GUEST EDITORIAL

Studies on the Parasitism of Mollusks as Models for Comparative Pathology

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Mollusks serve as hosts for certain helminths and other parasites which affect the mollusk’s body. Pathological alterations in these mollusks, which are associated with parasitic infections, are mainly histopathological and physiological as well as biochemical. Some parasites, however, do not cause recognizable disease in mollusks, especially if present in small numbers.


Studies of pathology in mollusks as a result of parasitic diseases have been multidisciplinary including those which are morphological, biochemical, physiological, histopathological, autoradiographic, and ecological. Such studies cover the entire spectrum of pathogenic organisms and are still in progress. Molluscan pathology is, no doubt, of great use when applied to invertebrate pathology in general.

Among economically important mollusks,
viruses or virus-like particles cause edematous nodular tumors, mainly on the tentacles of octopuses, and in oysters they cause dilatation of digestive diverticula, cellular infiltrates in the tissues surrounding the hemolymph sinuses. Several species of bacteria cause diffuse cell infiltration, and tissue necrosis leading to mortality of larval and adult bivalves including the American oyster, *Crassostrea virginica*, and the European oyster, *Ostrea edulis*. The fungus *Labyrinthomyxa (= Dermocystidium) marina* causes fatal epizootics in oysters on the Gulf Coast as well as along the southern Atlantic Coast. Infected *C. virginica*, *Ostrea frons*, and *O. equestris* show inhibited gonadal development, retarded growth, and general emaciation as a result of invasion of all tissues and formation of multiple abscesses. An important and often fatal protozoan pathogen of shellfish is *Minchinia nelsoni* (originally known as MSX, i.e., multinucleated sphere unknown) occurs from Connecticut to North Carolina. The multinucleated plasmodium stage of the life cycle of this protozoan is the most common in diseased oyster tissues and results in leukocytosis. The histopathology is not dramatic and it is believed that the mortality of the oysters is due to environmental and physiological stresses on the mollusks weakened by infection with *M. nelsoni*. An evident histopathology in marine mollusks of economical importance accompanies infections with certain helminths, but most of these are not lethal to these mollusks.

As to gastropods, infections with certain pathogenic bacteria have been reported whereby the bacilli cause swelling of the tentacles, necrosis, and hemorrhage. A species of *Mycobacterium*, an acid-fast bacillus, is pathogenic and has been isolated from naturally parasitized snails, *Helisoma anceps* (E. H. Michelson, *Amer. J. Trop. Med. Hyg.* 10, 423–427, 1961). The bacillus can be cultivated and transmitted to other species of planorbid snails by stab wounds or by dispersion in the water containing the snails. The histopathology reveals the bacteria inside amoebocytes, the latter aggregating, and they are eventually walled off by fibroblasts to form tubercles. Similar nodules surrounded by fibroblasts occur in certain species of snails and are caused by pathogenic protozoa, *Harimarrella biparia* and *H. quadriparia* (C. S. Richards, *J. Protozool.* 15, 651–656, 1968). These amoebas occur intracellularly in amoebocytes in various organs of the snail, and the pathology affects the growth and reproduction of the snail and causes mortality of some individuals. It is not certain that the pathology produced by the above bacteria and protozoans can be made use of in the biological control of the snails.

The effect on gastropods of parasitism by digenetic trematodes starts a few weeks after penetration of the miracidium when the migration and, later, the maturation of daughter sporocysts or rediae take place. Congestion of the blood sinuses in the visceral mass is the first noticeable alteration. In the digestive gland of the mollusk significant destruction and pathology take place caused by pressure exerted by the parasites; lysis caused by the parasites' excreta; and changes in enzymes and autolysis due to deprivation of nutritive material (T. C. Cheng and R. W. Snyder, *Trans. Amer. Microsc. Soc.* 81, 327–331, 1962). Histopathological alterations in the digestive glands of mollusks as a result of parasitism by larval trematodes include mechanical compression and rupturing of cells, displacement of the tubules and loss of their branched structure, degeneration of the tubules' epithelium and its alteration from columnar to cuboidal or squamous, increase in the number of cytoplasmic vacuoles, and reduction in the amount of stored glycogen in cells. The latter effect can be determined by employing histochemistry and biochemistry. The reduction in glycogen is not limited to the digestive gland but all the glycogen in the body is reduced.

A reduction in the fecundity of mollusks takes place when they become parasitized by larval trematodes. Rediae invade the
mollusk’s gonad, be it an ovotestis, an ovary, or a testis, and actually ingest gonadal tissue, thus causing castration of the organisms. Parasitic castration caused by sporocysts and rediae of trematodes also affects other parts of the reproductive system, especially the albumin gland, prostate, uterus, and sperm duct and thus indirectly affects the reproductive capacity of the parasitized gastropod.

Changes in the structure of connective tissue constituents also take place, whereby there is a marked hyperplasia of fibroblasts. The above histopathologic alterations concerning the digestive gland, gonad, and connective tissue have been reported in several papers by the writer in the case of infection of the snail Biomphalaria alexandrina with Schistosoma mansoni, Bulinus (Physopsis) ugandae infected with S. bovis, Bulinus (Bulinus) truncatus infected with S. haematobium, and Helisoma corpulentum infected with either the echiostome Peta-siger chandleri or the strigeid Uvulifer ambloplitis (E. A. Malek, Bull. WHO 18, 785–818, 1958; Ann. Trop. Med. Parasitol. 63, 501–513, 1969). In the case of the snail Helisoma corpulentum infected with Peta-siger chandleri, whose rediae possess a relatively long gut, there was a considerable reduction in the number of tubules of the digestive gland; in one section there were only 27 tubules, with 141 rediae. Moreover, no less than 95% of the acini of the ovotestis were occupied by rediae, and the reproductive structures were either digested completely or in a degenerate condition. On the other hand, infection with Uvulifer ambloplitis did not involve or damage the ovotestis; the sporocysts of this trematode could not invade the acini but were found in the connective tissue between them. Similarly schistosome sporocysts invaded the connective tissue around and between the acini of the ovotestis but not the acini themselves. Thus the influence of these schistosome sporocysts on the gonad of their snail hosts is not the result of mechanical damage but, rather, by induced physiological conditions that tend to disturb the metabolism of the snail host. In my experiments on infections of the respective snail host with Schistosoma mansoni, S. haematobium, or S. bovis, there was a pronounced reduction in egg laying as compared to uninfected snails, but egg laying was never permanently inhibited. That other parts of the genitalia such as the albumin gland, the prostate, the uterus, and the sperm duct were also affected by parasitism shows that these parasites indirectly affect the reproductive capacity of the parasitized snail.

In nonsusceptible snails, encapsulation of the trematode miracidium takes place shortly after its penetration into the snail’s tissues, or shortly after it commences its transformation into a mother sporocyst (W. L. Newton, J. Parasitol. 38, 362–366, 1952; E. A. Malek, Amer. J. Trop. Med. Hyg. 16, 715–717, 1967). The capsule, which causes the death of the miracidium, is composed of concentric layers of fibroblasts, leukocytes, and/or muscle cells which are the result of innate cellular resistance of such mollusks against noncompatible trematodes. Such trematodes normally develop to patency, i.e., to the stage of production of cercariae in susceptible snails without significant cellular resistance. However, in infections with Schistosoma mansoni in the snail Biomphalaria glabrata, which is a compatible snail for this species of blood fluke, the sporocysts of S. mansoni at a late stage of the infection provoke some focal as well as generalized tissue response. Degenerative changes, including lysis, occur in the wall of some of the sporocysts as well as in the germinal elements inside the sporocysts, and thus infection is terminated in some individual snails of an infected group. I have observed and reported “spontaneous cure” of snail hosts infected with S. mansoni, S. haematobium, or S. bovis (E. A. Malek, Bull. Tulane Univ. Med. Fac. 20, 181–207, 1961; Ann. Trop. Med. Parasitol. 63, 501–513, 1969).

It should be noted that larvae of certain nematodes are encapsulated in the tissues of their molluscan intermediate host as a
result of host-tissue response. Such is the case in land snails, freshwater snails, and slugs infected with the larvae of *Angiostrongylus cantonensis*, the lung nematode of rats (T. C. Cheng, and J. E. Alicata, *Malacologia* 2, 267–274, 1965). In spite of the fact that such capsules are comparatively thick and formed of several concentric layers of fibroblasts, muscle cells, and leukocytes, the larvae remain viable inside each capsule until the mollusk is eaten by, and the larvae are infective to, the mammalian hosts.

The usefulness of information on parasitism in mollusks and its application in comparative invertebrate pathobiology become evident if we consider a few examples of infection of certain other invertebrates with some parasites. As is the case in mollusks, we find that the development of nematode parasites in their arthropod vectors is affected considerably by external environmental, as well as by host factors. Within certain limits, the higher the temperature and the humidity, the more rapid is the development of larvae in their vectors.

Microfilariae of several filarial species, for example, those of *Brugia* and *Wucheraria*, penetrate the midgut and gain access to the hemocoel of the mosquito vector between 5 and 15 min following engorgement of the blood meal. The site of penetration of the midgut is usually the area near the attachment of the hindgut. In about another 15 min the microfilariae proceed to the thorax and invade the flight muscles. A peritrophic membrane is secreted around the blood in the midgut of most hematophagous insects; it can trap some of the microfilariae and prevent them from penetrating into the hemocoel. Thus large numbers of microfilariae of *Onchocerca volvulus* do not considerably affect the black fly *Simulium damnosum* (D. J. Lewis, *Bull. Entomol. Res.* 43, 597–644, 1953). However, there are reports that the more delicate peritrophic membrane in mosquitoes can be destroyed by microfilariae of certain filarial nematodes. As is the case in certain mollusks, we find that the sex of the arthropod does not appreciably limit the growth of the nematode parasites; thus *Dipetalonema vitae* develops in male and female ticks and *Littomosoides carinii* develops in male and female mites (G. S. Nelson, In “Symposium: Host–Parasite Relationships in Invertebrate Hosts,” pp. 75–119, Blackwell Scientific Publications, Oxford, 1964). Biochemical factors in the arthropod host affect the development of nematode larvae, and techniques for in vitro and in vivo studies are continually clarifying the fundamental requirements of the parasites.

Considerable pathological changes occur in the arthropod vector when large numbers of microfilariae penetrate the tissues, while there is a less severe damage in non susceptible vectors, especially if the microfilariae perish or fail to develop. The damage to mosquitoes, for example, involves the midgut epithelium, the muscles, the subcuticular fat bodies, and the Malpighian tubules. Necrosis and liquefaction take place in the tissues around the developing larvae as is the case with the filariae *Wucheraria bancrofti*, *Brugia pahangi*, and *Dirofilaria immitis*, all in mosquitoes, with *Dipetalonema vitae* in the muscles of ticks, with *Onchocerca volvulus* in the flight muscles of *Simulium damnosum*, and with *Loa loa* in the fat body of *Chrysops* (A. G. Chabaud, *Ann. Parasitol.* 29, 42–88, 206–249, 1954; M. M. J. Lavoipierre, *Ann. Trop. Med. Parasi tol.* 52, 103–121, 1958).

An interesting pathological feature of filarial infections in mosquitoes is the pig mental encapsulation of microfilariae in the hemocoel, fat bodies, and other tissues (L. Kartman, *Rev. Brasil. Malariol.* 5, 1–42, 1957; J. H. Esslinger, *Amer. J. Trop. Med. Hyg.* 11, 749–758, 1962), and, as is the case in mollusks, this encapsulation is the response of the arthropod vector to the presence of the parasite as a means of defense mechanism. However, it takes place more quickly than in mollusks, sometimes occurring within 15 min after engorgement of the blood meal. The process of encapsulation in mosquitoes sometimes also takes up to 48 hr, the same period of time necessary
for the development of the reaction in snails which are not susceptible to certain trematodes. Unlike the condition in mollusks, escape of the microfilariae from the capsules is possible. The encapsulating material in mollusks is known to be formed of leukocytes, fibroblasts, and/or muscle cells; however, the nature of the material in arthropods is not definitely known, but work on various insects suggests that the pigment formation around parasites or foreign bodies is related to cell damage, with the release of substances permitting tyrosinase to react with a chromogen.

Several structural similarities between mollusks and certain other invertebrates support the contention that parasitism in mollusks can be used as models for invertebrate comparative pathobiology. In mollusks, as well as in several other invertebrates, circulation is mainly undertaken through an open vascular system. Aside from a heart and a few hemolymph vessels there are intercellular spaces and hemocoelic sinuses, and the hemolymph moves slowly back from these sinuses to the heart, from which it leaves in arteries for the tissue spaces and sinuses. This fluid system not only functions in circulation but also provides rigidity to certain organs (hydrostatic skeletons), such as tentacles, arms, and penis in mollusks. The control of the pressure in the open systems is closely correlated with motor activity than by the heart, and the velocity of circulation in animals such as crayfishes and lobsters is therefore very low compared to higher animals such as primates, including man.

The circulatory system and hemolymph of gastropods act as vehicles for the transport of early and advanced larval digenetic trematodes (E. A. Malek, Amer. Midl. Natur. 54, 394–404, 1955). After its penetration of the anterior, i.e., proximal, organs of susceptible snails, the miracidium settles and develops to form a mother sporocyst. Inside these larval sacs, daughter sporocysts or rediae, depending on the trematode species, are formed and, on account of their motile nature but mainly helped by the circulating hemolymph, they migrate posteriad (distad) and become located principally in the area of the digestive gland and ovotestis. When cercariae are developed later from these sporocysts or rediae they migrate anteriad (proximad) with the hemolymph, in its normal route, where they are seen in considerable numbers in the large rectal hemolymph sinus. They gain access to the water outside the snail by penetration through a thin epithelial layer at the mantle collar.

With regard to the endocrine system we find that in insects and crustaceans it is functionally similar to that in vertebrates. In such invertebrates there are integrative functions between nervous and endocrine systems and coordinated activity of the two. The endocrine mechanisms are more understood and studied in insects and crustaceans than in mollusks. Nevertheless, the few studies which have been undertaken on mollusks (J. Lever, C. M. de Vries, and J. C. Jager, Malacologia 2, 219–230, 1965) have demonstrated the similarity of such mechanisms among mollusks, insects, and crustaceans inasmuch as the endocrine systems of the above groups are functionally very closely related to the central nervous system. Neurosecretory cells in ganglia of the brain in the above invertebrates form hormonal material which is apparently transported within their axons to the places of their liberation into the blood.

What is needed in mollusks are an investigation and determination of the relationship between neurosecretions and various aspects of their parasitism with helminths, such as the effect of these secretions on the development of daughter sporocysts and rediae of digenetic trematodes and the mechanism and periodicity of the release of the cercariae of certain trematodes from their molluscan hosts. Also not understood in mollusks is whether there is any relationship between neurosecretions and reproductive regulations.

There is also a similarity between mollusks and various other invertebrates with regard to nitrogen excretion. Nitrogenous
wastes may come from proteins by way of amino acids and from the purine and pyrimidine bases of nucleic acids. Excretory products of protein degradation may be ammonia, urea, or uric acid, and thus the animals are called ammonotelic, ureotelic, or uricotelic, respectively. Mollusks share various enzymes for protein and purine breakdown with several invertebrate phyla such as annelids and arthropods. Among these enzymes are deaminases, urease, arginase, xanthine oxidase, uricase, allantoïnase, and allantoicase. In these invertebrates several enzymatic routes may lead to the same nitrogenous excretory product. Changes in nitrogen excretory pattern and pathways have some genetic basis but have also been reported to be influenced by life cycle stages, by diet, and by water deprivation.