of the alimentary canal of sheep. Parasit., v. 30:278-295.
- FISCHER, A. 1924.—Über den Kohlehydratstoffwechsel von *Ascaris megalocéphala*. Bioch. Ztschr., v. 144:224-228.
- FLURY, F. 1912.—Zur Chemie und Toxikologie der Ascariden. Arch. Exp. Path. & Pharm., v. 67:275-392.
- HARNISCH, O. 1933.—Untersuchungen zur Kennzeichnung des

- Sauerstoffverbrauchs von *Trienophorus nodulosus* (Cest.). und *Ascaris lumbricoides* (Nemat.). Ztschr. Vergleich. Physiol., v. 19:310-348.
- 1935.—Daten zur Beurteilung des Sauerstoffverbrauchs von *Ascaris lumbricoides* (nach Messungen an isolierten Organen). Ztschr. Vergleich. Physiol., v. 22: 50-66.
- 1936.—Primäre und sekundäre Oxybiose der Larve von *Chironomus thummi*. Ztschr. Vergleich. Physiol., v. 23: 391-419.
- 1937.—Zellfrei arbeitendes Oxydans im Gaswechsel von *Ascaris lumbricoides* und einigen Cestoden. Ztschr. Vergleich. Physiol., v. 24:667-686.
- HARWOOD, P. D. and BROWN, H. W. 1934.—A preliminary report on the *in vitro* consumption of oxygen by parasitic nematodes. J. Parasit., v. 20:128.
- HIRSCH, G. C. and BRETSCHNEIDER, L. H. 1937.—Der intraplasmatische Stoffwechsel in den Darmzellen von *Ascaris lumbricoides*. Teil II. Die Adsorption von Eisen und die Beteiligung der Golgikörper dabei. Protoplasma, v. 29: 9-30.
- HOFFMANN, R. 1934.—Untersuchungen über die Wärmeentwicklung von *Ascaris lumbricoides* bei Fütterung mit Glykose, Fruktose und Galaktose. Ztschr. Biol. v. 95:390-400.
- JOST, H. 1928.—Vergleichende Physiologie des Stoffwechsels. Bethes Handb. Physiol., v. 5:377-466.
- KEMPNER, W. 1937.—The effect of oxygen tension on cellular metabolism. J. Cell. & Comp. Physiol., v. 10:339-364.
- KRÜGER, F. 1936.—Untersuchungen zur Kenntnis des aeroben und anaeroben Stoffwechsels des Schweinespulwurms (*Ascaris suilla*). Zool. Jahrb. Abt. Allg. Zool. & Physiol., v. 57:1-56.
- 1937.—Bestimmungen über den aeroben und anaeroben Stoffumsatz beim Schweinespulwurm mit einem neuen Respirationsapparat. Ztschr. Vergleich. Physiol., v. 24: 687-719.
- 1940.—Die Beziehung des Sauerstoffverbrauches zur Körperoberfläche beim Schweinespulwurm (*Ascaris lumbricoides*). Z. Zool., v. 152:547-570.
- KRUMMACHER, O. 1919.—Untersuchungen über die Wärmeentwicklung der Spulwürmer. Ztschr. Biol., v. 69:293-321.
- MARSH, G. 1935.—Kinetics of an intracellular system for respiration and bioelectric potential at flux equilibrium. Plant Physiol., v. 10:681-697.
- MEIER, W. 1931.—Neuere Untersuchungen über die Wärmeentwicklung der Spulwürmer. Ztschr. Biol., v. 91:459-474.
- MUELLER, J. F. 1928-29.—Studies on the microscopical anatomy and physiology of *Ascaris lumbricoides* and *Ascaris megalocephala*. Ztschr. Zellforsch., v. 8:361-403.
- OESTERLIN, M. 1937.—Die von oxybiotisch gehaltenen Ascariden ausgeschiedenen Fettsäuren. Ztschr. Vergleich. Physiol., v. 25:88-91.
- SCHIMMELPFENNIG, G. 1903.—Über *Ascaris megalocephala*. Beiträge zur Biologie und physiologischen Chemie derselben. Arch. Wiss. & Prakt. Tierheilk., v. 29:332-376.
- SCHULTE, H. 1917.—Versuche über Stoffwechselforgänge bei *Ascaris lumbricoides*. Pflüger's Archiv., v. 166:1-44.
- SLATER, W. K. 1925.—The nature of the metabolic processes in *Ascaris lumbricoides*. Biochem. J., v. 19:604-610.
- 1928.—Anaerobic life in animals. Biol. Rev., v. 3: 303-328.
- TOKYU, Y. 1934.—Contributions to the physiology of the *Ascaris*. II. The respiratory exchange in the *Ascaris*, *Ascaris megalocephala* (Clog. Sc. Rpt. Tohoku Imp. Univ., 4th. Ser., v. 9:61-70).
- 1935.—Idem. III. Survival and glycogen content of the *Ascaris*, *Ascaris megalocephala* Clog. in presence and absence of oxygen. Ibid., v. 10:361-375.
- 1936a.—Idem. IV. Products from glycogen during anaerobic and aerobic existence of the *Ascaris*, *Ascaris megalocephala* Clog. Ibid., v. 10:687-696.
- 1936b.—Idem. V. Survival and respiratory exchange of the *Ascaris*, *Ascaris megalocephala* Clog. intercepted from light in presence and absence of oxygen. Ibid., v. 11:1-17.
- WAECHTER, J. 1934.—Über die Natur der beim Stoffwechsel der Spulwürmer ausgeschiedenen Fettsäuren. Ztschr. Biol., v. 95:497-501.
- WEINLAND, E. 1901.—Über Kohlehydratzersetzung ohne Sauerstoffaufnahme bei *Ascaris*, einen tierischen Gärungsprozess. Ztschr. Biol., v. 42:55-90.
- 1902.—Über ausgepresste Extrakte von *Ascaris lumbricoides* und ihre Wirkung. Ibid., v. 43:86-111.
- 1904a.—Über die von *Ascaris lumbricoides* ausgeschiedenen Fettsäuren. Ibid., v. 45:113-116.
- 1904b.—Über die Zersetzung stickstoffhaltiger Substanz bei *Ascaris*. Ibid., v. 45:517-531.
- WEINLAND, E. and RITZER, A. 1902.—Über die Bildung von Glykogen aus Kohlehydraten bei *Ascaris*. Ztschr. Biol., v. 43:490-502.
- WELLS, H. S. 1931.—Observations on the blood sucking activities of the hookworm, *Ancylostoma caninum*. J. Parasit., v. 17:167-182.

#### METABOLISM OF EGGS AND LARVAE

- BATAILLON, E. 1910.—Contribution à l'analyse expérimentale des phénomènes karyocinétiques chez *Ascaris megalocephala*. Arch. Entwicklungsmech., v. 30(1):24-44.
- VON BRAND, TH. 1938.—Physiological observations on a larval *Eustrongylides* (Nematoda). J. Parasit., v. 24:445-451.
- BROWN, H. W. 1928.—A quantitative study of the influence of oxygen and temperature on the embryonic development of the eggs of the pig ascarid (*Ascaris suum* Goeze). J. Parasit., v. 14:141-160.
- CHITWOOD, B. G. 1938.—Further studies on nemie skeletoids and their significance in the chemical control of nemie pests. Proc. Helm. Soc. Wash., v. 5:68-75.
- CORT, W. W. 1925.—Investigations on the control of hookworm disease. XXXIV. General summary of results. Am. J. Hyg., v. 5:49-89.
- DINNIK, J. A. and DINNIK, N. N. 1937.—Influence de la température, de l'absence d'oxygène et du dessèchement sur les oeufs de *Trichocephalus trichiurus* (L.). Med. Parasit. & Parasitic Dis., v. 5:603-618. [Russian with French summary.]
- DYRDOWSKA, M. 1931.—Recherches sur le comportement du glycogène et des graisses dans les oeufs d'*Ascaris megalocephala* à l'état normal et dans une atmosphère d'azote. Comp. Rend. Soc. Biol., Paris, v. 108:593-596.
- FAURÉ-FREMIET, E. 1912.—Graisse et glycogène dans le développement de l'oeuf de *Ascaris megalocephala*. Bull. Soc. Zool. France, v. 37:233-234.
- 1913a.—Le cycle germinatif chez *Ascaris megalocephala*. Arch. Anat. Mier., v. 15:435-757.
- 1913b.—La segmentation de l'oeuf d'*Ascaris* au point de vue énergétique. Comp. Rend. Soc. Biol., Paris, v. 75:90-92.
- FENWICK, D. W. 1938.—The oxygen consumption of newly-hatched larvae of *Ascaris suum*. Proc. Zool. Soc. London, Ser. A, v. 108, Part 1:85-100.
- FRIEDHEIM, E. A. H. 1933.—Das Pigment von *Halla parthenopea*, ein akzessorischer Atmungskatalysator. Biochem. Ztschr., v. 259:257-268.
- GIOVANNOLA, A. 1936.—Energy and food reserve in the development of nematodes. J. Parasit., v. 22:207-218.
- HUFF, G. C. 1936.—Experimental studies of factors influencing the development of the eggs of pig ascarid (*Ascaris suum* Goeze). J. Parasit., v. 22:455-463.
- HUFF, G. C. and BOELL, E. J. 1936.—Effect of ultracentrifuging on oxygen consumption of the eggs of *Ascaris suum*, Goeze. Proc. Soc. Exp. Biol. & Med., v. 34:626-628.
- JACOBS, L. and JONES, M. F. 1939.—Studies on oxyuriasis. XXI. The chemistry of the membranes of the pinworm egg. Proc. Helm. Soc. Wash., v. 6:57-60.
- KOSMIN, N. 1928.—Zur Frage über den Stickstoffwechsel der Eier von *Ascaris megalocephala*. Tr. Lab. Exper. Biol. Zoopark, Moscow, v. 4:207-218. [Russian with German summary.]

- LOOSS, A. 1911.—The anatomy and life history of *Ancylostoma duodenale* Duj. Part II. The development in the free stage. Rec. Egypt. Govt. Sch. Med., v. 4:163-613. [Not seen.]
- MCCOY, O. R. 1930.—The influence of temperature, hydrogen-ion concentration, and oxygen tension on the development of the eggs and larvae of the dog hookworm, *Ancylostoma caninum*. Am. J. Hyg., v. 11:413-448.
- MCCOY, Q. R., DOWNING, V. F. and VAN VOORHIS, S. N. 1941.—The penetration of radioactive phosphorus into encysted *Trichinella* larvae. J. Parasit., v. 27:53-58.
- NOLF, L. O. 1932.—Experimental studies on certain factors influencing the development and viability of the ova of the human *Trichuris* as compared with those of the human *Ascaris*. Am. J. Hyg., v. 16:288-322.
- PAYNE, F. K. 1923.—Investigations on the control of hookworm disease. XXX. Studies on factors involved in migration of hookworm larvae in soil. Am. J. Hyg., v. 3:547-583.
- PINTNER, TH. 1922.—Die vermutliche Bedeutung der Helminthenwanderungen. Sitzungsber. Akad. Wiss. Wien, Math.-Naturw. Kl., Abt. I, v. 131:129-138.
- ROGERS, W. P. 1939.—The physiological ageing of *Ancylostoma* larvae. J. Helm., v. 17:195-202.
- SCHALIMOV, L. G. 1931.—A contribution to the biology of *Oxyuris equi*. Tr. Dynamics Develop., v. 6:181-196. [Russian with English summary.]
- SCHMIDT, W. J. 1937.—Doppelbrechung und Feinbau der Eischale von *Ascaris megalocephala*. Ein Vergleich des Feinbaues faserigen und filmartigen Chitins. Ztschr. Zellforsch., v. 25:181-203.
- STANNARD, J. N., MCCOY, O. R., and LATCHFORD, W. B. 1938.—Studies on the metabolism of *Trichinella spiralis* larvae. Am. J. Hyg., v. 27:666-682.
- STEPANOW-GRIGORIEW, J. and HOEPLI, R. 1926.—Über Beziehungen zwischen Glykogeengehalt parasitischer Nematodenlarven und ihrer Wanderung im Wirtskörper. Arch. Schiffs- u. Tropen Hyg., v. 30:577-585.
- SZWEJKOWSKA, G. 1929.—Recherches sur la physiologie de la maturation des oeufs de *Ascaris*. Bull. Internat. Acad. Polon. Sc. & Lett., Ser. B, 1928:489-519.
- WENDT, H. 1936.—Beiträge zum Entwicklungszyklus bei *Oxyuris vermicularis*. Ztschr. Kinderheilk., v. 58:375-387. [Not seen.]
- WOTTGE, K. 1937.—Die stofflichen Veränderungen in der Eizelle von *Ascaris megalocephala* nach der Befruchtung. Protoplasma, v. 29:31-59.
- ZAWADOWSKY, M. 1916.—Rôle de l'oxygène dans le processus de segmentation des oeufs de *Ascaris megalocephala*. (Note préliminaire.) Compt. Rend. Soc. Biol., Paris, v. 68:595-598.  
1926.—Zum Mechanismus der Wirkung von Zyankalium auf die lebende Zelle (Eier von *Ascaris megalocephala*). Biologia Generalis, v. 2:442-456.  
1928.—The nature of the egg-shell of various species of *Ascaris* eggs (*Toxascaris limbata* Reillet et Henry, *Belascaris mystax* Zeder, *Belascaris marginata* Rud., *Ascaris suilla* Duj.). Tr. Lab. Exper. Biol. Zoopark, Moscow, v. 4:201-206. [Russian with English summary.]
- ZAWADOWSKY, M. and SCHALIMOV, L. G. 1929.—Is autoinvasion possible given the presence of *Enterobius (Oxyuris) vermicularis* in the intestine? Tr. Lab. Exper. Biol. Zoopark, Moscow, v. 5:1-42. [Russian with English summary.]
- ZAWADOWSKY, M. and ORLOW, A. P. 1927.—Is there any possibility of autoinvasion during Ascariasis? Tr. Lab. Exper. Zoopark, Moscow, v. 3:99-116. [Russian with English summary.]
- ZVIAGINZEV, S. N. 1934.—Contribution to the history of development of *Nematodirus helveticus*. Tr. Dynamics Develop., v. 8:186-202. [Russian with English summary.]

## CORRECTIONS

We are indebted to Dr. G. L. Graham for his assistance in compiling this table of errors:

| Page | Column | Line   | Page | Column | Line  |
|------|--------|--|------|--------|---|
| 125  | 1      | 25— <i>Strongyles</i> to read <i>Strongylus</i> .  | 183  | 2      | 36—permeable to read permeable.   |
| 125  | 2      | 43—adherants to read adherents.  | 186  | 1      | 30— <i>Gonglonema</i> to read <i>Gongylonema</i> .                                    |
| 128  | 2      | 50— <i>Rhabdias</i> spp. to read <i>Rhabditis</i> .  | 187  | 1      | 1st line of 2nd paragraph—Dioctophymatoidea.  |
| 130  | 1      | 11-12—identifield to read identified.  | 187  | 1      | Bibliog. under Ackert— <i>Ascaridea</i> to read <i>Ascaridia</i> .                    |
| 132  | 1      | under Adenophori, line 4— <i>Enoploidea</i> to read Eno-<br>ploidea.                                     | 188  | 2      | Bibliog. under Skinker—salmanoid to read salmoid.                                     |
| 132  | 2      | 51—Hagmeir to read Hagmeier.   | 189  | 1      | Bibliog. under Steiner 1937—Jubileum to read Ju-<br>bileum.                           |
| 134  | 1      | bibliog. under Mueller 1927—Anisakis to read<br><i>Anisakis</i> .  | 189  | 1      | Bibliog. under Zawadowsky and Shalimov—En-<br>wicklungsbedihungen.                    |
| 145  | 1      | 8— <i>macramphidium</i> to read <i>macramphidum</i> .  | 189  | 2      | Bibliog. under Huff—Jour. Parasit., v. 36?  |
| 145  | 2      | 27-28—Critical studies <i>are due</i> .  | 190  | 2      | Under Annelid—Chaetognath Nematelminth The-<br>ory.                                   |
| 149  | 1      | 14— <i>kunkeli</i> to read <i>Kunekeli</i> .   |      |        | II. Plathyhelminthes—etc.   |
| 153  | 2      | 18-19— <i>infecta</i> to read <i>infectum</i> .  | 191  | 1      | 1 footnote—3rd line between to read between.  |
| 154  | 1      | 7—Comma between <i>Chromadora</i> and <i>Mono-<br/>posthia</i> .   | 192  | 2      | 12—descendent to read descendant.   |
| 154  | 1      | 8— <i>Griceffida</i> to read <i>Griffitha</i>  | 193  | 1      | 40—cloace to read cloaca.   |
| 155  | 1      | 22—Insert asterisk after <i>parasitifera</i> .   | 203  | 2      | under Cholodowsky Weiblichen to read Weiblichen.                                      |
| 157  | 2      | under Schneider, A. 1858—Gefässsystem to read<br>Gefässsystem.   | 204  | 1      | under Remane 1928—Ostee to read Ostsee.   |
| 165  | 1      | 36— <i>Spinonoura</i> to read <i>Spironouca</i> .  | 204  | 2      | under Zeder-Naturgeseschichte to read Naturges<br>chichte.                            |
| 171  | 1      | 14 15—posteriad to read posteriad.   | 205  | 2      | 3rd line from bottom—intercallation to read in<br>tercallation.                       |
| 173  | 1      | under Joseph 1883a—Erklärungen to read Erk-<br>lärunge.  | 214  | 2      | under Held 1912—Geselleesh to read Gesellseh.   |
| 174  | 1      | Acknowledgments—Mantor to read Maunter.  | 221  | 2      | 77-77—descendents to read descendants.  |
| 177  | 1      | 54—sculptored to read sculptured.  | 223  | 2      | footnote—line 3—divison to read division.   |
| 177  | 1      | 59—sculptoring to read sculpturing.  | 229  | 1      | 21—subsequal to read subequal.  |
| 177  | 1      | caption, Fig. 135 I.— <i>Syphacia</i> to read <i>Syphacia</i> .  | 229  | 2      | 18—Fig. 158J to read Fig. 157J.   |
| 166  | 1      | caption Fig. 135 R. <i>Pseudonymus</i> to read <i>Pscu-<br/>donymus</i> .                                | 231  | 1      | 6-7—Delete Fig. 156W.   |
| 177  | 1      | caption Fig. 135 III.— <i>fillicolis</i> to read <i>flicollis</i> ;<br>Also 182, col. 2, line 4.         | 231  | 1      | Caption Fig. 158—4th line E-H— <i>Ancylostoma</i> to<br>read <i>Ancylostoma</i> .     |
| 177  | 2      | 8—sculptoring to read sculpturing.   | 232  | 1      | 12—Esophagael to read Esophageal.   |
| 178  | 2      | 44—Trichostrongylidae.   | 235  | 1      | 16-18—caecae to read ceca.  |
| 179  | 2      | 4th line in next to last paragraph under Ovovivi-<br>parity— <i>macrocera</i> to read <i>macrocera</i> . | 239  | 2      | under Pai 1928—Beeinflussing to read Beeinflus-<br>sung.                              |
| 183  | 1      | 17—Thelostomatidae to read Thelastomatidae.  | 240  | 1      | under Schwartz and Alicata 1935— <i>Longistriati</i> to<br>read <i>Longistriata</i> . |
| 183  | 2      | 22-33— <i>Ascaridea</i> to read <i>Ascaridia</i> .   | 240  | 2      | under Wehr 1935—superfamily Filarioidae to read<br>Filariodea.                        |





---

---

ANNOUNCING SECTION II, PART III  
AN INTRODUCTION TO NEMATODOLOGY

J. R. CHRISTIE, EDITOR

| <i>Chapter</i> | <i>Title</i>  | <i>Authors</i>   |
|----------------|---|--|
| XII.           | Life History. Vagantia and Phytoparasitica .....                                | J. R. Christie   |
| XIII.          | Ecology, symptoms, control and treatment for Vagantia and Phytoparasitica ..... | A. L. Taylor,<br>W. L. Courtney,<br>G. Thorne,<br>B. G. Chitwood<br>and J. L. Bassen |
| XIV.           | Feeding habits of nematodes. Vagantia and Phytoparasitica .....                 | J. R. Christie,<br>G. Thorne and<br>B. G. Chitwood                                   |
| XV.            | Immunity due to nematodes.....  | Norman R. Stoll  |

All parts are published in editions of 500 copies unless prepublication orders indicate a greater demand. Prepublication price to be adjusted to cost.

PRICE LIST AN INTRODUCTION TO NEMATODOLOGY

|                                       | <i>per copy 5 copies</i> |                  |
|---------------------------------------|--------------------------|------------------|
| Sec. I, part 2.....                   | 3.00                     | 12.75            |
| Sec. I, part 3 & Sec. II, part 1..... | 4.50                     | 19.50            |
| Sec. II, part 2.....                  | 6.00                     | 25.50            |
| The set to date.....                  | 16.00                    | 68.00 per 5 sets |

Sec. I, part 1, not available except in sets. Price subject to change without notice; quantity very limited.

Sole by

M. B. CHITWOOD,

P. O. Box 425, Babylon, N. Y., U. S. A.

---

---