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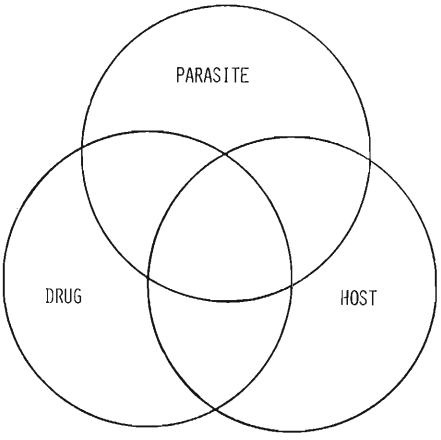

The Control of Parasites

The 500th meeting of the Helminthological Society of Washington was held on May 15, 1976, at New Bolton Center, School of Veterinary Medicine, University of Pennsylvania. The historical associations for this quincentennial meeting to be held at the University of Pennsylvania were strong; it was the bicentennial year with its focus on Philadelphia; Joseph Leidy the father of American parasitology was a professor at the University and the first Medical School in the country was established in Philadelphia. And yet the event came naturally, in the regular sequence of the monthly meetings of the Society. In the same uncontrived manner it was a consequence that the meeting was devoted to a consideration of the control of parasites; in a way, a self-examination by the Society of the progress it had made after 499 meetings, but more importantly of looking forward beyond the 500th meeting. The process of looking forward, of speculating, of enumerating unsolved problems and of tentatively programming for control measures of the future was approached from the holistic interrelationship of host, parasite and drug symbolized by the three interlocking circles in the logo above.

Consideration of the interaction between host, parasite and drug is not new, it was begun long ago by Paul Ehrlich, but we still have the need, frequently, to acknowledge Ehrlich's contribution to the understanding of the integrated system.

Three papers were presented, Dr. Thomas C. Cheng on the role of the parasite, Dr. William C. Campbell on the role of drugs and Dr. E. J. L. Soulsby on the role of the hosts. These are published in this number of the Proceedings. Collectively, they form a point of departure for new approaches to the control of parasites, since as each contribution shows, it is no longer possible to ignore the interlinkages symbolized by the three circles.—E. J. L. SOULSBY

The Control of Parasites: The Role of the Parasite[1]

Uptake Mechanisms and Metabolic Interference in Parasites as Related to Chemotherapy[2]

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ABSTRACT: By employing examples selected from among intra- and intercellular protozoan and helminth parasites, the idea that the specific uptake mechanisms of these dependent organisms may select for or against the delivery of drugs is being advanced. Furthermore it is being pointed out that parasiticidal properties of certain drugs may be based on their ability to alter the uptake mechanisms of parasites.

Although not generally considered to be effectual by the pharmaceutical industry, the idea of searching for specialized metabolic pathways unique to parasites and inhibiting these to their detriment is being reemphasized to be a more scientifically challenging approach to the development of new drugs. Also, the idea of taking advantage of quantitative differences favoring disruption of biochemical and physiological processes in the parasite should be considered in drug development.

In the control of parasites by employing drugs, the selective physiologic vulnerability and chemical receptibility of the parasites play important roles. In other words, ideally, the drug should readily enter and adversely affect the parasite and at the same time not be deleterious to the host. In this contribution I have attempted to focus in on some of the properties of selected parasites which conceivably could serve to regulate or control their receptivity to drugs.

Ecologically speaking the host's internal environment is the endoparasite's habitat. Consequently, in order for a drug to enter the parasite, it must be taken up from its habitat. In the case of most protozoans and astomate helminths, this means molecular uptake through the body surface. On the other hand, in the case of such helminths as the digenetic trematodes and nematodes, the uptake of drugs theoretically could occur either as the result of ingestion, passage through the body surface, or both. Thus the uptake mechanism employed by a specific parasite most probably influences or governs its selective receptivity to a specific drug. In view of this, a brief review of the uptake mechanisms of selected parasites is presented.

Uptake Mechanisms

INTRACELLULAR PROTOZOANS. The uptake mechanisms by intracellular protozoan parasites are intimately associated with how they acquire nutrients. Among members of the classes Telosporea, Piroplasmea, and Zoomastigophorea that are intracellular parasites, there is a cytostome through which molecules from their habitat can be ingested; however, among intracellular members of the classes Microsporea and Haplosporea, such an aperture is absent and hence other uptake mechanisms must be operative.

The cytosomes of telosporeans and piroplasmeans are essentially circular indentations in the pellicle (Fig. 1). When observed *en face*, each cytostome appears as two electron-dense concentric rings (Fig. 2), and when viewed in cross-section, the lining of each cytostome has been interpreted to consist of two electron-dense membranes (Fig. 3). The inner of these is continuous with the parasite's surfacial unit membrane while the outer one is attached to the inner one. The diameter of the cytostome varies not only among members of different genera but also among different species of the same genera (Aikawa, 1971).

[1] An address presented at the 500th meeting of the Helminthological Society of Washington held at New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, on May 15, 1976.

[2] The original information included herein has resulted from research supported by a grant (AI 12355-02-A1) from the National Institute of Allergy and Infectious Diseases.

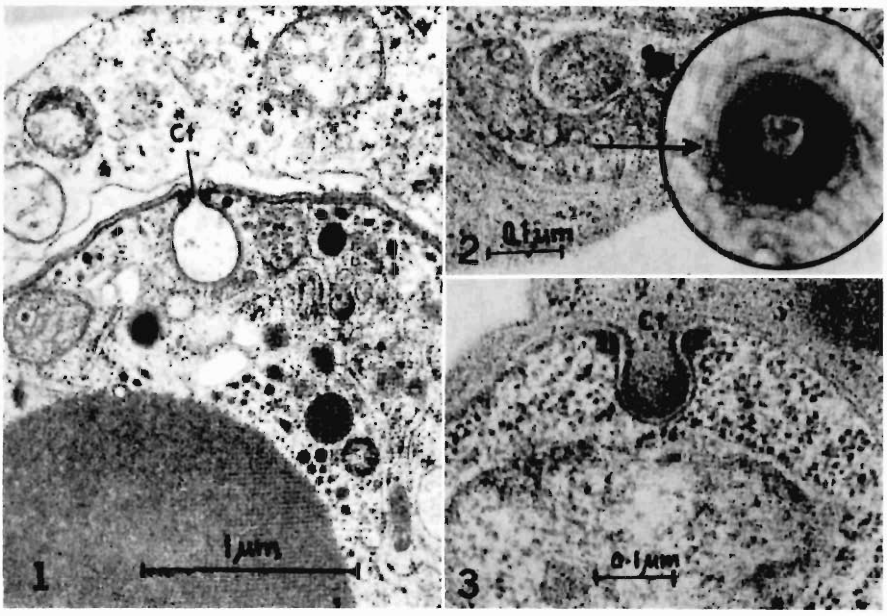


Figure 1. Electron micrograph of intracellular sporozoite of *Eimeria ninakohlyakimovae* showing cytostome (Ct). (After Kelley and Hammond, 1972).

Figure 2. Electron micrograph of *en face* view of cytostome of trophozoite of *Plasmodium cathermerium* (arrow) showing electron-dense concentric rings surrounding aperture. Negative staining technique. (After Aikawa, 1967).

Figure 3. Electron micrograph of portion of uninucleate trophozoite of *Plasmodium cathemerium* ingesting host cell cytoplasm through cytostome (Ct). (After Aikawa et al., 1966).

Nevertheless, the basic architecture is similar among telosporeans and piroplasmeans.

According to Aikawa et al. (1966), Hammond et al. (1967), and Sampson and Hammond (1971), the cytostome only becomes functional after these parasites become established in their intracellular positions. Furthermore, there appear to be differences as to how cytostomes function. For example, Aikawa et al. (1967) have reported that in *Plasmodium elongatum* most of the ingested material is taken into the parasitic cell by the cytostomal cavity, after which the cavity is pinched off from the surrounding host cytoplasm by closure of the cytostomal orifice, followed by the formation of a membrane over the orifice. Subsequent to these events, the food vacuole, i.e., the original cytostomal cavity, migrates to and is eventually incorporated within a digestive vacuole where complete digestion of the ingested material occurs. On the other hand, in several other species of avian haemosporidians, Aikawa et al. (1966) and Sterling and Aikawa (1973) have found that the ingested material is taken into the parasitic cell by small vacuoles pinched off from the cytostomal cavity, and these are subsequently incorporated into a digestive vacuole where digestion of the ingested material occurs (Fig. 4).

Another variation is that found in *Leucocytozoon simondi*. Sterling and Aikawa (1973) have found that the cytostome in this haemosporidian appears to be a transient structure. Specifically, they have reported that the cytostomal rings are only temporary in this parasite of birds, being only visible during the earliest stages of cytostomal ingestion, and disappearing as ingestion advances. Furthermore, the digestion of the ingested material occurs within the cytostomal cavity rather than in a separate digestive vacuole.

It is noted that a cytostome does not occur in oocysts of any of the members of the class Telosporea that have been studied. These

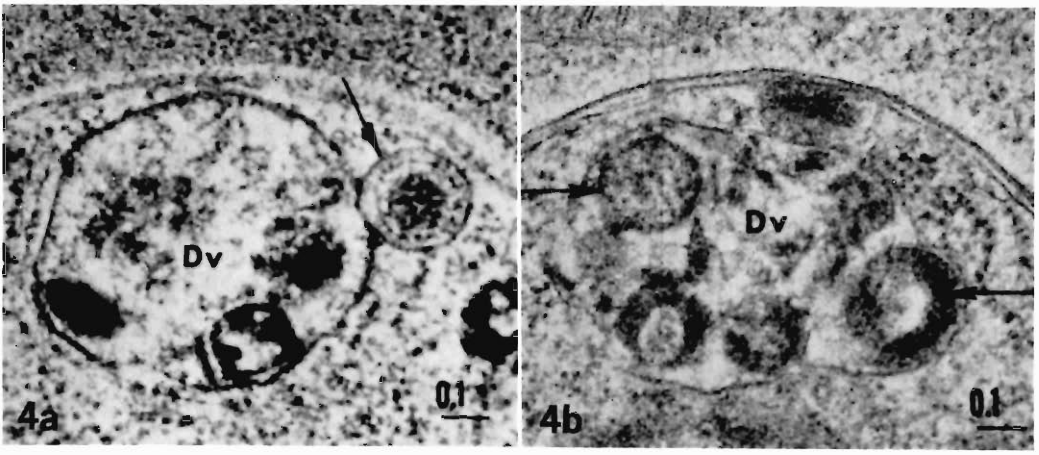


Figure 4. Ingestion and digestion of host cytoplasm by *Plasmodium elongatum*. (a) Food vacuole (phagosome) (arrow) migrating to digestive vacuole (Dv). (b) Several food vacuoles (arrows) within a digestive vacuole (Dv). (After Aikawa et al., 1967).

oocysts are surrounded by a thick electron-dense tunic, from the inner surface of which are pinched off small spherical bodies of medium electron density (Fig. 5). Terzakis et al. (1967) and Vanderberg et al. (1967) have suggested that these bodies may supply nutrients to the oocyst.

Measurements of the cytostomal aperture of various species of intracellular protozoans that possess cytostomes in published electron micrographs by various authors (Aikawa, 1967; Aikawa et al., 1967; Kelley and Hammond, 1972; Sterling and Aikawa, 1973; Aikawa and Sterling, 1974) have revealed that the diameters range between 0.05 and 0.25 μm. These dimensions are sufficiently large to permit the uptake of molecules of high molecular weights, at least as high as 7×10^4 or more. This figure is based on the molecular weight of mammalian hemoglobin, which is known to be taken in by the cytostomes of haemosporidians. Consequently the intake of most drugs is possible through the cytostomal mechanism. It must be noted, however, that since the cytostome is primarily an organelle of intracellular parasitic protozoa; specifically, members of the Telcosporea, Piroplasmea, and Zoomastigophorea, molecules that are taken in by this mechanism by necessity must pass through the surface membrane of the enveloping host cell before they can reach the parasite. Whether this can occur or not and what the facilitating mechanism(s) is are other aspects of cell biology that parasitologists should be concerned with (Fig. 6). For example, it is of interest to speculate whether macromolecules could also be taken into host cells by phagocytosis if these cells are leucocytes. For example, the host cells of *Leucocytozoon simondi* are known to be duck leucocytes, which are capable to phagocytosis.

It is noted that although the uptake of molecules via a cytostome has been reported to occur in the exoerythrocytic stages of *Haemoproteus metchnikovi* by Sterling and DeGiusti (1972) and those of *Plasmodium gallinaceum* by Aikawa et al. (1968), it remains to be ascertained whether this mechanism occurs in the exoerythrocytic stages of other species of haemosporidians. According to Hepler et al. (1966), simple diffusion is responsible for the entry of certain molecules into the exoerythrocytic stages of *Plasmodium fallax*. This mechanism undoubtedly is the one that occurs among the microsporeans and haplosporeans, which are without a surfacial aperture. Furthermore, simple diffusion may and probably does complement other specialized uptake mechanisms in those species with a cytostome. It remains unknown, however, whether simple diffusion is a useful mechanism for the uptake of drugs, since the relatively

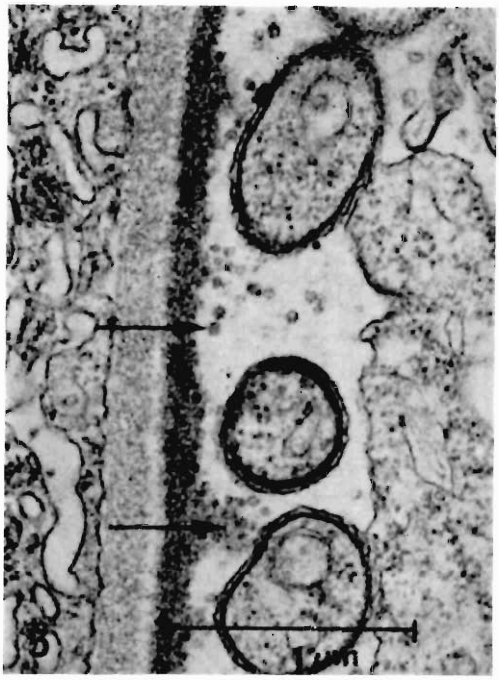


Figure 5. Electron micrograph of oocyst of *Plasmodium berghei* showing capsular material being pinched off from the internal surface (arrows). (After Aikawa and Sterling, 1974).

high molecular weights as well as other physiochemical features, such as surface charges, of these compounds may prevent their passage through membranes by diffusion.

While on the topic of surface permeability, it is of interest to note that the cidal mechanism of certain drugs is known to involve interference with carbohydrate absorption by host cells or by the parasite surfaces. For example, Cenedella and Jarrell (1970) have reported that 4,4′-diaminodiphenylsulfone is cidal to *Plasmodium berghei* as a result of disarrangement of the glucose transport mechanism in the membrane of the host erythrocyte. An example of the alternative mechanism has been contributed by Ghosh and Chatterjee (1961) who have reported that the antibiotic nystatin possibly interferes with the absorption of essential exogenous substrates by *Leishmania*.

Another method by which unicellular parasites may take in molecules of relatively high molecular weight is by pinocytosis along their

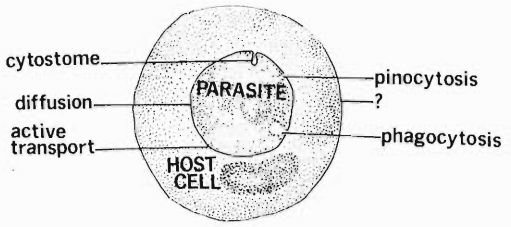


Figure 6. Diagrammatic drawing illustrating known mechanisms by which intracellular protozoans can take in molecules and the lack of information on how such molecules, including drugs, enter the host cell.

cell surfaces. Scalzi and Bahr (1968) and Cox and Vickerman (1966) have demonstrated the occurrence of small pinocytotic vesicles along the surfaces of the erythrocytic merozoites of *Plasmodium chabaudi* and *P. vinckei*, although these malarial parasites also utilize cytostomes for uptake. Also, Stehbens (1966) and Snigirevskaya and Cheissin (1969) have demonstrated that *Lankesterella* and *Eimeria* apparently ingest material in the surrounding parasitophorous vacuole by pinocytosis, respectively. It is noted that Hammond et al. (1967) have also reported the occurrence of pinocytotic vesicles along the lining of small V-shaped invaginations in the pellicle of *Eimeria auburnensis* macrogametocytes. Of course, pinocytosis could serve as a portal mechanism for the intake of drugs. But, again, since these parasites are intracellular, the drug must be able to pass through the delimiting surface of the host cell. One could speculate whether compounds which would inhibit or retard development of cytostomes and pinocytotic vesicles would be deleterious to intracellular parasites and could be considered in chemotherapy. However, before such compounds can be synthesized, we need to know a great deal more about the cell biology of these organelles.

EXTRACELLULAR PROTOZOANS. Prior to the discovery by Steinert and Novikoff (1960) that there is a "cytostome" in cultured amastigotes of *Trypanosoma mega*, it was generally thought that this and related extracellular hemoflagellates took in exogenous materials by diffusion through their pellicles. It is now known that in addition to absorption, cytostomes play a role in uptake. However, the

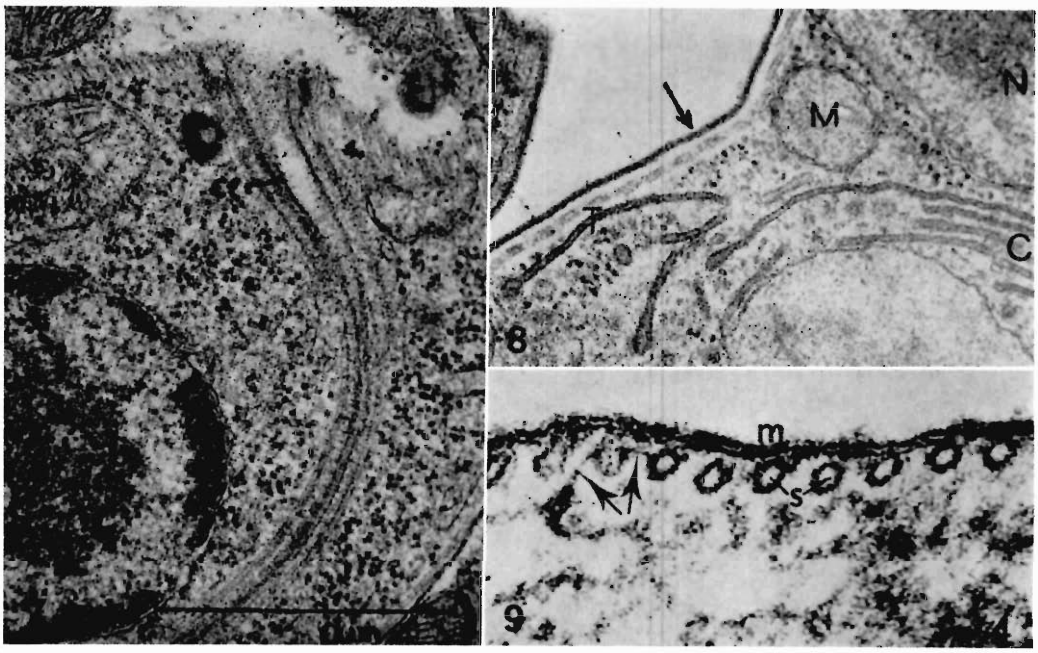


Figure 7. Electron micrograph showing cytostome (Ct) of *Trypanosoma cruzi* (= *Schizotrypanum cruzi*) extending deep into the protozoan's cytoplasm. (After Aikawa and Sterling, 1974).

Figure 8. Electron micrograph of a portion of the surface of *Trypanosoma brucei* showing electron-dense noncellular material (arrow) overlaying surface membrane. × 48,000. C, collecting membrane system; M, mitochondrion; N, nucleus; T, collecting tubule. (After Langreth and Balber, 1975).

Figure 9. Electron micrograph of a portion of the surface of *Leishmania hoogstraali* promastigote showing trilaminate cell membrane (m) and subpellicular microtubules (s). The arrows indicate where the microtubules are connected to the endoplasmic reticulum. × 351,000 (After Lewis, 1975).

so-called cytostomes of trypanosomes are structurally different from those of intracellular protozoans. Specifically, each cytostome consists of a cylinder of fibrils that are continuous with the pellicular fibrillar system. This invagination invades deeply into the cytoplasm, and the cell membrane delimiting the opening to the exterior is depressed, forming a conical pit (Fig. 7). Most of the length of the tubular cytostome is filled with cytoplasm. Furthermore, minute pinocytotic vesicles, each measuring about 0.2 μm in diameter, and delimited by a unit membrane, commonly occur along its length. Steinert and Novikoff (1960) and subsequent investigators have demonstrated that ferritin incorporated into the medium will enter the tubular cytostome and eventually become concentrated in these pinocytotic vesicles, thus indicating that they serve as organelles for uptake. Although no evidence is available at this time relative to drugs, it would appear that this is a possible vehicle for the introduction of such compounds into these extracellular protozoan parasites.

As stated, in addition to employing cytostomes and associated pinocytotic vesicles for the uptake of molecules, absorption through the pellicular surface also occurs. Consequently, some consideration is being given to the fine structure of the pellicle of flagellates.

In the bloodstream form of trypanosomes and most other parasitic flagellates, there is a coat of electron-dense noncellular material overlaying the surface unit membrane (Vickerman and Luckins, 1969, and others) (Fig. 8). This layer is not as substantial in the intracellular flagellates, such as the promastigotes of *Leishmania* (Lewis, 1975). In either case,

this surface coat is chemically a polysaccharide considered by Luft (1971) to be a constituent of the surface coats of nearly all animal cells. The cell membrane is typically trilaminate, and lying immediately beneath it is an array of longitudinally arranged subpellicular microtubules (Fig. 9). The exact organization of these microtubules has been described in *Trypanosoma brucei* and *T. gambiense* by Fuge (1968), and the pattern appears to hold true in other species. There are cross connections between parallel microtubules, and in most species there are "lollipop-like" structures arranged along the length of the microtubules. According to Brooker (1971), who studied *Crithidia fasciculata*, these structures are associated with the uptake of macromolecules such as proteins.

As far as I have been able to determine, although no mechanistic studies have yet been carried out on how drugs enter through the pellicle of protozoans, such compounds obviously do, since the story of drug-resistant forms of extracellular protozoans is well documented. This, of course, would necessitate molecular contact between the drug and the organism's DNA to result in mutations. Furthermore, as Rohatgi and Krawiec (1973) and Kay et al. (1974) have demonstrated, such antimicrobial compounds as chloramphenicol and ethidium bromide will enter the pellicle and the mitochondrial membranes of such protozoans as *Tetrahymena pyriformis* to effect morphometric changes. Thus, there is little doubt that the fine structure of protozoan pellicles is physiologically associated with the permeation of molecules, and conceivably could serve as a mechanism which could influence entry of drugs.

DIGENEA. The mechanisms utilized by helminths, including digenetic trematodes, for the uptake of materials from their environments have been investigated extensively at the fine structural and physiological levels. This, as is the case with protozoans, has come about primarily because of interest in how these parasites obtain their nutrients. Nevertheless, the discovered uptake mechanisms are equally applicable to other categories of molecules, including drugs.

It is now "ancient history" that the surfaces of digeneans are covered with a syncytial cytoplasmic layer known as the tegument, rather than a noncellular cuticle. Information pertaining to the fine structure of the tegument has been reviewed by Lee (1966, 1972) and Cheng (1973), among others. In brief, the general functional architecture is as follows.

The digenean tegument is comprised of two zones. The outer zone, which is separated from the environment by a unit membrane, consists of cytoplasmic syncytium (Fig. 10). Embedded in this layer are mitochondria, endoplasmic reticulum, various types of vacuoles, and, in some instances, glycogen granules and other types of inclusions.

The outer surface is usually thrown into folds to form microvilli. These undulations not only serve to increase the absorptive surface but pinocytotic vesicles are also formed in the crypts between adjacent microvillae which presumably serve for the intake of large molecules and particulate materials. Also embedded in the outer zone of some species are tegumentary spines. These are overlayed with the surfacial plasma membrane and undoubtedly serve as ancillary holdfast mechanisms *in situ*.

The outer syncytial zone is connected by cytoplasmic bridges to nucleated cells, known as cytons, embedded deeper in the parenchyma. The cytons, collectively designated as the inner tegumentary zone, include vacuoles, endoplasmic reticulum, mitochondria, Golgi bodies, glycogen deposits, and various types of vesicles in addition to the nucleus (Fig. 10).

In the region between the outer and inner tegumentary zones are found several other types of tissues. Specifically, lying immediately mediad to the outer tegumentary zone, and separated from it by a unit membrane, is a thin basal lamina. Beneath this are found a series of circular muscles and mediad to these are the fasicles of longitudinal muscles (Fig. 10).

Investigations into the chemical composition of the adult digenean tegument have revealed the presence of glycogen, nonglycogenic polysaccharides, lipids, acid mucopolysaccharides, and mucoproteins (Pantelouris, 1964; Öhman, 1965; and others). The occurrence of acid mucopolysaccharides is of particular significance since these molecules are known to be capable of inhibiting various digestive enzymes and their presence and possible secretion onto

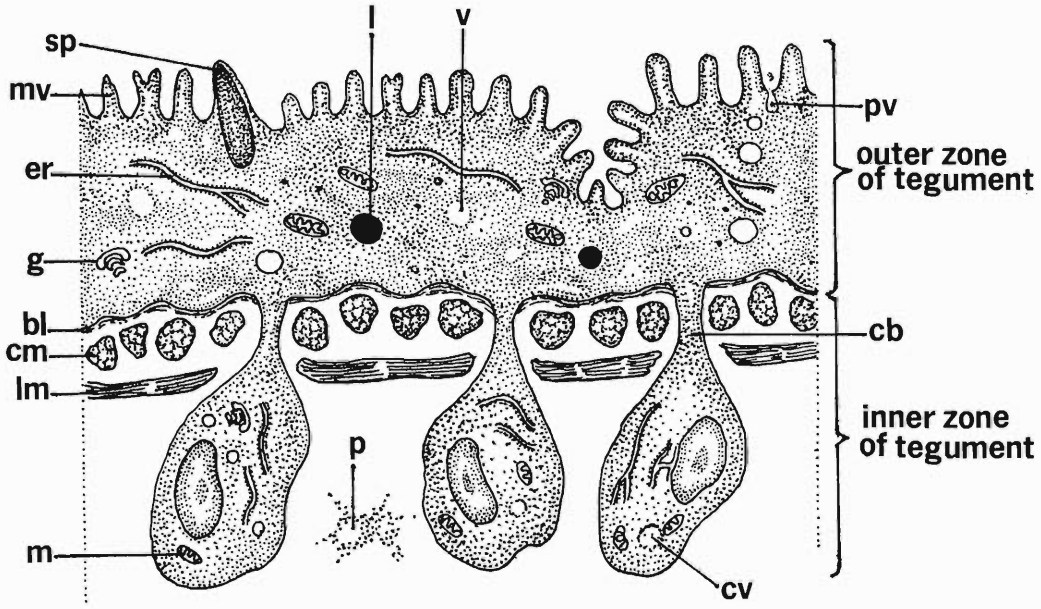


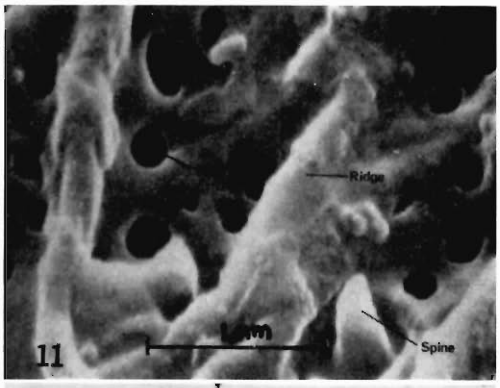
Figure 10. Diagrammatic drawing showing fine structure of the tegument of a digenetic trematode. bl, basal lamina; cb, cytoplasmic bridge; cm, circular muscle; cv, cytoplasmic vacuole; er, endoplasmic reticulum; g, Golgi apparatus; lm, longitudinal muscle; m, mitochondrion; mv, microvillus; p, parenchyma; pv, pinocytotic vesicle; sp, tegumental spine.

the body surface may account for why the intestinal trematodes are not digested by their hosts' enzymes.

In addition to the chemical entities listed, several enzymes have been detected in the trematode tegument. Both acid and alkaline phosphatases have been reported (Yamao, 1954; Lewert and Dusanic, 1961), and esterases have also been detected (Nimmo-Smith and Standen, 1963). In certain specialized cases, other hydrolytic enzymes are associated with certain regions of the tegument. For example, Erasmus and Öhman (1963) have reported the occurrence of aminopeptidase associated with the body surface in the adhesive organ region of *Cyathocotyle bushiensis*, a caecal parasite of ducks. In this case the enzyme is secreted by underlying cells and is active in extracorporeal digestion, i.e., the cells of the host's caecum are partially predigested outside of the parasite's body prior to ingestion.

A few additional words should be said about the tegument of adult schistosomes, which live in an essentially liquid environment. It is noted that information pertaining to the fine structure of the tegument of *Schistosoma*, both larval and adult, has been reviewed comprehensively by Hockley (1973).

Scanning electron microscopy has revealed that the surfaces of adult schistosomes are different between species as well as between sexes. However, all have a basic spongy appearance, i.e., there are ridges and pits (Fig. 11). Furthermore, spines are interspersed over the surfaces. When this topography is analyzed by transmission electron microscopy, the pits have been determined to be tortuous channels, which may be branched and interconnected (Fig. 12). Hockley (1973), as the result of incubating *S. mansoni* in colloidal iron or thorium, has demonstrated that these channels are open to the exterior, thus providing a large surface area. It is noted that the schistosome tegument is thinner than that of most other digenetic trematodes, being approximately 4 μm thick in *S. mansoni* and *S. japonicum* (Morris and Threadgold, 1968; Smith et



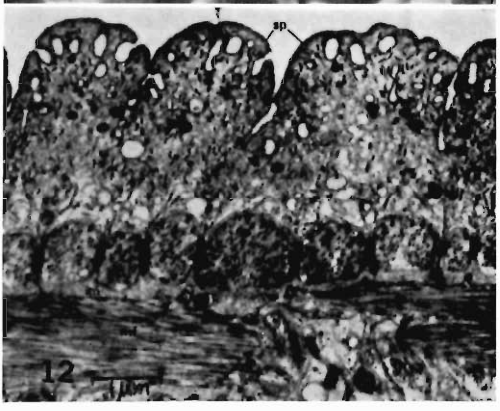


Figure 11. Scanning electron micrograph of portion of the tegument of adult male *Schistosoma mansoni* showing pits, ridges, and spines. (After Hockley, 1973).

Figure 12. Transmission electron micrograph of body surface of adult male *Schistosoma mansoni* showing profiles of interconnected tortuous channels. (After Hockley, 1973).

al., 1969; Silk et al., 1969; Hockley, 1970; Inatomi et al., 1970). For comparison, it is noted that the tegument of *Fasciola hepatica* measures 15–20 μm thick (Threadgold, 1963).

Based on these somewhat specialized features of the schistosome tegument, it was generally assumed that, as is the case with other species of digeneans, the tegument functions as an absorptive surface. However, Hockley (1973), as the result of comparing the structure of the schistosome tegument with that of its digestive epithelium, i.e., the syncytium lining the intestinal caeca, has concluded that the caecal wall is probably more efficient as uptake surfaces. However, a few words need to be said about the architecture of the membrane overlying the outer tegumental layer of schistosomes.

Smith et al. (1969) and Hockley and McLaren (1973) are responsible for the elucidation of the structure of the covering plasmalemma of schistosomes. Specifically, although the outer membrane, which extends over the entire pitted tegumental surface, has been described as being trilaminate and approximately 10 nm thick, Smith et al. (1969) have found that this membrane in the area of the gynocophoric groove is pentalaminate. This observation has been extended by Hockley and McLaren (1973) who, as a result of fixing specimens of *S. mansoni* with uranyl acetate, discovered that the surface membrane is essentially heptalaminate throughout, and is approximately 17 nm thick. Moreover, the heptalaminate nature of this membrane has its origin in the schistosomule and persists in the adult. According to Hockley's (1973) reasoning, the more complex nature of the surfacial membrane suggests that it is less efficient as an uptake surface. The surface membrane of the caeca syncytium is typically trilaminate.

It is noted that although it would appear from electron microscopical studies that the caecal cells are more efficient from the standpoint of uptake, this hypothesis may apply only to macromolecules. Molecules of relatively low molecular weights, including the amino acids glycine, proline, methionine, arginine, cysteine, glutamate, and tryptophan are taken into *S. mansoni* adults primarily through the tegument (Asch and Read, 1975b). Furthermore, kinetic studies by Asch and Read have revealed that these amino acids are taken in by different mechanisms. For example, cysteine is taken up solely by diffusion, proline is taken up only by active transport, while the other amino acids studied are taken up through a combination of diffusion and active transport. There is a highly specific transport locus for proline, and one for acidic amino acids, and there are probably at least two transport systems for most of the neutral amino acids. It is noted that earlier, Asch and Read (1975a) had reported that 80–100% of glycine and proline are taken up through the tegument, and Isseroff et al. (1972) have demonstrated that monosaccharides are also

primarily taken up through the tegument of *S. mansoni*.

What has been said about schistosomes relative to uptake probably also holds true for *Fasciola hepatica* and other digenetic trematodes since Mansour (1959) has shown that ligation of the mount of *F. hepatica* does not effect the rate of absorption of glucose, and Isseroff and Read (1969) have reported that amino acids are taken up by this parasite through the tegument. More recently, Hanna (1976) has reported that the monosaccharides galactose and glucose are also taken up primarily through the tegument of *F. hepatica*.

The point being made is that there is ample evidence that the teguments of *S. mansoni* and *F. hepatica* are actively involved in the uptake of molecules of low molecular weights; however, larger molecules are most probably taken up through the caecal epithelium. Consequently, in delivering drugs, which are commonly compounds of relative high molecular weights, to schistosomes and liver flukes, consideration must be given to the fact that its effective uptake can only occur through the digestive epithelium after ingestion.

It is noted that certain drugs are known to effect the target helminth parasites by interfering with their surfacial uptake mechanisms. For example, Bueding (1959, 1962) has demonstrated that the chemotherapeutic activity of alkyldiphenylamines can be explained by their ability to inhibit glucose absorption by *Schistosoma mansoni*. Similarly, Bueding et al. (1961) have reported that the therapeutic activity of a cyanine dye, dithiazanine, is the result of its ability to inhibit uptake of glucose by *Trichuris vulpis*, and Strufe and Gönnert (1967) are of the opinion that the cestodecidal mechanism of such drugs as dichlorophen and Yomesan most probably is also based on such a phenomenon.

At this point it would appear of interest to point out that there is some information available on the effect of drugs on the tegument of schistosomes. Gönnert (1955) has reported that Miracil D causes the vacuolation of *S. mansoni* tegument, Standen (1962) has reported that 1:7-bis(*p*-aminophenoxy) heptane, when injected into *S. mansoni*-infected mice, causes the parasite's tegument to disintegrate. Specifically, he found that initially there is the formation of small surface outgrowths from the tegument, followed by increasing vacuolation of the tegument, and the development of larger, balloonlike surface exudates. Eventually, the host's phagocytic cells become associated with these exudates, and as degeneration progresses, the phagocytes infiltrate through the parasite's degenerate tegument, and invade all of the tissues of the schistosome. Standen has expressed the opinion that the drug exerts a deleterious effect on the permeability and architecture of the parasite's tegument, and as a consequence, the uptake of nutrients through the tegument is partially or totally inhibited and this leads to the rapid senescence of the worm.

Bueding et al. (1967) have reported that a subcurative dose of tris(*p*-aminophenyl) carbonium chloride administered to mice infected with *S. mansoni* results in a reduction in the amount of glycogen in the dorsal tubercles of male worms, and this results in the flattening and eventual disappearance of the tubercles, but there is no direct effect on the tegument.

Finally, Hockley (1973) has reported that preliminary electron microscopical studies on *S. mansoni* recovered from a monkey 1 hour after treatment with the antimonial compound Triostam (sodium antimonygluconate) has revealed balloonlike swellings on the outer surface of the tegument. These swellings are 3–4 μm in diameter and each is jointed to the normal tegument by an isthmus. The outer membrane of the tegument is continuous over the swellings, but the inclusions in the tegumental syncytium do not extend into them.

A few studies are also available on the effects of drugs on the tegument of *Fasciola hepatica*. Dawes (1966a, b, 1967) has reported that there is vacuolation of the tegument of this parasite after the rat host had been treated with Bithinol, and Thorsell and Bjorkman (1966) have reported similar vacuolation of the tegument of this fluke after *in vitro* treatment with hexachlorophene and its dimethylether.

The question that must be raised is whether these pathological alterations of helminth teguments after drug treatment represent the direct effect of the drugs or reflect the moribund condition of the worms. That the latter is the answer appears to be supported by Dawes's

(1968) finding that the vacuolation was due to impending death after drug treatment. This interpretation is further supported by Dawes's (1963, 1964) reports that invasion of the tegument by host phagocytes occurs when specimens of *F. hepatica* are weakened by X-irradiation. In other words, drug treatment leads to chemical changes on the surfaces of these worms so that they no longer mimic their hosts immunologically (Smithers and Terry, 1967, 1969; Smithers et al., 1969; Damian, 1962, 1964), and consequently are recognized as nonself and are attacked by phagocytes. In conclusion, it may be said that from the limited data available, drugs known to be cidal to schistosomes and liver flukes apparently do cause cytopathological alterations in the parasites' teguments but these are secondary manifestations of the moribund condition of the parasites rather than the direct effect of the drugs. Therefore, the route of entry of the drug into the parasite has not been revealed by the studies reviewed above. As stated, from other available information, it is hypothesized that the drugs, all with relatively high molecular weights, are taken in orally.

In summary, it would appear that in the formulation of antiparasitic drugs, consideration should be given to: (1) whether the molecule will permeate the surfaces of the specific parasite, and (2) whether it will elicit normal function by the specialized uptake mechanisms of of the parasite, e.g., cytostomal uptake, pinocytosis, and other similar processes. Alternatively, the drug could be of such a nature that it would interfere with the uptake of essential exogenous substrates by the parasite.

Metabolic Interference

Ideally, the search for new parasiticidal drugs should involve the finding of new compounds which would interrupt some critical biochemical pathway in the parasite and at the same time not effect the host deleteriously. In other words, a systematic search for metabolic pathways unique to the target parasite should be conducted. This, of course, as we all recognize, is a long, tedious, and expensive process which cannot guarantee results. Consequently, those in pharmaceutical research know that research on parasiticidal compounds usually means the empirical screening of numerous compounds. For example, according to Standen (1967) approximately 250,000 compounds have been screened as possible antischistosomal drugs. It is usually only when an effective drug is found as the result of mass screening that the underlying cidal mechanism is investigated. The history of the development of chemotherapeutic agents for vaginal trichomiasis as presented by Jírovec and Petrů (1968) and that of the development of chemotherapeutic agents against Manson's schistosomiasis by Pellegrino and Katz (1968) attest to this route in parasiticidal drug research. This is not to say that empirical screening has not led to the discovery of useful drugs. As an example, the development of Miracil D, the first metal-free compound which proved to be of therapeutic value in human schistosomiasis (Kikuth et al., 1946; Kikuth and Gönnert, 1948) came about by modification of Miracil A, which, in turn, was selected from the screening of about 4000 substances. The discovery of Miracil D has led to the synthesis of Hycanthone (1-N-β-diethyleminoethylamino-4-hydroxymethylthioxanthone) (Rosi et al., 1965), which, despite the controversy, is in use in schistosomiasis endemic areas.

In this section is reiterated the concept of metabolic interference in the development of drugs for chemotherapy of parasitic diseases. This, of course, requires understanding the relationship between drugs, hosts, and parasites at the biochemical level.

CHEMOTHERAPY OF PROTOZOAN DISEASES. It is not my intent to present a critical review of either the metabolism of protozoan parasites or the available information on chemotherapy of protozoan-caused diseases in this section. The first topic has been reviewed brilliantly by von Brand (1973), and Fletcher and Maegraith (1972) have reviewed what is known in this area about *Plasmodium* spp. The second topic has been reviewed by Peters (1970) and others. Rather, it is my intent to reinforce the thoughts of many how basic understanding of the biochemistry of host and parasite could lead to a more rational approach to the chemotherapy of diseases caused by protozoa. Unfortunately, the development of new drugs based on this assumption has yet to become a reality. From what is known, the primary rea-

son for this appears to be that the parasites are biochemically "uncooperative," i.e., although qualitative and quantitative differences between the metabolism of host and parasite have been recorded, only a few clear-cut instances are yet known where the parasite includes a critical metabolic pathway that is absent from the host. A few selected examples of this situation are presented below.

Considerable interest has been focused on the pentose phosphate pathway of *Plasmodium* spp. and their host cells during the past two decades. Theoretically, it would appear that the malaria parasite could have an absolute requirement for this pathway since this is the principal, if not the only, pathway for the production of the pentose sugars essential for nucleic acid synthesis. In view of the fact that degradation of glucose via the pentose phosphate pathway leads to the formation of only 3 moles of ATP per mole of glucose utilized indicates that it is less important as an energy-producing mechanism than for the provision of compounds utilized in various syntheses.

Studies by Bowman et al. (1961), Fletcher and Maegraith (1962), Bryant et al. (1964), Herman et al. (1966), Theakston and Fletcher (1971a, b), and Luzzatto et al. (1969) all have suggested that the erythrocytic stages of various species of *Plasmodium* are completely dependent on the enzyme glucose-6-phosphate-dehydrogenase and possibly also 6-phosphogluconate-dehydrogenase of the host cells. These enzymes are essential for the operation of the pentose phosphate pathway. The point I wish to make is that if it was the other way around, i.e., the host cells were dependent on the parasite's enzymes, then an agent which would block the activities of these enzymes could theoretically be employed to inhibit the pentose phosphate pathway in the parasite and this presumably would lead to the death of both the parasite and the infected cell.

The reversed example cited above serves to illustrate what is meant by the biochemical "uncooperativeness" of parasites. But then, in view of the nature of parasitism and the evolutionary events leading to its development (Cheng, 1973), it is not surprising that if biochemical dependency is to be found in a host–parasite relationship, it is the parasite that is dependent on host erythrocytes for certain enzymes involved in the pentose phosphate pathway. One could go one step further and hypothesize that if simplified or abbreviated metabolic shunts are to be found in a host–parasite association, in most, if not all, instances the modified pathway would occur in the parasite since in their unique type of niche, parasites are exposed to restricted amounts of exogenous substrates, as are organisms that survive under other types of ecological stress, and this type of selective pressure, in my opinion, favors biochemical change from the complex to the simple. Consequently as is well documented by available data (von Brand, 1973), there are few clear-cut examples of the occurrence of essential metabolic pathways in parasites that are absent in the host.

Let us look at an example. It is well known that the classical Embden Meyerhoff glycolytic pathway leading from carbohydrate to lactic acid is independent of oxygen and therefore serves as the principal energy-producing pathway under anaerobic conditions. The wide distribution of many glycolytic enzymes among *Trypanosoma* spp. and *Entamoeba histolytica* (see von Brand, 1973 for review), and the demonstration of phosphorylated glycolytic intermediates in trypanosomes clearly indicate that their glycolytic sequences are in general typical of the classical ones. However, if there is a lack or a very low level of activity of a specific enzyme, a modification of the classical glycolytic pathway occurs. For example, the low level of enzymatic activity causing reduction of pyruvate to lactate results in the reoxidation of NADH formed during triose oxidation, and this process prevents the cessation of glycolysis. Now, among monomorphic trypanosomes of the *brucei* group, little or no lactic acid is produced, and phosphoglyceraldehyde (PGAL) acts as the hydrogen acceptor for the reoxidation of NADH, a process leading to the formation of phosphoglycerol. It is known that glycerol is one of the main anaerobic end products of these flagellates, and this molecule is the end product of the metabolism of phosphoglycerol mediated by glycerolphosphate dehydrogenase and phosphatase, which enzymes have been demonstrated in these hemoflagellates (Harvey, 1949; Grant and Sargent, 1960). In view of this, theoretically, an inhibitor of glycerolphosphate dehydrogenase

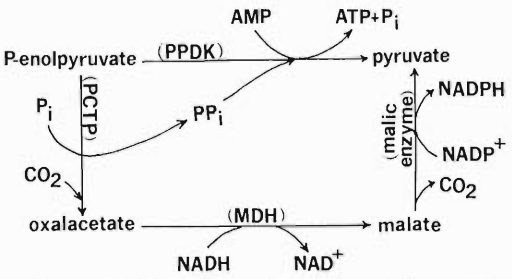


Figure 13. Interrelationship between the enzyme-mediated steps involved in glycolysis in *Entamoeba histolytica*. PPDK, phosphoenolpyruvic carboxytransphosphorylase; PCTP, phosphopyruvate carboxylase; MDH, malate dehydrogenase.

and phosphatase would block the liberation of glycerol to the detriment of these parasites.

Another example is considered at this point. In most organisms that produce lactic acid, phosphoenolpyruvate is transformed into pryuvate via a pathway mediated by pyruvate kinase as follows:

$$\text{Phosphoenolpyruvate} + \text{ADP} \underset{\text{pyruvate kinase}}{\rightleftharpoons} \text{pyruvate} + \text{ATP}$$

This enzyme, however, is lacking in *Entamoeba histolytica*, and the transformation is accomplished as a result of mediation by pyruvate-phosphate ligase (AMP, phosphoenolpyruvic carboxytransphorylase), which requires inorganic pyrophosphate as the substrate. This is illustrated by the following:

$$\text{Phosphoenolpyruvate} + \text{AMP} + \text{PP}_i \rightleftarrows \text{pyruvate} + \text{ATP} + \text{P}_i$$

The presence of the essential pyrophosphate is assured by the occurrence of the enzyme phosphopyruvate carboxylase, which catalyzes the formation of pyrophosphate and oxalacetate from phosphoenolpyruvate, CO_2, and inorganic phosphate. In conjunction with phosphopyruvate carboxylase, pyruvate and oxalacetate are produced from glucose and CO_2. The oxalacetate subsequently is converted to malate by a NAD-linked malate dehydrogenase, and malate is converted to pyruvate by a NADP-linked malate dehydrogenase known as the malic enzyme. The interplay of the four enzymes is depicted in Figure 13. Thus, theoretically, the introduction of inhibitors for pyruvatephosphate ligase, phosphopyruvate carboxylase, the NAD-linked malate dehydrogenase, and malic enzyme could be amoebicidal.

CHEMOTHERAPY OF HELMINTHIC DISEASES. Few examples are available of major metabolic differences between helminths and their hosts (see Bryant, 1970, for review). On the other hand, there appears to be some differences in the sensitivities of certain helminth enzymes and their counterparts in their hosts. This, of course, could be capitalized upon in the development of drugs; for example, the inhibition of schistosome phosphofructokinase by antimonials. Saz and Bueding (1966) have demonstrated that this enzyme from schistosomes differs from that of mammals in sensitivity to antimonials, with the former being more so. This is most probably the basis for chemotherapeutic properties of such antimonial compounds as potassium antimony tartrate, stibophen, and others. Trivalent antimonials are efficient in inhibiting phosphofructokinase in these parasites, and this enzyme is known to control the rate of glycolysis in schistosomes. Therefore, this is generally considered to be the cidal mechanism of antimonials, although these drugs may also affect other mechanisms essential for the survival of the parasites (Bueding, 1959).

It is noted that Cu^{++} is more effective at inhibiting succinic dehydrogenase activity of *Ascaris* than of the mollusc *Biomphalaria glabrata* (Cheng, unpubl.). This fact is being mentioned because it also serves to exemplify the concept that enzymes that catalyze the same process in different species of animals can be different in their sensitivities to inhibitors. It is also of interest to note that Cu^{++} will retard the normal development of larval *Schistosoma mansoni* in *Biomphalaria glabrata* when infected snails are exposed to 60 ppm of Cu in the form of $CuSO_4$ for 20 hours (Cheng, unpubl.). Although the mechanism for this phenomenon remains uninvestigated, it is, nevertheless, of interest to note that both antimony and copper, are schistosomidal. Since the cupric ion is essentially nontoxic to mammals, except for sheep, at low concentrations, i.e., in concentrations of parts per million, perhaps studies to determine the chemotherapeutic properties of copper compounds should be carried out. As far as I have been able to ascertain, such studies have not been done.

Of course, the inhibition of enzymes in helminths is not always brought about by metals. Tetramisole and thiabendazole, both nonmetal-containing drugs, interfere directly with fumarate reductase activity in *Ascaris* and *Haemonchus* (van den Bossche and Janssen, 1967, 1969; Prichard, 1970).

Finally, brief consideration is being given to another mechanism by which certain drugs cause the death of helminths. It is known that succinate production in *Ascaris* is considerably reduced when treated with piperazine. Bueding et al. (1959) have shown that this is not due to direct inhibition of one of the metabolic steps leading to the production of succinate. Rather, it is due to the fact that piperazine induces paralysis of the nematodes and this lowers their energy requirements.

In conclusion, my intent has been to point out that the formulation of effective drugs against parasites could depend on a variety of factors among which (1) entry into the parasite and that portion of the host harboring the parasite, (2) alteration of the uptake surface of the parasite, (3) inhibition of some specialized metabolic pathway unique to the parasite, and (4) quantitative differences favoring inhibiting of some biochemical or physiological process in the parasite are important ones.

ADDENDUM (added in proof). Since the submission of this paper, A. B. Clarkson Jr. and F. H. Brohn (1976. Science, 144:204–206) have published an excellent example of selective destruction of *Trypanosoma brucei brucei* in the mammalian host by salicyl hydroxamic acid and glycerol based on differences in host and parasite carbohydrate metabolism.

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The Control of Parasites: The Role of Drugs[1]

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". . . the play is the tragedy 'Man', and its hero, the Conqueror Worm"
Edgar Allan Poe

Poe's words were allegorical rather than parasitological; but parasite control might be regarded, in large measure, as an attempt to strip the worm of its role as conqueror.

Undoubtedly the major extrinsic factors in determining the extent and degree of parasitism are the prevailing social and political forces in the case of man, and the husbandry practices in the case of domestic animals. Of the mechanisms invoked intentionally to reduce parasitism, biological control and vaccination have played roles that have been overshadowed by the role of chemotherapy. It seems likely that antiparasitic drugs will continue to be of importance for some time.

Antiparasitic drugs actually play many roles —not just because there are many drugs, but because one drug in its time plays many parts. Some are general roles—the parts played by

[1] An address presented at the 500th meeting of the Helminthological Society of Washington, held at New Bolten Center, School of Veterinary Medicine, University of Pennsylvania, on May 15, 1976.

Table 1. Productivity of Merino sheep from age 6 months to age 18 months (50 sheep per group, all groups grazed together). Anthelmintic B has broader spectrum than A, and greater activity against immature worms. Both drugs were administered monthly. From Gordon, 1963.

Treatment	% sheep of 70 lb or over at 18 months old	Wool per head lb.	% fleeces showing defective wool
Controls—no anthelmintic treatment	16	6.7	32
Anthelmintic A	35	7.4	14
Anthelmintic B	62	7.9	4

drugs in the overall scheme of parasite control —and I shall say a little bit about those. There are innumerable specific roles: this drug kills *Haemonchus* in sheep, that drug kills *Ancylostoma* in man, and so on. I am not going to say anything about those roles. They have been recounted many times in recent years. There are specific roles of another kind—the biochemical roles. I do want to say something about those, partly because they are not often pulled together in one place, but mostly because I want to end by discussing how they might relate to the discovery of tomorrow's antiparasitic drugs.

The Role of Drugs in Livestock Parasitism

Although I will be focusing attention on helminth infections, the general role of drugs in controlling parasitic diseases in domestic animals is perhaps best illustrated by the situation in coccidiosis. Since the introduction of medicated diets for the control of coccidiosis in 1948, it has been virtually axiomatic that an effective coccidiostat is indispensable for successful intensive production of chickens—this, mind you, in a parasitic situation in which immunity to reinfection is easily demonstrated under both experimental and field conditions. Indeed the natural immunological protection is exploited in conjunction with chemotherapy, and the poultryman, in this day of sophisticated agriculture, takes a great interest in the degree to which a given coccidiostat will permit the development of natural immunity, especially in replacement chickens. Despite the very evident immunogenicity of the infection, vaccination has not become a significant means of control; and it has been the role of the antiparasitic drugs to make feasible the modern intensive production of an important food animal.

In helminthiasis we have to a lesser, but still significant extent, moved from the use of drugs for the salvage of sick animals to the prevention of disease. This is accomplished not by continuous chemoprophylaxis but by tactical treatment (administration of drugs whenever local conditions make a buildup of parasitism seem likely) or strategic treatment (administration of drugs according to a schedule based on the epidemiological conditions prevailing in a given region). Quite suddenly, as these things go, we no longer hear of devastating outbreaks of acute fascioliasis in the United Kingdom, or of haemonchosis in Australia. More important, perhaps, than the prevention of overt disaster is the prevention of the more subtle deleterious effects of parasitism on the quantity and quality of livestock products. In either case, anthelmintics, too, are currently playing the role of enhancer of agricultural production. Obviously the increase in productivity is easy to demonstrate under conditions favorable to clinical parasitism, and becomes more difficult and more controversial under conditions where parasitism is very much subclinical. An example of enhanced productivity may be found in the work of Gordon (1963); some of his data are given in Table 1. I want, however, to go beyond such well-documented effects and to consider a more recent and more comprehensive examination of the subject.

Only in recent years have attempts been made to determine whether the increased productivity associated with drugs necessarily results in economic benefit. Just last month a paper appeared that is, I think, a striking example of how parasitological and economic studies can be combined and focused on this point (Anderson et al., 1976). These workers determined the economic returns from 2 strategic schemes of helminth control in weaned lambs in Western Victoria, Australia; and they compared these with returns from flocks without a treatment scheme and, in part, with flocks subjected to unusually intensive treatment. The economic analysis included

Table 2. Maximum financial return (given favorable economic conditions) from flocks of lambs with no treatment schedule, a "traditional" strategic schedule, a "critical" strategic schedule, or an intense (biweekly) schedule. From Anderson, et al. 1976.

	Maximum return (Australian dollars)			
	None	Traditional	Critical	Intensive
Gross return per 100 sheep	1029	1115	1205	1441
Net return per 100 sheep*	1023†	1097	1186	1271
Percent return on cost of treatment‡	—	617	1254	151

* Gross return minus cost of treatment.
† Lambs received one treatment to control clinical parasitism.
‡ Cost in addition to cost of single treatment in flocks with no treatment schedule.

extrapolation of data to years in which economic conditions were more favorable or less favorable than the year in which the data were collected. The figures in Tables 2 and 3 have been extracted from the wealth of information in this paper to illustrate the following points. (1) All schemes of anthelmintic treatment gave better livestock production than no treatment scheme, and thus a higher gross financial return. (2) Under favorable economic conditions, all treatment schemes also gave a higher net return. Thus treatment always made economic sense, although the intensive (biweekly) treatment was not nearly so profitable as the more judicious schemes. (3) Under unfavorable economic conditions, both strategic treatment schedules were economically advantageous; but biweekly treatment resulted in financial loss in the face of production gains.

Thus it can be seen that anthelmintics can add plumpness not only to the carcass but also to the purse; and that the financial rewards are more or less proportional to the enlightenment of the designer of the control program.

The Role of Drugs in Parasitism of Man

First we must remember that antiparasitic drugs have played, and continue to play, a lifesaving role. I seem to recall from some introductory textbook that visceral leishmaniasis was about 95% fatal before the introduction of antimonial drugs, and about 95% nonfatal afterwards. The lifesaving role of the antimalarial drugs needs no elaboration. Control of morbidity on a population basis has also been of importance in protozoal and helminthic infections, but it must be admitted that the overall success rate has been disappointingly low, and the need for effective strategic control remains distressingly great.

The incentives of the marketplace ensure a steady search for new antiparasitic drugs for use in livestock, but the situation is less reassuring with respect to mankind. On the one hand there is the lowly pinworm, parasitizing the posteriors of our progeny, doing it in all climes and, most important, affecting the rich as well

Table 3. Minimum financial return (given unfavorable economic conditions) from flocks of lambs with no treatment schedule, a "traditional" strategic schedule, a "critical" strategic schedule, or an intense (biweekly) schedule. From Anderson, et al. 1976.

	Minimum return (Australian dollars)			
	None	Traditional	Critical	Intensive
Gross return per 100 sheep	333	368	398	451
Net return per 100 sheep*	327†	351	379	281
Percent return on cost of treatment‡	—	200	400	−28

* Gross return minus cost of treatment.
† Lambs received one treatment to control clinical parasitism.
‡ Cost in addition to cost of single treatment in flocks with no treatment schedule.

Table 4. Probable mode of action of common anthelmintics (from the literature). Compiled in collaboration with Dr. M. H. Fisher.

1. Neuromuscular anthelmintics
   a. Acetylcholinesterase inhibitors
      dichlorvos
      trichlorfon
   b. Choline mimetics
      bephenium
      thenium
      hycanthone
   c. Neuromuscular depolarizers
      methyridine
      pyrantel
      morantel
   d. Neuromuscular hyperpolarizers
      piperazine
   e. Ganglion stimulants
      levamisole
2. Inhibitors of fumarate reductase
   thiabendazole
   cambendazole
3. Inhibitors of glucose uptake
   mebendazole
   pyrvinium
   dithiazanine
   styrylpyridinium
4. Inhibitors of glucose metabolism
   antimonials
5. Disruptors of glycogen metabolism
   niridazole
6. Uncouplers of oxidative phosphorylation
   rafoxanide
   clioxanide
   oxyclosanide
   niclosamide
   bithionol
   hexachlorophene
   nitroxynil
   2,4-dinitrophenol
   aspidium oleoresin
7. Inhibitors of dihydrofolate reductase
   suramin
8. Others
   bitoscanate
   tetrachlorethylene
   phenothiazine
   organo-arsenic

as the poor. Sad to relate, the amount of money spent for the treatment of pinworm probably is more or less inversely proportional to its importance as a pathogen. On the other hand there are the many serious parasitic infections that plague the underdeveloped nations, especially those in tropical regions. Good drugs are available for some of these diseases but not for others. The commercial incentives for the development of new drugs for these diseases are, at best, uncertain. What can be done about them?

The World Health Organization recently published an important, and already widely quoted, document entitled "Tropical Diseases Today—The Challenge and the Opportunity" (Anonymous, 1975a). It discusses three tropical diseases with a combined prevalence of 600 million cases, and three other very serious but less common diseases of man. Five of these six diseases are caused by protozoa or helminths. The report stresses the urgent need for a coordinated research program aimed at the development of better control measures for these diseases. In the program proposed by WHO, "task forces" of internationally reputed scientists will define the needs, and plan and direct scientific operations to meet those needs. The operations will be carried out by a "network" of medical school and institutional laboratories in the tropics, mostly in Africa. The objective is to discover, develop and make available on a large scale, new and better remedies for these diseases before the end of the century. This strategy is indeed admirable, but are the tactics sound?

In commenting on the WHO report, Smith (1976) has pointed out that even if new drugs and vaccines are developed, they will have little impact in disease control in developing countries unless major efforts are made to solve the practical problems associated with their use under field conditions. That is true, but unfortunately there is a more immediate cause for concern. It seems to me that the WHO proposal does not offer the best opportunity for discovering the needed new drugs; and that is a subject to which I shall return.

Biochemical Roles of Antiparasitic Drugs

To review very briefly the biochemical roles of antiparasitic drugs, I have put them in categories, in collaboration with Dr. M. H. Fisher, as shown in Table 4. It must be emphasized that the actual mode of action is rarely if ever known with certainty. Of the many effects reported in the literature, those listed in the table seem at this date to be the most likely mechanisms of anthelmintic action.

In the first category there is a large group of anthelmintics that interfere with nervous control of muscle function, either at the neuromuscular junction or at the nerve ganglion. Some, such as the organophosphates, inhibit the deactivation of acetylcholine by acetylcholinesterase. Others, such as bephenium and

thenium, mimic this effect by usurping the acetylcholine receptor site, and thus foiling the esterase. Others interfere with neuromuscular transmission by altering the electric charge of the nerve cell membrane—either depolarizing it, in the case of methyridine, pyrantel, and morantel, or hyperpolarizing it, in the case of piperazine. The former action makes the worms uptight, while the latter makes them floppy. The final item in this category is levamisole, which probably acts directly on the nerve ganglion—producing a paralysis that is self-limiting *in vitro*. Levamisole also inhibits fumarate reductase (but it paralyzes worms that lack this enzyme) and inhibits alkaline phosphatase and acetylcholinesterase in mammalian test systems.

Most of the benzimidazole anthelmintics probably kill nematodes by inhibiting their fumarate reductase, thereby blocking carbohydrate metabolism. When the enzyme is extracted from benzimidazole-sensitive *Haemonchus*, it is inhibited by thiabendazole *in vitro*; but this is not the case when the enzyme is obtained from benzimidazole-resistant strains of the same worm.

Mebendazole apparently blocks carbohydrate metabolism in a way that is quite different from that of other benzimidazoles. Through inhibition of glucose uptake, or interference with some later step, there is a marked depletion of glycogen. There is also a rapid disruption of secretory organelles in the tegument of cestodes and in the intestinal cells of nematodes and these changes could well account for the death of the worms. The cyanine dye anthelmintics also inhibit the uptake of exogenous glucose, and probably act differently in aerobic and in anaerobic worm species.

The antimonial compounds interfere with glucose metabolism in schistosomes by inhibiting phosphofructokinase. The enzyme of the schistosome is more sensitive to such inhibition than is the isoenzyme of the host. Niridazole disrupts the glycogen metabolism of susceptible schistosomes by inhibition of two essential enzymes.

A large group of anthelmintics, consisting mainly of salicylanilides, bis-phenols and nitrophenols, probably act as uncouplers of oxidative phosphorylation. Thus they block energy production.

Suramin probably kills *Onchocerca* because the dihydrofolate reductase of the worm is more sensitive to the drug than is the isoenzyme of man. Mel W has an antimitotic effect on the embryogenesis of filarial worms, and this apparently causes the death of adult females. Hycanthone is an intercalator of DNA of bacterial origin, but there is no information as to whether it has a similar effect on schistosome DNA. It has recently been proposed that it is an acetylcholine blocker with a special affinity for the acetylcholine of schistosomes. For some drugs, such as the classic tetrachlorethylene and phenothiazine, there is no convincing evidence of a biochemical mode of action.

What Roles Do We Want Drugs to Play?

Foremost on the long list of desirable properties of an anthelmintic is efficacy against the target helminth or group of helminths. By this we usually mean that we want the drug to kill the worms. I believe we should think less about that. Worms that are merely paralyzed reversibly in the intestinal tract may be passed from the host before they can recover, so that a direct lethal action is not necessary. It is less often appreciated that such sublethal effects are probably sufficient in some extra-intestinal parasites. Schistosomes which are merely dislodged from the mesenteric vessels and swept into the liver will in many cases fail to recover, and their gradual demise in the liver sinuses may be even more desirable than their sudden destruction in the vessels. But I am thinking of something other than slow versus fast destruction of worms. Some diseases of medical and veterinary importance are due almost entirely to the reproductive products of the worms. Prominent examples are schistosomiasis and trichinosis. In both cases the adults are known to be relatively harmless (except when present in very large numbers) and indeed they may confer on the host some degree of immunity against reinfection. We would almost entirely suppress the pathogenicity of these worms if we were to suppress their reproduction. As it happens, in both cases we know of chemicals that will do precisely that—nicarbazin, thiosinamine, dapsone and others in the case of schistosomiasis (Campbell and Cuckler, 1967; Machado et al., 1970; Davies and Jackson, 1970; Pellegrino and Katz, 1975)

and thiabendazole in the case of trichinosis (Campbell and Cuckler, 1964). In the case of schistosomiasis the chemosterilizing drugs have not been put to practical use, but the potential for this general approach in schistosomiasis seems to warrant much more attention than it has received. In the case of trichinosis opportunities for use of such a drug would be very limited because the disease is usually diagnosed after the progeny have been produced. Yet the principle has actually been put into practice with apparent success (Gerwel et al., 1974). Among several people who were known to have eaten heavily contaminated pork, there was one who had eaten it completely raw and who had eaten a whole pound of it (containing an estimated 11,500 larvae). That person was in grave danger—yet treatment prevented any serious illness in that person or his less indulgent colleagues. The point I wish to make is not that chemosterilization of *Trichinella* is of great consequence (except in special cases) but that we should think carefully of the type of efficacy that could be most useful for whatever parasitic diseases we have in our sights. There seems to be no general agreement on the type of drug (if any) that would be most useful in the strategic control of hydatid disease or cysticercosis. We take for granted that it would be desirable to kill paramphistosomes in sheep, preferably the immature fluke in the small intestine. Do we stop to wonder whether we could block its pathogenicity by inducing the immature fluke to migrate prematurely to the rumen? Suppose that grazing cattle had a rumen "bullet" (of the type used for bloat control) that released a nonabsorbed compound that either caused *Fasciola* cysts to excyst in the rumen or prevented their subsequent excystment in the duodenum. We might then prevent all clinical phases of fascioliasis and be free of drug-related tissue residue and milk residues into the bargain. For all of our serious verminous diseases we should earnestly seek some subtle alternative to the direct vermicidal approach.

How Should We Look for New Antiparasitic Drugs?

Let us return for a moment to Smith's comments on the WHO proposal for disease control in the tropics (Smith, loc. cit.). Smith says, "There is little doubt—given time, sufficient resources and the harnessing of modern molecular biology, chemistry and immunology into well balanced laboratory teams—that the desirable new drugs and vaccines can be discovered." Two thoughts come to mind. First, we have not in fact been given much time, if we are to meet the WHO deadline of the end of the century. Second, I share Smith's confidence that the harnessing of molecular biology, biochemistry and immunology will eventually give us the vaccines we need, but I do not think it will give us the drugs we need in the meantime. How then should we look for new antiparasitic drugs?

This is the part that will make you wince. I'll come right out with it. I am a great believer in massive empirical screening. I am especially delighted this afternoon to have the opportunity to speak to so reputable an audience on a subject thought by so many to be so disreputable.

It seems to me that those who oppose empirical screening and favor the so-called rational approach do so largely because they find the idea of screening intellectually humiliating. This attitude may arise in part from a lack of incentive to evaluate the two approaches dispassionately. The incentive in terms of financial grants (to say nothing of professional esteem and advancement) is to focus on the rational approach and indeed to exclude any notion of empiricism.

There is one category of anthelmintic researcher that we can readily dismiss. They are those academicians who (taking an attitude attributed to E. Y. Davis [Leake, 1976]) apparently regard an anthelmintic as something that, when put into a parasitized animal, produces a scientific paper. Not surprisingly, the chemotherapeutic papers of such workers are often rubbish—the scientific acumen of the authors having been betrayed by their lack of interest in the subject matter. I was recently intrigued to discover a similar exasperation on the part of the editor of a journal devoted not to livestock health, but to human health. It appeared to him that "one of the more popular ways to achieve a research publication is to persuade a pharmaceutical company to supply, free of charge, a quantity of a new—or a frequently not so new—preparation, then give this to a number of patients who may or may

not be suitably chosen, make up some tables of results, hazard a chi square confirmation of scrappy results and, finally, proclaim in print a wonder drug with a most unlikely cure rate" (Anonymous, 1975b).

But my quarrel is with the serious and dedicated chemotherapists who are committed to the "rational approach" to the discovery of useful new anthelmintics. They are by no means limited to academic and governmental labs, but are even now hard at work in industrial labs (surrounded by empiricists of all sorts and conditions).

The rationalist hopes that by studying the basic physiology and biochemistry of helminths, he will discover a means to disrupt those processes selectively and thereby provide a novel chemotherapeutic agent. He may find that a worm has a high requirement for some substance that the host does not require at all. He would then select known antagonists or competitors of that substance for therapeutic trials, or he might design entirely new molecules for the same purpose. He may find in a helminth a metabolic step that is not known to occur in the host species—or, better yet, that is known not to occur in the host species. The enzyme responsible for a particular metabolic step in the helminth may be qualitatively or quantitatively different from the enzyme controlling the same step in the host. Whatever the difference, the objective of the therapist is a chemical that will disrupt the metabolism of the parasite but not that of the host.

Now what is wrong with all of that? Some say that what was wrong in the past was our infinitesimally small knowledge of helminth biochemistry; that the situation is improving constantly; and that we must strive for greater and greater knowledge to give us more and more chemotherapeutic targets to shoot at. I'll go along with the idea of greater basic information giving more targets, but I think this is not the important point. No, the trouble with the whole scheme is not just our inadequate knowledge of parasite biochemistry or host biochemistry. The big problem is our lack of knowledge of the hypothetical drug. Suppose we discovered almost everything about the metabolism of *Trichostrongylus axei*, and almost everything about the metabolism of *Bos taurus* (we can never discover everything); and suppose we selected a metabolic difference and devised a drug to exploit that difference to the detriment of the worm. Would we be heroes? Probably not, because the probability is high that the drug would do something else besides damaging the worm; and the probability is high that that something else would be a Bad Thing. It seems to me that we would be guilty of arrogance were we to assume that we could predict the total impact of any novel biodynamic substance on the host. Inevitably, the more novel the prospective drug, the more helpless we are in predicting all its effects. While the greatest danger is the rational selection of a chemical structure that turns out to have unsuspected host toxicity, the converse also imposes a limit on the potential success of the rationalist. The knowledge that dietary thiamine is required for the health of poultry might well have dissuaded one from proposing a thiamine antagonist as a continuous dietary medication for poultry coccidiosis. Fortunately, neither the anti-thiamine action of amprolium nor the dependence of coccidia on exogenous thiamine was known when amprolium was discovered as a coccidiostat! Thus, because of our present and future inability to predict all the effects, good and bad, of a new chemical structure, the rationalist takes a big chance.

But chance is supposed to be what empirical screening is all about! Chance, of course, plays a part in all scientific research (and has been particularly prominent in most of the particularly important scientific advances). It plays a larger than usual role in experimental chemotherapy, but on the whole I think that the role it plays in empirical screening is no whit greater than the role ascribed to it above in the rational approach. (The glaringly obvious element of chance in blind screening *in vivo* is offset to a slight extent by the fact that one gets at least a crude indication of differential toxicity right off the bat.)

The other thing that gives screening a bad name is its routine aspect. What is often forgotten is that a bioassay is simply a sorting device, as is a fractionation column, an amino acid analyzer, or a computer program for any kind of data analysis. A scientist who spends all of his time cranking the handle of a chemotherapeutic assay is mistaking the tool for the trade. So is any other scientist who is too busy collecting data to have time to do anything with them. Nor does the problem lie in the

fact that the chemotherapist appears to use so little of the data that goes through his sorting machine. After all, the work that lead to the discovery of quasars involved in the collection of astronomical numbers of (astronomically) uninteresting astronomical facts. The chemotherapeutic empiricist must concentrate on the nature of the bioassay to be used and on the results that come out of it. As in other fields, he may use an assay that has previously been established; he may wish to devise a novel assay; or he may have to devise a novel assay. The decision is of crucial importance; and if new methodology is to be involved, the exercise may be scientifically demanding.

A good example of the rational exploitation of empirical screening is the tetramisole story. Anthelmintic screening has traditionally been carried out in mice or rats, but the original predecessor of tetramisole (the initial lead) was inactive against helminths in these animals. Fortunately, the screening in this case had actually been carried out in chickens in which this predecessor was apparently active (Raeymaekers et al., 1966). As it turned out, it was the chicken, not the scientist, that synthesized the anthelmintic. But it was the scientist who discovered it, who postulated, sought and identified in the feces of treated chickens a metabolite active in mammals; and it was the scientist who made a more active analog of the metabolite, and who found a less toxic isomer of the analog. Another important example is the exploitation of the early thioxanthone lead to yield hycanthone—a development that made use of the chemical prowess of a mold (Archer and Yarinsky, 1972). Although these two examples involved nonhuman chemistry in unusual ways, it must be remembered that it is the work of the human chemist that is of paramount importance in these and all other examples of the exploitation of empirical leads. Virtually all modern anthelmintics represent vast improvement over initial discoveries—improvement achieved by an elaborate interplay of molecule manipulation and biological evaluation.

Perhaps the biggest thing that empiricism has going for it is its past success. So far as I know, all antiparasitic and antibacterial drugs had their origin either in empirical screening or in a chance observation made in the clinic or in the laboratory. We should continue to employ empiricism, not because future successes are guaranteed but because they are probable and because we can influence their probability.

It seems to me that the WHO report to which I have referred fails to take into account these aspects of drug discovery. There are, of course, strong arguments for placing the effort in non-industrial laboratories in non-industrial nations. The WHO is undoubtedly right in saying that solutions developed in the tropics would be more acceptable in the tropics. Further, commercial organizations find little or no commercial incentive for tackling the diseases in question. Yet, because of the inherent frailties of the proposed "task force" and "network" system, we must continue the search for an acceptable alternative approach to the immense problem of tropical disease.

It is a question of probabilities; and I believe the probability of finding the drugs needed by the developing nations could be increased by bringing the industrial-type of drug screening into the overall picture. Under some circumstances non-commercial agencies can mount a prodigious screening effort, such as the antimalarial program of the United States government (Kinnamon and Rothe, 1975). Alternatively it should be possible to develop a system in which private industry would do the screening and primary evaluation of drugs, while the further evaluation and development of promising candidates would be assigned to the "task forces" and "networks" of the nonindustrial sector. In this way the balance between investment risk and potential profit might be tipped in favor of a really intensive industrial screening program. It would not be easy to put such a dual (industrial and nonindustrial) approach into practice but it seems to me that such an approach offers the only hope for achieving reasonable control reasonably soon.

I hope no one will come to the conclusion that I am disparaging the value of biochemical research with respect to the chemotherapy of parasites. I would emphasize its value. In the first place, the high odds against the rational approach do not mean that the approach should not be tried. (In any case the urge to try it is irresistible.) But the greater value of biochemical research in chemotherapy lies elsewhere.

One of the roles played by antiparasitic

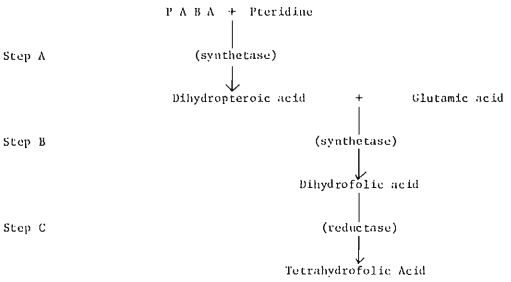

Figure 1. Major metabolic steps in the biogenesis of folic acid in bacteria and probably in coccidia.

drugs is that of educator. The study of empirically discovered drugs has taught us far more about basic chemotherapeutic mechanisms than has basic study of the parasites themselves. This is not only important in terms of the progress of biological science (a worthy end in its own right) but opens the way for the semirational approach to chemotherapy. While the triumphs of this method have been few and far between, the principle is clear and attractive: empirical discovery of a drug is followed by elucidation of its mode of action, which in turn provides a basis for looking for other compounds that will achieve the same therapeutic effect while yielding benefits in terms of improved spectrum, efficacy, patentability or whatever. Often this approach leads to the serendipitous discovery of compounds having radically different therapeutic effect, but sometimes it works as intended. A particularly pertinent type of semirational approach is the antimetabolite attack exemplified by the work of Hitchings (1972) and Rogers et al. (1964) in bacterial and protozoal infections but apparently not yet used successfully against helminths.

Once it became known that the antibacterial activity of sulfonamides was due to antagonism of the bacterial metabolite *p*-aminobenzoic acid (PABA), attempts were made to achieve similar antagonism and similar therapeutic efficacy by using analogs of PABA, such as 4-amino-2-chloro benzoic acid (Wyss et al., 1943). Indeed such analogs were effective to some extent against some bacteria. Because of this, and because sulfur and sulfonamides were known to be active against coccidia in chickens, similar PABA analogs were tested in coccidiosis (Rogert et al., loc. cit.). In contrast to the situation in bacteria, they not only worked but some of them were many times more potent than the sulfonamides (and a derivative of one of them, ethopabate, became a highly successful commercial coccidiostat). Here, knowledge of the mode of action of empirically discovered coccidiostats led to the testing (and thence to the discovery) of a drug that was therapeutically more effective. Note that the rationale rested on the mere presumption that folic acid synthesis in coccidia was the same as in bacteria. Actually, the mode of action of the newer drug is probably not quite the same as that of the original. It is true that the activity of ethopabate is reversed by the addition of PABA to the diet of infected chickens; and it is possible that (in chickens on a normal diet) ethopabate simply blocks the reaction between pteridine and PABA (Step A in Figure 1). However, it is considered more likely that ethopabate blocks Step B, i.e., that, in competition with PABA, it reacts with pteridine to form the ethopabate analog of dihydropteroic acid; and that this analog (2′-ethoxydihydropteroic acid) is sterically incapable of being coupled to glutamic acid to form dihydrofolic acid (Rogers et al., loc. cit.).

The next step in the folic acid pathway (Step C in Figure 1) may be the Achilles' heel for many parasites, yet so far it has apparently been struck only by the arrows of the empiricists. Jaffe (1972) has recently reviewed the presence of dihydrofolate reductase in parasites and their differential sensitivity to folate analogs and "antifol" drugs such as pyrimethamine and trimethoprim. He cited the detection of dihydrofolate reductase in plasmodia, trypanosomes, schistosomes, *Nippostrongylus brasiliensis*, and four genera of filarial worms. The reductase of *Onchocerca volvulus* turns out to be highly sensitive to inhibition by suramin—offering a possible retrospective explanation of the clinical efficacy of suramin in the treatment of onchocerciasis in man. Even more recently, the enzyme has been detected in coccidia thus offering a retrospective biochemical explanation of the action of pyrimethamine in avian coccidiosis (Wang et al., 1975).

Obviously there is a continuous gradation between empirical and rational methods, and the so-called "semirational" approach can in

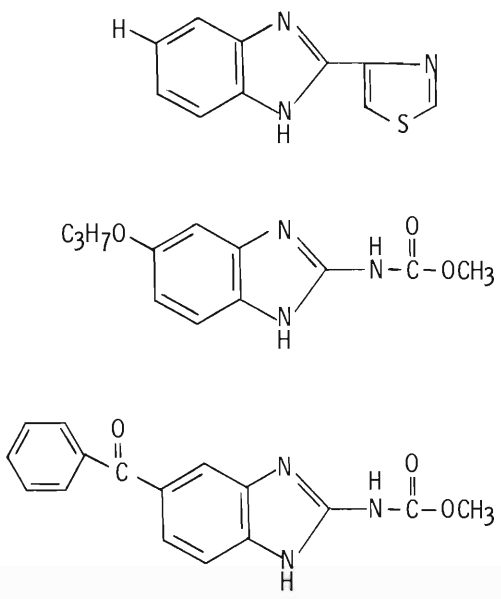

Figure 2. Chemical structure of thiabendazole (top), oxibendazole (middle), and mebendazole (bottom).

practice have either a low or a high ratio of empirical to rational content. The antimetabolite method has been developed by Hitchings (1972) and by Rogers (1976) into a general approach to therapeutic problems. Once a parasite metabolite is known (whether identified as a result of drug studies or not), known or suspected antagonists may be tested as chemotherapeutic agents. The degree of rationality in a particular case depends on the specificity of the available information. Compounds may even be screened randomly for antagonism to a given metabolite. Further, compounds known to be antimetabolites in one biological system may be tested "empirically" in a system in which a role for the metabolite in question has not been demonstrated. Thus empirical and rational components can be overlapping and mutually reinforcing.

The semirational approach has its own hazards. It has been proposed (as already indicated) that the anthelmintic activity of thiabendazole derives in some way from its inhibition of fumarate reductase in the parasite (Prichard, 1973). The effect of the drug on this enzyme can be demonstrated *in vitro*. One might then have designed or selected chemical structures with this property, or have screened randomly for structures with this property. In doing so one might well have discovered some of the more potent benzimidazoles that have in fact appeared. The trouble with that kind of approach is that one tends to find drugs that are basically similar to the original in terms of efficacy, though they are likely to be different (for better or worse) in other respects. They are, therefore, likely to share weaknesses as well as strengths in therapeutic spectrum, and to share problems of drug resistance. But the most instructive aspect of this hypothetical example of semirational drug development, is that one would not have discovered the one benzimidazole anthelmintic that does differ radically from the original.

Mebendazole apparently does not inhibit the fumarate reductase of *Haemonchus* (Prichard, 1973). Consider three examples of benzimidazole anthelmintics (Figure 2). All share the basic benzimidazole structure, but it is at once apparent that thiabendazole is very different from oxibendazole and from mebendazole, while the latter two compounds are very similar to each other. Yet in terms of anthelmintic spectrum I believe it is fair to say that thiabendazole and oxibendazole are more similar than oxibendazole and mebendazole. It is reported that mebendazole acts by inhibiting glucose uptake (Van den Bossche, 1972). Dr. R. O. McCracken, working in my laboratory with *Hymenolepis diminuta*, observed a drastic depletion in glycogen content following mebendazole treatment, but was unable to demonstrate an inhibition of glucose uptake (suggesting an impaired glycogenesis or glycogenolysis). We are thus confronted with the possibility that a group of chemically related compounds might contain anthelmintics that operate in two entirely distinct ways; and that a given drug might affect different helminths in entirely different ways.

We need to know how the existing drugs work—not to get more of the same, but to get more understanding. We need insight into the host parasite relationship so that we can select radically different and exciting targets for the empiricists to shoot at. The practical utility of discovering the site-location mechanism of a helminth within the host would probably not lie in the design of a molecule that would block

that mechanism—but in the creation of a screening assay to detect compounds that would block that mechanism. We need to know why some members of a worm population are not removed by a treatment that removes most of them. Many years ago Stoll (1962) cautioned us against the presumption that it is desirable to remove 100% of the worms present. We have not yet come to grips with that problem (perhaps the drug-resistance and immunity components of the problem are in conflict). We need to know about extrinsic factors that affect the success of treatment. About 100 years ago, the renowned Kuchenmeister examined his own feces for pinworm for 329 successive days, and correlated his findings with the phases of the moon in order to determine the best time for treatment (Kuchenmeister, 1857). We need to follow his example —in principle. We need biochemical exploitation of the innumerable mysterious observations that fly from the empiricist's benchlike sparks from a blacksmith's forge. Usually they fade away without igniting any interests on the part of those who could best make use of them. Most new observations of antiparasitic efficacy do not lead to useful new drugs—but they might teach us a great deal. This is especially so when high therapeutic efficacy is exhibited by a chemical that is relatively simple and biochemically well characterized. Similarly we might learn much from a chemical that kills one species but not another of the same genus, or a chemical that destroys the vitellaria of one trematode but not another. Such observations, which must abound wherever screening is conducted, are not to be regarded as crumbs for the jaws of Lazarus but as grain for the grist mills of the biochemists and physiologists.

If we are really to resolve the three-ring conundrum that we discuss today, we must make use of all kinds of research—all disciplines and all approaches. It is from such wide-ranging research that the big revolutions will emerge—not just some new drug. It is in the nature of things that we cannot imagine the nature of those revolutions any more than Galvani could imagine the lights of Times Square.

We have come a long way since the time when a person with worms might have a ram's tail inserted in his rectum so that the worms could gnaw at that, and so be easily removed —but we still have a long way to go.

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The Control of Parasites: The Role of the Host[1]

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"It takes all the running *you* can do to keep in the same place.

"If you want to get somewhere else, you must run at least twice as fast as that.

Red Queen to Alice.

The complex relationship between parasite and host poses problems of comprehension which become more acute every year. It is with an increasing degree of trepidation that one gets out of the rut of ones supposed competence to take a holistic view of parasitism and to formulate concepts on the control of parasitic infections. Nevertheless we are doing that today and my particular task is to examine the role of the host in the control of parasitic infections. In doing so I intend to consider, briefly though it may be: some of the host mechanisms which permit parasitism to occur in the first place and which, if manipulated, might provide the basis for control; the host mechanisms which may be abrogated or avoided by parasites to permit their survival and finally the application of the host responses to the control of infections.

[1] An address presented at the 500th meeting of the Helminthological Society of Washington, held at New Bolton Center, School of Veterinary Medicine, University of Pennsylvania on May 15, 1976.

Host Factors Determining Parasitism

One of the major unanswered questions of biology is that of the molecular biological basis of host–parasite specificity. Since a large proportion of the environment of a parasite is supplied by the host, and assuming that host and parasite have coevolved, the degree of integration between host and parasite probably determines the breadth, or narrowness, of the host range. Exploitation of the determinants of host compatibility could represent a novel and important approach to control which as far as is known, has not been extensively explored for the internal parasites. It is axiomatic that there are key events in the initiation of parasitism: inability to accomplish these by a parasite will lead, clearly, to failure of the parasite mission. Hitherto, control by drugs has focussed on the parasite after it has successfully integrated itself with the host molecular biological systems. Control by management, hygiene and public health techniques attempts to prevent contact in the first place, while immunological control represents a host-generated response which, under all but abnormal conditions, probably keeps parasite population to acceptable levels. However, none of these conventional approaches attempt to "misguide" the parasite in a way comparable, for example, to the use of pheromones for the control of insect populations.

An understanding of the molecular basis for host-parasite compatibility has progressed further with the protozoa than other forms. An example of this is the recent work of Miller et al. (1975) which indicates that red cell susceptibility to *Plasmodium vivax* may be associated with Duffy blood group determinants (Fy^a or Fy^b) which function as erythrocyte receptors for the parasite. Evidence that Duffy blood group negative human erythrocytes are resistant to infection with *Plasmodium knowlesi* has been correlated with the resistance of West Africans and American blacks to *Plasmodium vivax* which corresponds to the distribution of Duffy negative erythrocytes in the world. Other human malarias do not appear to depend on this factor and it is possible that the Duffy factor, alone or in association with another determinant, serves as a receptor for the merozoites of *P. vivax*. The uniqueness of the erythrocyte receptor suggests a similar uniqueness for the parasite determinant complementary to the receptor, and isolation of the compound concerned may provide an opportunity to immunize against it or to prepare a chemical antagonist with the intent of blocking the interaction.

The need for a recognition factor, or receptor, for other intracellular parasitisms is illustrated by studies of the *Leishmania* species by Dwyer, Langreth and Dwyer (1974). These authors have demonstrated that polysaccharides on the surface of the promastigotes of *L. donovani* (e.g. α –1, 4 and α 1, 6 glycan bonded polysaccharides) may be responsible for this recognition and internalization of the parasite in macrophages, and furthermore such surface saccharides may impart resistance to lysosomal enzymes once the organism has entered the cell (See also below). Further evidence that they are important comes from the work of Dawidowicz et al. (1975) who demonstrated a concomitant loss of surface saccharides and infectivity during *in vitro* culture of *Leishmania* species.

Other host factors which determine red cell susceptibility, for example to malaria parasites, are well known. Thus the single gene mutation responsible for the production of sickle-cell hemoglobin (hemoglobin-s), which differs from the normal by the substitution of valine for glutamic acid, confers partial resistance to *Plasmodium falciparum* malaria in that it limits intraerythrocytic multiplication (Allison, 1963). Similarly, impaired production of the α or β hemoglobin chains in thalassemia and glucose-6-phosphate dehydrogenase deficiency are other host factors which determine susceptibility to malaria (Review, Brown, 1976). The biochemical basis of the protective action of these abnormalities is not fully understood, though ATP levels of erythrocytes may play a role, especially in thalassemia. It is interesting that a potentially lethal genetic pathway for resistance to malaria evolved presumably under the pressure of that infection: the legacy of this is apparent in the American black today.

As well as genetically controlled events, nutrition may play a role in host receptivity for malaria. Thus rats and rhesus monkeys on a milk diet are resistant to *Plasmodium berghei* and *P. knowlesi* infection, respectively, an effect which can be reversed by the addition of

p-amino-benzoic acid to the diet (Hawking, 1953). While the biochemical needs of the parasite are denied in the case of PABA deficiency, in other situations they appear to be induced by the parasite. For example Oelshlagel, Sander and Brewer (1975) have produced evidence that malaria parasites introduce a pyruvate kinase isozyme into the host red cell in amounts sufficient to alter red cell glycolysis, thus increasing red cell adenosine triphosphate (ATP). ATP is needed by the parasite and for example parasitemia rates are correlated with red cell ATP values. Oelshlagel et al. (1975) speculated that *in vivo* "pyruvate kinase perturbations" may be a general host parasite phenomenon, and of course the phenomenon may extend to other enzyme systems, in which case new approaches to the control of parasites might be based on the concept of preventing the introduction of these into host cells or tissues.

The ability of a parasite to reorganize its environment for its own physiological needs is increasingly apparent: intracellular protozoa represent good examples of this. Thus *Toxoplasma gondii* an obligate intracellular parasite, must penetrate the cell, this is achieved by a process of phagocytosis (Hirsch, Jones and Len, 1974), but the important aspect of this is that the organisms induce a phagocytic mechanism both in cells normally capable of phagocytosis and in those ordinarily non phagocytic, for example, fibroblasts. The property of the toxoplasm responsible for this effect remains to be identified. Once *T. gondii* has entered the cell it then further modifies cell function so that it is not destroyed. The aspect is further discussed under the topic of abrogation of the immune response by parasites.

A further example of reorganization of cell function is evident in lymphoid cells infected with macroschizonts of *Theliera parva*, the causal agent of East Coast Fever of cattle. In normal *Theileria*-infected lymphoblast cultures macroschizont and cell division are synchronous and the mean number of macroschizont nuclear parasites remains constant. The *Theileria* body is closely associated with the mitotic apparatus and is pulled apart and distributed to the daughter lymphoid cells in late mitosis (Hulliger, Brown and Wilde, 1965). There are many indications that the organism induces lymphoid cells to enter the mitotic cycle, but dependence on the mitotic cycle of the host cell is not absolute since interference with normal growth, for example, the arrest of cell division, has no effect on the parasite and macroschizont division proceeds unimpeded. Indeed, with irradiated cells (Irvin, Brown and Stagg, 1975a) there was an increased uptake of tritiated thymidine by macroschizonts, indicating a greater than expected multiplication of the parasite which Irvin et al. (1975b) have ascribed to indirect activation of parasite DNA synthesis caused by radiation induced changes in the cell. Hitherto, the *Theileria*-lymphoblast system was considered to be somewhat uniquely host specific and cell dependent; little or no cross infection occurred between allogeneic cells and through infected bovine lymphoblasts will grow in whole body irradiated mice to produce tumorlike masses, there is no evidence that mouse cells are invaded by the parasite (Irvin et al., 1973). However, this has now been circumvented in that Irvin et al. (1975b) have been able to transfer the parasite to cells of a different species. This was achieved by the fusion of bovine lymphoid cells from a culture line parasitized with *T. parva* to monolayers of mouse heart and baby hamster kidney cells using inactivated Sendai virus. Heterokaryons formed in the cultures were infected with macroschizonts, but also cells that apparently contained only mouse heart or baby hamster kidney cell nuclei were also infected. In addition there was apparent activation of the macroschizont multiplication in the heterokaryons. An additional aspect of this study, is that it may lead to new information on the host cell mechanisms which are associated with transformation of macroschizonts to microschizonts. In the bovine, this event occurs after the macroschizonts have undergone a fixed number of divisions (Jarrett, Crighton and Pirie, 1967) however, in culture, microschizont production is not evident unless the culture is manipulated in some way. For example, Hulliger, Brown, and Wilde (1966) have obtained microschizont development by incubating infected lymphoblast culture at 42 C. At this temperature, host cell multiplication is minimal, but the parasite continues to replicate producing stages containing many small nuclear particles. The role of the higher temperature requirements for microschizont production in culture is not

clear, though it is interesting that microschizont production occurs in the bovine when body temperature is elevated (Hulliger et al., 1966). It is possible that perturbations of the mechanisms controlling the host cell nucleus, as result from the irradiation of cells and the creation of heterokaryons, but which may also be induced by physiological responses to infection, play a critical role in the life cycle of the parasite.

One of the most intriguing modifications of the host environment induced by parasitism is the change that occurs in the muscle fiber as a result of penetration by larvae of *Trichinella spiralis*. Following invasion, the cell becomes functionally and structurally quite distinct and serves as a "nurse cell" for the larva (Despommier, 1975). This unique series of cellular events consists in the first 8 days of infection of the muscle fiber, of a loss of contractile elements, nuclear enlargement, hypertrophy of sarcoplasmic reticulum and vacuolation of mitochondria. After this, as the nurse cell evolves, hyperinvolution of the plasma membrane occurs and a marked increase of rough endoplasmic reticulum is evident in the region of the larval cuticle and the nurse cell plasma membrane (Despommier, 1975). The nurse cell remains alive for the life of the larva and since this may be for an extended period of time, it is clear that there is a close interdependence between larva and host cell. Despommier (1975) suggests that the nurse cell functions as a "nutrient gather" and that metabolites destined for the larva must first traverse the membrane and the cytoplasm of the nurse cell and then finally cross the membrane and the cuticle at the nurse cell-parasite interface. There is no evidence that muscle stage larvae of *T. spiralis* ingest or otherwise utilize directly the cell constituents induced by the infection.

Factors which determine host-parasite compatibility for metozoan parasites have been less extensively investigated than is the case for the protozoa. Nevertheless, it is evident that the same finely tuned type of mechanism occurs, in which a host factor triggers activation of the parasite process. The majority of the infective stages of helminths have a low resting metabolism and are in a state of semidormancy (Lackie, 1975) requiring some specific host stimulus for activation. In the case of *Schistocephalus solidus*, a diphyllobothriid tapeworm of birds, larval stages (plerocercoids) harvested from fish will become sexually mature even in nonnutrient saline, when incubated at bird body temperature (Smyth, 1966). However, more complex host signals are necessary for the hatching of eggs and the evagination of larvae of taeniid cestodes (Smyth, 1969). Furthermore, the composition of bile, along with other factors, may determine whether or not a given species of cestode can develop in a particular host; in this case bile represents a biochemical determinant of host specificity.

With the infective stages of the majority of parasite nematodes excystment or ecdysis is an active process on the part of the parasite, induced by the chemical and physiological factors present in that part of the bowel where the development stages commence their parasitic existence. Thus exsheathment of infective larvae of *Haemonchus contortus* and *Trichostrongylus axei*, which normally occurs in the rumen, is optimal at a pH near neutrality, whereas the exsheathment conditions for *Trichostrongylus colubriformis* are at a pH which corresponds to the abomasal environment (Rogers, 1966). Furthermore, the exsheathing fluids induced by the physical conditions above, and responsible for the release of infective larvae from the sheath are highly specific. These are enzymes which attack a special region on the inside of the sheath; they are leucine amino peptidases which are highly specific in that they will attack only the substrate of the sheath of the species which produces the exsheathing fluid (Rogers, 1966). Rogers (1966) has also demonstrated that exsheathment of larvae of *H. contortus* and *T. colubriformis* can be inhibited by treatment with low molar concentrations of iodine, presumably through the inhibition of the receptors with which carbon dioxide reacts to stimulate exsheathment. This effect is reversible by subsequent treatment with hydrogen sulphide water. Similar requirements for the hatching of nematode eggs have been demonstrated by Fairbairn (1961). A general consideration of the host recognition signals for some gastrointestinal nematodes has been presented by Whitlock (1966) and McLaren (1976) has reviewed the structural and functional aspects

of the sense organs of nematodes which may be concerned with the recognition signals.

Despite the increasing evidence that parasite populations are under the tight constraint of a variety of host derived signals, and the fact that if these are not received by the infective stage of the parasite, parasitism cannot occur, there has been little study of control methods based on the abrogation of these signals. Possibly the simplest application of this approach is in the use of mixed grazing to control gastrointestinal parasitism in domestic animals. For example, the sheep does not provide the appropriate signal for horse parasites.

The host–parasite relationship is not simply a question of capability to infect. Within the parasitized host, a variety of physiological parameters are apt to determine the behavior of a parasitic infection. Circadian and other rhythms are important regulators of a variety of parasitisms (Hawking, 1975), the most fascinating of which is the periodicity of microfilariae of *Wuchereria bancrofti*. It was recognized in early studies that this periodicity coincided with the feeding time of the mosquito vector, but the control mechanisms of this still are not fully understood. Indeed the host influences which determine the distribution of microfilariae, which in turn often correspond to the preferential feeding site of the insect vector are in need of further examination.

Endocrine status is often an important host determinant of the level of parasitism. This complicated area has been reviewed by Solomon (1969) and there is need for intensive study of host hormonal regulation mechanisms in parasitism. The exquisite relationship between host and parasite in the case of the rabbit flea *Spilopsyllus cuniculi* (Rothschild, 1965) perhaps will serve as a beacon to reaffirm that parasites are very finely tuned to their hosts.

Substantial progress has been made over the last several years in the understanding of seasonal variations of nematode populations in domestic livestock, much of which can be traced to phenomena associated with parturition and lactation. The role of these events in the immunological response to infection is discussed later.

Evasion of the Host Response by Parasites

In any study of the control of parasitic infections by the host, a consideration of how a parasite may survive for extended periods in immunologically competent hosts is pertinent. Various mechanisms are used to accomplish this; some are concerned with the abrogation of the affector or effector arms of the host response, while others are associated with adaptive changes on the part of the parasite. Smithers and Terry (1976) have reviewed the evidence for the existence of antigens shared between schistosomes and their hosts and which are purported to be responsible for the antigenic disguise that protects the parasites from immunological recognition. Both host derived and parasite derived "shared" antigens seem to exist. Host derived blood group antigens are acquired by schistosomules when they are grown *in vitro* in the presence of erythrocytes of various A, B, or O specificities, but not with cells of other specificities such as Rh or Mn (Dean, 1974). There is also strong evidence for shared antigens of host origin. For example Damian, Greene, and Hubbard (1973) have demonstrated an antigenic determinant on the surface of *Schistosoma mansoni* which cross reacts with mouse $\alpha 2$ microglobulin. Smithers and Terry (1976) suggest that host antigens of parasite origin reduce the overall immunogenicity of the parasite, while those of host origin provide the immunological disguise for the adult worm. However, Smithers and Terry (1976) note that there is no direct evidence that host antigens serve to protect the parasite from the host.

Blood group antigens are known to be part of the normal antigenic mosiac of several parasitic helminths, including *Ascaris suum* (Soulsby and Coombs, 1959). There is no evidence that these antigens in any way serve to antagonize the infection, but it is possible, that as surface antigens, they may facilitate establishment by providing a mask of host protein, namely antibody, on the surface of larval stages (Soulsby, 1971). In situations where one might expect host derived materials to play a decisive role in the persistence of infection, for example in filarial infections, which are especially long lived, there is no evidence that host derived or parasite derived "hostlike" antigens play a role

in the establishment, or the persistence of the parasite.

Reduced reactivity of parasites upon their entry into a host may be an alternative mechanism for survival and Leventhal and Soulsby (1975) have shown that the early larval stages of *A. suum* have reduced reactivity in terms of binding immunoglobulins, activation of complement and the attachments of phagocytic cells. This might be interpreted as a mechanism which would permit a parasite to gain a foothold, an initial advantage, which would assure its establishment in the host.

Host induced modulation of antigenicity of the nematode *Nippostrongylus brasiliensis* has been reported by Ogilvie (1974). In this instance adults of *N. brasiliensis* derived from a second infection, or from trickle infections, are less immunogenic than worms from an initial infection. Such worms are termed "adapted" and they have a characteristic acetylcholinesterase isozyme pattern which is apparently induced by anti-acetylcholinesterase antibodies present in the infected rat.

Evasion of the immune response by parasites has been more extensively studied with the intracellular protozoa than with the helminths. For example, following internalization of the trophozoite of *T. gondii* the fusion of lysosome with the phagocytic vacuole is inhibited (Hirsch et al., 1974) though the mechanism of this is not fully understood. Indeed the mechanism is not uniform for the various intracellular organisms. Inhibition of lysosomal fusion, as occurs with *Toxoplasma*, is seen in macrophages parasitized by *Mycobacterium tuberculosis* (Armstrong and Hart, 1971), *Encephalitozoon cuniculi* (Weidner, 1975) and L cells parasitized by *Chlamydia psittaci* (Friis, 1972). However, when degenerating organisms of the above occur in the parasitophorous vacuole lysosomal material is discharged into it. In the case of *T. gondii*, compromise of the ability of the organism to prevent fusion of lysosomes is evident when it is killed or when it is exposed to specific antiserum before entry into the macrophage (Jones, Len and Hirsch, 1975). However other intracellular organisms survive in macrophages in spite of lysosomal fusion. For example, with *Mycobacterium lepraemurium* (Hart et al., 1972) and *Leishmania mexicana* (Alexander and Vickerman, 1975) lysosomal fusion occurs normally in infected macrophages but organisms are not killed by this. Reduced acid phosphatase activity is seen in vacuoles containing living parasites and it has been suggested that the pellicle of the organism (e.g. *Leishmania*) is resistent to lysosome mediated attack. Surface membrane glycosaminoglycans might play a role in this (Dwyer et al., 1974). Loss of surface glycans is associated with a loss of infectivity (Dawidowicz et al., 1975).

Still a further mechanism may occur with *Trypanosoma cruzi* and in which organisms proliferate in the cytoplasm of macrophages (Kress et al., 1975). In this case the parasite initially is in an endocytic vacuole, but this is lost and the organism lies in direct contact with the host cytoplasm; hence it is not exposed to secretions of the lysosomes. Organisms which fail to escape from the phagocytic vacuoles show degeneration and are surrounded by secondary lysosomal material. Evans and Levy (1973) have demonstrated similar intracellular, cytoplasmic, development for *Mycobacterium leprae*.

This intracellular parasitism, which is a familiar phenomenon of viruses and bacteria (Mims, 1964), is related to the problem of persistance of infection and, in turn, the failure of the host response to curtail the survival of the organisms. It is important to recognize that some of the intracellular forms are destroyed while others are not: macrophages from guinea pigs immune to *Leishmania enriettii* do not destroy that organism *in vitro*, but readily destroy others, such as *Listeria monocytogenes* (Mauel et al., 1974).

Parasites have evolved a number of adaptations to ensure their survival and transmission to a new host. One of the classical examples is the antigenic variation of African trypanosomes, where variant antigens, glycoprotein in nature and located in the surface coat which overlies the plasma membrane of the organism, are responsible for the survival of a trypanosome population in an immunologically responsive host (Vickerman, 1974). Antigenic variation is seen in the malarias (Brown, 1974) and Ogilvie and Wilson (1976) review the present status of knowledge on this.

Parasite induced immunodepression is now a well documented phenomenon in a variety of parasitic infections. Its occurrence in malaria and trypanosomiasis has been reviewed by

Greenwood (1974) and in parasitic infections, in general, by Ogilvie and Wilson (1976). A variety of mechanisms, apparently parasite in origin, can depress the immune response of the host and this may be accomplished either through a suppression of the affector response as for example in *Trichinella spiralis* infection or through suppression of the effector response, examples of which are found in the host response to larval cestodes (Hammerberg et al., 1976).

Factors of particular importance in the evasion of the host response to parasitic infection are those associated with age or with pregnancy, parturition and lactation. The inability of young sheep to respond in a protective manner to, for example, *H. contortus* infection, is well known. This may represent a basic inability of lambs to respond to helminth antigens and thus helminth antigens might be placed in the heirarchy of immunogenicity which Silverstein et al. (1963) have demonstrated for a variety of other antigens. The inability to induce an immune response to certain antigens does not imply an absence of immunological competence, indeed, in *H. contortus* infection of sheep, antibody responses can be stimulated by neonatal infection with infective larvae and the "self cure" reaction can be elicited in lambs of a few weeks of age (Varela-Diaz, 1970). Protective immunity is however a different matter, and competence for this is acquired much later in sheep (Urquhart et al., 1966). The basis for this is unclear, but cell mediated immunity mechanisms may be pertinent here since Chen (1972) has shown that peripheral blood lymphocytes of *H. contortus* infected lambs respond poorly to antigens of *H. contortus* until they are several months of age. The antigen induced responsiveness of peripheral lymphocytes is markedly reduced at the onset of lactation and is associated with a significant increase in fecal egg output at that time (Chen and Soulsby, 1976). In situations where lactation does not occur in the parturient animal, antigen induced lymphocyte responsiveness and fecal egg output are unchanged. There is now strong evidence that lactation is an important determinant, both in the "postparturient" rise in fecal egg output of gastrointestinal nematode infection of domestic animals and in experimental situations, such as rats infected with *Nippostrongylus brasiliensis* (Ogilvie and Love, 1974).

Physiological changes associated with reproduction are known to play an important role in parasitic infections in general. For example, *Trichomonas vaginalis* fails to develop in the female genital tract before puberty (Hawking, 1974) and gametocytes of *Leucocytozoon simondi* appear in the blood of ducks at the beginning of the breeding season (Chernin, 1952). The best known of these phenomenon is the "spring rise" in fecal egg output in sheep, which is variously known as the postparturient rise or lactational rise. Michel (1976) has summarized the various studies of this phenomenon and has concluded that there is a clear relationship between physiological status of the ewe and the "activation" of larvae arrested in their development, these having been acquired in the previous grazing season. In the past an understanding of the "spring rise" has been obscured by the fact that increases in fecal egg output of nonpregnant ewes, males, and castrated males in a flock of sheep often parallel those of parturient and lactating females. Michel (1976) has proposed that parasite populations are controlled by a biorhythm of parasite origin, in that arrested (hypobiotic) larvae in sheep of all descriptions mature at the same time, but in parturient and lactating animals parasites are retained longer than in barren animals. This explanation is consistent with the parasitological and immunological data available on the spring rise phenomenon.

Utilization of the Host Response for Control

The approaches to immunological prophylaxis in parasite infections can be listed as follows:

Passive Immunization
Active Immunization
- Use of standardized doses of normal infective stages.
- Use of related species with reduced virulence.
- Use of attenuated infective stages.
- Use of *in vitro* cultured materials.

Passive Immunization

Passive immunization in protozoal infections has been reviewed by Cohen (1975) and its

practical use is largely restricted to *Plasmodium falciparum* malaria in man (Cohen, McGregor and Carrington, 1961). *In vitro* studies of the effect of antibody in malaria indicate that antibody is directed against the merozoites and prevents reinvasion of the red cell by them, possibly by blocking the attachment of the merozoite to the erythrocyte membrane. This effect is variant specific predominantly, (Butcher and Cohen, 1972) though antibody to other variants is also present in low titer and is probably associated with the lack of clinical signs on challenge with antigenic variants.

More recent studies by Miller, Aikawa, and Dvorak (1975) on the interrelationship of immunity and the surface coat of merozoites of *Plasmodium knowlesi* indicate that agglutination of merozoites, caused by the binding of surface coats of adjacent parasites by homologous immune serum is a crucial factor in the reduced invasion of erythrocytes by merozoites. The general aspects of immunity to malaria have been reviewed by Brown (1976).

Under natural circumstances, passive immunization plays a substantial role in the control of infections due to *Babesia* spp. (Mahoney, 1972) and other protozoan infections of domestic animals.

Passive immunity appears to play a limited role in the control of helminth infections, except in the case of the larval cestodes. With the latter, studies in laboratory rodents clearly indicate that passive transfer of immunity occurs *in utero* and *via* the colostrum (Lloyd, 1975). The effective antibodies in passive transfer vary according to the host: thus in rats protective antibodies occur in the IgG_{2a} subclass of immunoglobulins while in mice the comparable effective antibody is of the IgG_1 subclass.

The successful passive transfer of immunity in the rodent system would suggest that larval cestodes of domesticated animals could be controlled by passive immunization procedures. Both field and experimental evidence indicate this is so. Thus Gemmell, Blundell-Hasell and Macnamara (1969) demonstrated that colostrum from hyperimmunized ewes conferred protection against infection with metacestodes of *Taenia hydatigena* in lambs. Similarly, colostral transfer of immunity from ewes to lambs has been reported by Rickard and Arundel (1974) for *Taenia ovis*. In the latter system immunization of ewes with an *in vitro* produced "secretory" antigen of *T. ovis* was effective in stimulating transfer of immunity (Rickard and Bell, 1974).

Passive immunization to the metacestode of *Taenia saginata*, hitherto, has proved unsuccessful (Urquhart, 1961; Froyd, 1964) however recent studies (Lloyd, 1975), have indicated that hyperimmune serum or hyperimmune colostrum, when fed to neonatal calves, will result in highly significant degree of protection. Hyperimmune colostrum is produced by the introduction of activated oncospheres into the mammary tissue, which results in local synthesis of protective antibodies. Evidence from the rodent system indicates that IgA is the putative immunoglobulin in immunity transferred by the colostral route (Lloyd, 1975) and studies are in progress to determine the responsible immunoglobulins in the bovine system. There are strong indications that the gut associated lymphoid tissue (GALT) is concerned with both intestinal and mammary gland immune response and a practical application of this to the control of metacestode infection might be to attempt oral immunization of animals in late pregnancy.

Active Immunization

The use of standardized doses of normal infective larval stages

Under natural conditions this is a process which is achieved by good husbandry practices and in which a parasitic infection undergoes normal development and the immunizing antigens are produced and presented to the host by the various developmental stages. The obvious drawbacks to this are that the potential for disease is always present and host physiological processes (e.g. lactation) or the vicissitudes of the weather may alter the transmission rate dramatically. Nevertheless it must be a reasonably effective approach to immunization since otherwise only a minority of animals would survive, whereas the majority do so!

A modification of this approach is to terminate a normal infection by an appropriate antiparasitic drug. This technique has been used in the field to control infection by *Dictyocaulus viviparus* in cattle (parasitic bronchitis) in that

animals are treated with diethycarbamazine as soon as the first clinical signs appear (Parker et al., 1959). Experimentally, this has been accomplished also with *Ascaris suum*, infection being terminated after a few days with high doses of thiabendazole (Campbell and Timinski, 1965). Very encouraging progress using this technique has been accomplished in immunization against *Theileria parva* infection of cattle (East Coast Fever) (Radley et al., 1975a). Cattle are immunized by injecting infective stages obtained from ticks (ground up tick supernate, which can be stored at –140 C or –80 C) and the infection is controlled by daily intramuscular injections of oxytetracycline for the first four days after infection. This method has been shown to be useful also when a combination of strains of *Theileria* organisms is used (Radley et al., 1975b).

A further modification of this approach is to administer infective stages by an abnormal route. Soulsby (1957) induced satisfactory immunity to *A. suum* by the subcutaneous injection of infective eggs. At this site a minority of eggs hatch, and larvae undergo local migration, a few eventually reaching the lungs. Otherwise lethal doses of eggs can be administered by this route with impunity, and a high degree of immunity produced.

Gemmell (1966) applied this technique to immunize against the larval stages of *Echinococcus granulosus* and other cestodes. For this, hatched and activated hexacanth embryos are injected intramuscularly, these produce a local colony of metacestodes which will immunize against homologous challenge and also heterologous challenge, provided a parasite of similar host assemblage is used. Intramuscular injection of activated embryos of *E. granulosus* will also induce some protection against the adult phase of the parasite in the intestine (Gemmell and Macnamara, 1972). Active immunization against bovine cysticercosis has been achieved using parenterally administered eggs of *Taenia saginata* (Soulsby, 1961) or activated oncospheres of *T. saginata* or *T. hydatigena* (Wikerhauser, Zuković and Dzakula, 1971; Sewell and Gallie, 1974). In the latter studies development of a focus of metacestodes at the site of injection was necessary for a good protective response, though occasionally metacestodes from the immunizing injection were distributed systematically (Sewell and Gallie, 1974). The use of oncospheres of *T. saginata* attenuated by X-irradiation induced a significant degree of protection and avoided the development of a postvaccinal colony of metacestodes (Wikerhauser et al., 1974).

Extended survival of the metacestodes of *T. saginata* may occur in endemic areas and this is probably associated with neonatal infection of calves (Urqhart, 1961). Experimental infection of neonates induces a poor antibody response and a failure of animals to develop protective immunity to a second infection (Soulsby, 1963), however, such neonatal infection does not interfere with the establishment of a primary immune response following reinfection, even though the metacestodes from a primary neonatal infection remain viable in the tissues (Sewell and Gallie, 1974).

The use of related species of parasites or species with reduced virulence

Though this method of immunization has been used widely in other infections, its use in parasitic diseases has been limited.

Cross protection between the sheep metacestodes *T. ovis* and *T. hydatigena* has been demonstrated by Gemmell (1966) and additionally these species cross protect against the larval phase of *Echinococcus granulosus* in sheep. It is particularly interesting that heterologous protection with metacestodes is expressed most satisfactorily with species of the same host assemblage. For example canine-sheep related forms show more relationship to each other than to canine-rodent related forms an example of which is that *E. granulosus* and *E. multilocularis* fail to share common protective antigens (see Gemmell and Macnamara, 1972).

This approach to immunization possibly is best expressed by the concept of zooprophylaxis, advanced by Nelson, Teesdale and Highton (1962) and reviewed by Nelson (1974), who defined it as "the prevention or amelioration of disease in man as a result of previous exposure to heterologous infections of animal origin." Nelson (1974) provides a number of examples of this with the protozoa and helminths, and, with viruses, an outstanding example is the protection induced by cowpox against smallpox.

Zooprophylaxis with the protozoa probably occurs with the malaria parasites and an attempted application of the concept of zooprophylaxis was that by Manson-Bahr (1963) who used a ground squirrel strain of *Leishmania* to induce immunity to a human form. Other examples occur with malaria, babesiosis and trypanosomiasis (Nelson, 1974).

With helminth infections, zooprophylaxis is best exemplified in the studies of cross immunization between human and animal schistosomes (Nelson, 1974; Taylor, 1975). For example, partial immunity against *Schistosoma mansoni* can be produced by immunizing rhesus monkeys with bovine schistosome cercariae, which die before reaching maturity (Amin and Nelson, 1969). Conversely, calves and sheep immunized with cercariae of *S. mansoni* partially resist challenge with *Schistosoma mattheei*. Other examples of this are given by Taylor (1975). An important observation in these studies was that immunity to challenge was not dependent on the presence of adult worms of a previous infection and consequently the use of developmental stages of homologous parasites suitably attenuated so that they will not mature or heterologous parasites which do not mature, is a valid approach to immunization against *S. mansoni*. A similar approach may well be possible for *Schistosoma japonicum* since Sadun et al. (1961) have demonstrated that the Formosan (non-human) strain of *S. japonicum* will induce immunity in monkeys against the Chinese and Japanese (human) forms of the parasite.

Nelson (1974) has reviewed the evidence for zooprophylaxis in filarial infection, stating that the situation is more speculative. Nevertheless, he notes that many of the infective larvae of filarids in man-biting insects are of non-human origin and hence there is likely to be continuing sensitization to these in endemic areas. The impact of this on infection and the manifestation of human filarial disease is not known.

The use of species with reduced "virulence" has been little explored with regard to helminths. Allen and Samson (1961) identified a strain of *Haemonchus contortus* from the pronghorn antelope that was relatively nonpathogenic to domestic sheep, but this has not been exploited as a mode of immunization. There is need to examine the less virulent members of a genus, possibly from wildlife, for their potential use as immunizing agents against the more pathogenic species of the genus.

The use of artificially attenuated infective stages

Exposure of infective stages of parasites to X or gamma radiation is the most usual approach to attenuation. Early studies by Tyzzer and Honeij (1916) demonstrated that exposure of *Trichinella spiralis* larvae to radium rendered them noninfective to mice and subsequent work by Levine and Evans (1942) indicated that immunity to *T. spiralis* infection could be induced in rats by feeding irradiated larvae. Practical application of this resulted in the development of a highly successful vaccine for the prevention of lungworm (*Dictyocaulus viviparus*) infection of cattle (Jarrett et al., 1960) and similar vaccines have been developed for the sheep lungworm (*Dictyocaulus filaria*) (Sokolić et al., 1965) and the dog hookworm (*Ancylostoma caninum*) (Miller, 1971). In the case of the vaccine against *A. caninum*, protection is achieved also against other members of the genus (Miller, 1967). The application of radiation attenuated vaccines against other helminth infections has been summarized by Mulligan (1975).

Chemical attenuation of infective stages of helminths has been studied by Cornwell and Jones (1970). These workers showed that infective larvae of *D. viviparus* exposed to triethylene melamine, a cytotoxic agent, would induce an immunity comparable to that of radiation attenuated larvae.

With the protozoa, immunization, with radiation attenuated sporozoites of malaria (Nussenzweig, Vanderberg and Most, 1969) offers a promising approach to the immunoprophylaxis of malaria. Radiation attenuated parasitized erythrocytes have also been shown to induce satisfactory immunity in malaria (Wellde and Sadum, 1967). Immunization against malaria is discussed in greater detail by Brown (1976).

Immunization against *Trypanosoma cruzi* infection using radiated attenuated (gamma radiation from a ^{60}Co source) parasites from cell cultures has been reported by Hanson et al. (1974). Resistance to homologous challenge has been produced in rats and monkeys by inoculation with irradiated *Trypanosoma rho-*

desiense (Duxbury et al., 1972) and in cattle (Wellde et al., 1973) by similar techniques. Phillips (1971) was able to induce immunity in mice and rats by inoculation of ^{60}Co irradiated *Babesia rodhaini* infected red cell, but Lemma and Cole (1974) were unable to induce immunity against *Leishmania enriettii* in guinea pigs using irradiated promastigotes of the parasite.

The use of *in vitro* produced materials

Though no vaccine of this nature is available for use, the method of immunization offers the greatest promise for the future.

With malaria, immunity against the erythrocytic phase of the infection is directed against the merozoite and Miller, Aikawa, and Dvorak (1975) have provided evidence that the surface coat on extracellular merozoites is important in immunity and antibodies directed against it are important for reduction in the invasive capacity of merozoites for erythrocytes, at least *in vitro*. The successful *in vitro* cultivation of *Plasmodium falciparum* (Trager and Jensen, 1976) opens the way for the harvest of substances, such as the surface coat of merozoites, for practical immunization.

Another approach which may follow the successful *in vitro* cultivation of malaria is immunization against the sexual stages of the parasite. Gwadz (1976) has demonstrated that gametocyte infectivity and oocyst development of *Plasmodium gallinaceum* can be reduced or eliminated in mosquitoes by immunizing chickens on which the mosquitoes feed, with infected erythrocytes. Immobilization of microgametes occurs in the mosquito gut and this is associated with the immunoglobulin G fraction of serum.

As with studies of the protozoa, there is an increasing preoccupation on the part of workers in the field of helminthology to produce antigens which are meanfully related to the development cycle and/or the biological activity of the parasite. Examples of such are the specific schistosome membrane antigens (Kusel et al., 1975), granules of stichocyte cells of *Trichinella spiralis* (Despommier and Muller, 1970), the soluble egg antigen of schistosomes (Boros and Warren, 1970), secretory antigens produced by adult *Echinococcus granulosus* in culture (Herd, 1975) and acetylcholinesterase secreted from the oesophageal glands of several nematodes (Ogilvie et al., 1973).

There have been many attempts to induce protective immunity with antigens prepared from nonviable helminths, but with few exceptions they have been unsuccessful. Reasons for these failures include the probability that protective antigens are associated with excretory and secretory materials to do with physiological functions of the parasite. These might be expected to occur in minimal amounts in nonviable material. Two decades ago Chandler (1953) suggested that the protective antigens were enzymatic in nature and in some cases, e.g., in *Ascaris suum* infection, immunization with malic hydrogenase isolated from the parasite, induced specific antibodies which inhibited activity of the enzyme and also induced protection against infection, in guinea pigs (Rhodes, Nayak, and Kelley, 1965).

Secretory materials derived from *E. granulosus* (Herd, 1975), *Oesophagostomum radiatum* (Keith and Bremner, 1973) and *Ancylostoma caninum* (Thorson, 1956) have been shown to induce immunity to infection. Furthermore, with *Nippostrongylus brasiliensis*, antiacetylcholinesterase antibodies modulate the production of this enzyme by the parasite (Jones and Ogilvie, 1972) and this evidence would seem to indicate that such substances are important candidates for protective antigens. However, the situation is still in need of clarification since recent reports by Neilson (1975) and Rothwell and Merritt (1975) indicate that immunization with the metabolic antigens of *H. contortus* and acetylcholinesterase of *Trichostrongylus colubriformis*, respectively, fails to induce immunity in experimental hosts.

More recent studies of *in vitro* produced immunogens of *A. suum* have demonstrated that material produced at the time of moulting from the third to the fourth larval stage is capable of inducing significant protective immunity in guinea pigs (Stromberg and Soulsby, 1976). The likelihood that this is an antigen associated with a physiological function of the parasite is good, since *in vitro* metamorphosis from L3 to L4 in *A. suum* is associated with a switch from aerobic to anaerobic metabolism (Sylk, 1969). *In vitro* cultures of larvae which do not undergo metamorphosis do not produce the

immunogen. It is now possible to perform the above cultures in a completely defined medium (Stromberg and Soulsby, 1976) and hence this will provide an unusual opportunity for preparative and analytical studies of the immunogen.

Conclusion

In this review a full comprehension of the host parasite relationship may have suffered under abbreviated treatment of the multifaceted nature of the situation. Nevertheless, it seems evident that there are many places in this relationship which may be exploitable for control purposes. The traditional approaches have been immunization to prevent parasitism and therapy to cure parasitism once it has occurred. The middle area of manipulating the microenvironment of a parasite and the triggers a parasite needs to receive from this may represent fruitful areas of development in the coming years.

The 1000th meeting of the Helminthological Society of Washington may well be a time to judge whether at the time of this 500th meeting we have provided a significant point of departure for such future studies.

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Editor's Note

Authors submitting manuscripts of a survey or taxonomic nature for publication in the Proceedings of the Helminthological Society of Washington are urged to deposit representative specimens in a recognized depository such as the National Parasite Collection at Beltsville, Maryland and include the accession numbers in the manuscript.

Notes on the Biology of *Plagiorchis noblei* Park, 1936 (Trematoda: Plagiorchiidae)

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ABSTRACT: Several aspects of the parasite-host relationships of larval and adult *Plagiorchis noblei* were investigated. In the laboratory, eggs and metacercariae were infective for a longer time than had previously been reported. Cercariae of *P. noblei* from naturally infected lymnaeid snails showed marked nocturnal periodicity. Examination of more than 4,000 naturally occurring adult *Lymnaea stagnalis* and *Stagnicola reflexa* revealed that the highest prevalence of infection with this plagiorchiid trematode occurred from August to early October. Prevalence of adult worms in red-winged and yellow-headed blackbirds collected in the same area peaked in late June to early August.

Digenetic trematodes of the genus *Plagiorchis* Lühe, 1899, are intestinal parasites found in nearly every class of vertebrates, but particularly in birds and mammals. Most known life histories of *Plagiorchis* involve lymnaeid snails and aquatic insects as first and second intermediate hosts, respectively. Knowledge of life cycles of members of this genus was summarized by Buttner and Vacher (1960).

Plagiorchis noblei was described by Park (1936) from a collection of 20 specimens recovered from the small intestines of red-winged blackbirds, *Agelaius phoeniceus*. Of the more than 45 species included in the genus *Plagiorchis* at that time, *P. noblei* most closely resembled *P. maculosus* (Rudolphi, 1802) Braun, 1902, from mice. Certain aspects of the life history of *Plagiorchis noblei* have been studied by Williams (1964a, b), Daniell (1964) and Daniell and Ulmer (1964). These studies, however, have been published only in the form of brief abstracts. Additional life cycle data resulting from experimental studies on host-parasite relationships of *P. noblei* are presented in this study.

Materials and Methods

Eggs of *Plagiorchis noblei* were recovered from naturally infected red-winged blackbirds (*Agelaius phoeniceus*). Gravid worms were placed in petri dishes containing boiled lake water and teased apart to obtain the eggs. Laboratory-reared *Stagnicola reflexa* and *Lymnaea stagnalis* were exposed to fully embryonated eggs incubated at room temperature in filtered lake water. Snails were reared and maintained in aquaria on a diet of lettuce, fish pellets and oyster shells.

Cercariae of *P. noblei*, showing nocturnal periodicity, were obtained by first placing infected snails under fluorescent light for 24 hours and then in total darkness for an additional hour. Various species of aquatic insect larvae were exposed to these cercariae. Mosquitoes and certain chironomids were reared from eggs; other species were collected from tertiary ponds at the Ames Sewage Plant (Ames, Iowa). Because these ponds contained no lymnaeid snails, naturally occurring infections of *Plagiorchis* were not present. Dragonfly naiads, individually isolated in finger bowls to prevent cannibalism, were fed small aquatic arthropods obtained from the same ponds.

Birds used in this study were either reared from eggs under helminth-free conditions (house sparrows) or were purchased as newly hatched young from suppliers (domestic chicks). Newly hatched birds were placed in artificial nests and transferred to a simple incubator constructed of wire mesh, wood and a heating pad. Adults were maintained on a mixture of coarse game-scratch and 26% grain balancer (nonantibiotic). Occasionally, insect larvae (*Tenebrio molitor* and *Musca domestica*) were used to supplement this diet.

Summary of Life Cycle

The life cycle of *Plagiorchis noblei* involves at least two species of snails, *Stagnicola reflexa* and *Lymnaea stagnalis*. Following ingestion of

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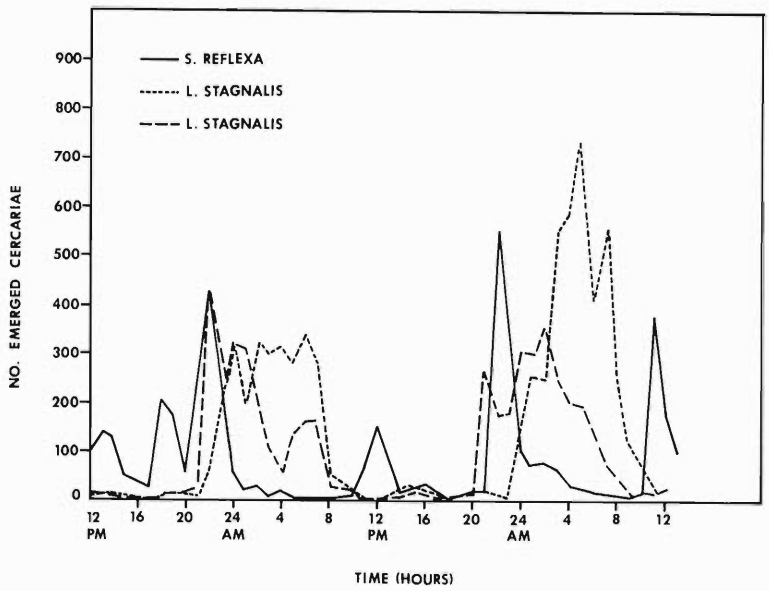

Figure 1. Cercarial emergence of *P. noblei* from three naturally infected lymnaeid snails at hourly intervals during a continuous 48 hours of natural light and darkness.

embryonated eggs, miracidia hatch, penetrate the gastropod's digestive tract, then elongate to form mother sporocysts on the outer surface of the intestine. Daniell (1964) observed fully developed miracidia within the eggs two days after they were expelled from adult flukes.

Daughter sporocysts produced by mother sporocysts migrate to the hepatopancreas of the snail and produce large numbers of xiphidiocercariae approximately 40 days after the egg was ingested. Daniell (1964) and Williams (1964b) observed nocturnal periodicity of cercariae shed from *Stagnicola reflexa.* Cercariae penetrate and encyst in a variety of aquatic second intermediate hosts including odonates, dipterans, caddisflies, mayflies and amphipods (Daniell, 1964; Williams, 1964a).

According to Daniell and Ulmer (1964), metacercariae of *P. noblei* are infective seven days or less after cercarial penetration into immature insects (damselfly and dragonfly naiads and midge larvae). Williams (1964a), working with the same trematode species, found metacercariae to be infective four to six days after cercarial penetration of a mosquito, *Aedes aegypti.* Metacercariae encysted in dragonfly naiads for 36 days were still infective when experimentally fed to yellow-headed blackbirds (Daniell, 1964).

Definitive hosts acquire *P. noblei* by ingestion of infected second intermediate hosts. Experimental hosts include domestic chicks (*Gallus gallus*), yellow-headed blackbirds (*Xanthocephalus xanthocephalus*) and red-winged blackbirds (*Agelaius phoeniceus*) (Daniell, 1964). Blankespoor (1974) found that at least 17 species of birds and mammals are capable of harboring experimental infections of this species.

Experimental Results

Egg

Data from this study indicate that laboratory-reared *S. reflexa* became infected when exposed to eggs of *P. noblei* 84 hours following extrusion from adult worms. Furthermore, experimental infections could still be established in snails 43 days after eggs had been maintained in the laboratory at room temperature.

Cercaria

DIEL PERIODICITY: The following investigation was undertaken to obtain more precise information on cercarial emergence of *P. noblei*

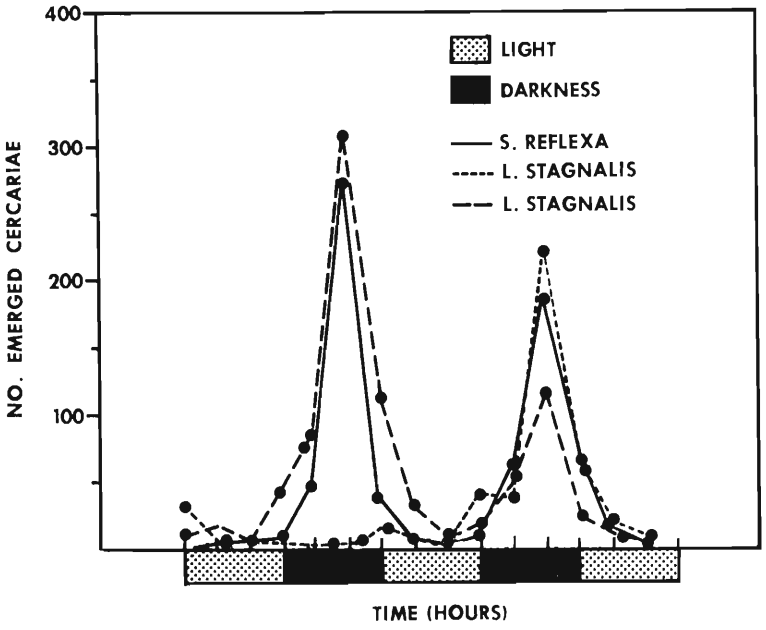

Figure 2. Cercarial emergence of *P. noblei* from three naturally infected lymnaeid snails during alternative three-hour periods of light and darkness.

during alternating periods of light and darkness. Two days before hourly counts of cercarial emergence began, three naturally infected lymnaeid snails were individually isolated in finger bowls containing lake water and were then maintained under natural conditions of light and darkness. At hourly intervals for two consecutive days, each snail was transferred to another bowl containing fresh water at the same temperature. Cercariae that had emerged during the previous hour were then fixed in 5% formalin and subsequently counted.

As shown in Figure 1, cercariae of *P. noblei* exhibit pronounced nocturnal periodicity, but the time of maximum emergence appears to vary with individual gastropod hosts. Of a total of 15,218 cercariae from these snails, 10,957 (72%) were shed between 8:30 PM and 5:30 AM. In these experiments, water temperatures varied in accordance with ambient temperatures. To determine if declining temperatures at night relate to nocturnal periodicity, another set of experiments was undertaken. The three snails used in the above experiment were again individually isolated in finger bowls and then subjected to alternating three-hour periods of light and darkness. However, during this experiment, a constant temperature of 19–20 C was maintained. Cercariae emerged from these snails almost entirely during periods of darkness (Fig. 2).

SEASONAL PERIODICITY: During the summer and fall of 1969 (June–October), 4,361 snails, representing two lymnaeid species (*S. reflexa* and *L. stagnalis*) were collected in nature, individually isolated in the laboratory, and examined for natural infections of *P. noblei*. Twenty-two of 2,691 (0.82%) *S. reflexa* and 19 of 1,676 (1.13%) *L. stagnalis* harbored natural infections of *P. noblei*. Natural infections from both species of snails over the entire period averaged 0.94% (41 of 4,361). Percentages of infection are based, however, on numbers of snails actually shedding cercariae and do not take into account all those with prepatent infections. Figure 3 summarizes monthly percentages of naturally infected snails. Infections remained below one per cent until August and September. Rate of natural

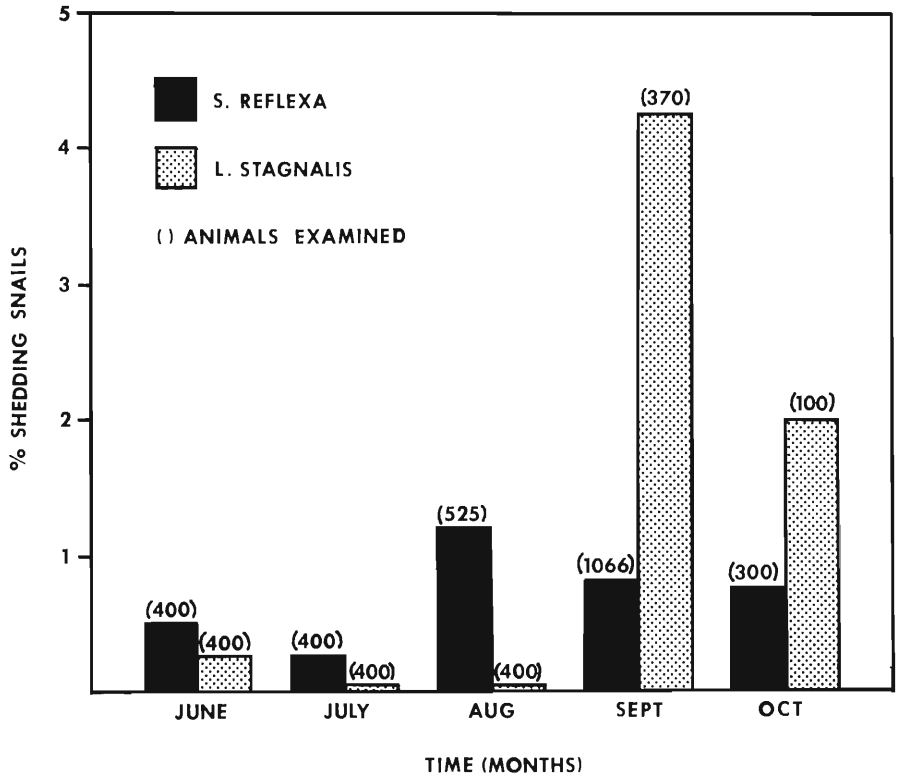

Figure 3. Percentage of naturally infected *Stagnicola reflexa* and *Lymnaea stagnalis* shedding cercariae of *P. noblei*.

infection in S. *reflexa* reached a peak of 1.14% (6 of 525) in August, whereas 4.33% (16 of 370) of *L. stagnalis* harbored infections of *P. noblei* in September. Infections of *P. noblei* in both species of mollusks declined in late September and October.

EFFECT OF TEMPERATURE OF LONGEVITY AND INFECTIVITY: Cercariae of *P. noblei* may live as long as 30 hours in lake water at room temperature. However, the ability of cercariae to penetrate the cuticle of second intermediate hosts ceases approximately 12 hours after emerging from the snail. In a study to determine the longevity and length of infectivity of these cercariae maintained at various temperatures (4, 16 and 30 C), approximately 250 were isolated in each of three petri dishes containing filtered lake water. Temperature has a very pronounced effect on longevity of these cercariae. Those maintained at 4 C lived for nearly 10 days, whereas those maintained at temperatures of 16 and 30 C survived for only 90 and 18 hours, respectively. Cercariae maintained in water at 4, 16 and 30 C were unable to penetrate second intermediate hosts after 38, 18 and 6 hours, respectively.

Metacercaria

The range of infectivity of metacercariae of *P. noblei* in dragonfly naiads (*Aeschna*) was shown to vary from 66 hours to at least 80 days. Domestic chicks (three to seven days old) served as definitive hosts during this experiment.

Adults

LOCATION OF DEFINITIVE HOST: In nearly all natural and experimental infections of *P. noblei*, adults were recovered from the posterior por-

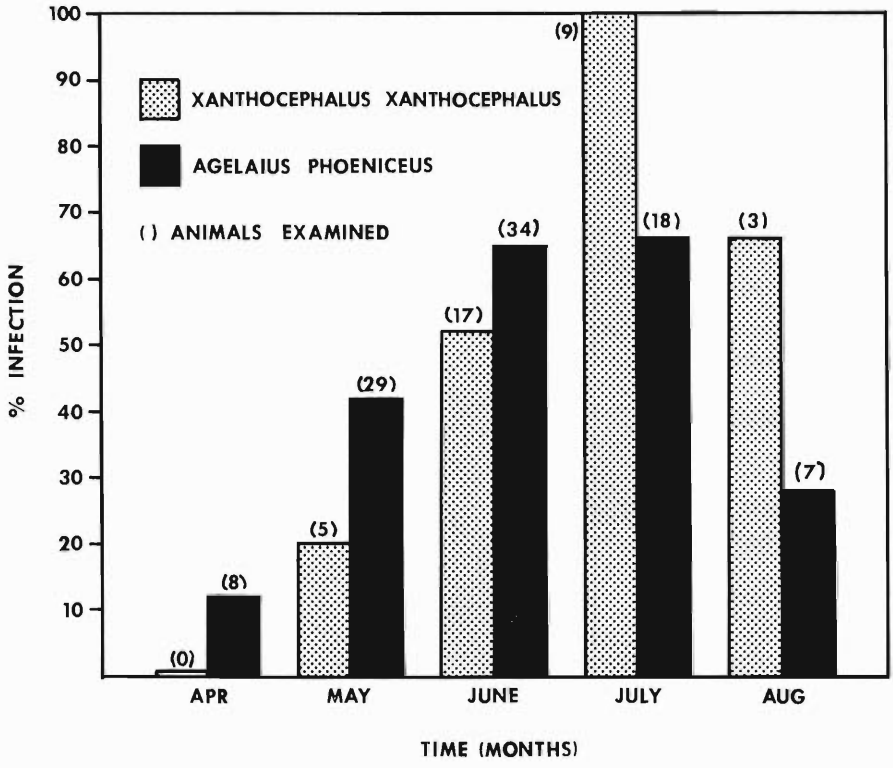

Figure 4. Seasonal prevalence of *P. noblei* in two species of blackbirds, 1967–69.

tion of the host's intestine. However, in experimentally infected chicks, worms often gathered in that area of the intestine to which the residual yolk was attached. In heavy experimental infections, adult flukes were recovered along the entire length of the intestine, and occasionally a few worms were found in the intestinal ceca.

LONGEVITY: The longevity of adult *P. noblei* in experimental infections varied markedly among different species of avian hosts and was longer in passerine than in galliform hosts. For example, adults in young domestic chicks usually disappeared after eight or nine days. To determine the life span of this plagiorchiid species in passerine birds, house sparrows (*Passer domesticus*) were used. Laboratory-reared chironomids (*Chironomus tentans*), infected with approximately 15 metacercariae each, were exposed to each of ten sparrows collected on the Iowa State University campus. These birds had been maintained in captivity for at least three months before exposures were made. Fecal examinations of these birds, as well as autopsies of six additional sparrows collected from the same site and used as controls, revealed no plagiorchiid infections. Beginning three weeks after exposure of these hosts to metacercariae, sparrows were sacrificed at weekly intervals. Results of these experiments (Table 1) indicate that infections of *P. noblei* can remain in adult house sparrows for at least 60 days.

SEASONAL PERIODICITY: During a three-year study (1967–69), 130 red-winged and yellow-headed blackbirds were examined for natural infections of adult *P. noblei*. Sixty-two percent (80 of 130) harbored these trematodes. Forty-eight of 93 (52%) red-winged blackbirds and 32 of 37 (87%) yellow-headed blackbirds were

Table 1. Longevity of experimental infections of adult *P. noblei* in adult house sparrows (*Passer domesticus*).*

Date of examination	Number adults recovered	Days post exposure
January 21	5	22
January 27	3	28
February 2	5	34
February 8	0	40
February 14	0	46
February 20	0	53
February 28	12	61
March 6	0	68
March 13	0	75
March 20	0	82

* All exposures made on December 30, 1967.

infected. Monthly percentages of natural infections of these hosts are shown in Figure 4.

Birds were collected and examined weekly from the time they arrived in northwestern Iowa (April and May) until they migrated (August and September) to their southern wintering grounds in the fall. Of 19 red-winged blackbirds examined before May 10, only a single bird harbored one adult (immature) *P. noblei*. The earliest natural infection with this trematode in yellow-headed blackbirds was recovered on May 25. These data suggest that birds migrating northward in the spring are uninfected, and that infections are acquired at the nesting grounds. Infected gastropods (*S. reflexa* and *L. stagnalis*) or overwintering dragonfly naiads harboring metacercariae of *P. noblei* probably serve as sources of infection in the spring.

Percentages of naturally infected blackbirds continue to increase through July; all nine yellow-headed blackbirds and 12 of 18 red-winged blackbirds examined in July were infected. This increase probably resulted from greater consumption of naturally infected second intermediate hosts.

After July, fewer red-winged and yellow-headed blackbirds were available for examination. Nearly all of them, having completed their nesting season by early August, had congregated in preparation for fall migration. Because the summer residence of many of these birds was unknown, only those in established territories were collected.

Discussion

The genus *Plagiorchis* represents more than 140 species that are cosmopolitan in distribution. The success of *P. noblei*, a typical representative of the genus, is based on the relatively long infective periods of the egg and metacercariae, low degree of host specificity of larvae and adults, reduced pathogenicity of adult worms, and its ability to develop in commonly occurring molluscan, arthropod and vertebrate hosts. Lymnaeid snails, insects and passerine birds, all associated with an aquatic environment, serve as first, second, and definitive hosts, respectively.

It has been established that light and darkness are important factors controlling cercarial emergence of many digenetic trematodes. Macy (1960), working with *Plagiorchis vespertilionis parorchis*, concluded that darkness preceded by light was necessary to induce shedding of cercariae from the snail. Cercariae of *Plagiorchis micracanthos* from *Lymnaea exilis* emerge during darkness (Wagenbach and Alldredge, 1974). Similarly, cercariae of *P. noblei* show marked diel and seasonal periodicity. In the present study, nocturnal periodicity of *P. noblei* was demonstrated by subjecting infected lymnaeid snails to normal diel and to alternating three-hour periods of light and darkness.

During the summers of 1967–69, more than 4,000 adult *Lymnaea stagnalis* and *Stagnicola reflexa* were examined for natural cercarial infections of *P. noblei*. These data show that the incidence of cercarial infections declined from May to July, but then peaked in August and early September, and suggest that snails that harbor over-wintering sporocyst infections shed cercariae in the spring and early summer, but die with the advent of warmer temperatures. In the meantime, young snails recruit infections during the breeding season of the definitive hosts. In midsummer, these newly acquired infections begin producing cercariae.

Although adults of *P. noblei* show a low degree of host specificity, the length of time they remain in the definitive host varies considerably with the host species. In passerine birds, presumed to be the natural final hosts, experimental infections remained for at least two months. However, in galliforms, adult worms were never found after ten days post

exposure. Furthermore, infections of *P. noblei* only become established in very young chicks.

Dogiel et al. (1964) categorized parasites of migrating birds into four groups: (1) ubiquitous species parasitizing their host throughout the year in the southern (wintering grounds) and northern (nesting grounds) areas; (2) southern forms infecting birds only in the wintering grounds; (3) northern parasites infecting their hosts only in the nesting habitats; and (4) species infecting birds only during migration flights. It is apparent from the present study that adults of *Plagiorchis noblei* fall into category three. During the summers (1967–69), the incidence of natural infections in both the red-winged and the yellow-headed blackbirds continued to rise through July. However, infections decreased in late summer and early fall. These trends are anticipated because the blackbirds begin changing their diet from arthropods to seeds after the breeding season (July).

Acknowledgments

This study was supported in part by NSF grant GB 5465X and NIH Predoctoral Fellowship grant 1-F1-GM-38, 055-01. Appreciation is extended to Dr. Martin J. Ulmer for supervising this project and to Drs. W. F. Hollander and W. J. Miller of the Genetics Department (Iowa State University) for providing mice. Finally, thanks are due to Mr. M. L. Williams of De Kalb Hatchery of Roland, Iowa for supplying large numbers of chicks.

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Six New Species of Tetraphyllidean Cestodes, Including a New Genus, from a Marine Stingray *Himantura schmardae* (Werner, 1904) from Colombia[1]

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ABSTRACT: Six species of tetraphyllidean cestodes are described from the marine stingray *Himantura schmardae* from the Caribbean coast of Colombia. The new genus *Acanthobothroides* is proposed for *A. thorsoni* which has outer bothridial hooks characteristic of members of *Acanthobothrium* and inner bothridial hooks similar to those of species of *Onchobothrium*. *Acanthobothrium himanturi* differs from *A. southwelli* by having an average of 47 rather than 34 testes per proglottid, a larger cirrus sac, and unequal ovarian lobes; it differs from *A. brevissime* in having hooks averaging 138 μm rather than 115 μm in total length, 47 rather than 25 testes, and a distinct ovarian isthmus, and by lacking a prominent genital atrium. *Acanthobothrium tasajerasi* differs from *A. brevissime* by having hooks averaging 165 μm rather than 115 μm and larger bothridia; it differs from *A. dujardinii* by having smaller hooks, a prominent genital atrium and an indistinct ovarian isthmus. *Caulobothrium anacolum* differs from all other members of the genus by having a cephalic peduncle which is considerably shorter than the strobila, acraspedote proglottids, and quadrate rather than canoe-shaped bothridia which lack a median longitudinal septum. *Rhinebothrium magniphallum* has a cirrus sac which is relatively much larger than any other known species. It resembles *R. monodi* and *R. scobinae* in number of bothridial loculi, *R. hawaiiensis*, *R. euzeti*, and *R. cadenati* in number of testes per proglottid, and *R. scobinae* and *R. cadenati* in lacking a median longitudinal septum. *Rhinebothrium tetralobatum* resembles *R. spinicephalum* by having two testes and craspedote proglottids, but differs by having 50 to 54 rather than 32 to 34 bothridial loculi and 82 to 100 rather than 36 to 49 proglottids per strobila. The ovary resembles that of *R. lintoni*, but has four lobes rather than six to eight.

The genus *Himantura* Müller and Henle, 1837 (Chondrichthyes: Dasyatidae) comprises approximately 12 species of marine and estuarine stingrays distributed throughout the western Indo-Pacific Ocean and Red Sea regions, one species from the Pacific coast of Costa Rica, and one species from the Caribbean Sea. Little is known about their parasitic helminths: Williams (1964) described two species of tetraphyllidean cestodes from *Himantura granulosa* (Macleay) from Australia and Diaz-Ungria (1973) reported a nematode, *Echinocephalus ungriai* Troncy, 1969 (erroneously reported from *Potamotrygon hystrix*), from *H. schmardae* (Werner) from Lake Maracaibo in Venezuela. This report describes six new species of tetraphyllideans from *H. schmardae* from the Caribbean coast of Colombia.

[1] Funds for this study were provided through a grant from the National Geographic Society to Dr. T. B. Thorson, University of Nebraska.
Part of this study was conducted in cooperation with the U.S. Department of Commerce, NOAA, Office of Sea Grant, under Grant No. 04-5-158-54. The U.S. government is authorized to produce and distribute reprints for governmental purposes notwithstanding any copyright notation that may appear hereon.

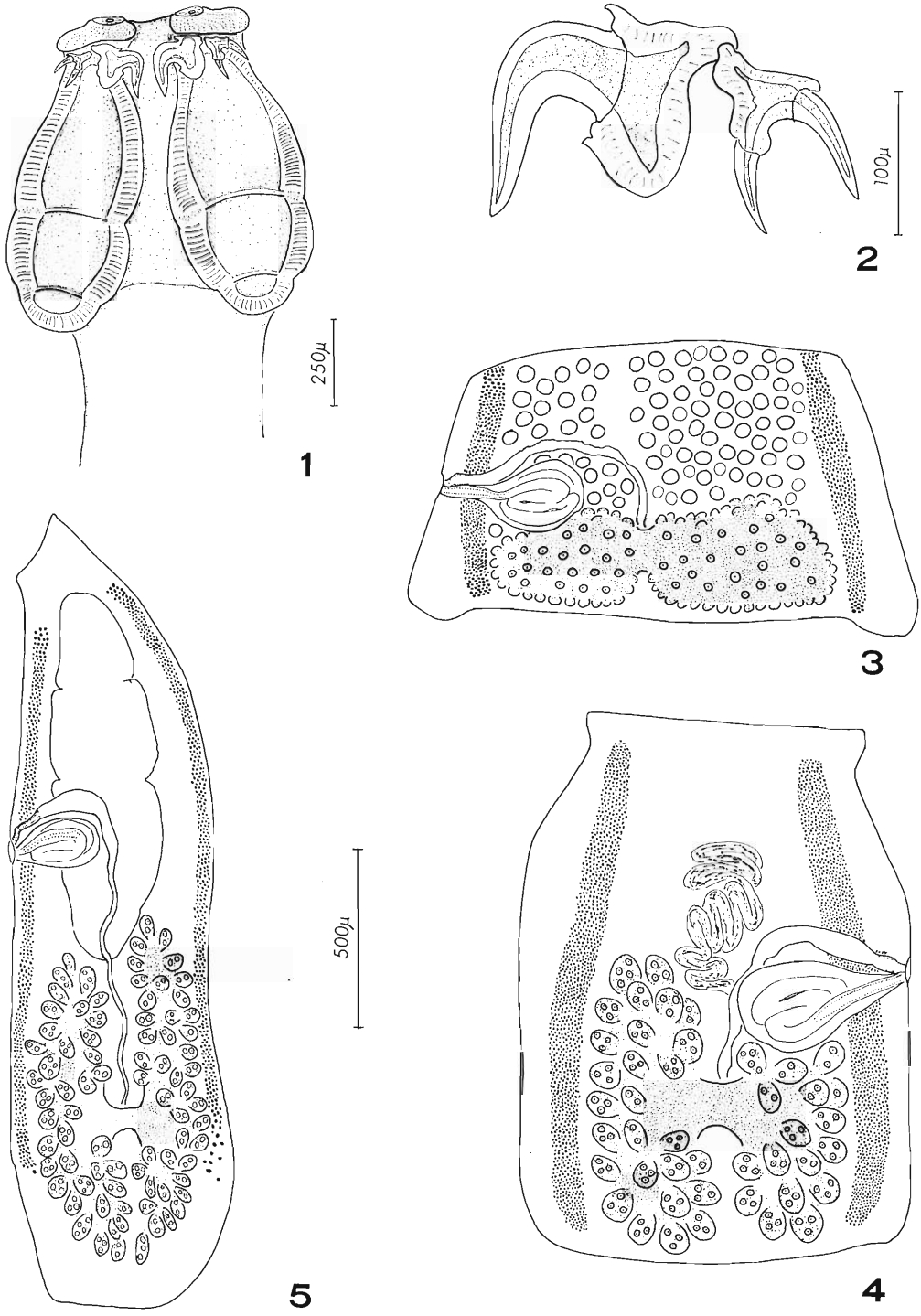
Materials and Methods

Local fishermen harpooned rays at night in the Caribbean Sea 15 kilometers west of La Cienaga, Departamento Magdalena, Colombia and kept them alive in seawater over night. Worms were fixed *in situ* or removed from the host, relaxed in cold seawater and fixed with AFA. All were stored in 70% ethanol. Whole mounts were stained with Ehrlich's acid hematoxylin and mounted in Histoclad. Serial cross-sections, cut at 8 micra and stained with hematoxylin-eosin, were used to confirm some aspects of proglottid morphology. Mean values and standard deviations are included for some characters. Measurements are in micra unless otherwise stated; figures were drawn with the aid of a drawing tube.

Cestoidea

Acanthobothroides gen. n.

DIAGNOSIS: Onchobothriidae. Scolex with four sessile, triseptate bothridia each with apical sucker and pad armed with pair of dissimilar



Figures 1–5. *Acanthobothroides thorsoni*. 1. Scolex. 2. Bothridial hooks. 3. Proglottid with testes. 4. Proglottid without testes but with sperm in vas deferens. 5. Gravid proglottid.

hooks; outer bothridial hooks bifid with handle, inner hooks with single prong and base. Genital pores marginal, irregularly alternating. Ovary bilobed in frontal view, X-shaped in cross-section. Vitellaria follicular, in marginal area of proglottid. Parasites of elasmobranchs. Type and only species:

Acanthobothroides thorsoni sp. n. (Figs. 1–5)

DESCRIPTION (based on 6 specimens): Strobila slightly craspedote, anapolytic, up to 400 mm long; composed of up to 700 proglottids. Internal musculature as follows: single layer with bundles of longitudinal muscles immediately below basement membrane of tegument; few poorly developed and poorly organized circular muscles in cortex; ring of longitudinal muscle bundles central to circular muscles. Scolex 665–1080 long by 820–1150 wide. Bothridia 675–880 long by 160–220 wide; anterior loculus 410–513 long, middle loculus 190–247, posterior loculus 76–95. Ratio of loculi lengths 1: 0.5: 0.2. Outer bothridial hooks 168–198 long; handle 66–90, inner prong 90–108, outer prong 90–108. Inner bothridial hooks 220–288 long; base 162–168 long. Cephalic peduncle 4.5–20 mm long. Immature proglottids wider than long. Proglottids containing testes 852–876 long by 1560–1680 wide. Testes occupying anterior ½ of proglottid, 87–97 in number, 90–145 in diameter; 12–15 postporally, 26–36 preporally, 46–50 antiporally. Cirrus sac near mid-proglottid, 415–504 long by 180–240 wide, containing spined eversible cirrus. Genital pore 50% of proglottid length from anterior end. Vagina anterior to cirrus sac; vaginal sphincter present. Ovary follicular, 468–852 long by 648–1080 wide at isthmus. Vitelline follicles extending entire length of proglottid. Proglottids not containing testes but with sperm in the vas deferens 948–2160 long by 900–1500 wide. Genital pore 41–45% of proglottid length from anterior end. Gravid proglottids 1220–2200 long by 636–720 wide. Genital pore 36–41% of proglottid length from anterior end. Ovary 780–960 long by 420–580 wide. Vitelline follicles extending from level of ovarian isthmus to near anterior end. Uterus saccate with irregular shallow constrictions, occupying most available preovarian space. Eggs 10–20, unembryonated.

HOST: *Himantura schmardae*.

SITE: Spiral valve.

LOCALITY: Caribbean Sea, 15 km. west of La Cienaga, Magdalena, Colombia.

HOLOTYPE: USNM Helm. Coll. No. 73959. Paratypes: USNM Helm. Coll. No. 73960; Univ. Neb. State Mus., H. W. Manter Lab. No. 20259.

ETYMOLOGY: The generic name means *Acanthobothrium*-like, and is masculine in gender. The species is named in honor of Dr. Thomas B. Thorson, University of Nebraska.

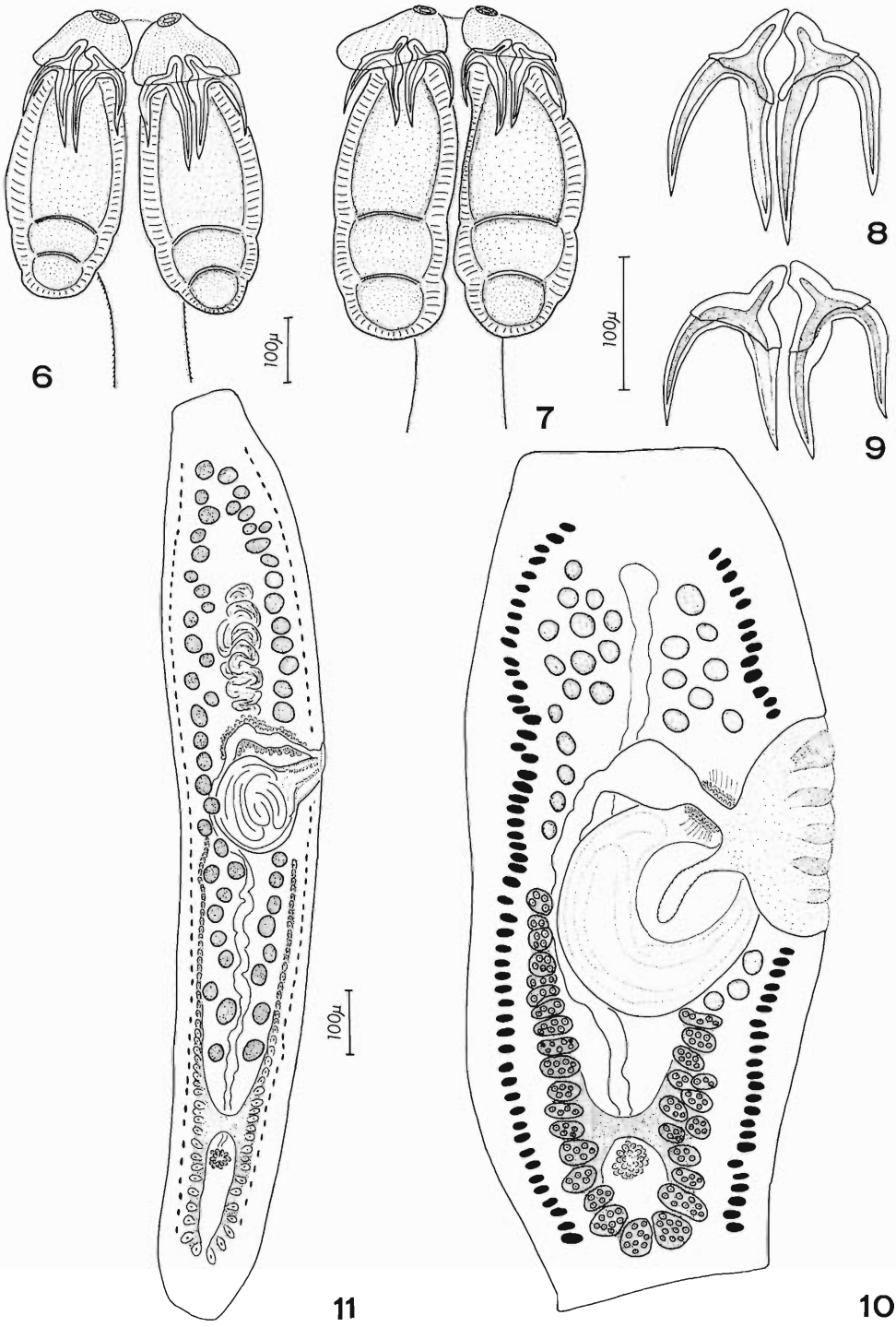
Three known genera of tetraphyllidean cestodes possess armed, triseptate bothridia, vitelline follicles restricted to the lateral margins of the proglottids, and ovaries which appear X-shaped when seen in cross-section: *Onchobothrium* Blainville, 1828; *Acanthobothrium* Beneden, 1850; and *Calliobothrium* Beneden, 1850. The morphology of the outer bothridial hooks of *Acanthobothroides thorsoni* is typical of species of *Acanthobothrium* while the inner hooks resemble those of members of *Onchobothrium*. The new genus is proposed because of this composite nature of the bothridial armature.

Acanthobothrium himanturi sp. n. (Figs. 7, 9, 11)

DESCRIPTION (n = number of measurements used): Strobila acraspedote, apolytic, 3.84–9.30 mm long; composed of 17–26 (n = 40) proglottids. Scolex 240–350 ($\bar{x}$ = 300, n = 20) long by 247–360 ($\bar{x}$ = 310, n = 20) wide, composed of four sessile, triseptate bothridia; each bothridium with apical sucker and pad, armed with pair of bifid hooks. Bothridia 297–432 ($\bar{x}$ = 372, n = 40) long by 111–185 ($\bar{x}$ = 161, n = 40) wide; anterior loculus 142–185 ($\bar{x}$ = 163) long, middle loculus 42–111 ($\bar{x}$ = 73), posterior loculus 31–86 ($\bar{x}$ = 64). Ratio of loculi lengths 1: 0.45: 0.40. Apical sucker 20–62 ($\bar{x}$ = 45) in diameter, pad 105–167 ($\bar{x}$ = 157) in diameter. Hook formula (modified from that of Euzet, 1956) for 40 hooks:

$$\frac{43\text{–}61\ (52 \pm 4)\quad 86\text{–}116\ (101 \pm 7)\quad 73\text{–}99\ (86 \pm 6)}{119\text{–}157\ (138 \pm 10)}.$$

Cephalic peduncle 0.3–1.0 mm long. Immature proglottids wider than long; mature ones 0.78–1.26 mm long by 0.18–0.34 mm wide. Testes in anterior ⅔ of proglottid, 38–57 (47 ± 5, n = 90) in number, 29–58 in diameter; 6–12 (9 ± 1.5) postporally, 9–17 (13 ± 2) preporally,



Figures 6–11. *Acanthobothrium himanturi* and *A. tasajerasi*. 6. Scolex of *A. tasajerasi*. 7. Scolex of *A. himanturi*. 8. Bothridial hooks of *A. tasajerasi*. 9. Bothridial hooks of *A. himanturi*. 10. Mature proglottid of *A. tasajerasi*. 11. Mature proglottid of *A. himanturi*.

18–30 (25 ± 2) antiporally. Cirrus sac near midproglottid, 120–180 long by 96–144 wide, containing spined eversible cirrus. Genital atrium indistinct. Genital pore 40–48% of proglottid length from anterior end, irregularly alternating. Vagina anterior to cirrus sac; vaginal sphincter present. Ovary near posterior end of proglottid, bilobed in frontal view, X-shaped in cross-section, 480–600 long by 120–180 wide at isthmus in terminal proglottids; ovarian lobes unequal length, aporal lobes reaching level of anterior margin of cirrus sac, poral lobes reaching posterior margin of cirrus sac. Isthmus near posterior end of ovary. Vitelline follicles extending from level of isthmus to near anterior end, 10–15 in diameter.

HOST: *Himantura schmardae.*

SITE: Spiral valve.

LOCALITY: Caribbean Sea, 15 km. west of La Cienaga, Magdalena, Colombia.

HOLOTYPE: USNM Helm. Coll. No. 73963. Paratypes: USNM Helm. Coll. No. 73964; Univ. Neb. State Mus., H. W. Manter Lab. No. 20260.

ETYMOLOGY: The specific name refers to the genus of host.

Acanthobothrium himanturi most closely resembles *A. brevissime* Linton, 1908 as redescribed by Goldstein (1964) and Campbell (1969) and *A. southwelli* Subhapradha, 1955. It differs from *A. southwelli* by possessing a much larger cirrus sac, an average 47 rather than 34 testes per proglottid, and unequal ovarian lobes. It differs from *A. brevissime* by having an average of 47 rather than 25 testes per proglottid, an indistinct genital atrium, and hooks averaging 138 micra in total length rather than 115 micra.

Acanthobothrium tasajerasi sp. n. (Figs. 6, 8, 10)

DESCRIPTION (n = number of measurements used): Strobila acraspedote, apolytic, 2.5–5.5 mm long, composed of 11–18 proglottids (n = 20). Scolex 309–384 ($\bar{x}$ = 340, n = 20) long by 240–371 ($\bar{x}$ = 310, n = 20) wide, composed of four sessile, triseptate bothridia; bothridia each with apical sucker and pad, armed with pair of bifid hooks. Bothridia 336–429 ($\bar{x}$ = 387, n = 10) long by 123–167 ($\bar{x}$ = 154, n = 10) wide; anterior loculus 175–215 ($\bar{x}$ = 190) long, middle loculus 80–130 ($\bar{x}$ = 100), posterior loculus 70–100 ($\bar{x}$ = 80). Ratio of loculi lengths 1: 0.5: 0.4. Apical sucker 37–62 ($\bar{x}$ = 50) in diameter, pad 111–185 ($\bar{x}$ = 130) in diameter. Hook formula (modified from that of Euzet, 1956) for 60 hooks:

$$\frac{52\text{–}64\,(58 \pm 3)\quad 110\text{–}130\,(119 \pm 4)\quad 90\text{–}114\,(102 \pm 6)}{152\text{–}178\,(165 \pm 6)}$$

Cephalic peduncle 144–240 long. Scolex and peduncle spinose. Immature proglottids wider than long; mature ones 468–840 long by 264–360 wide. Testes in anterior ⅔ of proglottid, 19–33 (26 ± 3, n = 70) in number, 15–30 in diameter; 2–5 (3 ± 1) postporally, 5–12 (9 ± 2) preporally, 9–20 (15 ± 2) antiporally. Cirrus sac spherical, 120–180 in diameter, containing spined eversible cirrus. Genital atrium prominent. Genital pore 37–43% of proglottid length from anterior end, irregularly alternating. Vagina anterior to cirrus sac, vaginal sphincter present. Ovary bilobed in frontal view, X-shaped in cross-section, 240–384 long by 60–144 wide at isthmus in terminal proglottids; isthmus not distinct; ovarian lobes unequal, aporal lobes reaching level of anterior margin of cirrus sac, poral lobes reaching posterior margin of cirrus sac. Vitelline follicles extending length of proglottid, 15–20 in diameter.

HOST: *Himantura schmardae.*

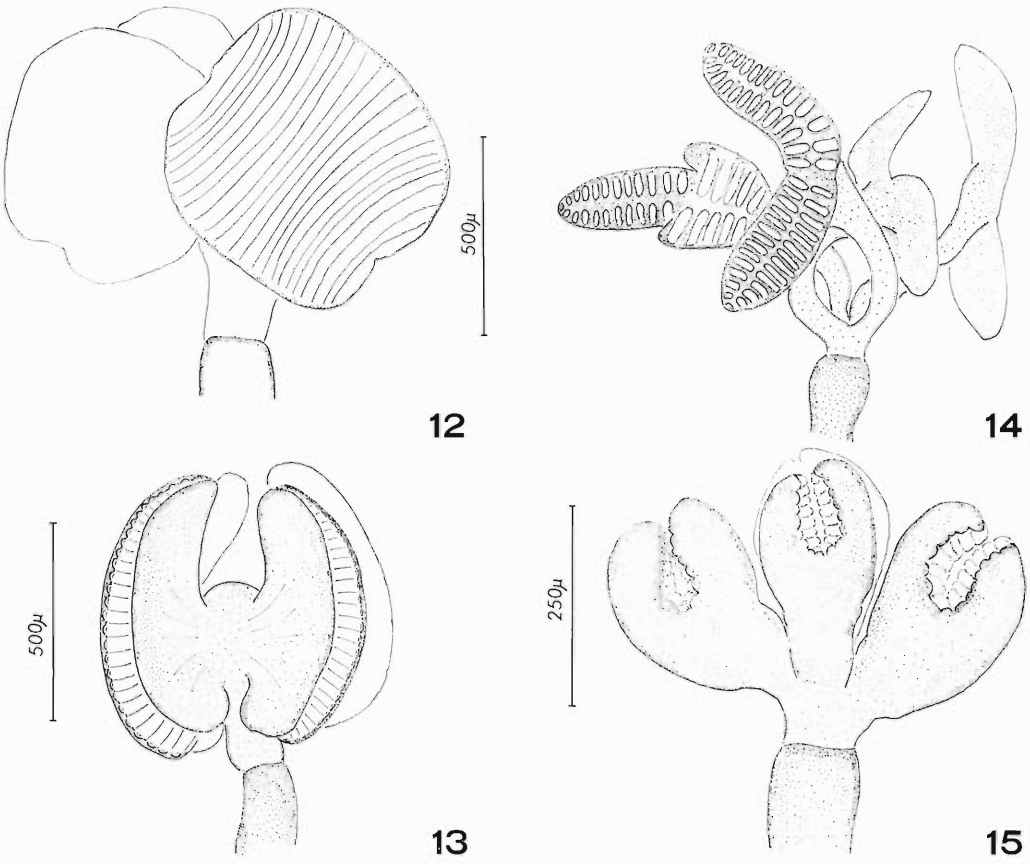
SITE: Spiral valve.

LOCALITY: Caribbean Sea, 15 km. west of La Cienaga, Magdalena, Colombia.

HOLOTYPE: USNM Helm. Coll. No. 73961. Paratypes: USNM Helm. Coll. No. 73962; Univ. Neb. State Mus., H. W. Manter Lab. No. 20261.

ETYMOLOGY: The specific name is derived from the Tasajeras Fishing Cooperative, where the author obtained the hosts.

This species also bears some resemblance to *Acanthobothrium brevissime.* It has a similar number of testes per proglottid, lacks a distinct ovarian isthmus, and has a prominent genital atrium. The hooks of *A. tasajerasi* average 165 micra in total length while those of *A. brevissime* average 115 micra. *Acanthobothrium tasajerasi* further differs from *A. dujardinii* by possessing a prominent genital atrium and lacking a distinct ovarian isthmus; additionally, *A. dujardinii* has hooks 180 to 210 micra long rather than 152 to 178 micra.



Figures 12–13. Scolex of *Caulobothrium anacolum*. 12. Bothridia relaxed. 13. Bothridia contracted. Figure 14. Scolex of *Rhinebothrium tetralobatum*. Figure 15. Scolex of *Rhinebothrium magniphallum*.

Caulobothrium anacolum sp. n. (Figs. 12–13, 19)

DESCRIPTION (n = number of measurements used): Strobila acraspedote, apolytic, 6.8–15.4 mm long, composed of 13–32 (n = 30) proglottids. Scolex with four pedicellated, quadrate bothridia; pedicels 50–150 long, wider at bothridium than at trunk; bothridia 648–804 long by 324–700 wide, with 22–23 transverse septa forming 23–24 total loculi; median longitudinal septum absent. Cephalic peduncle 120–360 long. Immature proglottids wider than long; mature ones 1.14–2.54 mm long by 0.38 mm wide. Testes in anterior ¾ of proglottid, 34–45 ($\bar{x}$ = 41, n = 20) in number, 54–138 in diameter; 10–14 (12) postporally, 5–7 (6) preporally, 18–27 (23) antiporally. Cirrus sac in anterior ⅓ of proglottid, 144–216 long by 108–178 wide, containing spined eversible cirrus. Genital atrium inconspicuous. Genital pore 19–29% of proglottid length from anterior end, irregularly alternating. Vagina anterior to cirrus sac; vaginal sphincter present; posterior portion expanded to form seminal receptacle. Ovary bilobed in frontal view, X-shaped in cross-section, follicular, 144–312 long by 96–300 wide at isthmus in terminal proglottids. Vitelline follicles extending length of proglottid, 12–60 in diameter.

HOST: *Himantura schmardae*.

SITE: Spiral valve.

LOCALITY: Caribbean Sea, 15 km. west of La Cienaga, Magdalena, Colombia.

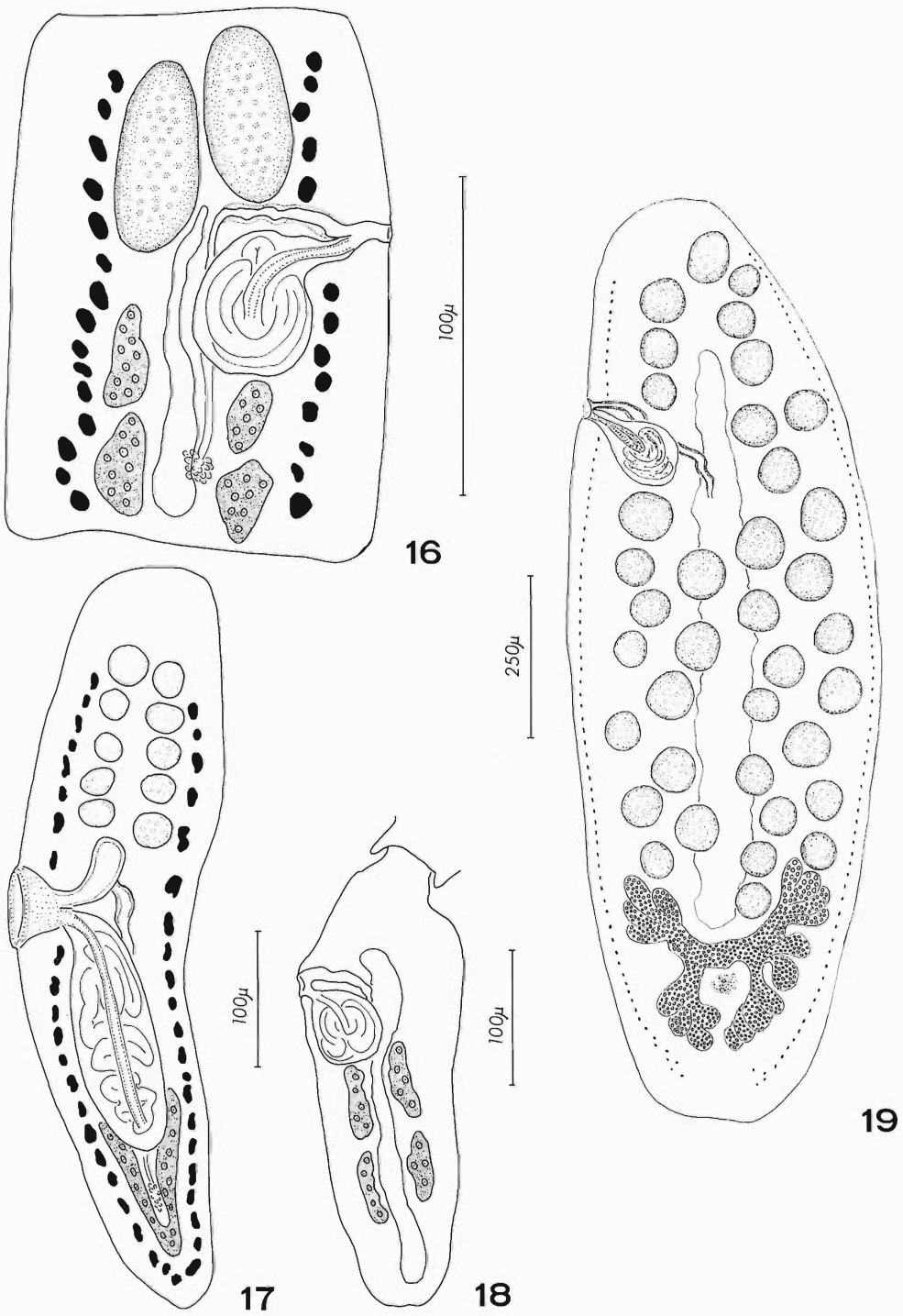


Figure 16. Mature proglottid of *Rhinebothrium tetralobatum*. **Figure 17.** Mature proglottid of *Rhinebothrium magniphallum*. **Figure 18.** Advanced mature proglottid of *Rhinebothrium tetralobatum*, drawn to same scale as 17. **Figure 19.** Mature proglottid of *Caulobothrium anacolum*.

HOLOTYPE: USNM Helm. Coll. No. 73969. Paratypes: USNM Helm. Coll. No. 73970; Univ. Neb. State Mus., H. W. Manter Lab. No. 20265.

ETYMOLOGY: The specific name is derived from Greek "anakolos" meaning stunted or shortened, and refers to the relative length of the peduncle.

Baer (1948) erected the genus *Caulobothrium* for species resembling *Rhinebothrium* but possessing extremely long cephalic peduncles, craspedote proglottids, and postvaginal testes. Five species have previously been described. *Caulobothrium anacolum* differs from all five by having a cephalic peduncle which is considerably shorter than the strobila, acraspedote proglottids, and quadrate rather than elongate bothridia which lack a median longitudinal septum, but is retained in the genus since it does possess postvaginal testes and a cephalic peduncle of some size. *Caulobothrium insignia* (Southwell, 1911) Baer, 1948 has 18 to 26 testes per proglottid while *C. anacolum* has 34 to 45; all other species have more than 100. The new species bears some resemblance to *C. opisthorchis* Riser, 1955 in size of strobila and number of proglottids, and in position of genital pore.

Rhinebothrium magniphallum sp. n. (Figs. 15, 17)

DESCRIPTION (n = number of measurements used): Strobila acraspedote, apolytic, 2.28–3.06 mm long, composed of 8–12 (n = 40) proglottids. Scolex composed of four pedicellated, bilobed, elongate bothridia with indistinct hingelike constriction between lobes; pedicels up to 22 long; bothridia 264–488 long by 128–144 wide, divided transversely by 15–17 transverse septa forming 16–18 total loculi; median longitudinal septum absent. Cephalic peduncle 24–60 long. Immature proglottids wider than long to longer than wide; mature ones 444–912 long by 108–204 wide. Testes in anterior ½ of proglottid, in two longitudinal rows, 10–15 ($\bar{x}$ = 12, n = 21) in number, 17–41 in diameter. Cirrus sac at midproglottid, 145–195 long by 46–75 wide, containing spined eversible cirrus. Genital atrium prominent. Genital pore 36–48% of proglottid length from anterior end, irregularly alternating. Vagina anterior to cirrus sac; vaginal sphincter present. Ovary near posterior end of proglottid, lobes fused posteriorly, X-shaped in cross-section, 116–238 long by 29–87 wide at isthmus in terminal proglottids. Vitelline follicles extending entire length of proglottid, may be confluent posterior to ovary, 10–35 in diameter.

HOST: *Himantura schmardae*.

SITE: Spiral valve.

LOCALITY: Caribbean Sea, 15 km. west of La Cienaga, Magdalena, Colombia.

HOLOTYPE: USNM Helm. Coll. No. 73965. Paratypes: USNM Helm. Coll. No. 73966; Univ. Neb. State Mus., H. W. Manter Lab. No. 20262.

ETYMOLOGY: The species name refers to the relative size of the cirrus sac.

Rhinebothrium magniphallum resembles *R. monodi* Euzet, 1954 (17) and *R. scobinae* Euzet and Carvajal, 1973 (19) in number of bothridial loculi; *R. hawaiiensis* Cornford, 1974 (11–13), *R. euzeti* Williams, 1958 (12) and *R. cadenati* Euzet, 1954 (12–14) in number of testes per proglottid; and *R. scobinae* and *R. cadenati* in lacking a median longitudinal septum in each bothridium. The cirrus sac of *R. magniphallum* is relatively much larger than that of any other species.

Rhinebothrium tetralobatum sp. n. (Figs. 14, 16, 18)

DESCRIPTION (based on six specimens): Strobila craspedote, apolytic, 15–30 mm long, composed of 82–100 proglottids. Scolex composed of four pedicellated, bilobed, canoe-shaped bothridia with distinct hingelike constriction between lobes; pedicels 144–200 long; bothridia 564–804 long by 228–264 wide, divided longitudinally by median septum, transversely by 25–27 septa forming two parallel rows of 24–26 loculi with single loculus at tip of each lobe; total number of loculi 50–54. Cephalic peduncle 96–144 long. Immature proglottids wider than long; mature ones 360–564 long by 96–156 wide. Testes in anterior ⅓ to ½ of proglottid, 2 in number, 35–58 in diameter. Cirrus sac in anterior ½ of proglottid, spherical, 50–70 in diameter, containing spined eversible cirrus. Genital atrium indistinct, 44–48% of proglottid length from anterior end, irregularly alternating. Ovary composed of paired anterior and posterior lobes in frontal view, paired dorsal and ventral lobes in cross-

section, 198–300 long by 60–90 wide at isthmus in terminal proglottids; isthmus indistinct. Vitelline follicles extending length of proglottid, 30–40 in diameter.

HOST: *Himantura schmardae.*

SITE: Spiral valve.

LOCALITY: Caribbean Sea, 15 km. west of La Cienaga, Magdalena, Colombia.

HOLOTYPE: USNM Helm. Coll. No. 73967. Paratypes: USNM Helm. Coll. No. 73968; Univ. Neb. State Mus., H. W. Manter Lab. No. 20253, 20266.

ETYMOLOGY: The specific name refers to the four-lobed appearance of the ovary.

By having two testes per proglottid and craspedote proglottids, *Rhinebothrium tetralobatum* closely resembles *R. spinicephalum* Campbell, 1970. It differs from that species by having 50 to 54 bothridial loculi and 82–100 proglottids per strobila rather than 34 to 36 loculi and 36 to 49 proglottids. *Rhinebothrium himanturi* Williams, 1964, *R. burgeri* Baer, 1948, and *R. lintoni* Campbell, 1970 all have similar numbers of bothridial loculi. The ovary of *R. tetralobatum* is similar to that of *R. lintoni*, but has only four lobes rather than six to eight.

Acknowledgments

The author expresses appreciation to Dr. Thomas B. Thorson for providing the opportunity to collect the specimens; Alvaro Boada, INDERENA, Ministry of Agriculture in Santa Marta for his aid in procuring the hosts; Tom E. Mattis for the loan of specimens of *Rhinebothrium lintoni* and his helpful suggestions; Dr. Ronald A. Campbell for his aid and suggestions; Dr. Robin M. Overstreet for his aid in the preparation of this manuscript; and Ms. Anne Langenfeld for her aid in assembling the plates.

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In Memoriam

MARIO MOLLARI

February 18, 1977

Member 1928–1961

Hymenolepis asketus sp. n. (Cestoidea: Hymenolepididae) from the short-tailed shrew, *Blarina brevicauda* Say, from Nebraska

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ABSTRACT: *Hymenolepis asketus* sp. n. from *Blarina brevicauda* in Nebraska most closely resembles *H. longi*, *H. pauciproglottis*, and *H. parvissima* but differs by having a terminal proglottid which is as large as the rest of the strobila. It resembles *H. parvissima* by having 10 rather than 8 or 12 rostellar hooks and *H. longi* in hook morphology.

Examination of frozen intestinal tracts of the short-tailed shrew, *Blarina brevicauda* Say collected in Nebraska produced specimens of an extremely small hymenolepidid cestode. Sixteen of the specimens were suitable for study and form the basis of this report. Worms were removed from the intestine and stored in 70% ethanol, then stained with Mayer's hematoxylin and mounted in Canada balsam. Measurements of rostellar hooks are given as the following lengths: A–D, B–C, B–D (Fig. 1). Measurements are in micra unless otherwise stated; figures were drawn with the aid of a drawing tube.

Hymenolepis asketus sp. n. (Figs. 2–5)

DESCRIPTION (based on 16 specimens): Strobila 1.2–2.0 mm long, composed of 6–8 proglottids. Scolex 136–142 in width, with four suckers and armed retractable rostellum; suckers in dorsal and ventral pairs, 52–87 long by 35–52 wide; rostellar bulb 40–75 long by 29–61 wide; rostellar hooks 10, measuring 13–18, 10–14, 11–15. Immature proglottids 29–73 long by 67–160 wide. Mature proglottids 85–157 long by 87–275 wide. Testes three, linear, 15–58 long by 23–52 wide. Cirrus sac near anterior end of proglottid, 73–160 long, extending slightly beyond midline, containing spined eversible cirrus and internal seminal vesicle; cirrus spines 2–3 long. External seminal vesicle saccate, 20–30 long. Genital pore ⅐ proglottid length from anterior end, irregularly alternating. Vagina posterior to cirrus sac; vaginal sphincter present. Ovary dumbbell-shaped, 29–52 long by 73–174 wide. Vitellarium submedian, compact, pretesticular, 30–58 in diameter. Two testes porally, one aporally to vitellarium. Gravid proglottids 238–1015 long by 168–450 wide. Uterus saccate, occupying all available space. Eggs 23–35 in diameter; oncospheres 20–29 in diameter.

HOST: *Blarina brevicauda* Say, short-tailed shrew.

SITE: Intestine.

LOCALITY: vic. Lincoln, Nebraska.

HOLOTYPE: USNM Helm. Coll. No. 73971. Paratypes: USNM Helm. Coll. No. 73972; Univ. Neb. State Mus., H. W. Manter Lab. No. 20284.

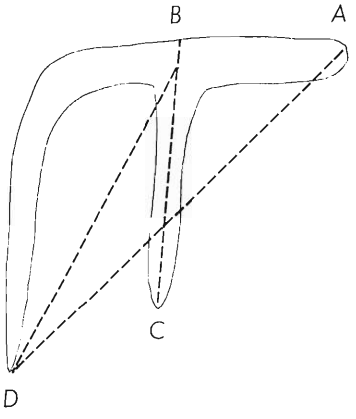
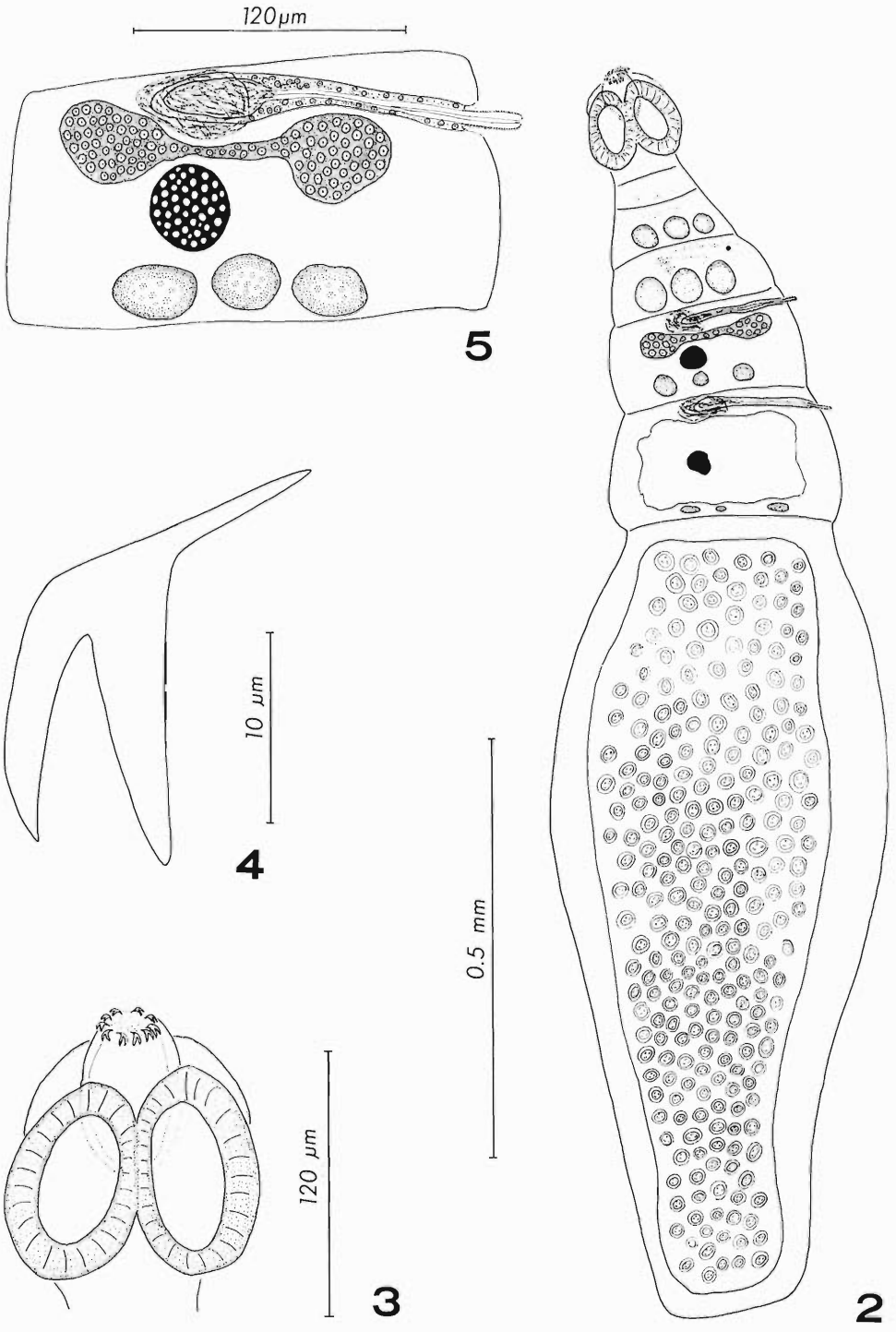


Figure 1. Generalized rostellar hook of *Hymenolepis* spp.



Figures 2–5. ***Hymenolepis asketus.*** **2. Entire worm, holotype. 3. Scolex. 4. Rostellar hook. 5. Mature proglottid.**

ETYMOLOGY: The specific name is derived from Greek "asketos," meaning little gem, and is masculine in gender.

Hymenolepis asketus most closely resembles *H. longi* Oswald, 1951, *H. pauciproglottis* Neiland, 1952, and *H. parvissima* Voge, 1953. It differs from *H. longi* by having 10 rather than 8 rostellar hooks which are smaller (13–18 vs. 20–24) and a dumbbell-shaped rather than flat ovary. *Hymenolepis pauciproglottis* has 12 rostellar hooks 12 to 14 micra long and a median rather than submedian vitellarium; *H. parvissima* has 10 hooks 20 to 24 micra long and a rostellar bulb which is relatively much larger than that of *H. asketus*. The new species differs from all three by having a terminal gravid proglottid which is as large or larger than the rest of the strobila. The rostellar hooks of *H. longi* are similar in shape to those of *H. asketus*.

Oswald (1951) described *Hymenolepis longi* from the smoky shrew *Sorex fumeus* in Tennessee. Neiland (1953) reported the species from *Sorex bendirei palmeri* in Oregon but stated that his specimens uniformly possessed 10 rather than 8 rostellar hooks; he suggested that closer study of the specimens might show them to be another species. Senger (1955) stated that Neiland's specimens were probably *H. parvissima* which Voge (1953) had described earlier from *S. bendirei bendirei* in California. We have not examined any of Neiland's material, but in view of the close similarity in hook shape between *H. longi* and *H. asketus*, it is possible that Neiland's specimens were the latter.

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Parasites of the Clapper Rail, *Rallus longirostris* Boddaert. III. Description of *Notocotylus schmidti* sp. n. (Digenea: Notocotylidae)[1]

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ABSTRACT: *Notocotylus schmidti* sp. n. is described from the clapper rail, *Rallus longirostris*, from Mississippi and Georgia. It is most similar to *N. gibbus*, *N. pacifera*, and *N. regis* from which it differs by having four ventral glands in each of two outer rows and three glands in the inner row. By having a short esophagus, vitellaria extending into the anterior ⅓ of the body, and lobate gonads, *N. schmidti* most closely resembles *N. regis*, but has a shorter, stouter cirrus and cirrus sac, and is less than ½ as large.

Twenty-seven species of digeneans, four of cestodes, one of nematode, and one of acanthocephalan have been reported from clapper rails, *Rallus longirostris* Boddaert from North America (Heard, 1968, 1970; Deblock and Heard, 1969; Nickol and Heard, 1970; Bates and Meade, 1972). One of the digeneans, *Notocotylus* sp. of Heard, 1970, is herein described as new.

Materials and methods follow those of Heard (1968, 1970). All measurements are in micra unless otherwise noted; figures were drawn with the aid of a drawing tube.

Notocotylus schmidti sp. n. (Figs. 1–4)

DESCRIPTION (based on 25 specimens): Monostomate. Body robust, ovoid, 1.80–2.40 mm long by 0.90–1.45 mm wide; widest point 60–75% of body length from anterior end. Tegument aspinous, with three longitudinal rows of ventral glands in posterior ⅔ of body; outer rows comprising 4 glands, inner 3. Oral sucker subterminal, 171–247 in diameter. Esophagus 38–76 long; cecal bifurcation 13–16% of body length from anterior end, ceca extending to within 6–10% of body length from posterior end.

Testes symmetrical, extracecal, highly lobed, 12.5–17.5% of body length from posterior end; left testis 285–380 long by 228–304 wide, right testis 247–437 by 228–304. Cirrus sac postbifurcal, 247–418 long by 114–248 wide, containing seminal vesicle, prostatic complex and eversible cirrus; everted cirrus 165 long by 61 wide, tuberculated. Genital pore immediately postbifurcal.

Ovary intercecal, deeply lobed, between testes, 209–323 long by 190–323 wide. Ootype immediately anterior to ovary; Mehlis' gland and Laurer's canal present. Uterus a series of transverse loops occupying intercecal space between ootype and posterior margin of cirrus sac; space occupied equal to 32–41% of total body length. Metraterm 285–456 long. Vitellaria follicular, extending from 27–46% of body length from anterior end to 20–27% of body length from posterior end; some follicles ventral to testes; vitelline ducts anterior to ovary, reservoir median. Eggs 20–23 long by 11–14 wide, filaments 4–6 times longer than eggs.

Excretory pore subterminal, dorsal, median; excretory vesicle saccate, anterior extent not seen.

HOST: *Rallus longirostris* Boddaert, clapper rail.

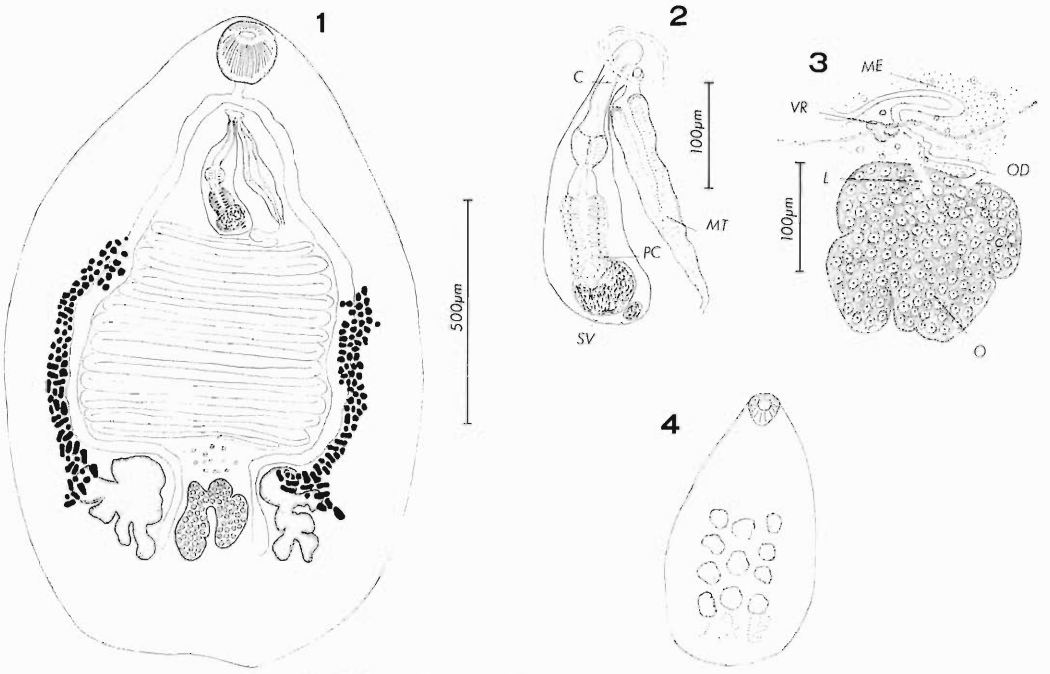
LOCALITIES OF TYPE MATERIAL: Ocean Springs, Mississippi (type locality); Savannah, Georgia.

SITE: Intestinal ceca.

HOLOTYPE: USNM Helm. Coll. No. 73973. Paratypes: USNM Helm. Coll. No. 73974; Univ. Neb. State Mus. H. W. Manter Lab. No. 20282, 20283.

ETYMOLOGY: This species is named in honor

[1] This study was conducted in cooperation with the U.S. Department of Commerce, NOAA, National Marine Fisheries Service, under PL 88-309, Project No. 2-262-R and NOAA, Office of Sea Grant, under Grant No. 04-6-158-44060. The U.S. Government is authorized to produce and distribute reprints for governmental purposes notwithstanding any copyright notation that may appear hereon.



Figures 1–4. ***Notocotylus schmidti.*** **1. Ventral view of holotype. 2. Terminal genitalia. 3. Ootype region. 4. Freehand drawing of ventral surface of living worm. Legend: C = cirrus; L = Laurer's canal; ME = Mehlis' gland; MT = metraterm; O = ovary; OD = oviduct; PC = prostate complex; SV = seminal vesicle; VR = vitelline reservoir.**

of Dr. Gerald D. Schmidt in recognition of his contributions to helminth taxonomy.

Notocotylus schmidti is most similar to *N. gibbus* (Mehlis in Creplin, 1846) Kossack, 1911, *N. pacifera* (Noble, 1933) Harwood, 1939, and *N. regis* Harwood, 1939. Lumdsen and Zischke (1963) reported the latter two species from birds in Louisiana and discussed the taxonomic status of *N. gibbus*, *N. pacifera*, and *N. regis*. The new species differs from the previous three in the number and arrangement of the ventral glands: *N. schmidti* has four glands in the outer rows and three in the inner; *N. pacifera* has 11 in the outer rows and 4 in the inner; *N. gibbus* has 6 to 8 in each row; and *N. regis* has 10 in each row. *Notocotylus schmidti* further differs from *N. gibbus* and *N. pacifera* by having a relatively shorter esophagus, vitelline follicles which extend further anteriorly, and highly lobate gonads. It resembles *N. regis* from *Rallus elegans* in Texas and Louisiana and *R. longirostris* in Mississippi in the above characteristics, but is less than half as large. The single specimen of *N. regis* collected from *Rallus longirostris* in Mississippi and reported by Heard (1970) is more than 5 mm long, while no specimen of *N. schmidti* found was more than 2.4 mm long; additionally, the cirrus sac of our specimen of *N. regis* is relatively more elongate than that of *N. schmidti*, and contains a cirrus which is much longer and more slender. *Uniserialis breviserialis* Stunkard, 1967 has a similar number and arrangement of ventral glands as *N. schmidti*, but has the genital pore located near the oral sucker and a much-abbreviated cirrus sac.

Much of the taxonomy of notocotylids is based on the structure and arrangement of the ventral glands, which are difficult or impossible to observe in mounted specimens. Harwood (1939) described *Notocotylus porzanae* which differs from *N. regis* only by possessing an apparently smaller number of ventral glands in the middle row of glands; Harwood (1939)

reported that it was not possible to ascertain the number of glands in the middle row of his specimens. Clearly, these two species need to be re-examined.

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Allocorrigia filiformis gen. et sp. n. (Trematoda: Dicrocoeliidae) from the Crayfish, *Procambarus clarkii* (Girard, 1852)

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ABSTRACT: *Allocorrigia filiformis* gen. et sp. n. is described from infections in the antennal glands of 57 of 88 crayfish, *Procambarus clarkii*, collected in the vicinity of Baton Rouge, Louisiana. Unlike most dicrocoeliids, this species attains sexual maturity in an invertebrate host. *Allocorrigia* resembles *Corrigia* Strom, 1940, but differs in the relative size of its suckers, extent of the ceca and uterus, the absence of a seminal receptacle and character of the vitellaria.

Dicrocoeliid trematode infections have been noted in the swamp crayfish, *Procambarus clarkii* (Girard, 1852) collected in the vicinity of Baton Rouge, Louisiana. Of the 88 specimens of *P. clarkii* examined, 57 had one to three live worms threaded through the interstices of their antennal or green glands. In 34 of the 57 crustaceans infected, both glands were involved. The worms ranged in development from mature to fully gravid. Other species of crayfishes examined from the same or similar habitats have not been found to be infected with this species of worm. Worms were dissected out of the antennal gland and studied live or fixed under coverslip pressure in AFA and examined as whole mounts. Infected, whole antennal glands were removed and studied in section. The following description is based on 20 worms. The drawing was made with the aid of a microprojector and all measurements are given in microns unless indicated otherwise.

Allocorrigia gen. n.

DESCRIPTION: Body filiform, spinous. Acetabulum very near anterior end, postbifurcal, smaller than oral sucker. Prepharynx short. Esophagus short or absent. Ceca to near posterior end. Genital pore at level of or slightly anterior to cecal bifurcation. Common genital

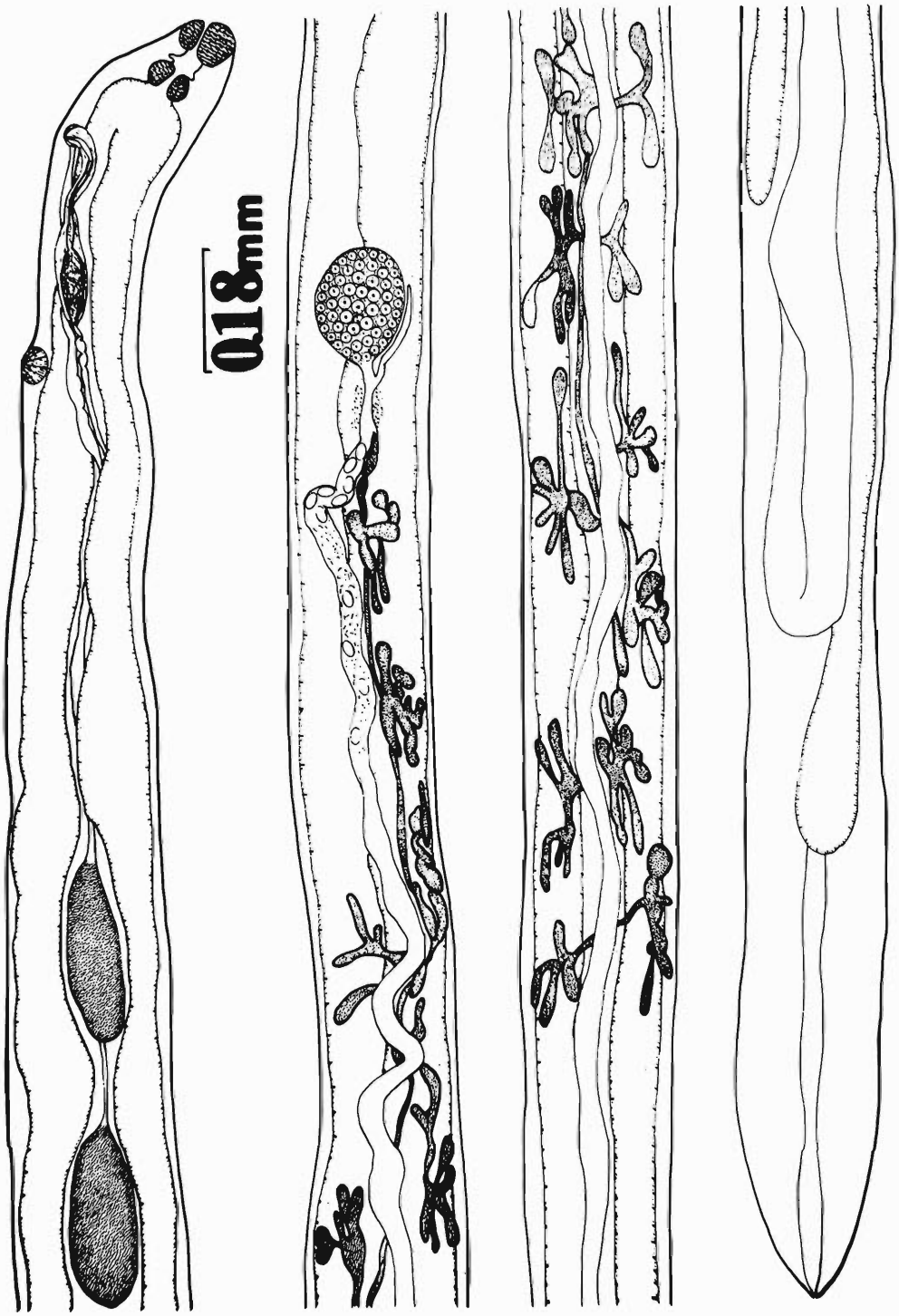

Figure 1. ***Allocorrigia filiformis*** **gen. et sp. n. from** ***Procambarus clarkii.*** **Adult worm.**

atrium present. Cirrus pouch preacetabular, seminal vesicle internal. Testes separate, tandem in anterior fourth of body. Ovary posttesticular in anterior of mid-third of body. Seminal receptacle absent. Laurer's canal present. Uterus with single posterior and anterior loop. Vitellaria postovarian with lobed follicles confined to mid-third of body. Excretory bladder tubular, reaching level of vitelline field.

Allocorrigia filiformis sp. n. (Fig. 1)

DESCRIPTION: Body filiform, 6.29–11.6 mm long by 0.09–0.28 mm wide. Tegument with minute spines extending only to level of pharynx. Anterior sucker terminal, 52–100 by 56–112. Mouth slightly subterminal. Acetabulum in anterior sixteenth of body, postbifurcal, weakly formed, 44–78 by 42–70. Prepharynx short, 54 by 50. Pharynx muscular, 48 by 68. Esophagus short or absent. Ceca unequal in length, exceeding vitelline field and extent of uterus but not reaching posterior end of body. Genital pore median at level of or slightly anterior to cecal bifurcation. Common atrium present. Cirrus pouch preacetabular, elongate, 291 by 43. Seminal vesicle elongate, cirrus not spined. Testes elongate, tandem in anterior fourth of body, not separated by uterine coils. Anterior testis, 256 by 111. Posterior testis, 307 by 114. Vasa efferentia unite midway before entering cirrus pouch. Ovary posttesticular, in anterior of mid-third of body, 233 by 150. Seminal receptacle absent, basal portion of uterus congested with sperm. Laurer's canal present, directed anteriorly and extending to mid level of ovary. Uterus with single loop extending to near posterior end of body before proceeding anteriorly as a single loop. Eggs 25–30 by 12–14. Vitellaria postovarian with large lobose follicles confined to mid-third of body. Mehlis' gland poorly defined. Excretory bladder tubular, extending to level of vitelline field before receiving primary trunks.

TYPE HOST: *Procambarus clarkii* (Girard, 1852).

HABITAT: Antennal gland.

TYPE LOCALITY: Sorrento, Ascension Parish, Louisiana.

TYPE SPECIMENS: USNM Helm. Coll.: Holotype: No. 74053.

PARATYPES: No. 74054.

Discussion

On morphological grounds, *Allocorrigia filiformis* most nearly resembles members of the genus *Corrigia* Strom, 1940. It differs, however, in the following characters: anterior sucker larger than acetabulum; ceca not extending to posterior extremity; seminal receptacle absent; vitellaria large, lobose follicles; uterus not exceeding posterior level of ceca. There is a general similarity between *Allocorrigia* and *Skrjabinosomum* Evranova, 1944, but a disparity exists in relative size of the acetabulum. Further comparison will be difficult until more descriptive information becomes available relative to the latter genus.

Allocorrigia filiformis is one of the few dicrocoeliids known to attain maturity and reproductivity in an invertebrate host. A similar kind of infection was described from Malaysia by Macy and Basch (1972) in which a dragonfly served as host. Like the species in Malaysia, *A. filiformis* is not encysted within the arthropod. Unlike the Asian form, it would not seem necessary for the crayfish to be eaten by a predator before the eggs could be liberated. Eggs containing active miracidia were frequently observed in the gravid worms, and in sectioned antennal glands a few eggs were observed in the excretory tubules. It could be assumed that eggs readily pass from the crayfish by way of the excretory pore of the antennal gland. The possible role of a vertebrate cannot be completely ruled out, however, for what we know of other dicrocoeliid life cycles indicates a strong linkage between an arthropod and vertebrate host. In any event, it is probable that an aquatic snail host is involved in the cycle and, as pointed out by Macy and Basch (1972), even though most dicrocoeliids have a terrestrial cycle, closely related plagiorchiids use an aquatic mollusc.

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Studies on the Population Structures of Two Species of *Haematoloechus* Looss, 1899 (Digenea: Plagiorchiidae) in Raniid Frogs in New Mexico

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ABSTRACT: Three hundred and seventy-four *Rana catesbeiana* and 112 *Rana pipiens* collected from Sierra County, New Mexico were examined for *Haematoloechus breviplexus* and *Haematoloechus coloradensis* respectively. *Rana catesbeiana* smaller than 20 and larger than 460 g, and *R. pipiens* smaller than 10 and larger than 150 g were not infected with lung flukes. One hundred and seventy-three *R. catesbeiana* collected were males of which 92 (53%) were infected with *H. breviplexus* and 201 were females of which 110 (55%) were infected. Fifty-one *R. pipiens* collected were males of which 37 (73%) were infected with *H. coloradensis* and 61 were females of which 43 (70%) were infected. Stomach analysis indicated there was a relationship between the volume of second intermediate hosts present in ingesta and the incidence and intensity of *Haematoloechus* spp. infections in these two species of frogs. Incidence and intensity of infections and the percentage of odonates by volume in ingesta increased from zero with size in smaller frogs, reached a high in medium sized frogs and then decreased to zero again with size in the largest frogs. Twenty-six *R. catesbeiana* smaller than 20 g and 22 *R. pipiens* smaller than 10 g exposed in the laboratory became infected with their respective lung flukes, while none of eight *R. catesbeiana* larger than 460 g and four *R. pipiens* larger than 200 g became infected. These results along with field observations indicated that in addition to food preferences, other factors such as physiological status, age, immunological responses, seasonal timing and inter- and intraspecific competition, may affect the susceptibility of some sizes of the frogs.

Relationships between parasite burdens and size (age) of definitive hosts have been observed by many parasitologists. However, relatively few studies have examined these relationships in detail. Dogiel (1966) and more recently, Noble and Noble (1971) have published general accounts of the literature concerning the ecology of parasites, which included sections on these relationships.

Many definitive hosts become infected with their parasites *via* food chains. There is little doubt that in such cases the feeding habits or preferences of hosts play a major role in both incidence and intensity of infection. Turner (1958) reported that larger *Rana pretiosa* had more parasites than smaller ones. He felt this was in part due to feeding preferences of the frog. As Dogiel (1966) has summarized, some parasitologists have suggested the following general ideas: (1) the density of parasites will increase with the quantity of food ingested by the host; (2) the number of some of the parasites present will increase as the host becomes older while others will decrease in number and (3) not all age groups of definitive hosts are necessarily parasitized by the same species of parasites.

Another area of interest to parasitologists is the influence of the sex of the host on incidence and intensity of infection. Hollis (1972) demonstrated a significant difference between the burdens of *Haematoloechus medioplexus* in male and female frogs.

The objectives of this study were: (1) to examine the relationship between the size (age) and feeding preferences of *Rana catesbeiana* and *R. pipiens* and the incidence and intensity of infection for their respective lung flukes, *Haematoloechus breviplexus* and *H. coloradensis*; and (2) to examine the relationship between the sex of these frogs and the incidence and intensity of infection. The life cycles of these two lung flukes have been reported by Schell (1968) and Dronen (1975a) respectively.

Materials and Methods

Preliminary field observations in southern New Mexico indicated that *R. catesbeiana* smaller than 20 and larger than 460 g, and *R.*

Table 1. Infections of Experimental Definitive Hosts with *Haematoloechus breviplexus* and *Haematoloechus coloradensis*.*

Definitive host	Frog wt (g)	*Haematoloechus* species	Odonate hosts	Length of infection (days)	No. of flukes recovered
R. catesbiana[1]	18.2	*H. breviplexus*	*Libellula* sp.	5	14
	18.5			5	9
	18.5			5	9
	17.5			5	12
	19.2			5	21
	18.3			5	17
	18.4			30	23
	18.1			30	10
	19.8			30	16
	17.9			30	21
	468.3			5	0
	475.1			5	0
	487.1			5	0
	491.2			5	0
	466.1			30	0
	470.0			30	0
	497.0			30	0
	512.3			30	0
R. catesbeiana[2]	17.7	*H. breviplexus*	*Libellula* sp.	5	23
	17.8			5	12
	17.9			5	16
	17.9			5	11
	17.8			30	12
	17.9			30	19
	17.9			30	13
	18.1			30	14
	18.2			30	9
	18.2			30	6
	18.3			30	11
	18.3			30	15
	18.4			30	17
	18.5			30	24
	18.8			30	10
	19.1			30	16
R. pipiens[1]	7.1	*H. coloradensis*	*Anax* sp.	5	2
	7.3			5	6
	7.4			5	3
	8.7			5	18
	9.3			5	10
	7.8		*Enallagma* sp.	30	14
	8.1			30	17
	8.2			30	18
	8.9			30	12
	9.4			30	20
	7.2		*Tramea* sp.	30	13
	7.4			30	10
	7.6			30	7
	7.8			30	11
	213.8		*Anax* sp.	30	0
	241.3			30	0
	253.5			30	0
	271.1			30	0
R. pipiens[2]	8.7	*H. coloradensis*	*Tramea* sp.	5	18
	8.8			5	13
	8.9			5	21
	8.3		*Anax* sp.	5	10
	9.9		*Enallagma* sp.	5	7
	8.5		*Anax* sp.	30	12
	8.8			30	12
	8.8			30	17
	8.9			30	14
	9.7		*Enallagma* sp.	30	4

* All metacercariae used in infection of experimental hosts were 10 to 12 days old.
[1] Definitive hosts were collected from nature.
[2] Definitive hosts were laboratory reared.

pipiens smaller than 10 and larger than 150 g, were rarely infected in nature with *Haematoloechus* spp.

In the current study a total of 374 *Rana catesbeiana* Shaw, ranging from 8 to 532 g, and 112 *Rana pipiens* Schreber, ranging from 7 to 382 g, were collected over a two year period from a series of 11 permanent ponds in Sierra

Table 2. The incidence and intensity of *Haematoloechus breviplexus* of *Rana catesbeiana* (in 40 g increments) compared to the percent odonates ingested. Frogs larger than 460 g and smaller than 20 g were not infected at this research area.

Host weights (g)	Per cent infected	No. of flukes per frog	No. of flukes per infected frog	Per cent odonates in ingesta	Sample size
L.T. 20	0	0		0	39
20 to 59.9	57	5.1	10.3 (7 to 25)	21	107
60 to 99.9	87	6.5	8.3 (3 to 20)	31	71
100 to 139.9	91	10.0	11.1 (2 to 30)	29	20
140 to 179.9	88	8.2	9.4 (6 to 19)	23	16
180 to 219.9	79	9.8	13.7 (5 to 21)	20	28
220 to 259.9	53	6.1	11.0 (4 to 19)	13	18
260 to 299.9	34	2.2	6.6 (3 to 11)	8	15
300 to 339.9	42	1.2	2.8 (1 to 4)	4	14
340 to 379.9	18	0.9	6.0 (3 to 11)	2	13
380 to 419.9	17	0.4	3.2 (2 to 5)	0	8
420 to 459.9	8	0.7	7.7 (3 to 15)	4	11
G.T. 459.9	0	0		0	14

L.T. = less than, G.T. = greater than, g = grams, No. = number.

County, New Mexico. All frogs were necropsied within 20 hr after collection, catalogued by body weight and sex, and the number of *Haematoloechus* spp. present in the lungs recorded. The stomach contents of frogs were analyzed to determine the percentage by volume of nymphal and adult odonates ingested by different weight classes of these frogs, since odonates are the second intermediate host for these parasites.

To determine the susceptibility of bullfrogs in these size categories, 26 weighing less than 20 g (16 laboratory reared and 10 from nature), were exposed to *H. breviplexus* metacercariae (Table 1). Also, 8 bullfrogs from nature, weighing more than 460 g were exposed to *H. breviplexus* (Table 1).

Similarly, 22 *R. pipiens*, weighing less than 10 g (10 laboratory reared and 12 from nature), were exposed to *H. coloradensis* (Table 1). Also, 4 leopard frogs from nature weighing more than 200 g were exposed to *H. coloradensis* (Table 1). Uninfected odonate nymphs collected in early spring and maintained in the laboratory were exposed to the cercariae of each parasite to establish a source of metacercariae for exposure of frogs. These odonates were maintained in the laboratory for 10 to 12 days after exposure before being force fed to experimental frogs as shown in Table 1. All experimental frogs from nature were collected in early spring when metacercariae of *Haematoloechus* spp. could not be demonstrated in odonates, and kept for at least two weeks prior to exposure to allow any flukes which might have been acquired naturally to mature enough to be separated from experimental infections (Dronen, 1975b). Frogs were necropsied at the intervals shown in Table 1. Relationships between host sizes and data were examined by a simple linear regression.

Results

Although *R. pipiens* has been reported as a suitable host for *H. breviplexus*, in this study area *H. breviplexus* occurred only in *R. catesbeiana* and *H. coloradensis* occurred only in *R. pipiens*. No other parasites were found in the lungs of these hosts. None of the *R. catesbeiana* smaller than 20 g and larger than 460 g or *R. pipiens* smaller than 10 g and larger than 150 g collected from the New Mexico research area were infected with these two species of *Haematoloechus*. However, an additional *R. catesbeiana* weighing 694 g which I collected from Brazos County, Texas was infected with three large adult specimens of what appeared to be *Haematoloechus breviplexus*.

The incidence and intensity of infection with *H. breviplexus* and *H. coloradensis* as a whole had neither a significant positive nor a negative correlation with increasing size (age) of frogs. Rather, the incidence of infection, the number of parasites per frog and the percent by volume of odonates in ingesta of frogs increased with size (age) for both species of *Haematoloechus* in smaller frogs, reached a high in medium sized frogs, than decreased with size (age) in larger frogs, reaching zero in the largest size groups (Tables 2 and 3). It should be pointed out that the pattern of rise, decline and establishment of the highest incidence of infection

Table 3. The incidence and intensity of *Haematoloechus coloradensis* of *Rana pipiens* (in 20 g increments) compared to the percent odonates ingested. Frogs larger than 150 g and smaller than 10 g were not infected at this research area.

Host weights (g)	Per cent infected	No. of flukes per frog	No. of flukes per infected frog	Per cent odonates in ingesta	Sample size
L.T. 10	0	0		0	17
10 to 29.9	90	7.4	8.3 (3 to 17)	22	30
30 to 49.9	100	7.7	7.7 (3 to 20)	38	21
50 to 69.9	100	7.2	7.2 (3 to 11)	34	10
70 to 89.9	100	7.7	7.7 (3 to 10)	35	12
90 to 109.9	79	6.3	8.1 (4 to 11)	13	9
110 to 129.9	68	6.3	9.5 (2 to 15)	11	6
130 to 149.9	33	4.0	12	7	3
G.T. 149.9	0	0		0	4

L.T. = less than, G.T. = greater than, g = grams, No. = number.

and greatest intensity occurred in the same relative size ranges as the rise, decline and establishment of the highest levels of odonates ingested.

Results of frog susceptibility studies on these two species of *Haematoloechus* in different weights of frogs are shown in Table 1. *Rana catesbeiana* weighing less than 20 g and *R. pipiens* weighing less than 10 g, which were not normally infected in nature, were easily infected in the laboratory. All 26 experimental *R. catesbeiana* weighing less than 10 g became infected with *H. coloradensis*. Stomach analysis indicated that frogs in these smaller size categories rarely ingested odonates (Tables 2 and 3). None of the experimental *R. catesbeiana* weighing more than 460 g became infected with *H. breviplexus* and none of the experimental *R. pipiens* weighing more than 200 g became infected with *H. coloradensis*. Stomach analysis of larger frogs from nature showed a distinct preference for food items larger than odonates.

Stomach analysis of *R. catesbeiana* indicated approximately 14% of ingesta by volume was odonates of which 83% was nymphs. In *R. pipiens* approximately 22% of ingesta by volume was odonates of which only about 10% was nymphs. Apparently the bullfrog, being the larger and more aggressive of the two frogs, dominates the pond proper forcing the leopard frog to spend an abnormally large portion of its time away from the pond. Because of this, adult odonates are more available as a food item to the leopard frog than nymphs and therefore, were the major source of lung fluke infection for that host.

Of the 374 *R. catesbeiana* collected 173 were males and 201 were females. Ninety-two of the males (53%) and 110 of the females (55%) were infected with *H. breviplexus*. Of the 112 *R. pipiens* collected, 51 were males and 61 were females. Thirty-seven of the males (73%) and 43 of the females (70%) were infected with *H. coloradensis*. The intensity of *H. breviplexus* in *R. catesbeiana* was 4.4 flukes per frog in males and 5.2 females. For *H. coloradensis* in *R. pipiens* it was 5.4 in males and 6.3 in females.

Discussion

Laboratory experiments showed that *R. catesbeiana* smaller than 20 g and *R. pipiens* smaller than 10 g, which were not infected in nature, were easily infected in the laboratory. This indicates that their low level of infection in nature was due to lack of exposure, not resistance. Food preferences may play a role in keeping these smaller frogs from being infected by *Haematoloechus* spp. in nature. However, frogs in these size groups are newly metamorphosed and although some frogs metamorphose at other times, the majority metamorphose in late summer when infected odonates are becoming less available. These newly metamorphosed frogs usually are not exposed to *Haematoloechus* spp. until the following year (Dronen, unpublished data).

Rana catesbeiana larger than 410 g and *R. pipiens* larger than 150 g were also not infected by *Haematoloechus* spp. in the research area. Although stomach analysis of large size classes of these frogs showed a distinct preference for food items larger than odonates, laboratory experiments suggested that these frogs were resistant to infection by *Haemato-*

loechus spp. This apparent resistance could be due to extended contact with these helminths which might stimulate acquired immunological resistance or it could be due to physiological factors innate in the larger frogs. In any case, a number of factors are probably involved.

Hollis (1972) found a significant difference in seasonal incidence of *Haematoloechus medioplexus* between male and female *R. pipiens*. In the current study there was no significant difference between males and females for *Haematoloechus* spp. in either *R. catesbeiana* or *R. pipiens*. The time of the year collections are made, the sample size, experimental techniques used, or other parameters may account for nonagreement of the two studies; however, there may also be a great deal of difference in parasite faunas in different geographic regions. This is demonstrated by the differences between *Haematoloechus* sp. infections in larger *R. catesbeiana* collected in Sierra County, New Mexico and Brazos County, Texas.

Some general trends are apparent in these two species of *Haematoloechus* which may be applicable to other parasites as well: (1) the incidence of infection and number of parasites per host may increase in some age groups of host and decrease in others due to a combination of factors such as physiological status, feeding preferences, inter- and intra-specific competition, immunological responses and seasonal timing of these different age groups of hosts. (2) In hosts acquiring their parasites through food chains, the incidence of infection and number of parasites per host may for some parasites increase with the volume of the intermediate host ingested.

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ANNOUNCEMENT: HONORARY MEMBER

At the 500th Meeting of the Society Norman R. Stoll was elected to Honorary Membership.

Some Digenetic Trematodes from Fishes of the Bering Sea with the Descriptions of *Prosorhynchus mizellei* sp. n. (Bucephalidae) and *Pseudopecoelus nossamani* sp. n. (Opecoelidae)

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ABSTRACT: *Prosorhynchus mizellei* sp. n. (Bucephalidae), described from *Aptocyclus ventricosus* (Pallas), differs from *P. squamatus* Odhner, 1905, in the size of the pharynx and distribution of the vitellaria. *Pseudopecoelus nossamani* sp. n. (Opecoelidae), described from *Hippoglossus stenolepis* Schmidt, is most similar to *P. japonicus* (Yamaguti, 1938) Von Wicklen, 1946, and *P. vulgaris* (Manter, 1934) Von Wicklen, 1946, from which it differs conspicuously in the sucker ratio. *Derogenes varicus* (O. F. Müller, 1784) Looss, 1901 (Hemiuridae), is reported from three new hosts: *Ronquilus jordani* (Gilbert), *Lycodes palearis* Gilbert, *Hemilepidotus hemilepidotus* (Tilesius).

This report is based on digeneans collected in 1973 by Dr. John D. Mizelle from fishes taken near Amchitka. They were found during routine examination for monogeneans using methods described by Mizelle (1938).

The trematodes were fixed and stored in 70% ethanol; whole mounts were stained with Mayer's hematoxylin; two specimens of the bucephalid were sectioned at 6 microns and stained with hematoxylin and eosin; all material was dehydrated, cleared in xylene, and mounted in Canada balsam. Drawings were made with the aid of a camera lucida; measurements are in microns with averages in parentheses.

Bucephalidae

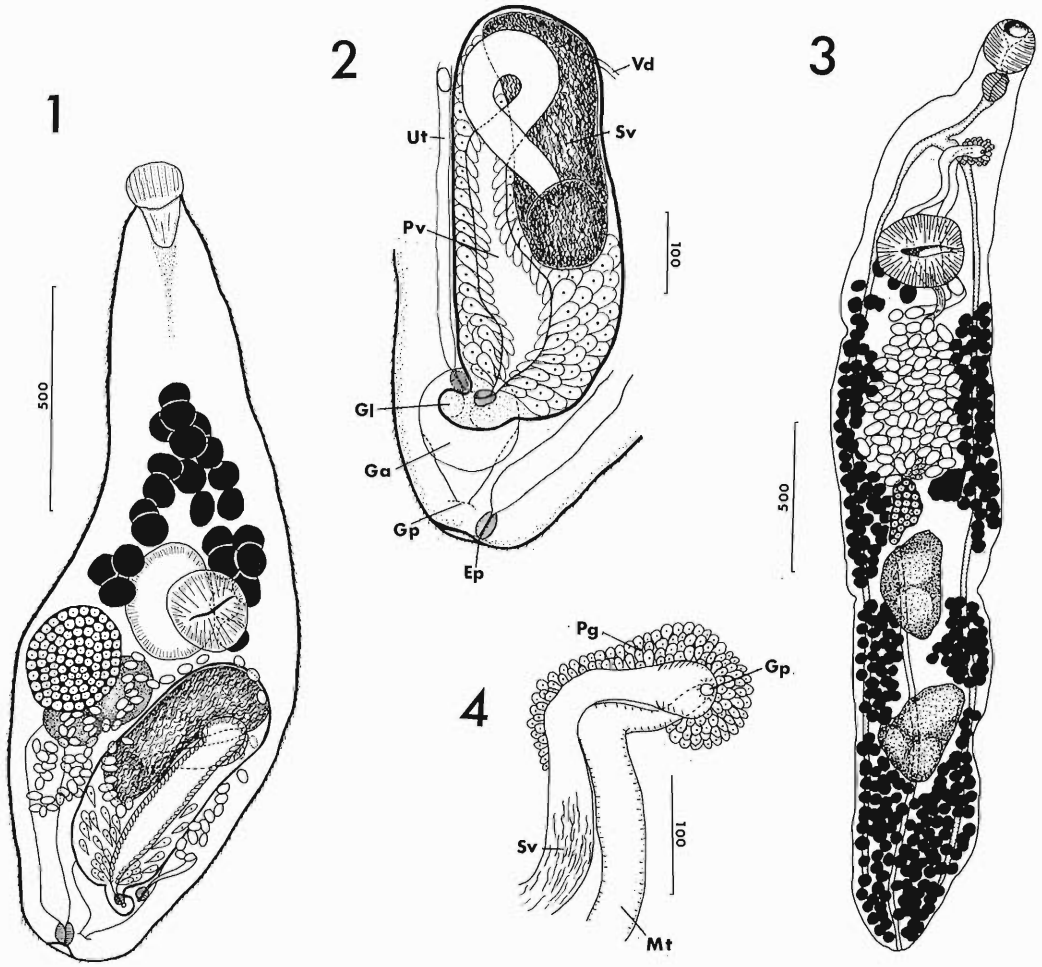
Prosorhynchus mizellei sp. n. (Figs. 1 and 2)

HOST: *Aptocyclus ventricosus* (Pallas), Cyclopteridae; Smooth lumpsucker; 9 specimens from 1 host.

TYPE SPECIMENS DEPOSITED: USNM Helminth. Coll. No. 74119 (holotype), 74120, Univ. Nebraska State Mus., H. W. Manter Lab. No. 20314.

DESCRIPTION (based on 9 specimens): Body subpyriform, 1665–2438 (1879) long by 449–685 (598) wide, tapered anteriorly, rounded posteriorly; tegument spined. Rhynchus conical, 171–196 (180) long by 96–146 (120) wide. Mouth near midbody; pharynx large, round, 130–196 (164) in diameter; esophagus short, indistinct, apparently contracted; intestine saccular, extending anterodorsally. Testes dextral, rounded, 152–184 (167) in diameter, oblique or tandem, close or overlapping; postesticular space 441–586 (511), about ¼ body length. Cirrus sac sinistral, 400–578 (528) long by 168–240 (200) wide, extending to near pharynx; seminal vesicle occupying anterior half or two-thirds of cirrus sac, sometimes recurved distally, 280–460 (364) long by 104–128 (116) wide; sperm-free duct joining well-developed prostatic complex; genital lobe curved; genital atrium spacious; genital pore subterminal. Ovary 192–224 (211) in diameter, larger than testes and anterior or anterolateral to testes, lateral or posterolateral to pharynx. Vitelline follicles rounded, 25–29 in number, forming arc from level of pharynx halfway to base of rhynchus. Uterine coils few, usually postpharyngeal (extending slightly anterior to pharynx in 2 specimens), joining genital atrium laterally. Eggs 32–40 (37) long by 20 wide. Excretory vesicle extending to posterior testis, dextral to cirrus sac; excretory pore terminal.

DISCUSSION: This species of *Prosorhynchus* is named in honor of Dr. Mizelle. It is most similar to *P. squamatus* Odhner, 1905, which has also been reported from Arctic localities. Both species have the vitellaria forming an arc in the anterior half of the body, the gonads contiguous on the right side, a relatively large



Figures 1 and 2. ***Prosorhynchus mizellei*** **sp. n. 1. Holotype, ventral view. 2. Terminal genitalia, paratype, dorsal view.**

Figures 3 and 4. ***Pseudopecoelus nossamani*** **sp. n. 3. Holotype, ventral view. 4. Terminal genitalia, paratype, ventral view.**

Abbreviations: Ep, excretory pore; Ga, genital atrium; Gl, genital lobe; Gp, genital pore; Mt, metraterm; Pg, prostatic gland cells; Pv, prostatic vesicle; Sv, seminal vesicle; Ut, uterus; Vd, vas deferens.

cirrus sac, and the mouth anterior to the cirrus sac; but *P. mizellei* differs from *P. squamatus* in having a more elongated body, a conspicuously larger pharynx, larger eggs, and the vitellaria extending posteriorly to the pharyngeal level (i.e., almost to the anterior of cirrus sac).

A second species of *Prosorhynchus* was represented by a single specimen from *Hemilepidotus hemilepidotus* (Tilesius), Cottidae; it could not be identified.

Opecoelidae

Pseudopecoelus nossamani sp. n. (Figs. 3 and 4)

HOST: *Hippoglossus stenolepis* Schmidt, Pleuronectidae, Pacific halibut; 2 specimens from 1 host.

TYPE SPECIMENS DEPOSITED: USNM Helminth. Coll. No. 74121 (holotype), 74122.

DESCRIPTION: Body elongate, 2531–3191

long by 465–539 wide at ovarian level. Tegument unspined. Forebody conical, ¼ body length. Oral sucker subterminal, 140–160 long by 92–160 wide. Acetabulum slightly protrusible, nonpapillate, 204–224 long by 244–276 wide. Sucker ratio 1:1.72. Prepharynx short; pharynx 92–120 long by 76–96 wide; esophagus 156–228 long; intestinal bifurcation midway between suckers; ceca ending blindly near posterior end of body. Genital pore ventral, slightly to left of cecal bifurcation. Testes tandem, intercecal, 236–332 long by 176–232 wide; posttesticular space 375–510. Seminal vesicle narrow, sinuous extending from near genital pore posteriorly to region near ovary; cirrus short; cirrus sac absent; prostatic gland cells in region of genital pore. Ovary trilobed, pretesticular, median, 80–112 by 172–180 wide; seminal receptacle lacking; Mehlis' gland preovarian; vitelline follicles circumcecal extending from level of acetabulum to posterior end of body, interrupted lateral to testes, filling posttesticular space. Uterus preovarian, extending to acetabulum; metraterm weakly muscular; uncollapsed uterine eggs 80 to 84 long by 44 wide. Excretory vesicle tubular, extending to ovary.

DISCUSSION: The eggs in these specimens are larger than those of all other known species of *Pseudopecoelus* except for *P. japonicus* (Yamaguti, 1938) Von Wicklen, 1946 (63–84 long by 36–54 wide) and *P. vulgaris* (Manter, 1934) Von Wicklen, 1946 (young worms: 78–80 long by 40 wide; older worms: 90–127 long by 50–76 wide). The sucker ratio of *Pseudopecoelus japonicus* is smaller (1:1.2–1.3) and that of *P. vulgaris* is larger (1:2–3). The species is named in honor of Bob J. Nossaman, York College, professor of biology.

A second species of *Pseudopecoelus* was represented by a single specimen from *Myxocephalus polyacanthocephalus* (Pallas), Cottidae, but it was macerated and cannot be described.

Hemiuridae

Derogenes varicus (O. F. Müller, 1784) Looss, 1901

HOSTS: *Ronquilus jordani* (Gilbert), Bathymasteridae, Northern ronquil; 4 specimens from 1 host.—New host record.

Lycodes palearis Gilbert, Zoarcidae, Wattled eelpout; 4 specimens from 1 host.—New host record.

Hemilepidotus hemilepidotus (Tilesius), Cottidae, Red Irish lord; 35 specimens from 8 hosts.—New host record.

Hippoglossus stenolepis Schmidt, Pleuronectidae, Pacific halibut; 18 specimens from 4 hosts.

Lepidopsetta bilineata (Ayres), Pleuronectidae, Rock sole; 1 specimen from 1 host.

SPECIMENS DEPOSITED: Univ. Nebraska State Mus., H. W. Manter Lab. No. 20316 through 20319.

DISCUSSION: *Derogenes varicus* has been reported with a sucker ratio of 1:1.8 to 2.4. The present specimens have a smaller sucker ratio, 1:1.42 to 1.84 (1:1.62), as a result of having a relatively smaller acetabulum, 237–392 (313). *Derogenes varicus* has been reported from over 120 hosts and three fish in this report are new host records.

Most reports of this parasite are from the northern hemisphere, but *Derogenes varicus* is also found in the southern hemisphere. Prudhoe and Bray (1973) reported it from several sub-Antarctic localities which is in agreement with Manter's (1954) suggestion that *D. varicus* has a bipolar distribution. It is very common in colder regions but limited to fishes in deeper (colder) waters in the subtropical and tropical regions.

Szidat (1950) described *D. parvus* from *Eleginops maclovinus* (Cuvier and Valenciennes) of Tierra del Fuego and reported it again in 1965 in *Notothenia neglecta* Nybelin (1 specimen—Szidat says that it was probably an accidental infection) from the Melchior Archipelago (Antarctic Peninsula) and in *Urophycis brasiliensis* from Puerto Quequén (Argentina). He admitted that it was difficult to distinguish the two species, but indicated that *D. parvus* differed from *D. varicus* only in the superficially lobed vitellaria. The measurements of *D. varicus* given in this report fall into the range for *D. parvus*. The similarity of the two species and the increasing reports of *D. varicus* from the southern hemisphere suggest a synonymy of the two species.

The life-history of *Derogenes varicus* is not fully known, but Manter (1954) suggested that the geographical distribution is probably due

to the temperature limitations of the intermediate host(s) which are found only in colder waters.

Only a few individuals of *D. varicus* are usually found in each host (Manter, 1954; Shotter, 1973). One host in this report (*Hemilepidotus hemilepidotus*) harbored 13 mature specimens of this parasite.

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Histochemical Studies of Abomasal Tissue from Calves with Monospecific and Dual Species Infections of *Ostertagia ostertagi* and *Trichostrongylus axei*

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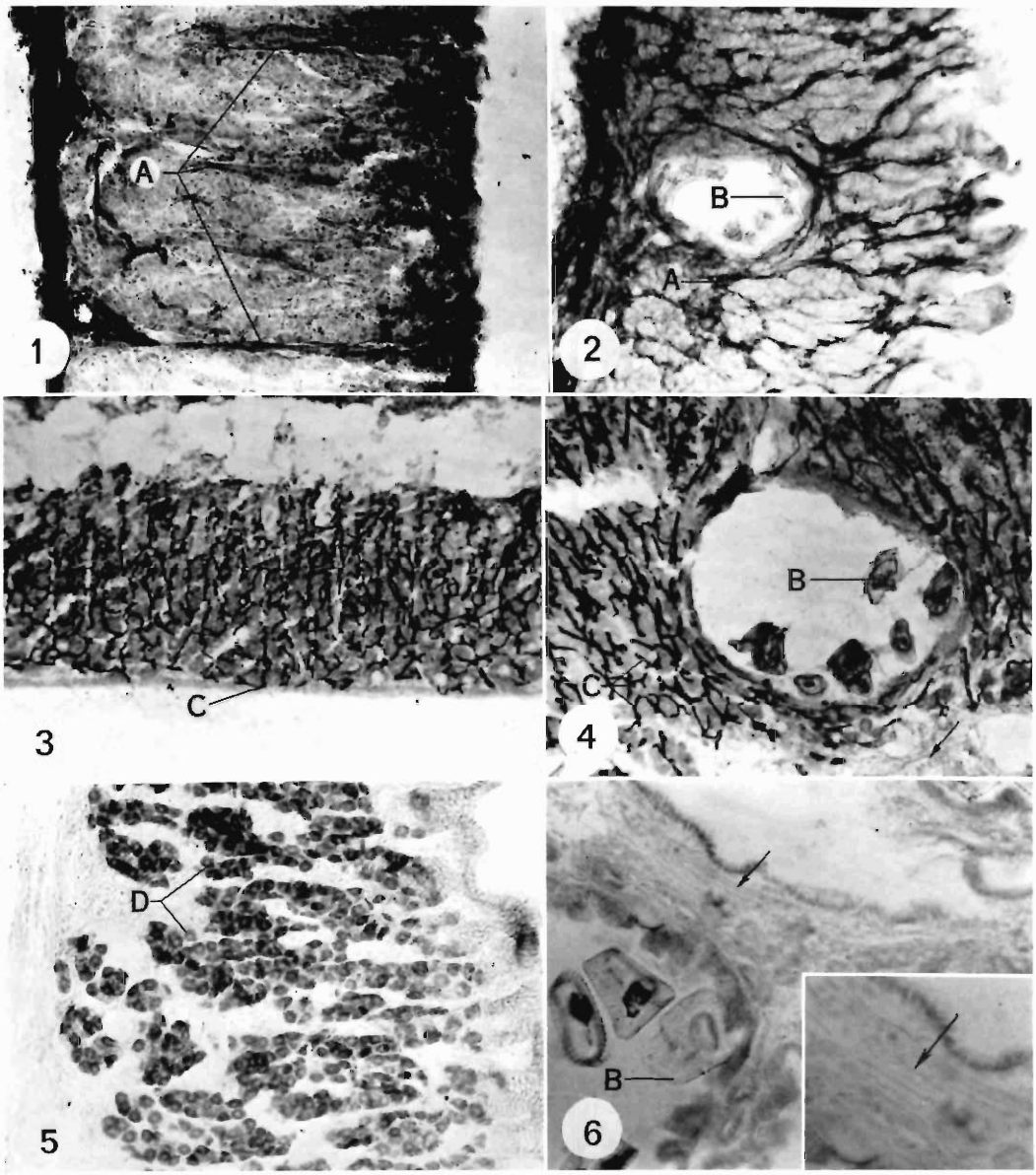
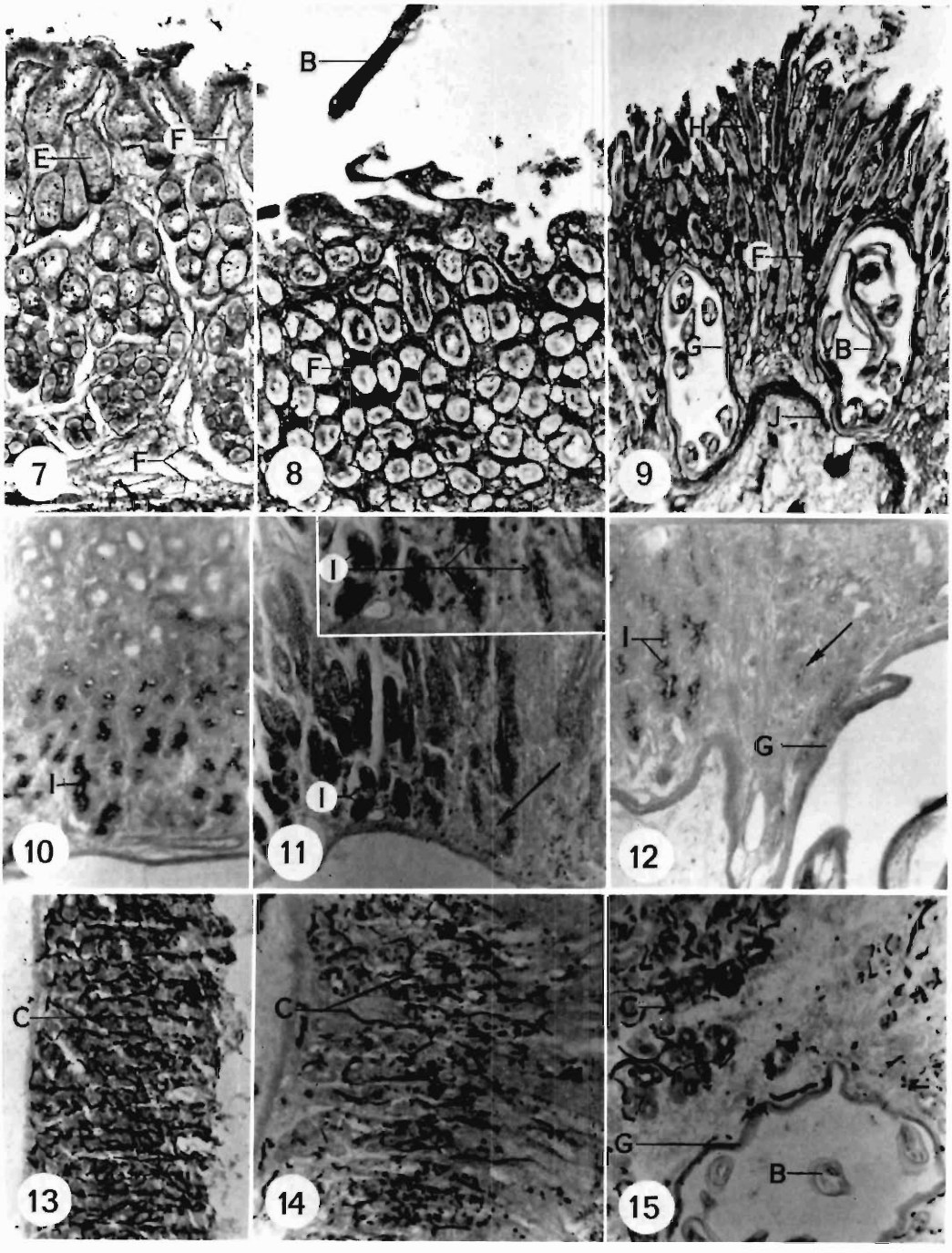
ABSTRACT: Abomasal mucosa from three groups of calves each infected with 250,000 larvae of *Ostertagia ostertagi*, 250,000 *Trichostrongylus axei*, or 125,000 each of *O. ostertagi* and *T. axei* was studied with histochemical techniques at 3, 5, 7, 14, and 22 days after infection (DAI). *O. ostertagi*: The capillary network, stained with methods for alkaline phosphatase, was disrupted about the infected gastric gland compared with uninfected mucosa. Methods for carbonic anhydrase detected less stainable activity in areas about the infected gastric gland than in uninfected and adjacent mucosa because parietal cells were replaced by collagen fibers. *T. axei*: Cytoplasmic RNA of chief cells stained similarly; whereas, large areas lacked chief cell zymogen granules in infected compared with uninfected mucosa. The capillary network of infected mucosa was compressed because of mucoid hyperplasia; the stainable carbonic anhydrase activity of parietal cells was similar in infected and uninfected mucosa. *O. ostertagi* plus *T. axei*: Cytoplasmic RNA and zymogen granules in chief cells stained similar to calves infected with *O. ostertagi*. The capillary network was compressed and disrupted about the gastric gland infected with *O. ostertagi*; and, where hyperplastic mucus cells were abundant, the capillary network was compressed. The stainable carbonic anhydrase activity of parietal cells about the gastric gland was variable depending upon the extent of mucoid hyperplasia.

The histopathology of monospecific infections of calves infected with *Ostertagia ostertagi* and *Trichostrongylus axei* and dual species infections were described by Ritchie et al. (1966), Ross et al. (1967, 1968) and Ross et al. (1968); however, no extensive histochemical studies have been carried out other than those by Stringfellow (1974) with *O. ostertagi*.



Figures 1–6. Stained sections of bovine fundus infected with *Ostertagia ostertagi*. 1. The distribution of smooth muscle bundles of uninfected fundus—ATPase. 2. Smooth muscle bundles compressed by the dilated gastric gland infected with *O. ostertagi* at 22 DAI. 3. Distribution of the capillary network of uninfected fundus—Alkaline phosphatase. 4. Distribution of the capillary network about the infected gastric gland of fundus infected with *O. ostertagi* at 22 DAI showing the disrupted (arrow) and compressed capillary network. 5. Parietal cells in uninfected, fundus stained darkly with methods for carbonic anhydrase—Häusler's method. 6. The absence of staining of parietal cells with methods for carbonic anhydrase because of replacement of parietal cells with collagen fibers (arrow). Inset magnified to show enhanced collagen fibers. Figs. 1, 5, 6, (125×); Figs. 2–4 (60×). Abbreviations: A, Smooth muscle; B, worm; C, capillary; D, parietal cells.



Results of histochemical studies on calf abomasal mucosa infected with monospecific and dual species infections with *O. ostertagi* and *T. axei* are reported herein; they are interpreted relative to the known pathology.

Materials and Methods

Three groups each consisting of five 3-month-old castrated Holstein calves infected with the following dose of infective larvae were killed with a captive bolt gun at 3, 5, 7, 14, and 22 DAI: Group 1, *O. ostertagi* (250,000); Group 2, *T. axei* (250,000); and Group 3, *O. ostertagi* (125,000) plus *T. axei* (125,000), referred to as the combined infection. Three 3-month-old calves served as uninfected controls. Fundic mucosa from the three groups was sectioned (8 μ), stained (H & E), and the pathology studied. The following histochemical methods, selectively staining certain structures in fundic mucosa, were used to further interpret the pathology. They were also used for their histochemical value in detecting tissue components and enzymes of functional significance to fundic mucosa.

O. ostertagi: Methods given previously were used to study the nonenzymic histochemistry of calves infected with *O. ostertagi* (Stringfellow, 1974). The enzyme histochemistry was studied as follows: tissues excised from the fundus were quenched at –70 C in isopentance, frozen with dry ice, stored in screw-cap vials on dry ice, then studied with enzyme histochemical methods. All tissues were sectioned on the cryostat at 40 micrometers. Methods were used for detecting aminopeptidase, acid phosphatase, alkaline phosphatase, 5′-nucleotidase, the Wachstein-Meisel adenosine triphosphatase technique, and nonspecific esterase (Barka and Anderson, 1965; Pearse, 1968). The method for alkaline phosphatase, which specifically stained the capillary network, was run to visualize vascular damage; and, if so, its extent so that these data might be correlated with dye injection and biochemical studies. The substrates, L-leucyl-beta-naphthyl-amide, sodium-beta-glycerophosphate for both acid and alkaline phosphatases, adenosine-5-phosphate, adenosine triphosphate (ATP), and alpha-naphthyl acetate, were used in each of the above methods. Häusler's variant of Kurata's method (Lillie, 1965) was used to stain for carbonic anhydrase. Tissues, inhibited with Diamox[1] and incubated without sodium bicarbonate, served as controls. The following fixatives were used for the above procedures: cold acetone for aminopeptidase, acid and alkaline phosphatases, and carbonic anhydrase; and cold formol calcium for 5′-nucleotidase, ATPase, and nonspecific esterase. All methods were run without their respective substrates. Positive controls were calf small intestine for aminopeptidase, pancreas for carbonic anhydrase, and liver for the other enzymes. Despite the absence of specificity, 0.01 M KCN was used to inhibit aminopeptidase. Only an uninhibited test for esterase was desired, and Lugol's iodine was used to inhibit acid and alkaline phosphatase, 5′-nucleotidase and ATPase activity.

[1] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture, and does not imply its approval to the exclusion of other products that may be suitable.

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Figures 7–15. Stained sections of bovine fundus infected with *Trichostrongylus axei* only and *Ostertagia ostertagi* plus *T. axei*. 7. Distribution of reticulum fibers of uninfected calf fundus—Foot's modification of Bielschowsky's method. 8. Distribution of reticulum fibers beneath lining epithelium of fundus infected with *T. axei* at 22 DAI. 9. Distribution of reticulum fibers in fundus infected with *O. ostertagi* and *T. axei* at 14 DAI. 10. Darkly stained zymogen granules in chief cells of uninfected fundus—DMAB nitrate method. 11. Fundus infected with *T. axei* at 22 DAI showing area (arrow) denuded of zymogen granules compared with adjacent mucosa. Inset shows clusters of chief cells with fewer zymogen granules from left to right. 12. Fundus infected with *O. ostertagi* and *T. axei* at 14 DAI denuded of zymogen granules in chief cells about infected gastric gland (arrow). 13. Distribution of the vasculature of uninfected fundus—Alkaline phosphatase. 14. Altered vasculature of fundus infected with *T. axei* at 22 DAI. 15. Disrupted vasculature about infected gastric gland of fundus infected with *O. ostertagi* and *T. axei* at 14 DAI. Figs. 7–9; 13–15 (60×); Figs. 10–12 (125×). Note that all infected fundic mucosa are hypertrophied relative to uninfected tissues. Abbreviations: B, worm; C, capillary; E, mucus cells; F, reticulum fibers; G, metaplastic mucus cells; H, hyperplastic mucus cells; I, zymogen granules; J, muscularis mucosa.

T. axei: The following nonenzymic and enzymic histochemical tests replaced or were added to those previously described by Stringfellow (1974). Masson's trichrome stain and Lillie's method replaced Mallory's aniline blue collagen stain and the methyl green pyronin stain for nucleic acids, respectively. Lillie's allochrome method detected basement membranes and Bodian's method detected nerve fibers and endings (Armed Forces Institute of Pathology, 1968). Sudan B replaced Oil red 0 as a general lipid stain (Barka and Anderson, 1965). The method for succinic dehydrogenase was run to specifically stain parietal cells (Barka and Anderson, 1965). Unfixed calf mucosa as well as kidney incubated in the presence and absence of the substrate served as controls.

O. ostertagi and *T. axei*: Both nonenzymic and enzymic histochemical tests were run as described for *T. axei*.

Results

O. ostertagi: The nonenzymic histochemistry was described previously (Stringfellow, 1974). The enzymic histochemical results from monospecific and dual species infections of calves killed at 3, 5, and 7 DAI were similar to those obtained at 14 and 22 DAI. Methods for aminopeptidase stained mucus cells of uninfected and some metaplastic mucus cells of infected fundic mucosa faintly at 14 DAI. Methods for acid phosphatase stained smooth muscle (Figs. 1, 2) and some metaplastic mucus cells intensely in uninfected and infected mucosa. ATPase was detected in elastica interna of arteries, faintly in mucus cells at the fundic lumen, and in mucus cells of uninfected and metaplastic mucus cells of infected mucosa. The capillary network of uninfected and infected mucosa stained intensely for alkaline phosphatase (Figs. 3, 4); and smooth muscle and tunica adventitia of arteries stained intensely for 5′-nucleotidase. The smooth muscle and capillary network were markedly compressed and disrupted respectively about the infected gastric gland (Figs. 2, 4). The method for nonspecific esterase intensely stained smooth muscle and arteries, cell membranes of fat cells, the border surrounding the mucus droplet of mucus cells, and metaplastic mucus cells. Because parietal cells (Fig. 5) were replaced with collagen fibers and mucus cells, less stainable carbonic anhydrase activity was detected about the infected gastric gland at 22 DAI (Fig. 6).

T. axei: Nonenzymic histochemical results similar to those reported for *O. ostertagi* (Stringfellow, 1974) are not reported here; so, only those results which differed using the same procedures or obtained with different procedures are reported. Few, if any, collagen fibers were deposited in fundic mucosa from infected calves. Compared with uninfected mucosa (Fig. 7), reticulum fibers condensed at the eroding superficial mucosa beginning at 3 DAI progressed increasingly to 22 DAI (Fig. 8). In uninfected and infected mucosa zymogen granules of chief cells stained for tryptophane, SS bonds, and SH groups (Fig. 10). Large areas were degranulated whether or not the worm was embedded beneath the superficial epithelium (Fig. 11). RNA in chief cells stained similarly from uninfected and infected mucosa. Nerve fibers were intact and there was about the same distribution of fat between uninfected and infected mucosa.

Aminopeptidase, acid phosphatase, and nonspecific esterase was distributed similar to that reported above for *O. ostertagi*. The capillary network of uninfected (Fig. 13) and infected (Fig. 14) mucosa stained intensely for alkaline phosphatase; and smooth muscle and tunica adventitia of arteries stained intensely for 5′-nucleotidase and ATPase. The hyperplastic mucus cells compressed the fundic smooth muscle. The carbonic anhydrase activity of parietal cells stained similarly in uninfected and infected mucosa. Purple granules, stained intensely in parietal cells with methods for succinic dehydrogenase, were similar in parietal cells of uninfected and infected mucosa.

O. ostertagi plus *T. axei*: There was such overlap in the pathology and histochemistry of both the combined and monospecific infections that only the distinguishing features are reported here. Compared with uninfected mucosa (Fig. 7), structural fibers staining with reticulum stains were condensed beneath the lining mucus cells of the infected mucosa (Fig. 9). Compared with uninfected (Fig. 10) and adjacent mucosa, fewer zymogen granules in chief cells stained for tryptophane, SS bonds, and SH groups about the dilated gastric gland from 3–22 DAI (Fig. 12). Cytoplasmic RNA of chief cells stained intensely in uninfected

and adjacent mucosa: whereas, RNA of chief cells degenerating about the dilated gastric gland stained less intensely at 14–22 DAI. The capillary network of uninfected (Fig. 13) and infected mucosa was disrupted and compressed about the dilated gastric gland (Fig. 15). Where hyperplastic mucus cells were abundant, the capillary network was compressed. Parietal cells, stained with methods for carbonic anhydrase, stained variably depending upon the extent of mucoid hyperplasia. Fewer parietal cells were detected about the dilated gastric gland with methods for succinic dehydrogenase.

Discussion

These three types of infections were characterized by: (1) mucus cells which proliferated in all cases; (2) chief and parietal cells which degenerated in *O. ostertagi* and the combined infections and; (3) chief cells which degenerated but not parietal cells (*T. axei*). Secreted fibers and mucus cells usually infiltrate an area of localized ischemia induced by a disrupted vascular supply to the tissue; cellular and extracellular elements that can survive under those conditions do, whereas those that cannot degenerate (Stringfellow, 1974). Chief cells were considered degenerated if fewer zymogen granules were detected (Figs. 11, 12), less stainable RNA was detected with histochemical methods, or if they were replaced by fibers or mucus cells. Parietal cells, thought to secrete HCl mediated by the enzyme carbonic anhydrase (Cross, 1970; Carter, 1972), were considered degenerated if less stainable carbonic anhydrase was observed (Fig. 6) when they too were replaced by fibers or mucus cells. The present studies show that the vascular supply of the infected mucosa was disrupted (Figs. 4, 14, 15). Presumably this would interfere with the ability of chief cells to secrete pepsinogen, shown by areas void of zymogen granules and decreased stainable RNA, and the ability of parietal cells to secrete HCl shown by decreased stainable carbonic anhydrase when those cells were replaced by collagen fibers (Fig. 6). These results support the pathogenic mechanisms reviewed for *O. ostertagi* by Armour (1974).

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Revision of the Genus *Rauschiella* Babero, 1951 (Digenea: Plagiorchiidae) with a Redescription of *R. palmipedis* (Lutz, 1928) n. comb. from Venezuelan Frogs[1]

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ABSTRACT: *Glypthelmins palmipedis* (Lutz, 1928), redescribed from *Leptodactylus bolivianus* Boulenger collected in northeastern Venezuela, *G. proximus* Teixeira de Freitas, 1941, and *G. sera* Cordero, 1944, are transferred to *Rauschiella* Babero, 1951, on the bases of an intercecal uterus with well-developed pretesticular uterine coiling and a Y-shaped excretory bladder. *Repandum* Byrd and Maples, 1963, is considered a synonym of *Rauschiella*. Additionally, *R. tineri* Babero, 1951, and *R. repandum* (Rudolphi, 1819) Babero, 1951, are recognized as valid species. *Plagiorchis lenti* Teixeira de Freitas, 1941, is designated a synonym of *R. palmipedis*.

In September, 1970, 48 specimens of a digenetic trematode, morphologically consistent with *Haplometra palmipedis* Lutz, 1928, were recovered from the small intestines of four of seven *Leptodactylus bolivianus* Boulenger collected in Bordones, Sucre, Venezuela. One additional specimen was found in the small intestine of *Bufo marinus* (L.) in Cumaná, Venezuela.

Teixeira de Freitas (1941) recognized Travassos' (1930) placement of *H. palmipedis* in *Glypthelmins* Stafford, 1905, and synonymized *Metorchis leptodactylus* Savazzini, 1930, with it. Cheng (1959) transferred *Glypthelmins palmipedis* (Lutz, 1928) to *Margeana* Cort, 1919. Byrd and Maples (1963) and Nasir (1966) did not accept Cheng's reinstatement of *Margeana*; the former authors placed *G. palmipedis* in a new genus *Repandum*, while Nasir preferred to retain it in *Glypthelmins*, a position reiterated by Nasir and Diaz (1970).

Consisting only of a figure, Lutz' (1928) description of *G. palmipedis*, with Caracas as the type locality, has been supplemented by a written description of two large specimens (5.544–6.640 mm) from Maracay, Aragua, Venezuela (Caballero y C. et al., 1953). Although complete descriptions are available for Argentinian, Brazilian, Costa Rican and Panamanian *G. palmipedis*, the Venezuelan form is only known from three specimens. Consequently, a redescription of *G. palmipedis* from eastern Venezuela is presented. Comparison of these *G. palmipedis* with the type and paratype of *Rauschiella tineri* Babero, 1951 (USNM Helm. Coll. Nos. 47089 and 47090) indicates that *G. palmipedis* should be transferred to *Rauschiella* thus becoming *Rauschiella palmipedis* (Lutz, 1928) n. comb.

Trematodes were heat-killed in 0.7% saline under slight coverslip pressure, fixed in AFA, stained with Harris hematoxylin, and mounted in Permount. Figures were drawn with the aid of a Wild drawing tube. Unless stated otherwise, measurements are in micrometers with the mean in parentheses. Individual measurements of worms on which the description is based are available elsewhere (Sullivan, 1972). References for localities cited in Table 1 are listed as they appear in the Index Catalogue of Medical and Veterinary Zoology, U.S. Government Printing Office, Washington, D.C.

Rauschiella palmipedis (Lutz, 1928) n. comb. (Figs. 1–3)

SYNONYMS: *Haplometra palmipedis* Lutz, 1928; *Haplometra palmipedes*: Travassos, 1930

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Table 1. *Rauschiella palmipedis* (Lutz, 1928) n. comb. Hosts and localities (*new locality record).

Host	Locality	Author
Bufonidae		
Bufo marinus (L.)	Sao Paulo, Brazil	Teixeira de Freitas (1941)
	Chapernal, Puntarenas, Costa Rica	Brenes & Arroyo (1960)
	*Cumaná, Sucre, Venezuela	Present study
Leptodactylidae		
Leptodactylus bolivianus Boulenger	La Carrasquilla, Panama	Caballero y C. et al. (1956)
	*Bordones, Sucre, Venezuela	Present study
L. oscellatus (L.)	Cordoba Prov., Argentina	Savazzini (1930)
(= *L. caliginosus* Girard)	Estado de Mato Grosso, Brazil	Teixeira de Freitas (1941) Travassos & Teixeira de Freitas (1941b)
	Montevideo, Uruguay	Teixeira de Freitas (1941)
	Chaco-i Prov., Paraguay	Lent et al. (1946)
	Estado de Pernambuco, Brazil	Dobbin (1957)
L. pentadactylus (Laurenti)	Salvador (Bahia), Brazil	Fahel (1952)
	Estado de Pernambuco, Brazil	Dobbin (1957)
Hylidae		
Pseudis paradoxa (L.) (?)	Salobra, Estado de Mato Grosso, Brazil	Travassos & Teixeira de Freitas (1940, 1941a)
Ranidae		
Rana palmipes Spix	Caracas, Venezuela	Lutz (1928)
	Maracay, Aragua, Venezuela	Caballero y C. et al. (1956)
	Estado de Pernambuco, Brazil	Dobbin (1957)

[*sic*]; *Glypthelmins palmipedis* (Lutz, 1928)* and of Nasir and Diaz (1970), in part; *Metorchis leptodactylus* Savazzini, 1930; *Meorchis leptodactylus* Savazzini, 1930 [*sic*]; *Plagiorchis lenti* Teixeira de Freitas, 1941; *Glypthelmins pseudis* Fahel, 1952 [*sic*]; *Glypthelmins linguatula* (Rudolphi, 1819) of Caballero y C. et al. (1956) and of Nasir (1966), in part; *Margeana linguatula* (Rudolphi, 1819) Cheng, 1959, in part; *Repandum palmipedis* (Lutz, 1928) Byrd and Maples, 1963.

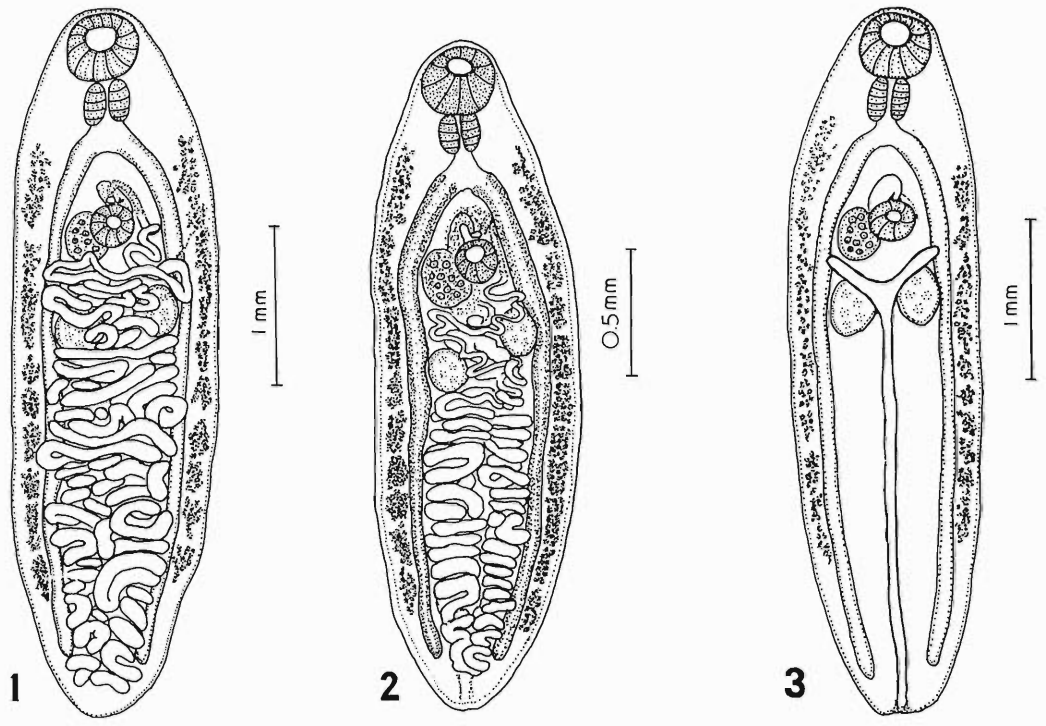
DESCRIPTION (measurements based on 35 specimens): Body elongate, subcylindrical, attenuated posteriorly, 1590–5200 (3690) long by 500–1570 (1100) wide. Tegument spined with spines disappearing in posterior quarter of body. Oral sucker subterminal, 190–450 (350) by 220–500 (410). Acetabulum medial, situated in second quarter of body, 120–290 (220) by 130–310 (240). Ratio of oral sucker to acetabulum 1:0.62 ± 0.02. Prepharynx short. Pharynx muscular, 110–320 (240) by 110–310 (230). Esophagus short. Ceca narrow, terminating near posterior extremity of body. Testes subspherical, regular in outline, diagonally to symmetrically arranged in second quarter of body; anterior testis sinistral, 170–430 (310) by 140–450 (300), posterior testis 180–470 (320) by 150–500 (320). Cirrus pouch elongate, 120–620 (360) by 70–190 (150), containing coiled seminal vesicle and unarmed eversible cirrus. Ovary spherical, dextral, in acetabular zone, 160–360 (290) by 150–370 (260). Seminal receptacle and Laurer's canal present. Uterus intercecal, ascending and descending limbs of uterus transversely coiled, uterine coils developed in pretesticular zone. Metraterm approximately as long as cirrus pouch. Genital pore medial, immediately preacetabular. Vitellaria follicular, occasionally grouped in discrete bunches, commencing in the region of esophageal bifurcation and terminating at varying distances posterior to testes, occasionally reaching cecal ends. Eggs operculated, 27–37 (31) by 10–15 (12). Excretory bladder Y-shaped, bifurcating between testes, arms reaching to ovarian level.

HOSTS AND LOCALITIES: Table 1.

SITE OF INFECTION: Small intestine.

SPECIMENS: USNM Helm. Coll. No. 72279. Other specimens in the author's collection.

* In view of Travassos' (1930) failure to properly designate the combination *Glypthelmins palmipedis* in transferring this species from *Haplometra* Looss, 1899, Travassos et al. (1969) suggested that reference to Travassos (1930), when used in connection with the name *G. palmipedis*, be enclosed in square brackets. If this suggestion is followed, the citation *Glypthelmins palmipedis* (Lutz, 1928) [Travassos, 1930] could generate confusion regarding the taxonomic history of the specific epithet, since the International Code of Zoological Nomenclature Recommendation 51A reserves square brackets to indicate the name of the author of a taxon which was originally published anonymously. Since the Code does not provide specific rules for formation of new combinations, an alternative to the suggestion of Travassos et al. (1969) would be citation of the author who first properly designated the combination.



Figures 1–3. ***Rauschiella palmipedis*** **(Lutz, 1928) n. comb. from northeastern Venezuela. 1. Ventral view of a specimen from** ***Leptodactylus bolivianus*****; note intercecal position of the uterus and pretesticular uterine coiling. 2. Specimen from** ***Bufo marinus*****; note relative size of gonads compared with Figs. 1 and 3. 3. Specimen showing Y-shaped excretory bladder.**

Discussion

Byrd and Maples (1963) established *Repandum* for *Glypthelmins repandum* (Rudolphi, 1819) Travassos, 1924, and included *G. palmipedis* and *G. sera* Cordero, 1944, in it. However, Babero (1951b) had previously erected *Rauschiella* in the Plagiorchiidae Lühe, 1901, for *R. tineri* and transferred *G. repandum* to that genus. Comparison of their respective generic diagnoses failed to indicate any differences between the two; therefore, *Repandum* is considered a synonym of *Rauschiella*.

The present author accepts Babero's (1951b) transfer of *G. repandum* to *Rauschiella* and agrees with Byrd and Maples (1963) in removing *G. palmipedis* and *G. sera* from *Glypthelmins* and allying them with *Rauschiella repandum* (= *Repandum r.*). Consequently, *G. sera* is designated *Rauschiella sera* (Cordero, 1944) n. comb.

Sullivan (1976) emended the diagnosis of *Glypthelmins* to include those forms with an I-shaped excretory bladder and an intercecal uterus without pretesticular uterine coiling. Although the form of its excretory bladder is unknown, *G. proximus* has an intercecal uterus but with pretesticular coiling which suggests a closer relationship to *Rauschiella* than to *Glypthelmins* (Byrd and Maples, 1963; Sullivan, 1976). Babero (1951a) suggested that *G. proximus* might be included in a genus characterized in part by pretesticular uterine coils, or alternatively synonymized with *G. repandum*. However, when Babero (1951b) transferred *G. repandum* to *Rauschiella*, he did not consider *G. proximus*, which is now transferred to *Rauschiella* and designated *Rauschiella proximus* (Teixeira de Freitas, 1941) n. comb. Comparison of specimens reported as *G. proximus* by Thatcher (1964)

from snakes in Tabasco, Mexico with the type of *R. tineri* indicates that the *G. proximus* of Thatcher (1964) is *R. tineri*.

Plagiorchis lenti Teixeira de Freitas, 1941, should be removed from *Plagiorchis* Lühe, 1899, because of its uterine configuration (Odening, 1959). This character and the others of Babero (1951b) refer *P. lenti* to *Rauschiella*. However, *P. lenti* only differs from *R. palmipedis* by the presence of the seminal receptacle in the latter. Since the seminal receptacle is visible in only 19 of the 35 specimens of *R. palmipedis* in the writer's collection, this character is not considered reliable for purposes of identification. Since the two forms are morphologically consistent otherwise, *P. lenti* is designated a synonym of *R. palmipedis*.

Rauschiella proximus differs from the other four species of *Rauschiella* in possessing a sacculate seminal vesicle as opposed to a strongly coiled seminal vesicle. Since *R. sera* is only known from a single specimen, its taxonomic position is uncertain; however, it is distinguished from the other species by ceca which do not reach the posterior extremity of the body. *Rauschiella repandum* differs from the other species in having vitellaria which commence anteriorly at or behind the level of the ovary. The position of the acetabulum with reference to the esophageal bifurcation separates *R. tineri* from *R. palmipedis*.

In view of this proposed revision of the genus *Rauschiella*, the following emended generic diagnosis is presented:

Rauschiella Babero, 1951 Char. Emend.

SYNONYM: *Repandum* Byrd and Maples, 1963.

DIAGNOSIS: Plagiorchiidae. Body elongate, cylindrical to subcylindrical. Tegument spined. Oral sucker subterminal, larger than acetabulum. Acetabulum medial, pre-equatorial. Pharynx muscular. Esophagus present, bifurcating at various levels between suckers. Ceca terminating in posterior quarter of body, often reaching posterior extremity. Testes diagonal to symmetrical, in anterior half of body. Cirrus pouch elongate, usually overlapped by acetabulum. Ovary pretesticular in acetabular zone. Seminal receptacle and Laurer's canal present. Uterus intercecal, usually reaching posterior extremity of body; uterine coils well-developed in pretesticular region. Metraterm nonmuscular. Genital pore preacetabular, medial. Vitellaria follicular, occasionally in discrete bunches, in lateral fields, sometimes overlapping ceca. Excretory bladder Y-shaped. Parasitic in intestine of amphibians and reptiles.

TYPE SPECIES: *Rauschiella tineri* Babero, 1951.

Acknowledgments

The author would like to thank the late Dr. Elon E. Byrd, Zoology Department, University of Georgia, Athens, for his comments and suggestions during the course of this study. The author also thanks Dr. Pir Nasir, Biology Department, Universidad de Oriente, Cumaná, Venezuela and his staff for their assistance, Drs. Vernon E. Thatcher, Biology Department, Universidad del Valle, Cali, Colombia, and J. Ralph Lichtenfels, U.S. National Museum, for the loan of specimens.

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Development of *Waltonella flexicauda*, a Filarial Parasite of *Rana catesbeiana*, in *Aedes aegypti* and other Culicine Mosquitoes

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ABSTRACT: *Waltonella flexicauda*, a filarial parasite of the bullfrog, *Rana catesbeiana*, completed its development to the third larval stage in laboratory colonies of *Aedes annandalei*, *A. atropalpus*, *A. aegypti*, *A. polynesiensis*, and *Culex pipiens quinquefasciatus*, but not in *A. seatoi*, *A. triseriatus*, or *A. malayensis*. Susceptibility in 12 geographic strains of *A. aegypti* varied from 0 to 81%. Starting with 19% susceptibility in the NEW-GKEP strain of *A. aegypti*, a susceptible (97%) and a refractory (4%) line were selected in four generations. Worm development in these two selected lines was contrasted. In the refractory line filarial development progressed to 36 hours post-infection and then stopped.

The concept of genetic factors controlling vectorial capacity is not new. Huff (1929) demonstrated that the level of susceptibility of a *Culex pipiens* population to *Plasmodium cathemerium* infection could be altered in the laboratory by selection and later concluded that susceptibility was controlled by a simple Mendelian factor (Huff 1931). Roubaud (1937) similarly reported that susceptibility of *Aedes aegypti* to *Dirofilaria immitis* was an heritable characteristic. Kartman (1953) successfully increased susceptibility in the same system after eight generations of selection. Zielke (1973) and McGreevy, McClelland, and Lavoipierre (1974) independently demonstrated that a sex-linked factor affected *A. aegypti* susceptibility to *D. immitis*. Macdonald (1962a, b) selected *A. aegypti* susceptible to sub-periodic *Brugia malayi* and then showed susceptibility to be controlled by a sex-linked, recessive gene designated f^m.

These previous genetic-susceptibility studies involved filariae developing in Malphigian

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Table 1. Susceptibility of eight culicine species to infection with *W. flexicauda*.

Experiment	Species	No. ♀♀ dissected	% susceptible
1	*A. malayensis*	14	0.0
	C. p. quinquefasciatus	37	73.0
	A. polynesiensis	38	97.4
	*A. aegypti**	12	75.0
2	*A. seatoi*	25	0.0
	A. triseriatus	25	0.0
	A. annandalei	17	47.1
	A. atropalpus	35	94.3
	*A. aegypti**	20	85.0

* Susceptible strain selected from NEW-GKEP served as the control.

tubes or flight muscles. The present investigation is an extension of this work to a fat body-developing filariid. Using *Waltonella flexicauda* (Schacher and Crans, 1973)*, a mosquito-transmitted filaria of the bullfrog, *Rana catesbeiana*, we studied inter- and intra-specific variation in mosquito ability to affect development of this parasite.

Materials and Methods

Approximately two dozen bullfrogs with naturally acquired *W. flexicauda* infections were field-collected in Morris County, New Jersey, and thereafter, maintained in the laboratory. Filarial identification was based on the microfilaria and the L_3. *W. flexicauda*'s development in the fat body of *A. aegypti* was similar to that described in *Culex territans*, the probable natural vector (Benach and Crans 1973).

Mosquito species and strains were selected from among stocks maintained at the W.H.O. International Reference Centre for *Aedes* by the Vector Biology Laboratory of the University of Notre Dame. Rearing materials, techniques, and conditions for maintenance of mosquitoes were similar to those of Kilama and Craig (1969). Five-day post-emerged females were used. The carbohydrate source, apple, was removed 24 hr before feeding and water was taken away 12 hr preinfection.

For exposure to infection with *W. flexicauda*, an unsedated bullfrog was restrained in a cheesecloth sock held tight by a rubber band. The frog was placed directly into the cage with the mosquitoes for 30–90 min. Only those mosquitoes engorging to stage 4 or higher (Pilitt and Jones 1972) were retained. After 12 days the infected mosquitoes were dissected in *Aedes* physiological saline (Hayes 1953) and scanned for *W. flexicauda* development using a stereoscope at 60 ×. An individual mosquito was scored as susceptible if filariae had developed to the late L_2 or L_3 stage; otherwise, it was considered refractory. Suscepti-

* *Waltonella flexicauda* (Schacher and Crans, 1973) = *Foleyella flexicauda* Schacher and Crans (1973).

Table 2. Susceptibility of twelve geographic strains of *A. aegypti* to infection with *W. flexicauda*.

	Strains tested					NEW-GKEP control			
	Infected		Dissected			Infected		Dissected	
	No.	% mortality	No.	% susceptible	Probability*	No.	% mortality	No.	% susceptible
JD†	66	31.8	45	0.0	4.70	61	47.5	32	15.6
GANDA	148	22.9	108	1.9	3.35	50	44.4	28	28.6
BAHAMA	112	48.7	58	8.6	1.47	34	23.5	26	19.2
CURACAO	58	41.4	34	8.8	1.42	61	23.0	47	17.0
BANGKOK‡	139	47.5	73	13.7	0.50	55	60.0	22	9.1
TAHITI	127	14.2	109	16.5	0.04	48	37.5	30	23.3
NEW-MERIDIAN	89	28.1	64	29.7	1.83	36	44.4	20	15.0
BUGURUNI	52	38.5	32	31.3	2.04	not recorded		24	16.7
MANDALAY‡	75	65.3	26	34.6	2.45				
DAHOMEY	52	63.5	19	63.2	5.88	not recorded		10	10.0
DHOW†	127	55.1	57	80.7	8.21				
NEW-GKEP§	345	44.1	229	18.8					

* Values exceeding 2.306 differ significantly from the control at the 0.05 level.
† JD and DHOW shared the same control.
‡ BANGKOK and MANDALAY shared the same control.
§ Total of the recorded control replicates.

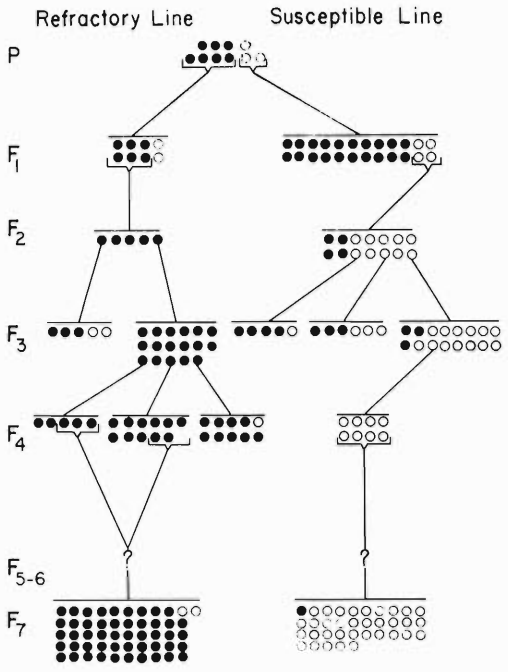

Figure 1. Pedigree showing selection of *A. aegypti* susceptible and refractory lines to infection with *W. flexicauda*. ● = without developing or mature *W. flexicauda* larvae after 12 days; ○ = with developing or mature *W. flexicauda* larvae.

bility of a mosquito species or strain was based on the number of individuals scored as susceptible out of the total number dissected.

The usual technique of counting microfilariae per volume of host peripheral blood was not used. Rather, parasite development after ingestion, not the numbers of circulating microfilariae, was the criterion for selecting the proper control. For this, *A. aegypti* strains of previously determined susceptibility to *W. flexicauda*, NEW-GKEP and the susceptible selected NEW-GKEP, were infected along with experimental mosquitoes. Susceptibility of these control strains was compared to expected susceptibility values to confirm the infectivity of the bloodfeeding.

Results and Discussion

VARIATION IN MOSQUITO SUSCEPTIBILITY. Thirteen culicine species were tested, but *Aedes togoi*, *Aedes albonotatus*, *Aedes mascarensis*, *Aedes albopictus*, and *Armigeres subalbatus* fed poorly or not at all on the bullfrog. Those species that fed adequately are shown in Table 1. The susceptible strain of *A. aegypti* served as the control. By the criterion of development to L_2–L_3, *Aedes malayensis*, *Aedes seatoi*, and *Aedes triseriatus* were not susceptible. *Aedes annandalei* showed moderate susceptibility, and *C. pipiens quinquefasciatus*, *Aedes polynesiensis*, and *Aedes atropalpus* were highly susceptible. It seems clear that susceptibility to *W. flexicauda* is widespread among species of culicinae.

Twelve geographic strains of *A. aegypti* were also tested for variation in susceptibility to *W. flexicauda* (Table 2). The unselected NEW-GKEP strain, which served as control, showed a mean of 18.8% susceptibility with a range of 9–29% for different exposure periods. In contrast, variability in susceptibility of the test strains was much greater, ranging from 0–81%. Using the test for comparison of single observations with the mean of a sample (Sokal and Rohlf 1969), 5 strains—JD, GANDA, MANDALAY, DAHOMEY, and DHOW—differed significantly ($t_{.05(8)} = 2.306$) from the control. This variability did not fit a geographic or subspecific pattern of distribution. Likewise, Kandall's test of rank correlation failed to indicate a correlation between susceptibility and mortality ($\tau = 0.184$, P. = 0.289) or sample size ($\tau = -0.138$, P. = 0.395). These data demonstrate substantial variation in susceptibility of *A. aegypti* to *W. flexicauda*. The selection procedure which follows was based on this variability.

SELECTION FOR SUSCEPTIBILITY. Susceptible and refractory lines were derived from the NEW-GKEP strain of *A. aegypti*. Ten progeny of a single female were tested, 3 were susceptible and 7 were refractory. These individuals became the parental generation from which the two lines were selected (Fig. 1). In all cases the females were mass-mated to their male sibs.

Refractory selection was initiated in the first 2 generations by culling the susceptible phenotypes. Susceptibility decreased from 30–0%. In the F_3 and F_4 the progeny of single females were tested. Susceptibility remained low. The pattern of selection was similar in

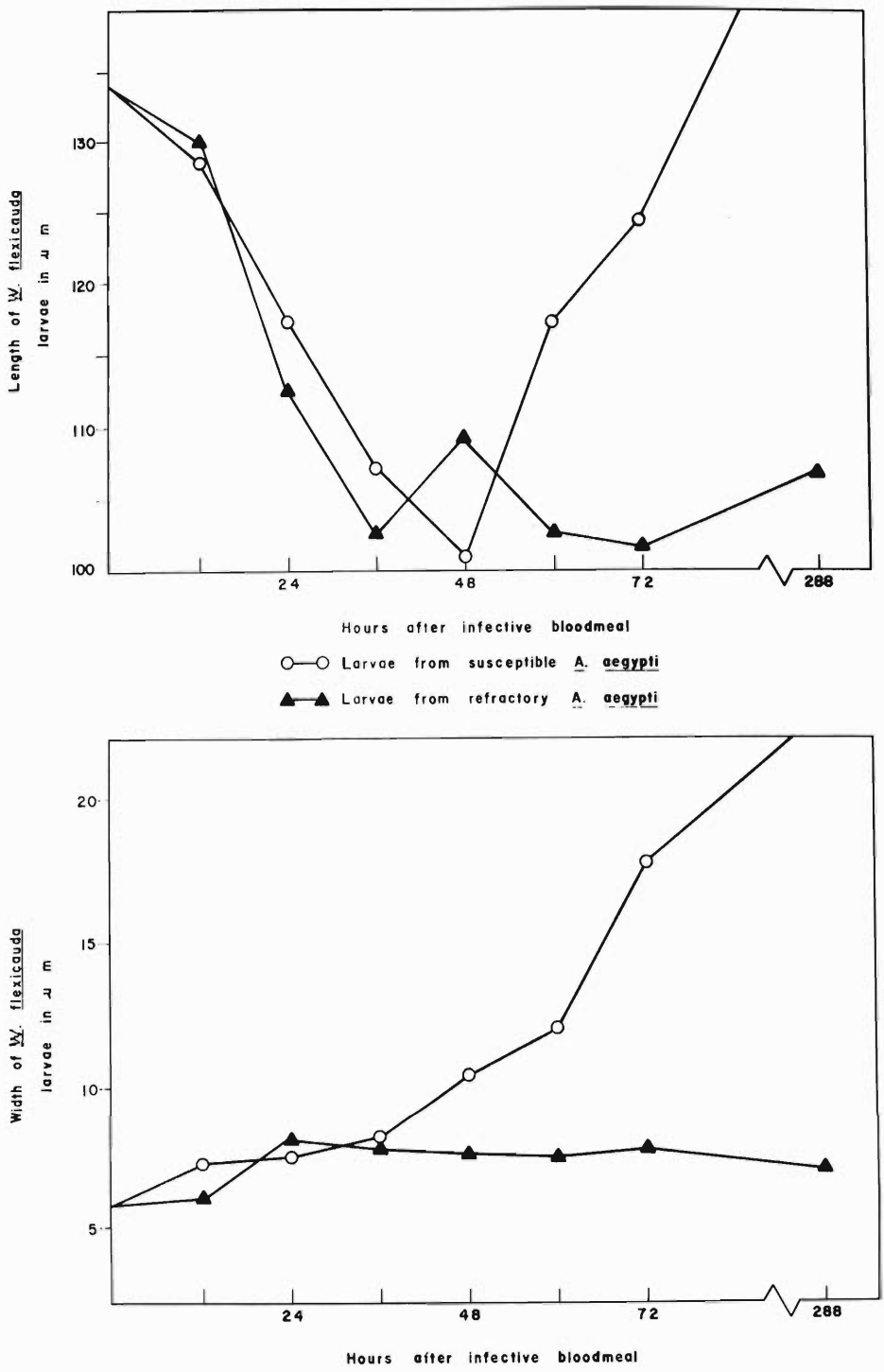

Figure 2. Graphs showing differences in length and width measurements of *W. flexicauda* larvae from susceptible and refractory *A. aegypti*.

the susceptible line. After 2 generations of culling, susceptibility reached 71% and continued to increase after 2 subsequent generations of testing the progeny of single females.

Reduction of progeny numbers by the F_4, probably due to inbreeding, was observed in the susceptible line. Both lines were mass-mated without selective pressure for 2 generations. A portion of the F_6 progeny from each line were tested. The refractory line remained at a low level of susceptibility (4%), while susceptibility in the second line remained high (97%). Thus, we successfully altered susceptibility to *W. flexicauda* in a laboratory strain of *A. aegypti*. The rapid response to artificial selection and the subsequent sustained levels of susceptibility support the hypothesis of inheritance via a simple genetic factor.

Defining refractoriness. Before attempting to analyze the mode of inheritance of this susceptibility factor, we decided to further define our susceptible and refractory phenotypes. The meaning of these two terms is too often left vague and ill defined. Most frequently susceptibility is accepted as the presence of L_3 worms in the mosquito head, while refractoriness is implied as: (1) no microfilariae reaching the site of development, (2) no developing filarial larvae, or (3) no L_3 in the host proboscis. Usage varies from one mosquito-filariid system to another and is dependent on the interests of the individual using the term, i.e. epidemiologist, physiologist, geneticist, etc.

Therefore, mosquitoes from both selected lines were allowed to engorge on a single, infective frog and dissected at 12-hr intervals from day 0–3 and on day 12. For each line at each time interval, measurements of 20 heat-killed larval worms were recorded (Fig. 2). Looking first at length, larvae from refractory mosquitoes were distinguished from their susceptible counterparts after 48 hr. More importantly, worms from refractory hosts did not significantly change length after 36 hr. On the basis of width measurements larvae from susceptible and refractory *A. aegypti* were differentiated after 36 hr. From 24 hr on, worms in refractory mosquitoes did not increase in width significantly. At 12 days post-infection, worms in refractory hosts were vacuolated and motionless. "Melanization" of larvae was not a characteristic of refractoriness, but appeared infrequently in both selected lines.

We have provided further evidence that parasitic development in the vector can be controlled by genetic factors, and that these factors can be selected for in the laboratory. In addition, *Aedes aegypti-Waltonella flexicauda* is a model system for further genetic investigation into mosquito-filariid relationships.

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Isoglaridacris chetekensis sp. n. and *I. wisconsinensis* sp. n. (Cestoda: Caryophyllaeidae) from Red Cedar River, Wisconsin Catostomid Fishes

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ABSTRACT: *Isoglaridacris chetekensis* sp. n. and *I. wisconsinensis* sp. n. parasitized 16 of 22 northern redhorse, *Moxostoma macrolepidotum* (LeSueur) and 17 of 42 northern hogsuckers, *Hypentelium nigricans* (LeSueur), respectively. By possessing a median row of vitellaria, *I. chetekensis* and *I. wisconsinensis* differ from all other species of *Isoglaridacris* except *I. etowani* Williams, 1975, which also shares this character. Differences in testes number, ovary shape, anterior extent of uterus, position of cirrus sac, and fish host serve to separate the above three species.

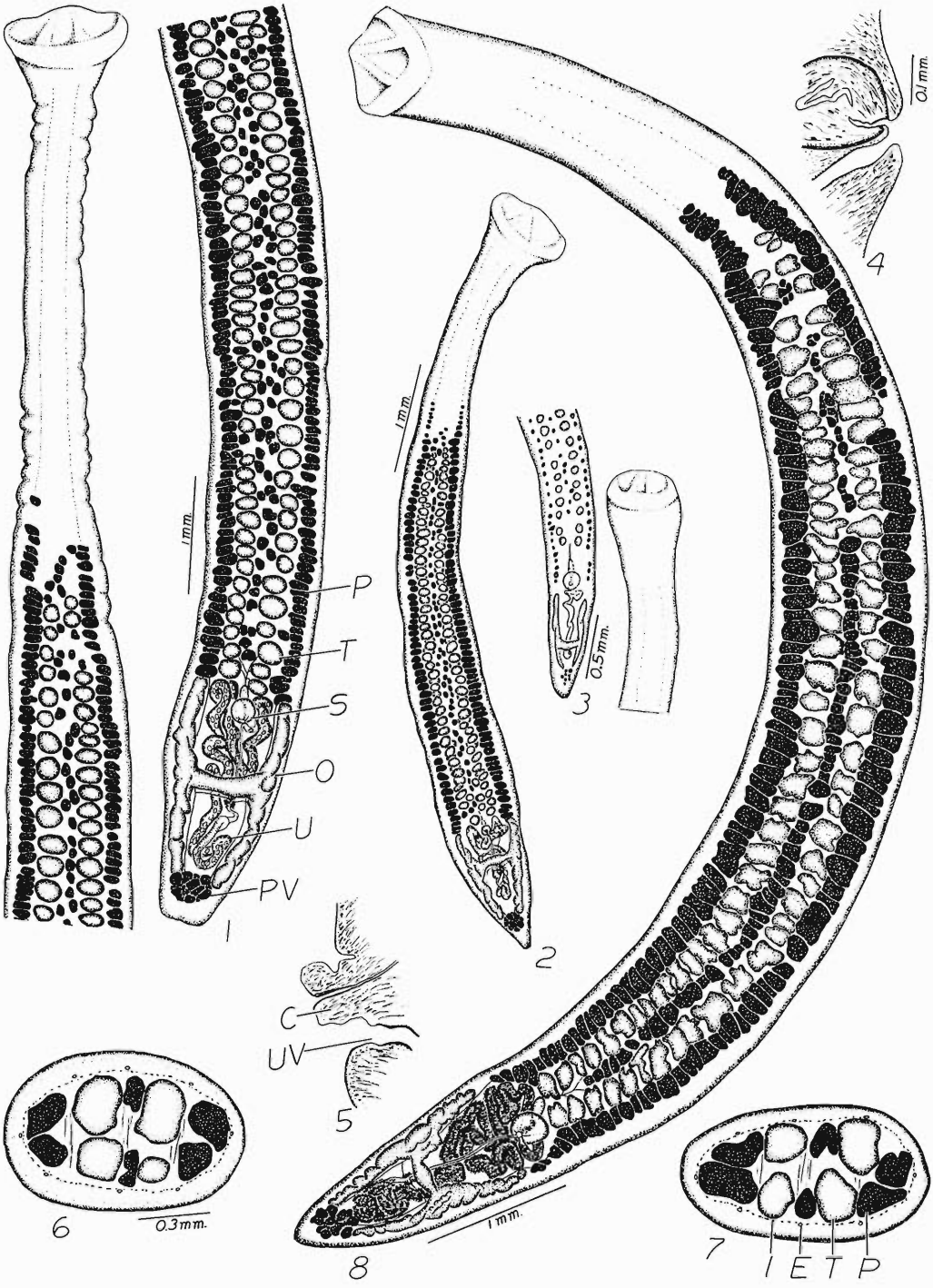
Eight species of caryophyllaeid cestodes comprise the genus *Isoglaridacris* Mackiewicz, 1965. A ninth species, *I. agminis* Williams and Rogers, 1972, is not considered valid (Mackiewicz, 1974). This paper describes two new species of the above genus and presents additional information about *Biacetabulum infrequens* Hunter, 1927.

Materials and Methods

Twenty-two northern redhorse, *Moxostoma macrolepidotum* (LeSueur), and 42 northern hogsuckers, *Hypentelium nigricans* (LeSueur), (13–72 cm in total length) were collected from the Red Cedar River between County Highway D and A bridges (Barron Co.) from March through September, 1968 and June, 1975. Fishes collected by seining and spearing, were examined immediately after capture. Caryophyllaeids were fixed by shaking in cold A.F.A. or 10% formalin, stored in 70% ethanol, stained in Mayer's paracarmine, and mounted in resinous media. Drawings were made with the aid of a microprojector. All measurements are expressed in microns unless otherwise indicated. Means are given with ranges in parentheses (N = sample size).

Isoglaridacris chetekensis sp. n. (Figs. 1, 2, 4, 6)

SPECIFIC DIAGNOSIS (measurement based on 10 gravid cestodes; 2 sectioned): Gravid helminths 11.3 (5.9–15.9) mm long by 0.99 (0.71–1.08) mm wide at gonopore. Scolex cuneiform, bearing three pairs of shallow loculi (Figs. 1, 2). Neck distinct, 4.9 (2.4–5.7) mm from scolex apex to anterior testes; 3.8 (1.9–4.4) mm from scolex apex to anterior vitelline follicles (Figs. 1, 2). Outer longitudinal muscles indistinct. Inner longitudinal muscles distinct, well developed, and arranged around cortical parenchyma (Fig. 6). Testes spherical to oval to irregular in shape, number 216 (201–228), average 222 (100–266) by 171 (80–226) (N = 35). External seminal vesicle present, oval in shape, 188 (80–320)



by 79 (60–112). Cirrus sac circular, 197 (142–232) (N = 8) in diameter, lying entirely to ¾ within anterior ovarian arms (Figs. 1, 2). Cirrus unarmed, eversible. Gonopore large. Gonopore joins uterovaginal canal on ventral surface. Ovary H-shaped, lobate; posterior ovarian arms converge medially but do not join. Ovary arms 1.63 (1.52–1.78) mm long by 0.15 (0.14–0.20) mm wide at isthmus. Vitellaria smaller than testes; preovarian round to irregular in shape in one medial and two lateral rows (Figs. 1, 2, 6); postovarian vitellaria round, consisting of 12–19 follicles, not continuous with preovarian. Vagina straight, 5–8 wide, 280–610 long. Uterus extends anterior of cirrus pouch. Uterine glands well developed. Eight osmoregulatory canals at midbody. Eggs unembryonated, 28 (26–29) by 22 (20–24) (N = 10) (measured in utero). Development unknown.

HABITAT: Anterior ⅓ of intestines, weakly attached.

HOST AND LOCALITY: Northern redhorse, *M. macrolepidotum*. Red Cedar River (Barron Co.), 1.8 km w. Chetek, Wisconsin.

TYPE SPECIMENS: Holotype, U.S.N.M. Helm. Coll. No. 73951; Paratype, U.S.N.M. Helm. Coll. No. 73952.

Remarks

With regard to length, this species exhibits some variation: gravid worms fixed in cold fixative were from 6 to 16 mm. This feature is not unusual among the Caryophyllidea, however. Different fixatives caused variation in the shape of the testes: those fixed in cold formalin were spherical whereas those in cold A.F.A. were irregularly shaped.

I. chetekensis parasitized 16 of 22 redhorse with one to seven cestodes; the mean being 3.2. Gravid adults were present in June and August but not in July. The fish examined during these three months were 13, 6, and 3, respectively. Studies are in progress on the seasonal periodicity of this cestode.

Isoglaridacris wisconsinensis sp. n. (Figs. 3, 5, 7, 8)

SPECIFIC DIAGNOSIS (measurement based on 12 gravid cestodes, 1 sectioned): Gravid cestodes 13.1 (9.3–15.6) mm long by 0.84 (0.75–1.01) mm wide at gonopore. Body tapers at posterior end. Scolex cuneiform to rounded, bearing three pair of shallow loculi (Figs. 3, 8). Neck distinct, 4.2 (3.9–4.7) mm from scolex apex to anterior testes; 3.3 (3.1–4.2) mm from scolex apex to anterior vitelline follicles. Outer longitudinal muscles indistinct. Inner longitudinal muscles, well developed, arranged around cortical parenchyma (Fig. 7). Testes spherical to lobate in shape, number 171 (154–194), average 215 (112–302) by 126 (100–210) (N = 35). External seminal vesicle present, oval in shape, 157 (142–196) by 77 (65–97). Cirrus sac oval to circular, 267 (160–284) (N = 9) in diameter, lying anterior to anterior ovarian arms (Fig. 8). Cirrus unarmed, eversible. Large common gonopore. Ovary H-shaped, lobate; posterior ovarian arms converge medially, but do not join. Ovarian arms 1.28 (0.96–1.56) mm long by 0.16 (0.14–0.18) mm wide at isthmus. Vitellaria smaller than testes; preovarian vitellaria round to irregular in shape: one median row and two lateral rows (Fig. 7); postovarian vitellaria round, consisting of 9–19 follicles. Vagina straight, 5–7 wide, 490–820 long. Uterus seldomly extending anterior to cirrus pouch. Uterine glands well developed. Seminal receptacle absent. Eight osmoregulatory canals at midbody region (Fig. 7). Eggs unembryonated, 34 (32–35) by 23 (21–24) (N = 10) (measured in utero). Development unknown.

HABITAT: Anterior ⅓ intestine, weakly attached.

←

Figures 1–8. All cestodes from Red Cedar River, Wisconsin. 1. Gravid *Isoglaridacris chetekensis*. 2. Gravid *I. chetekensis*. 3. Scolex and posterior of immature *I. wisconsinensis*. 4. Midsagittal section through gonopore of *I. chetekensis*. 5. Midsagittal section through gonopore of *I. wisconsinensis* showing everted cirrus. 6. Cross-section through mid-body of *I. chetekensis*. 7. Cross-section through mid-body of *I. wisconsinensis*. 8. Gravid *I. wisconsinensis*. Figs. 5 and 7 are drawn to scale shown in Figs. 4 and 6 resp. Abbreviations: C—cirrus; E—excretory (osmoregulatory) canal; I—inner longitudinal muscles; P—preovarian vitellaria; PV—postovarian vitellaria; S—cirrus sac; T—testis; U—uterus; UV—uterovaginal opening.

HOST AND LOCALITY: Northern hogsucker, *H. nigricans*. Red Cedar River (Barron Co.) 1.8 km w. Chetek, Wisconsin.

TYPE SPECIMENS: Holotype, U.S.N.M. Helm. Coll. No. 70702. Paratype, U.S.N.M. Helm. Coll. No. 70703.

Remarks

Although scolex variation was more prevalent in this species, variation in the length of gravid individuals was less than in *I. chetekensis*.

The incidence of parasitism varied from one immature to 33 gravid cestodes per infected fish ($\bar{x} = 5.6$). Immature cestodes were obtained in early spring collections (March–May). Gravid cestodes were obtained in all months March through September.

Only one other species of *Isoglaridacris*, *I. etowani*, Williams, 1975, (from the Alabama hogsucker, *H. etowanum* (Jordan)) also possesses a median row of preovarian vitellaria and is thus similar to *I. wisconsinensis* and *I. chetekensis*. *I. chetekensis* differs from *I. etowani* in having 201 or more testes, ovary H-shaped with open apex, uterus extending anterior of cirrus sac, and cirrus sac enclosed by anterior ovarian arms, while *I. etowani* possess 105 or fewer testes, ovary A-shaped normally with closed apex, uterus not extending anterior of the cirrus sac, and the cirrus sac not enclosed by anterior ovarian arms. *I. wisconsinensis* differs from *I. etowani* in having 154 to 194 testes, ovarian apex open; neck short, 0.3 or less of body length; and cirrus sac generally larger (160–284 μ), while *I. etowani* possesses 105 or fewer testes; ovarian apex closed; neck long, more than 0.3 of body length; and cirrus sac generally smaller (137–197 μ).

I. wisconsinensis differs from *I. chetekensis* in that the former has 194 or fewer testes, the cirrus is anterior to the anterior ovarian arms and thus, is not enclosed by them, the cirrus sac is larger, the uterus seldomly extends anterior to the cirrus sac, and the scolex is wedge-shaped to rounded and nearly the same width as its neck, while *I. chetekensis* has 201 or more testes, the cirrus sac is enclosed by the anterior ovarian arms, the cirrus sac is smaller (for cestodes of each species of the same total length), the uterus extends anterior of the cirrus sac, and the scolex definitely wedge-shaped, is always wider than its neck. In addition, both caryophyllaeids are host specific for different species of catostomids even though infected catostomids were collected in the same seine haul within yards of each other; northern hogsuckers in shallow water and northern redhorse in adjacent deeper water.

Biacetabulum infrequens Hunter, 1927

B. infrequens has been encountered in Iowa golden redhorse, *M. erythrurum* (Raf.) silver redhorse, *M. anisurum* (Raf.) (Calentine, 1965) and in Wisconsin greater redhorse, *M. rubreques* Hubbs, (Fischthal, 1947) but was reported absent in northern redhorse in these two studies. Its presence in northern redhorse, *M. macrolepidotum*, constitutes a new host record. *B. infrequens* is apparently a common helminth of redhorse (*Moxostoma*) and its catholic occurrence seemingly indicates a lack of definite host specificity for species of *Moxostoma*.

In this study it was a frequent parasite, infecting 12 of 22 (54.4%) northern redhorse. Variation in the number of cestodes (immature to gravid) per host varied from one to 37 ($\bar{x} = 5.7$). *B. infrequens* was present in 12 of 13 redhorse collected in June but absent in redhorse collected in July and August.

Discussion

Three species of caryophyllaeids are now known to parasitize northern redhorse: *I. longus* Fredrickson and Ulmer, 1967, in Iowa (Fredrickson and Ulmer, 1967); *I. chetekensis* in Wisconsin (this study); and *B. infrequens* in Wisconsin (this study). Four species of caryophyllaeids parasitize northern hogsuckers: *M. ulmeri* in Iowa (Calentine and Mackiewicz, 1966); *I. bulbocirrus* Mackiewicz, 1965 in five states (Mackiewicz, 1965); *I. wisconsinensis* in Wisconsin (this study); and *B. infrequens* in Tennessee (Mackiewicz, 1972). Mackiewicz (1965) was unable to substantiate the presence of *Glaridacris catostomi* Cooper, 1920, in Wisconsin northern hogsuckers, as reported by Fischthal (1947).

Acknowledgments

Appreciation is expressed to Messrs. J. M. Dennis, D. V. Williams, and R. Lindblad, all of Chetek, Wisconsin for their aid in collecting fishes.

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Announcement

In response to numerous requests for reprints of the publication, "Identification of Parasitic Metazoa in Tissue Sections," by MayBelle Chitwood and J. Ralph Lichtenfels has been reprinted by the U. S. Department of Agriculture. Copies are available from J. Ralph Lichtenfels, Animal Parasitology Institute, USDA, ARS, BARC East Bldg. 1180, Beltsville, Maryland 20705.

Research Note

Prevalence of Certain Endoparasitic Helminths of the Yellow Perch from Western Lake Erie

A number of studies of parasitism in Lake Erie fishes have been conducted. Early investigations are summarized in the extensive study of Dechtiar (1972. Great Lakes Fish. Comm. Tech. Rpt. No. 17. 20 p.). The purpose of this work was to discern the prevalence of endoparasitic helminths in the body cavity and viscera of yellow perch, *Perca flavescens*, from the island region of western Lake Erie.

A minimum of 100 yellow perch were collected by otter trawl on a twice monthly basis from June through October, 1974. The fish were transported to the laboratory alive or on ice and examined immediately for parasites. Identification was accomplished by studying living and preserved whole mount specimens under a compound microscope. Preserved specimens were prepared for identification following standard methods.

A total of nine species of helminth parasites were removed from the body cavity and visceral organs of the 735 yellow perch examined. These are listed in Table 1.

Two previous studies by Bangham and Hunter (1939. Zoologica 24:385–448) and Bangham (1972. Bull. Ohio Biol. Sur. n. s. 4:1–23) have been made of fish collected from the same areas as those in the present study. Table 1 compares the prevalence of the parasites collected during the present study with the prevalence of these parasites in yellow perch from the western basin of Lake Erie during 1927–1929 and 1957. Dechtiar (loc. cit.) reported 13 new host records for parasites of Lake Erie perch, but he did not distinguish records from fish collected in the eastern and western basins of the Lake. Comparisons with this study, therefore, are not possible.

Of the nine species of helminths encountered parasitizing the body cavity and visceral organs of yellow perch, it is evident that *T. nodulosus* and *E. tubifex* have increased in prevalence, *C. cooperi*, *Bothriocephalus* sp., *Proteocephalus* spp. and *D. cotylophora* have decreased in prevalence and *P. cylindracea* and *C. oxycephalus* exhibit no difference in prevalence (χ^2, 95% l.c. with 1 d.f.) since 1927–1929.

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Table 1. Prevalence of certain endoparasitic helminths of yellow perch from Western Lake Erie.

Parasite	Site of infection	Prevalence 1927–1929 n-60	Prevalence 1957 n-93	Prevalence 1974[a] n-735
Trematoda				
Crepidostomum cooperi Hopkins, 1931	intestine	20	65	0.8
Cestoda				
Bothriocephalus sp.[b]	intestine	11.6	15.1	3.3
Triaenophorus nodulosus Pallas, 1790[b]	liver, mesenteries	3.3	3.2	12.2
Proteocephalus spp.[b]	intestine	80	10.8	0.1
Nematoda				
Camallanus oxycephalus Ward and Magath, 1917	intestine	1.6	5.4	6.1
Dacnitoides cotylophora (Ward and Magath, 1917)	intestine	38.3	31.2	10.3
Eustrongylides tubifex (Nitzsch, 1819)[b]	mesenteries	0.0	8.6	41.4
Philometra cylindracea (Ward and Magath, 1917)	body cavity	1.6	0.0	8.4
Acanthocephala				
Neoechinorhynchus cylindratus (Van Cleave, 1913)	intestine	0.0	0.0	1.9

[a] Present study.
[b] Immature or larval forms.

Research Note

Prepatent and Patent Periods of the Bovine Coccidium *Eimeria subspherica* Christensen, 1941, with a Redescription of the Sporulated Oocyst

Eimeria subspherica was originally described from cattle in Alabama (Christensen, 1941, J. Parasit. 27: 203–220) and has since been reported from cattle throughout the world (Levine and Ivens, 1970, Ill. Biol. Monogr. 44: 71–72). The prepatent and patent periods for this species are reported here, along with a redescription of the sporulated oocysts.

Fecal samples were collected from the rectums of cattle with natural infections of *E. subspherica*, and the oocysts were recovered and sporulated by the methods of Soekardono et al. (1975, Vet. Parasit. 1: 19–33). Oocysts used to inoculate calves were from prior experimental infections. Seven 2- to 3-week-old calves were inoculated per os with 1 million to 58 million sporulated oocysts. Feces were collected daily from these calves and examined for oocysts by direct flotation with Sheather's sugar solution. For detailed microscopic examination, the sporulated oocysts were concentrated with Sheather's sugar solution and examined with a microscope fitted with a 100× planapochromatic oil immersion objective. One hundred sporulated oocysts and 100 sporocysts were measured. The number of layers in the oocyst wall was determined by carefully rolling the oocysts under gentle coverslip pressure until the outer layer peeled off. All measurements are in microns; the means are in parentheses after the ranges.

The prepatent and patent periods from the experimental infections are shown in Table 1.

Table 1. Prepatent and patent periods of *Eimeria subspherica*.

Number of oocysts given in millions	Prepatent period in days	Patent period in days
1	7	15
6	8	12
12	9	12
12	18	4
29	8	9
29	9	13
58	8	13

The mean prepatent period was 9.3 days, and the mean patent period was 11.1 days. The prepatent period was shorter than that reported for most bovine coccidia (Levine and Ivens, 1970, loc. cit.). One of the calves had an unusually long prepatent period of 18 days and a very short patent period of 4 days (Table 1). This may have been due to a natural infection.

From preliminary work (unpublished data), we found that it was necessary to give the calves at least one million sporulated oocysts to produce detectable patent infections with *E. subspherica*. Soekardono et al. (1975, loc. cit.) found it necessary to give 10 million oocysts of *E. alabamensis* to produce consistent detectable patent infections in calves. Further

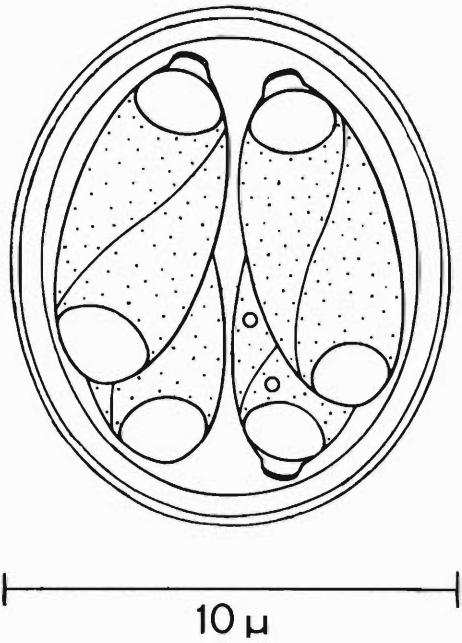

Figure 1. Drawing of the sporulated oocysts of *Eimeria subspherica*.

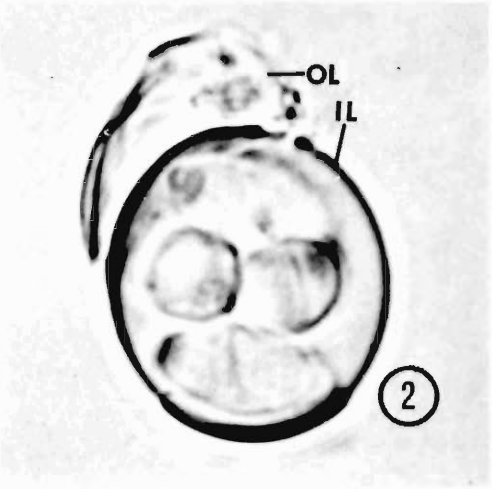

Figure 2. Photomicrograph of sporulated oocyst of *Eimeria subspherica* with the outer layer (OL) of the wall separated from the inner layer (IL). × 3,600.

investigation is needed to determine why it is necessary to give such large numbers of oocysts to produce detectable infections with some species of bovine coccidia.

Sporulated oocysts of *E. subsperica* were spherical to subspherical, rarely ellipsoidal (Fig. 1). The oocyst wall was smooth and consisted of 2 layers (Fig. 2) about 1.0 thick. When separated, the outer layer was brownish-yellow, 0.3 thick, and the inner layer was colorless, 0.7 thick, and lined by a dark membrane. Micropyle and micropylar cap were absent. The oocysts were 10–13 by 9–12 (11.0 by 10.5); their length–width ratios were 1.0–1.2 (1.06). Polar granule and oocyst residuum were absent. The sporocysts were elongate-ellipsoidal, 7–8 by 3–5 (7.7 by 3.1), with a Stieda body and without a substiedal body. The sporocyst residuum was absent or rarely present as a few scattered granules. The sporozoites were elongate, lying lengthwise in the sporocysts, partly curled around one another. A posterior refractile globule was present in each sporozoite. A refractile granule was usually present at the ends of sporocysts. The nuclear area was visible in most sporozoites.

Our observations of the oocysts of *E. subspherica* agree with those of previous workers (Levine and Ivens, 1970, loc. cit.) except most reports state that the oocyst wall of *E. subspherica* has only one layer. By careful manipulation however, we found that there are two layers. Bhatia et al. (1968, Acta Vet. Acad. Sci. Hungary 18:115–133) reported that the wall had two layers. They drew the oocyst wall with a thick outer layer and a thin inner one. We found that the wall actually had a thin outer layer and thick inner one.

The oocyst of *E. subspherica* is the smallest one described from cattle, and it can be easily overlooked during routine fecal examinations. To determine whether *E. subspherica* was present, we often found it necessary to examine fecal flotations at 400× magnification, especially when pollen grains were abundant in the feces.

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Research Note

Occurrence of Canine Hepatozoonosis in the Philippines

Hepatozoon canis was first described in the leucocytes of a dog in India by Bentley (1905, The Brit. Med. J. I: 988). In the same year, James (1905, Sci. Mem. Off. Med. and Sanit. Depts. Govt. India, N.S. 14: 1–12) also reported the parasite in India and originally assigned the name *Leucocytozoon canis*, a generic name already reserved for avian hemo-

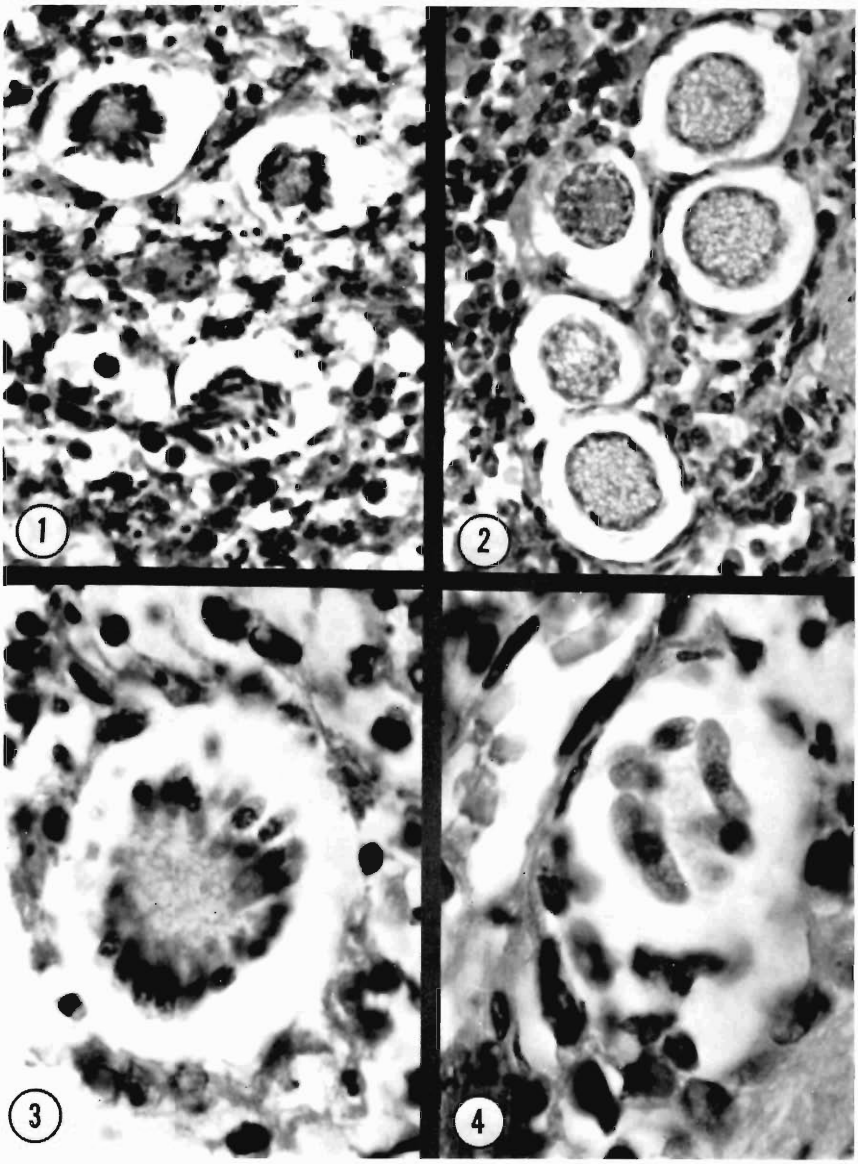

Figure 1. Case #3. Splenic necrosis with associated schizonts. H & E stain. × 600.
Figure 2. Case #3. Spleen with several schizonts. H & E stain. × 525.
Figure 3. Case #3. Higher magnification of schizont containing small merozoites. H & E stain. × 1310.
Figure 4. Case #3. Higher magnification of schizont containing several large merozoites. H & E stain. × 1310.

gregarines. The genus *Hepatozoon* was founded by Miller in 1908 for a parasite observed in the leucocytes of laboratory rats (1908, Bull. U.S. Hygiene Lab. 46: 1–48).

Although *H. canis* is a cosmopolitan parasite having been reported from Europe, Africa and parts of Southeast Asia, it has been the subject of little systematic review or experi-

mentation. The most comprehensive description of the genus *Hepatozoon* is by Wenyon (1926, Protozoology, Bailliere Tindall and Cox, London 1563 pp.).

The brown dog tick, *Rhipicephalus sanguineus* is considered the vector of the parasite. Natural infection occurs from ingestion of ticks harboring the organism. Schizogony occurs in the liver, spleen, bone marrow and other organs and the merozoites produced enter leucocytes, where they form the familiar gametocytes which have been described in the peripheral blood. They are subsequently taken into the body of the tick following a blood meal where sporogony and gametogony occur (Wenyon, 1926, loc. cit.).

Information is sparse regarding the pathogenicity of *H. canis*. Rau (1925, Vet. J. 81: 293–307) claimed to have infected dogs by injecting splenic material containing schizonts. He reported that parasites were found in the peripheral blood two to three weeks after infection and that the infection may bring about the death of the animal. Rahimuddin (1942, Ind. Vet. J. 19: 153–154) reported a serious disease syndrome in dogs leading to death which was presumably due to *H. canis*. A report from South Africa by Porter also indicated that this parasite may cause serious disease (1918, S. Afr. J. Sci. 15: 335–336). In 1954, Tuchmanyan cited by Galuzo (1965, Acad. Sci. Kazakh. S. S. R., ALMA-ATA pp. 328–342) examined 900 dogs in Tashkent, USSR and found *H. canis* in 20 of the animals. He also found that experimental infection in dogs resulted in clinical signs four to five months later with signs resembling those of visceral leishmaniasis. Hoogstral pointed out that the parasite is apparently able to cross the placental barrier (1961, Int. Rev. Trop. Med. 1: 247–267). Also, the possibility of *Hepatozoon* as a cause of disease in neonatal pups has been suggested by Jackson (1958, Vet. Rec. 70: 288–289).

In the Philippines, Carlos et al. (1971, Phil. J. of Vet. Med. 10: 181–189) identified gametocytes of *H. canis* in the leucocytes of eight dogs. Four showed clinical disease while the rest were apparently healthy. The death of one of the four sick dogs was attributed to concurrent dirofilariasis; however, a necropsy was not done. The other three dogs showed decreased parasitemia after treatment with antibiotics, iron and vitamins. One dog later became free of gametocytes on plain blood and buffy coat smear preparations.

This is the first histopathological description of three cases in which schizonts resembling those of *H. canis* were observed in tissues from dogs in the Philippines. One dog (Case 1) was euthanatized due to an intractable demodecosis, while two other cases died following a clinical syndrome resembling canine distemper. In Case 1, significant changes were in the liver and consisted of small focal areas of necrosis associated with *H. canis* organisms. Schizonts were also observed in the prescapular lymph node. In Case 2, the liver was also affected with sharply demarcated areas of coagulative necrosis and infiltrations of lymphocytes and macrophages. The splenic changes consisted of multifocal zones of necrosis and reticuloendothelial cell hyperplasia. There were many schizonts present in the liver and spleen in association with areas of necrosis. Other pathologic features were interstitial pneumonia and slight interstitial myocarditis. In Case 3, numerous schizonts were seen in the spleen often in association with large foci of necrosis (Figs. 1 and 2). Several types of schizonts varying in size and number of merozoites were observed (Figs. 3 and 4). Lesions in the liver were similar but less severe. Early pneumonitis was found in the lung; however, only one schizont was observed in the lung within an endothelial cell. Other tissues including the central nervous system were not remarkable.

Since we were unable to confirm a histopathologic diagnosis of canine distemper or other intercurrent disease, it appears that hepatozoonosis could have been the cause of death in cases 2 and 3. The finding of numerous *H. canis* developmental forms in tissues of three dogs in association with areas of focal necrosis indicates a direct injurious effect by the parasite. Although some authors consider *H. canis* of little pathologic significance, we feel that it can be responsible for severe lesions and death. This view is in agreement with a recent report by McCully et al. (1975, Onderstepoort J. Vet. Res. 42: 117–134) who described severe lesions in dogs due to *H. canis* which were seen in uncomplicated cases as well as in those cases complicated by intercurrent viral and protozoal disease. Further

studies are needed to determine the pathogenetic mechanisms involved in canine hepatozoonosis as well as the host specificity of the parasite in various carnivores.

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Research Note

Cestodes from *Rattus* Fischer in Taiwan

From a total of 206 rats, representing four species, collected in Taiwan and examined for helminth parasites, 173 individuals, or 84%, were infected with tapeworms.

The hosts included 82 specimens of *Rattus norvegicus* (Berkenhout), 80 of *R. rattus* subsp., 25 of *R. losea* (Swinhoe), and 17 of *R. c. coxinga* (Swinhoe), among each of which were infected specimens. Both of two individuals of *R. rattus mindanensis* Mearns examined were uninfected.

Four families of cestodes with a total of seven identifiable and two uncertain species were found (Table 1).

Of the seven species of cestodes identified, all except *Dipylidium caninum* (Linné, 1758) are common parasites of rats in that portion of Asia which includes China and its offshore islands (Tang, 1940, Biol. Bull. Fukien Christian Univ. 2: 73–88; Li and Hsü, 1948, Peking Nat. Hist. Bull. 16: 203–214; Tubangui, 1931, Philipp. J. Sci. 46: 537–591; Yeh, 1970, J. Taiwan Assoc. Anim. Husb. Vet. Med., No. 16, pp. 38–43).

The appearance of *Dipylidium caninum*, typically a parasite of canines and felines, and occasionally humans, in four specimens of *Rattus norvegicus* is the first instance noted by

Table 1. Cestodes from species of *Rattus* in Taiwan.

	Hosts							
	Rattus coxinga coxinga (17 examined) Infected		*Rattus losea* (25 examined) Infected		*Rattus norvegicus* (82 examined) Infected		*Rattus rattus* subsp. (80 examined) Infected	
Cestodes	No.	%	No.	%	No.	%	No.	%
Hymenolepidiidae								
Hymenolepis diminuta	8	47	8	32	26	31.7	19	23.7
Hymenolepis sp.	0	0	0	0	1	1.2	1	1.25
Rodentolepis straminea	3	17.5	0	0	2	2.4	4	5
Vampirolepis fraterna	9	53	0	0	3	3.7	4	5
Davaineidae								
Raillietina (Raillietina) celebensis	3	17.6	8	32	31	37.8	19	23.7
Raillietina (Paroniella) retractilis	0	0	0	0	2	2.4	1	1.25
Raillietina (R.) sp.*	2	11.7	8	32	18	22	19	23.7
Dilepidae								
Dipylidium caninum	0	0	0	0	4	4.8	0	0
Taeniidae								
Hydatigera taeniaeformis (strobilocercus)	0	0	1	4	4	4.8	1	1.25

* Probably *Raillietina (R.) celebensis.*

the writers in which this species, one of medical and veterinary importance, has been found in rats or any species of rodents. This report and those of Ivanitskii, Tysmbal, and Nosik (1940, Sbornik Trudov Kharkovskogo Vet. Inst. 19: 163–164) and Shimalov (1965, Vesti Akad. Navuk BSSR, s. Biial. Navuk (1), p. 120–123) of *D. caninum* in the intestine of pigs extend the range of definitive hosts of this cosmopolitan cestode.

Other species of cestodes found in this survey and known to occur in humans include *Vampirolepis fraterna* (Stiles, 1906), which occurred in three species of rats and *Raillietina* (*Raillietina*) *celebensis* (Janicki, 1904). *R.* (*R.*) sp., which could not be identified with certainty, but is probably *R.* (*R.*) *celebensis*, appeared in all four species of rats.

The four remaining species include *Rodentolepis straminea* (Goeze, 1872) from rats and mice, *Raillietina* (*Paroniella*) *retractilis* Stiles, 1925 from rabbits, hares and rats, *Hymenolepis diminuta* (Rudolphi, 1819) from rats and mice, and the strobilocercus of *Hydatigera taeniaeformis* (Batsch, 1876) whose adults are cosmopolitan parasites of cats.

Yeh (1970 loc. cit.) noted that the incidence of *R.* (*R.*) *celebensis* in rats had increased over the past 30 years in Taiwan. Aside from *Hymenolepis diminuta*, *R.* (*R.*) *celebensis* is the most abundant species found in the rats of this survey.

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Research Note

Anticoccidial Activity of Flutamide (Sch 13521) against *Eimeria tenella*

Flutamide (4'nitro 3'trifluoromethyl isobutyranilide, Sch 13521) is a nonsteroidal antiandrogen in rodents and dogs (Neri et al., 1972, Endocrinol. 91: 427–437) and causes penis displacement and inhibits Cowper's gland development in male swine fetuses (Dame and Campbell, 1974, Annual Meeting, Society for Reproduction). This report presents data evaluating the prophylactic and therapeutic anticoccidial activities of this compound against *Eimeria tenella*.

Hubbard, White Mountain cross cockerels were obtained at one day of age from a supplier in Pennsylvania and maintained under coccidia-free conditions until used at 7 days of age.

Flutamide (Sch 13521) was supplied as pure drug in micronized form. Appropriate amounts were weighed and mixed in a crude premix by hand and then mixed with a household mixer to obtain final concentrations of 0.1, 0.2 and 0.3% of diet. A prophylactic and a therapeutic evaluation were each conducted as described below.

Prophylactic study: Chicks 7 days of age were weighed, wingbanded and distributed by weight in groups of 10. Two days after starting on medicated feeds each bird was infected with 250,000 sporulated oocysts of *Eimeria tenella*.

In the prophylactic study, estimates of cecal oocyst numbers, cecal lesions, weight gains,

Table 1. Prophylactic activity of flutamide against experimental *Eimeria tenella* infections in chickens.

Drug level	Weight gain (survivors) grams	Weight gain (survivors) % UUC	Mortality/ Total	Lesion indices	Cecal oocysts Bird ($\times 10^6$)	Fecal score	Index
0.30	127.6	42.6	0/10	1.3	0.081	—	128.8
0.20	180.0	60.6	0/10	0.8	0.105	—	151.5
0.10	236.0	79.0	0/10	0.0	0.194	—	177.1
Uninfected Untreated Control	297.9	100.0	0/10	0.0	0.0	—	200.0
Amprolium (0.0125%)	253.4	84.0	0/10	0.1	0.0	—	183.9
Infected Untreated Control	141.3	47.6	3/10	1.8	3.401	+++	65.6

mortality and droppings scores were evaluated as described (Panitz, 1974a, b, Proc. Helm. Soc. Wash. 41: 111–112; J. Parasit. 60: 530–531). The anticoccidial index (ACI) described previously was calculated for each group.

There was no mortality in any birds treated prophylactically with flutamide (Table 1). Cecal oocyst estimates of each group of experimental birds was reduced by at least 95% when compared to the infected untreated control group. Cecal lesion indices increased in a direct relationship to drug level. This suggests that the drug may have affected the cecal appearance in the 0.2% and 0.3% treatment groups. Fecal scores were evaluated as severe in the IUC group only. Drug levels of 0.025% (Panitz, unpublished data) were ineffective in preventing *E. tenella* induced coccidiosis.

THERAPEUTIC STUDY: Birds were distributed as described above and infected with 250,000 sporulated *E. tenella*. Starting simultaneously with infection a group of chicks was placed on medicated feed of 0.1% diet. An additional group of chicks was placed on flutamide medicated feed (0.1% diet) on each of days 1, 2, and 3 after infection. Chicks remained on medicated feed until termination of the experiment. Birds were weighed and killed 10 days after infection and cecal lesions were evaluated as described (Panitz, 1974a, b, loc. cit.).

Estimates of daily and total oocyst production by each treatment group starting day 6 after treatment and terminating day 10 at necropsy were determined by the Long and Rowell (1958, Lab. Pract. 7: 515–518, 534) method. Cecal lesion indices, mortality, fecal scores and weight gains were evaluated. The ACI was calculated for each treatment group.

Weight gains of survivors in experimental groups decreased with the increased delay in

Table 2. Therapeutic activity of flutamide against experimental *Eimeria tenella* infections in chickens.

Drug level (% diet)	Time started (DPI)*	Weight gain (survivors) grams	Weight gain (survivors) % UUC	Mortality/ Total	Lesion index	Total oocysts/ group ($\times 10^6$)	Fecal score	Index
0.1	0	201.7	82.0	0/10	0.9	44.16	+	128.8
0.1	+1	215.8	88.0	2/10	1.7	91.18	++	59.8
0.1	+2	198.1	80.5	0/10	1.8	92.69	+++	69.8
0.1	+3	159.9	64.9	3/10	2.4	45.99	+++	65.0
Infected Untreated Control	—	210.1	85.0	0/10	1.8	106.0	++++	61.0
Uninfected Untreated Control	—	245.1	100.0	0/10	0.3	0.00	—	197.0

* DPI = days post infection.

Table 3. Effect of therapeutic administration of flutamide on *Eimeria tenella* oocyst release pattern.

Treatment group	Days after infection				
	6	7	8	9	10
Infected Untreated Control	5.7*	68.0	4.7	13.6	7.5
0**	2.3	59.0	19.2	13.6	5.7
+1	2.2	33.0	30.8	24.8	9.3
+2	10.8	37.8	20.2	21.8	8.6
+3	4.4	28.4	26.2	10.8	30.4

* Percent of total oocysts passed.
** Days after infection treatment started.

addition of drug (Table 2). Mean weights of all surviving experimental groups were approximately equal to or less than the infected untreated control group. No definitive mortality pattern was observed. Lesion indices were reduced only in birds treated starting day 0. Total oocyst production was reduced by 58%, 14%, 12% and 54% in groups receiving treatment on days 0, +1, +2 and +3 after infection respectively. Fecal dropping scores also increased in groups where drug administration was delayed after infection.

The pattern of daily oocyst shedding is of interest as the level of flutamide used in these studies appears to modify the shedding pattern, minimizing the peak passage of oocysts as is classically expected, and shown in the IUC group (Table 3).

The therapeutic study suggests the main mode of action of flutamide is on the early stages of the life cycle of *E. tenella*, either in the intestinal lumen or migrating within the intestinal wall. The pattern of oocyst excretion suggests a more subtle and less pronounced effect on later stages of the coccidian life cycle. This could be determined by appropriate *in vitro* or histologic evaluation.

The antiandrogenic activity of flutamide (Neri et al., 1972, loc. cit.; Dame and Campbell, 1974, loc. cit.) may have influenced the chicks' weight gain and cannot be overlooked. No attempt was made to evaluate flutamide effects on sex organs of the birds used in these studies nor were histologic preparations of the cecal lesions examined.

The chemical similarity between flutamide and corresponding 3,5 dinitrobenzamides such as zoalene and aklomide and 3 trifluoromethyl, 5 nitrobenzamides should also be noted.

Dr. Eli Gold synthesized the compound used in these studies.

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Research Note

Helminths of Wild Turkeys in West Texas

Helminth parasitism in the eastern subspecies of the wild turkey, *Meleagris gallopavo silvestris* Viellot, was reviewed and extensively studied by Prestwood (1968, Parasitism Among Wild Turkeys (*Meleagris gallopavo silvestris*) of the Mississippi Delta, Ph.D. dissertation, Univ. Ga., 67 p.) and Prestwood, Kellogg, and Doster (1973, *In* G. C. Sanderson and H. C. Schultz, Wild Turkey Management, Proc. Natl. Wild Turkey Symp. 2, Univ. Mo. Press, Columbia, Mo., 159–167). Recently, Hon, Forrester, and Williams (1975, Proc. Helm. Soc. Wash. 42: 119–127) examined 220 wild turkeys from Florida, recovered 34 species of helminths, and compared the helminth faunas of *Meleagris gallopavo osceola* Scott from peninsular Florida with that of *M. g. sylvestris* from the Mississippi Delta as elucidated by Prestwood (1968, loc. cit.). The only existing record of disease and parasitism in the Rio Grande subspecies, *Meleagris gallopavo intermedia* Sennett, from Texas is that of Thomas (1964, Wilson Bull. 76: 292) who found a single case each of blackhead (*Histomonas meleagridis*), scaly-leg (*Knemidokoptes mutans*), and fowl pox in 3 of 330 birds examined. The only record of

Table 1. Helminths from the Rio Grande Wild Turkey in West Texas.

Helminth	Prevalence		Number of worms per infection	
	Number infected	Percent	Mean	Range
Cestoda				
Metroliasthes lucida Ransom, 1900	12/12	100	21	1–159
Raillietina williamsi Fuhrmann, 1932	8/12	67	4	1–21
Liga braziliensis (Parona, 1901)	4/12	33	8	2–24
Nematoda				
Heterakis gallinarum (Schrank, 1788)	7/12	58	2	1–12

helminth parasitism in this subspecies is the study of Self and Bouchard (1950, J. Parasit. 36: 502–503) from the Wichita Mountains in southwestern Oklahoma.

Because of the dearth of information on the helminth fauna of this subspecies the present study was undertaken. The viscera of 12 *M. g. intermedia* were collected in November and December 1973 near Paint Rock, Concho County, Texas by the junior author. These consisted of 6 (4 juveniles and 2 adults) male and 6 (3 juveniles and 3 adults) female specimens. All organs were examined for gross lesions and helminths. Intestinal and cecal contents were sedimented in beerman glasses and the sediment examined under a dissecting microscope. The supernatant was passed through a series of fine meshed screens to facilitate recovery of small nematodes. Cestodes were fixed in AFA, stored in 70% ethyl alcohol and later stained with colestin blue B or Semichon's acetic carmine.

Three species of cestodes and one nematode were recovered (Table 1). Of these, only *Liga braziliensis* (Parona, 1901) represents a new host record for the wild turkey in North America. Ransom (1909, Bull. U.S. Nat. Mus. 69: 1–141) provided a history and redescription of this species based on specimens from the common flicker, *Colaptes auratus*, in Maryland. This species was also reported from *C. auratus* and *Dendrocopos villosus* in Oregon by Weatherly and Canaris (1960, J. Parasit. 47: 230). The former host is common in the open scrub live oak woodlands of West and Central Texas which may explain the source of infection for the wild turkey. The remaining species, *Metroliasthes lucida* Ransom, 1900, *Raillietina williamsi* Fuhrmann, 1932, and *Heterakis gallinarum* (Schrank, 1788) are common parasites of the wild turkey in the Eastern and Southeastern United States (Prestwood, et al., 1973, loc. cit.; Hon et al., 1975, loc. cit.). Except for *M. lucida* reported from Oklahoma by Self and Bouchard (1950, loc. cit.), these represent new host records for *M. g. intermedia*. *Echinoparyphium recurvatum* and *Zygocotyle lunata*, reported as the only other helminths encountered in the Wichita Mountains *M. g. intermedia* by these workers, are characteristic trematodes of waterfowl as pointed out by Hon et al. (1975, loc. cit.). Trematodes were not encountered in birds from the drier areas of West Texas in the present investigation.

This study indicates an apparent minor significance of helminthiasis in the Rio Grande subspecies of the Wild Turkey in West Texas. However, it reflects the need for a much larger sample size from several localities involving both poults and adults collected on a seasonal basis in order to more fully understand the implications of parasitism and associated diseases in this game species.

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Research Note

The Occurrence of a "Coiled" *Metamicrocotyla macracantha* on the Gills of the Mullet, *Mugil cephalus*

In January of 1974 a number of mullet (*Mugil cephalus*), 150 to 300 mm in length, were obtained from the Mississippi Gulf Coast near Ocean Springs, Mississippi. During routine examination for parasites, 3 immature *Metamicrocotyla macracantha* (Koratha) were found on the gills of one mullet. Two of the monogenetics were coiled around a filament much as a *Boa* coils around its prey. I found this behavior quite interesting and thought that my colleagues might enjoy seeing a picture of it.

Many thanks go to Dr. David E. Zwerner of the Virginia Institute of Marine Science for identifying the trematode.

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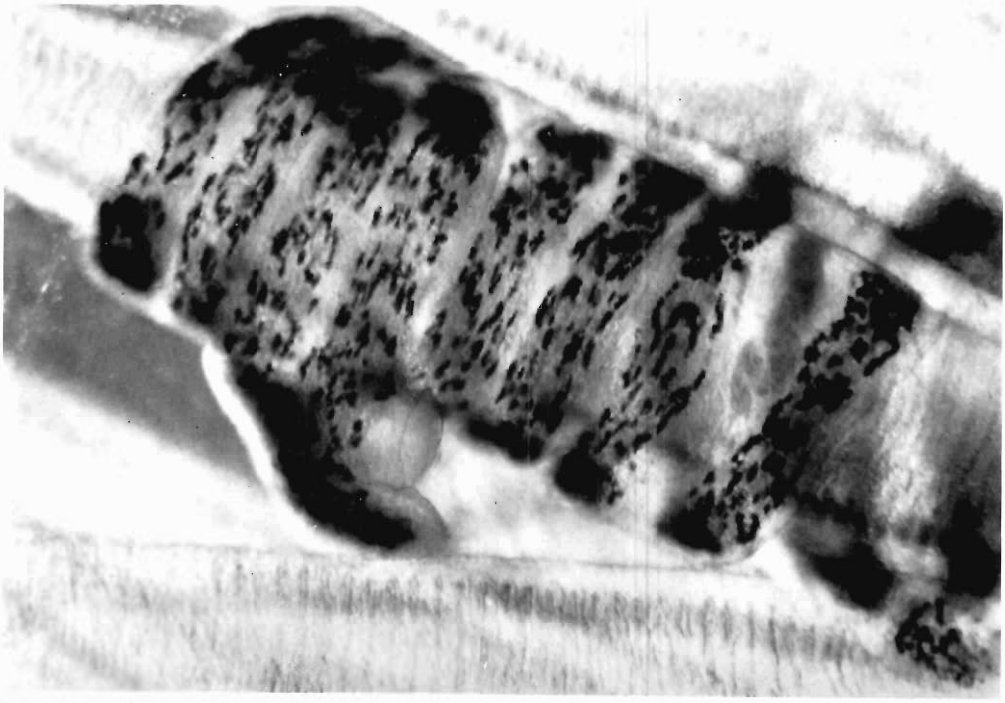

Figure 1. A photomicrograph of an immature *Metamicrocotyla macracantha* coiled around a gill filament from a mullet (*Mugil cephalis*).

Research Note

Helminth Parasites of White-tailed Deer (*Odocoileus virginianus*) from New Jersey and Oklahoma

During 1974, 20 white-tailed deer from New Jersey and Oklahoma were examined for helminth parasites. Deer originated from the Peaslee Wildlife Management Area, Cumberland County, New Jersey, and from the Cookson Refuge, Adair County, and the Pushmataha Wildlife Management Area, Pushmataha County, Oklahoma. Helminthologic findings are summarized in Table 1.

Seventeen species of helminths were recovered. New Jersey deer harbored 9 species, Oklahoma deer harbored 15 species, and seven species were common to deer of both states. Parasite faunas of New Jersey and Oklahoma deer were similar to those found previously in deer of the southeastern United States (Prestwood and Smith, 1969, J. Parasit. 55: 720–725; Prestwood et al., 1970, J. Parasit. 56: 123–127; Prestwood, 1971, J. Parasit. 57: 1292; Prestwood et al., 1971, J. Wildl. Dis. 7: 149–154; Prestwood et al., 1973, J. Am. Vet. Med. Assoc. 163: 556–561; Pursglove et al., 1974, J. Parasit. 60: 1059–1060; Pursglove et al., 1976, J. Am. Vet. Med. Assoc. 169: 896–900).

Setaria yehi has been reported previously from New Jersey deer (Becklund and Walker, 1969, J. Parasit. 55: 359–368), and *Eucyathostomum webbi*, *Oesophagostomum venulosum*, and *Parelaphostrongylus tenuis*, respectively, have been reported previously from Oklahoma deer (Pursglove, 1976, J. Parasit. 62: 574–578; Baker and Pursglove, 1976, J. Parasit. 62: 166–168; Carpenter et al., 1972, J. Wildl. Dis.

Table 1. Helminth parasites of white-tailed deer from New Jersey and Oklahoma.

Location/Parasite	New Jersey (10 deer†) No. infected	New Jersey (10 deer†) Worm count Range	New Jersey (10 deer†) Worm count Mean	Oklahoma (10 deer) No. infected	Oklahoma (10 deer) Worm count Range	Oklahoma (10 deer) Worm count Mean
Musculature						
Parelaphostrongylus andersoni Prestwood, 1972	1	0–1	0.2	—	—	—
Brain						
Parelaphostrongylus tenuis (Dougherty, 1945)	4*	0–3	1.4	9*	0–25	8.2
Lungs						
Dictyocaulus viviparus (Bloch, 1782)	3*	0–13	3.0	—	—	—
Abdominal Cavity						
Sertaria yehi Desset, 1966	3*	0–100	21.0	1	0–2	0.2
Esophagus						
Gongylonema pulchrum Molin, 1857	4*	0–61	39.8	10*	3–97	21.5
Rumen						
Gongylonema verrucosum (Giles, 1892)	—	—	—	6*	0–150	16.8
Abomasum						
Haemonchus contortus (Rudolphi, 1803)	—	—	—	1*	0–92	9.2
Ostertagia dikmansi Becklund and Walker, 1968	4*	0–97	19.3	4*	0–169	54.0
Ostertagia mossi Dikmans, 1931	8*	0–616	168.6	10*	37–1,264	474.6
Skrjabinagia odocoilei (Dikmans, 1931)	10*	14–1,233	327.1	10*	245–2,165	921.5
Trichostrongylus askivali Dunn, 1964	—	—	—	3*	0–388	94.0
Trichostrongylus axei (Cobbold, 1879)	—	—	—	1*	0–122	12.2
Small Intestine						
Moniezia sp.	—	—	—	1	0–1	0.1
Capillaria bovis (Schnyder, 1906)	1	0–1	0.2	6*	0–8	1.8
Cecum						
Trichuris sp.	—	—	—	1	0–1	0.1
Large Intestine						
Eucyathostomum webbi Pursglove, 1976	—	—	—	3*	0–7	1.4
Oesophagostomum venulosum (Rudolphi, 1809)	—	—	—	5*	0–103	13.4

† Only the abomasum of 5 of 10 deer were examined.
* Representative specimens deposited in the National Parasite Collection, Beltsville, Maryland, as USDA Par. Coll. Nos. 67184-67197.

8: 381–383). New locality records were established by the remaining deer helminth findings reported herein.

Two New Jersey deer had mild, interstitial pneumonia resulting from numerous, migrating larvae of *Parelaphostrongylus tenuis* and/or *P. andersoni* in conjunction with *Dictyocaulus viviparus* infections. Pleuritis and pulmonary adhesions were noted in both animals. A marked fibrinous peritonitis associated with a severe *Setaria yehi* infection also was evident in a young New Jersey deer. In addition, eosinophilic meningitis was associated with *Parelaphostrongylus tenuis* infections in two deer from Oklahoma.

This study was supported in part by an appropriation from the Congress of the United States. Funds were administered and research coordinated under the Federal Aid in Wildlife Restoration Act (50 Stat. 917) and through Contract No. 14-16-0008-707, Fish and Wildlife Service, U.S. Department of the Interior.

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PRESENTATION

1976 Anniversary Award of the Helminthological Society of Washington
501st Meeting, 15 October 1976
Dr. Leo A. Jachowski, Jr.

The Anniversary Award is presented to Dr. Leo A. Jachowski Jr. by Dr. George W. Luttermoser.

On behalf of the Helminthological Society, the Awards Committee and Executive Committee have the pleasure of presenting the 1976 Anniversary Award to Dr. Leo Jachowski. The recipient has contributed significantly to the science of Parasitology while carrying out a variety of related activities. Some of the highlights of his career follow.

Dr. Jachowski was born in Baltimore County, Maryland in 1918 and from his early boyhood days he has been an active "naturalist". His professional training in science began at the University of Maryland in 1937, and in 1939 he transferred to the University of Michigan where he received his B.S. in 1941 and later his M.S., on a thesis which dealt with a problem on protozoa in sewage. In 1953 he earned his Sc.D. at the Johns Hopkins School of Hygiene and Public Health with thesis work on filariasis.

In 1943, he entered the U. S. Navy as Research Parasitologist and rose to the rank

of Commander in the Medical Service Corps while completing the research, administrative and teaching assignments which are briefly summarized as follows:

1944—Instructor, Malariology, Naval Medical School.

1944–1948—Officer in Charge, Parasitology Research Program, Naval Medical Research Institute (NMRI): Investigations on insect repellents, vector biology and control and survey of filariasis in Samoa.

1948–1950—Officer in Charge, Filariasis Research Unit on epidemiology and control, Government of American Samoa.

1950–1957—Officer in Charge of Filariasis Research Unit, (NMRI), with evaluation of control procedures in American Samoa including chemotherapy of infections and readings on bionomics of mosquito vectors of disease.

1957–1960—Officer in Charge of Navy Research Unit and Department of Serology, Army Tropical Medical Research Lab in San Juan, Puerto Rico. This period resulted in reports with others on pathology and serology of filariasis, comparison of methods for diagnosis of schistosome infections and host serologic reactions.

1960–1964—As Staff Member of the NMRI until his retirement from the Navy in 1964, Jachowski extended his investigations on diagnosis, host immunity in filariasis and relationship of serological picture and immunity to schistosome infections in lab animals. As a result of his service accomplishments, Dr. Jachowski received the Commendation Ribbon with Pendant, Secretary of Navy, 1945 for research on filariasis and also Commendation Ribbon with Pendant, Secretary of the Army, 1959 for research on serology of parasitic diseases.

1964–present. Joining the University of Maryland Department of Zoology in 1964, he became full Professor in 1965. In addition to his part in the development of a strong graduate program in parasitology at Maryland, Leo has maintained an open-door policy for the many graduate students he has guided and encouraged along with his other academic responsibilities.

In reports of original research, Dr. Jachowski has contributed significantly to basic knowledge of several aspects of the field ecology of human filariasis and schistosomiasis, as well as life cycle observations on zoonoses and on interactions of concurrent parasitic infections. It is appropriate to note that Dr. Jachowski was the recipient of the American Society of Tropical Medicine and Hygiene "Bailey K. Ashford Award", in 1952, for epidemiological studies on filariasis in Samoa and the Puerto Rican Bilharzia Committee "Isaac Gonzalez-Martinez Award", 1959, for studies on the lab diagnosis of schistosomiasis. He has participated in several WHO Workshop/Conferences on filariasis and was a U. S. Delegate in 1949 to the South Pacific Commission and a conference member in 1951.

Dr. Jachowski is an active member of at least ten national/international scientific societies. Over the years he held the following offices in the Helm. Soc.: Recording Secretary, Vice-President, President (1965–66) and Chairmanship of several committees.

In presenting this Anniversary Award to you, Dr. Leo Jachowski, the Helminthological Society formally recognizes your contributions to Parasitology and to this Society. By your continuous dedication to research and teaching under diverse circumstances, you have accomplished most effectively the high achievements for this award. We congratulate you. (Awards Committee: George W. Luttermoser, Chairman, Richard L. Beaudoin, Halsey H. Vegors)

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Peters, L. E.
Schneider, C. R.

Minnesota
Ballard, N. B.
Vande Vusse, F. J.

Mississippi
Brooks, D. R.
Cake, E. W., Jr.
Holder, Ray, Jr.
Minchew, C. D.
Overstreet, R. M.
Wellborn, T. L., Jr.

* Life Member
** Honorary Member
† Spouse Member
‡ Life Member deceased

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