45

8

Molluscan Immunobiology[1]

CHRISTOPHER J. BAYNE

Department of Zoology
Oregon State University
Corvallis, Oregon

[1]I have been supported by NIH Contract 22-527, and these grants: NSF PCM-75-11911; NIH A1-13295, A1-16137, and RR-07079; and Medical Research Foundation of Oregon 282.

THE MOLLUSCA, VOL. 5
Physiology, Part 2

ISBN 0-12-751405-8

"It is wrong to think of invertebrates as simply producing large numbers of offspring so that those which become infected are considered expendable, or that invertebrates do not live long enough to justify an immune system [Dales, 1979]."

"Si l'on considère que la "reconnaisance en soi" et la "reconnaissance de structures non apparentées" sont les premiers échelons de la réponse immunitaire on peut affirmer qu'ils existent bien chez les mollusques [Golvan and Mougeot, 1973]."

I. Introduction

The study of molluscan immunobiology began with Haeckel (1862, cited by Malek and Cheng, 1974). Modern research in this field, started by Cuénot (1914), really got off the ground when Stauber (1950, 1959) and his student (Feng, 1958; Tripp, 1958) followed the fates of particulate and soluble foreign substances in the oyster *Crassostrea virginica*. The field was reviewed in 1970 (Tripp) and 1974 (Malek and Cheng).

In molluscs other than cephalopods, the body cavity is a hemocoel in which *hemolymph* circulates. Hemolymph and other elements which defend the body against and in the face of invasion by foreign agents constitute the internal defense systems. The *hemocytes* are leukocytic, i.e., without pigment, in most cases, and most are capable of ameboid locomotion and may therefore be referred to as *amebocytes*. The relative abundance of granules in the cytoplasm has led to the use of such terms as *granulocyte* and *hyalinocyte*, the latter referring to hemocytes with scarce granules.[2]

The fluid portion of molluscan hemolymph is incapable of firmly clotting, and is therefore referred to as *plasma*. In polyplacophoran, gastropod, and cephalopod molluscs, a predominant plasma component is commonly hemocyanin (HCY) or hemoglobin; bivalves generally lack respiratory pigments, although some occur in erythrocytes. Other plasma components are poorly characterized, although they are numerous; for example, *agglutinins*, substances which agglutinate particles, may occur and may have *opsonic* properties, i.e., be able to enhance phagocytosis or encapsulation as a result of coating a foreign object.

Finally, internal defense responses may be *innate* or *acquired*. Innate responses are those which occur on a first encounter with a foreign agent. Acquired responses are those which differ from an innate response when an agent is encountered a second or later time; they are, therefore, the result of earlier

[2]For a fuller review of gastropod hematology, refer to Sminia (1981).

experience with a foreign agent. An animal may react to a second encounter with an innate response; this would imply that the response was qualitatively and quantitatively identical to the primary response. In animals with advanced immune systems, both cellular and humoral responses may be either innate or acquired.

Within the phylum Mollusca, there is a great diversity of form and a great range of complexity; from *Neopilina* and *Chiton*, through *Mytilus*, *Thais*, *Helix*, and others, to *Octopus* and *Vampyromorpha*. In the phylum we also find a diversity of immune mechanisms. For this reason, I treat the molluscan classes separately. For the Aplacophora, Scaphopoda, and Monoplacophora, we are without any relevant information.

II. Internal Defenses of Polyplacophorans

Immunorecognition occurs when a defensive cell reacts (alters its steady state) in an advantageous manner (i.e., in a direction toward execution of its defensive function) as a result of encountering nonself material. The research of Hildemann and colleagues (Hildemann et al., 1977, Hildemann & Johnson, 1979) and others (e.g., Buss and Jackson, 1979; Francis, 1973) have conclusively shown that even the simplest metazoans are capable of immunorecognition. As expected, then, molluscs too can recognize and respond to foreign materials, and a high level of specificity occurs in such recognition.

The Australian chiton *Liolophura gaimardi* was found to clear injected bovine serum albumin (BSA) much more rapidly than injected hemolymph proteins from another specimen of *L. gaimardi* (Crichton et al., 1973). These studies, using fluorescein-conjugated protein, were followed by experiments using isotopes of iodine as labels, and the earlier results were confirmed (Crichton and Lafferty, 1975). A surprisingly high level of discrimination was evident (Fig. 1) when ^{125}I-labeled homologous *L. gaimardi* HCY was injected simultaneously with each of several ^{131}I-labeled heterologous HCYs. The rates at which these foreign HCYs were cleared from the hemolymph of *L. gaimardi* agreed exactly with the degree of antigenic similarity of these molecules revealed by immunodiffusion against rabbit anti-*L. gaimardi* HCY: The rabbit antibody formed strong precipitin lines with *Ischnoradsia* (I, a chiton) HCY and weaker precipitin lines with *Poneroplax* (P, another chiton) HCY. Keyhole limpet HCY (KL-HCY) was cleared more quickly than the three chitons HCYs but more slowly than the most unrelated HCY, that from a crayfish (C-HCY). Thus, the greater the difference between the injected protein antigen and the equivalent antigen of the chiton, the more rapidly the antigen was cleared.

The implied specificity of recognition was again evident when a heavy primary dose of human serum albumin (HSA) effected reduced clearance rates for secondary injections of HSA and BSA but failed to change the clearance rate of

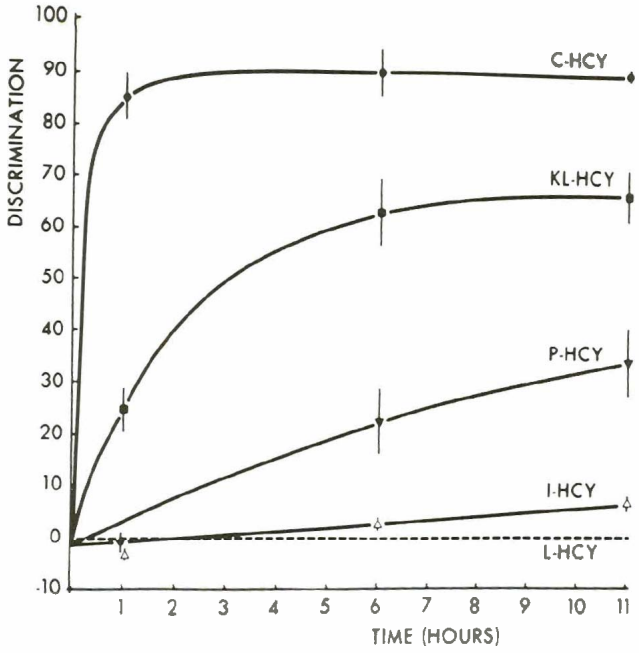


Fig. 1. Discrimination curves showing the rate of clearance of *Ischnoradisia* (I-HCY), *Poneroplex* (P-HCY), Keyhole limpet (KL-HCY) and crayfish (C-HCY) hemocyanin (HCY) relative to the clearance of *Liolophura* HCY (L-HCY). Groups of animals were injected simultaneously with 0.25 μg ^{125}I-labeled L-HCY and 0.25 μg ^{131}I-labeled test HCY. Each point is the mean value obtained in groups of four animals, and the vertical lines show the standard error of mean. (From Crichton and Lafferty, 1975, with permission.)

chiton (*Poneroplax*) HCY (Crichton and Lafferty, 1975). As stated by the authors, "The phagocytic system is capable of recognizing structural differences whose magnitude is comparable with differences detected by serological techniques."

In chitons (*L. gaimardi*), the cells responsible for the removal of foreign substances from the circulation are circulatory hemocytes and fixed phagocytes (Crichton et al., 1973; Cuénot, 1914; Killby et al., 1973). The latter occur in the widely distributed connective tissues and are especially abundant in highly vascularized areas such as the ctenidia and foot (Crichton et al., 1973) and the digestive gland (Cuénot, 1914). The hemocytes of *Liolophura* are apparently a population of morphologically similar, multifunctional cells capable of phagocytosis (Table I), synthesis, secretion, and storage. They resemble ameboid hemocytes of bivalves and gastropods, but the weakly basophilic cytoplasm lacks stainable inclusions (Azure II, methylene blue). The apparent dose dependence of the percentage of hemocytes containing *Staphylococcus aureus* (Table

I) was later confirmed. Yet, as also reported for gastropods (Bayne, 1974), only a small percentage of injected bacteria were evident in circulating hemocytes, implying that "the haemocyte was not the major cell responsible for the removal of foreign material in *Liolophura* [Crichton et al., 1973]." This conclusion was supported both histologically and ultrastructurally: More injected material appeared to accumulate in fixed phagocytes. Ultrastructural differences between these two cells may be due to the fact that they belong to distinct lineages or are different stages of a single lineage; this issue is unresolved (Killby et al., 1973). The presence of secondary lysosomes implies that digestion of injected material is another function of these phagocytes.

III. Internal Defenses of Bivalves

A. Responses to Tissue Damage

Within seconds of damage to the bivalve body wall, local muscles contract and thus reduce leakage of hemolymph (Sparks, 1972). Inflammation, the local accumulation of numerous hemocytes, occurs within hours of damage to the soft tissue or to the overlying shell. In oysters (*Crassostrea gigas*), the response to soft tissue damage is visible grossly as a local darkening and greening (possibly associated with copper; Ruddel, 1971a), beginning about 16 h after injury (DesVoigne and Sparks, 1968) and starting to fade after about 9 days, although the entire sequence of events is temperature dependent (Ruddell, 1971a). However, the cellular response is detectable microscopically as early as 4 h after wounding. Numerous hemocytes infiltrate the area, moving from the hemocoelic sinuses through the connective tissue. Depending on the extent of the damage, wounds are plugged by hemocytes within 1–6 days (DesVoigne and Sparks, 1968).

TABLE I

The Uptake of Colloidal Gold, Carbon, and Heat-Killed *Staphylococcus aureus* by *Liolophura* Hemocytes *in Vivo* at 24 h Postinjection[a]

Exp	Label	Dose	Total % labeled hemocytes (mean ± SE)
A	Gold	0.25 mg	16.9 ± 4.3
B	Carbon	2.0 mg	73.0 ± 2.2
C	Bacteria	5×10^9	25.7 ± 3.3
	(*S. aureus*)	3×10^9	18.9 ± 1.6
		3×10^8	4.3 ± 1.3

[a] Adapted from Crichton and Lafferty (1975) with permission.

Damaged cells are phagocytosed, and many of the originally spherical hemocytes become elongate. Collagen is formed within the scar tissue, and as the infiltrate later dissipates and the normal architecture is restored, the collagen is removed. In *C. gigas*, adductor muscle responds to and repairs damage more rapidly than does Leydig tissue (Pauley and Sparks, 1967).

Infiltrating hemocytes in oysters (*C. gigas*) are of three types: agranular amebocytes, basophilic granulocytes, and acidophilic granulocytes (Ruddell, 1971b). Similarly, in *Mytilus edulis* (Bubel et al., 1977) and *M. californianus* (Bayne et al., 1979), three types of hemocyte can be identified (Table II). In both *Mytilus* spp. and *C. gigas*, agranular amebocytes are the most common cell type early in the response. These predominantly small basophils infiltrate wounds (Bayne et al., 1979; Ruddell, 1971a). In *Mytilus* spp., basophils have macrophage-like properties, i.e., a good phagocytic capability and a diverse component of lysosomal hydrolases; in *Crassostrea* the agranular amebocytes appear to be most phagocytic (Ruddell, 1971a). After the initial cleanup of the wound, a healing phase follows in which acidophilic granulocytes accumulate and may release copper in the wound area, but the possible defensive significance of this

TABLE II

Properties of the Hemocytes of *Mytilus californianus*[a]

Cell type	Acidiphilic granulocyte	Small basophilic hyalinocyte	Large basophilic hyalinocyte
Terminology of Moore and Lowe (1977); Bubel et al. (1977)	Granulocyte (G)	Small basophil (SB)	Macrophage (M)
Diameter (μm)			
Cell	9.7 ± 1.2 (SD) (n = 30)	4.5 ± 0.6 (SD) (n = 30)	7.7 ± 1.4 (SD) (n = 30)
Nucleus	3.2 ± 0.4 (SD) (n = 30)	3.75 ± 0.5 (SD) (n = 30)	3.5 ± 0.7 (SD) (n = 30)
(Radius cell)^3/(radius nucleus)^3	27.8	1.7	10.6
Phagocytosis of colloidal carbon *in vivo*[b]	0/+[b]	+	+++[b]
Lysosomal enzymes[b]			
N-Acetyl-β-hexosaminidase	0/+		++/+++
Acid phosphatase	0	+	++[b]
β-Glucuronidase	0	+	++
Involvement in graft rejection	5/7–37 d	0–7 d	0–7 d

[a] From Bayne et al. (1979) with permission.

[b] 0 = Negative; + = present; ++ = moderate; +++ = strong.

process remains to be determined (Ruddell, 1971a). Healing proceeds from the interior of the lesion toward the body surface (Des Voigne and Sparks, 1968); during this period, the dense mass of hemocytes is replaced by normal connective tissue. "Fibroblasts" and myoblasts in amebocyte plugs may be "derived from wound agranular hemocytes [Ruddell, 1971b]." Mitoses occur in the neighboring epithelium which thereby grows out over the wound plug (Ruddell, 1971a). Epithelial mitoses were also reported in wounded *Mytilus* (Bayne et al., 1979) and *Crassostrea* (Hillman, 1963). When oysters are injected with gill extract (Bang, 1961), hemocytes may clump *in vivo*.

After shell damage in *Mytilus* spp., the sequence of cell populations infiltrating the underlying mantle (Bayne et al., 1979; Bubel et al., 1977) is similar to that in wounded oyster soft tissues (Ruddell, 1971a, 1971b). However, the shell must also be repaired, and to meet this need, hemocytes undergo *diapedesis*, or emigration through the mantle epithelium (Fig. 2). Diapedesis continues for weeks, during which time the epithelial cells change in size and shape (Bayne et al., 1979). RNA synthesis increases in small agranular basophils of shell-damaged mussels (Bubel et al., 1977).

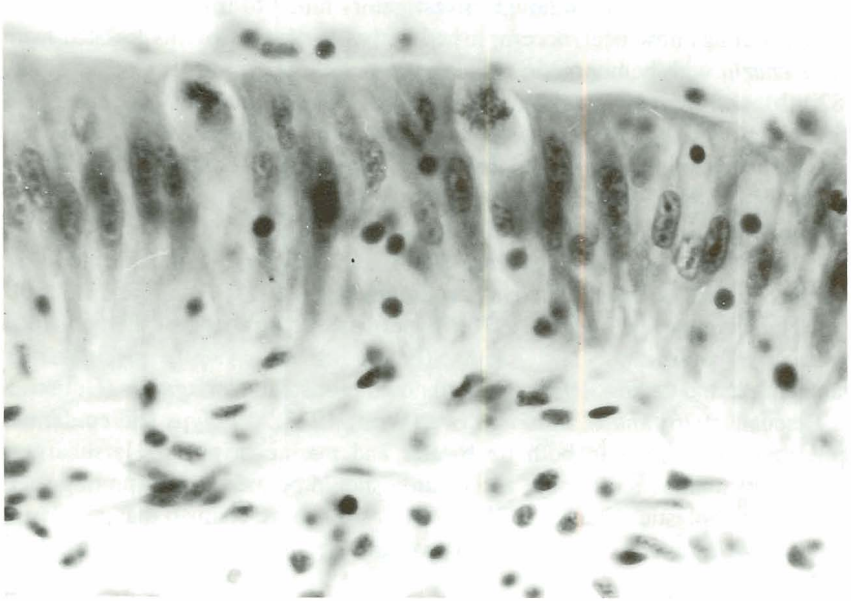


Fig. 2. Diapedesis of hemocytes through the palial surface epithelium of the *Mytilus californianus* mantle. The area from which this tissue was taken was near a damaged portion of the shell. The small, darkly stained nuclei of the hemocytes reveal their presence below, within, and exterior to the mantle epithelium. Two mitoses are seen in epithelial cells.

Regeneration of shell material in bivalves is reviewed by Watabe (Volume 4, Chapter 7).

B. Agents of Disease

Etiologic agents of oyster, clam, mussel, and scallop diseases were known in 1971 (Sprague) to include bacteria (*Achromobacter, Aeromonas/Vibrio, Myotomus*), fungi (*Labyrinthomyxa* or *Dermocystidium marinum,* i.e., *Perkinsus marinus, Monilia, Sirolpidium, Cladothrix, Nocardia*), sporozoans (*Minchinia* (*Haplosporidium*) *nelsoni* and *M. costalis, Haplosporidium tumefascientis, Chytridiopsis,* and *Nematopsis,* a gregarine), flagellates (*Hexamita*), ciliates (*Sphenophyra, Orchitophyra, Ancistrocoma*), amebae (*Valkampfia* or *Flabellula*), trematodes (*Bucephalus*), cestodes, nematodes, gastropods (*Odostomia* ectoparasites), and crustacea (*Mytilicola, Pinnotheres*). The bacterium, *Pseudomonas enalia,* was also known to cause disease in oysters (Colwell and Sparks, 1967). For several diseases, the etiologic agents were unknown. Noncommunicable diseases were reviewed by Sparks (1972).

A later list of oyster and clam diseases mentioned 10, two of which were of uncertain etiology (Sinderman, 1977). Among those of known etiology was herpes-type viral disease; although investigators failed to transmit this disease, transmission has now been accomplished with a B-type retrovirus isolated from *Mya arenaria* with hemopoietic neoplasia (Oprandy et al., 1981; Cooper et al., 1982,a,b), and Koch's postulates were satisfied. Comps et al. (1976) described a virus which wiped out *Crassostrea angulata* from the French coasts, and viral etiology has also been claimed for other pathological conditions in oysters (Comps and Masso, 1978; Elston, 1979).

Bivalve pathogens continue to be discovered (e.g., Elston, 1979; Gutierrez, 1977; Jones, 1981; Li and Clyburne, 1979), and previously unrepresented taxa have been added: a rickettsia in oysters (Comps et al., 1977; Comps and Deltreil, 1979; Harshbarger and Chang, 1976); hyperparasitic protozoa in the nematode *Sulcascaris* parasitizing surf clams (Payne et al., 1980); mycoplasmas (Harshbarger and Chang, 1976); and unknown protists in *C. angulata* (Gutierrez and Pascual, 1976) and *C. echinata* ova (Wolf, 1977). "Polyps and epidermal papillomas" described in both freshwater and marine bivalves (Harshbarger, 1976) are of unknown etiology, and caution should be used in interpreting such growths as neoplastic (Cheng, 1976). Indeed, some have claimed that neoplastic cells in bivalves could well have been protistan symbionts (Machin and Schlict, 1976).

C. Responses to Metazoan Symbionts

Organisms living in the mantle cavities of bivalves are essentially ectosymbionts. The oligochaete *Chaetogaster limnaei,* found on the gills of the Asiatic

clam *Corbicula manilensis,* elicits no obvious host response (Eng, 1976). However, another occupant of bivalve mantle cavities, the pea crab *Fabia subquadrata,* may induce watery cysts on the visceral walls of *Mytilus californianus* (Fig. 3) (M. N. Moore, T. H. Carefoot, R. J. Thompson, and C. J. Bayne, unpublished observations). The cysts may be caused by prolonged irritation, and it is likely that the crabs supplement their diets by including mussel tissue (Anderson, 1975). Similarly, in *Anodonta anatina,* the parasitic mite *Unionicola intermedia* appears to exploit a host response (Baker, 1976a). Mite damage to host gill filaments causes epithelial proliferation, edema, and hemocytic infiltration; numerous hemocytes are then ingested by the mite. Eventually, fibrous tissue forms where the mite pedipalps are embedded (Baker, 1976b). *Corbicula fluminea* is a freshwater bivalve in which the young are brooded in the marsupial inner demibranchs. Larvae which fail to escape from the marsupia undergo autolysis. This elicits a maternal "inflammatory and eventually an encapsulating process resulting in the formation of a granuloma [Morton, 1977]" The ctenidial epithelium, normally columnar and ciliated, becomes a nonciliated cuboidal or squamous epithelium; eventually, a thick, fibrotic coat surrounds the degenerating larval mass. Fibrocytes and amebocytes invade and resorb the larva, resulting in a complex, multilayered capsule (Fig. 4). In older clams, heavily affected portions of the inner demibranchs may be autotomized, a process in which an unusual granulocytic cell may actively digest ctenidial tissue (Britton et al., 1980). This closely resembles the events occurring around dead *Tylocephalum* in the marine clam *Tapes semidecussata* (Cheng and Rifkin, 1970).

Gut parasites of bivalves include *Mytilicola* spp. (*Copepoda*) (reviewed by Katkansky et al., 1967). In infected *Crassostrea gigas,* the normally ciliated columnar epithelium of the gut loses its cilia and changes to a cuboidal or even a squamous state near the parasites (Sparks, 1962). No leukocytosis was reported, but a fibrotic response in the compacted connective tissue is possibly a cellular response to this parasite.

True endoparasites of bivalves include several trematodes. Both larval sporocyst and adult *Proctoeces maculatus* occur in *Mylilus edulis* (Dupouy et al., 1973; Tripp and Turner, 1978), in which there is an absence of host cellular response to healthy sporocysts and to some adults. However, eggs, dead or dying sporocysts and adults, and even some active adults may be surrounded by numerous host hemocytes. These may encapsulate and break down moribund tissues, but they may also be fed upon by adult flukes located in the pericardial cavity (Fig. 5). The flukes "seem to 'graze' on the surface of the pericardial tissues, ingesting cells and mucus, and eliciting a strong hemocyte response [Tripp and Turner, 1978]," reminiscent of the exploitation by *Unionicola* in *Anodonta* (Baker, 1976a). Some freshwater mussels encapsulate the trematode *Aspidogaster conchicola* (Huehner and Etges, 1981). Capsules have distinct inner and outer layers, and "brown cells" occasionally between the layers. Although collagen is absent, "fine reticulum fibers" occur in the outer layer. Pathology of the diges-

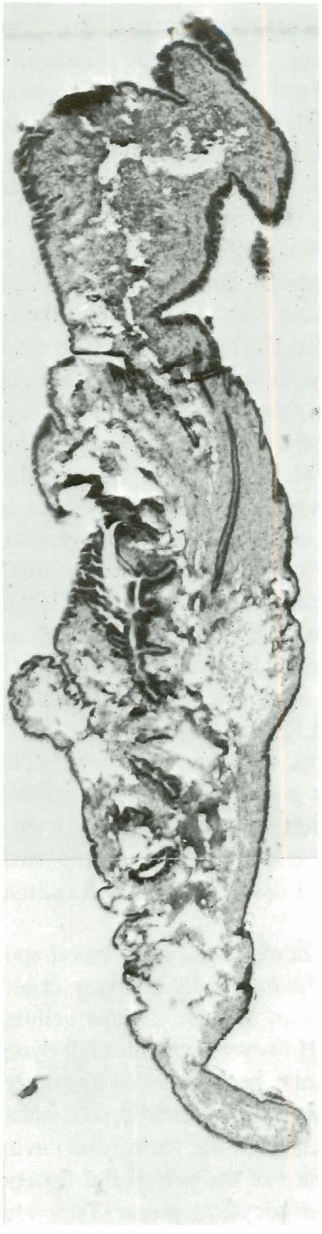


Fig. 3. A section through a watery cyst removed from the visceral body wall of *M. californianus*, which hosted a pea crab, *Fabia subquadrata*. The quite amorphous cyst is covered by a ciliated epithelium, contains depressions and pseudoducts, and is richly populated with hemocytes in the spongy sinuses of connective tissue.

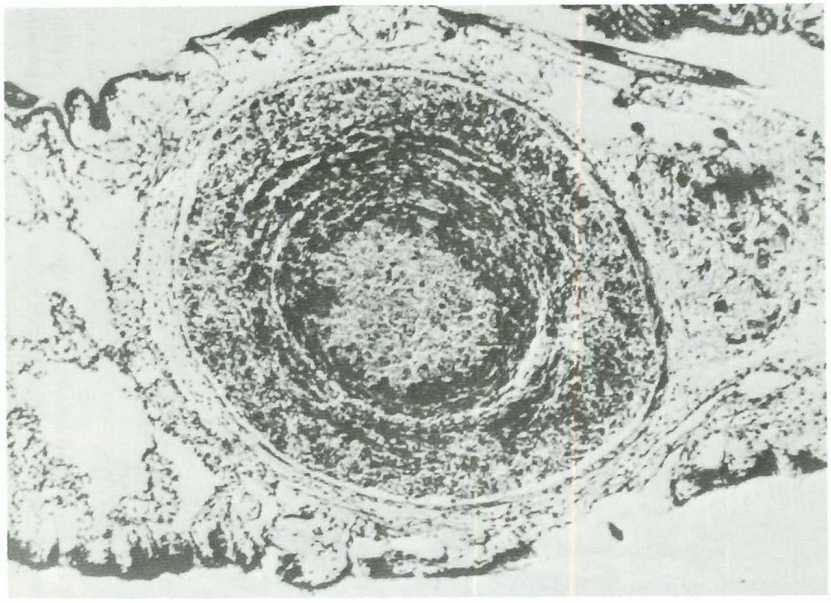


Fig. 4. A late-stage granuloma in *Corbicula fluminea* formed in response to a larva which failed to be released during the previous spawning season. Such tumor-like cysts develop in the inner demibranch (marsupium). Amebocytes probably reabsorb the larval cellular debris seen toward the center of the granuloma. (From Morton, 1977, with permission.)

tive epithelium induced by *A. conchicola* is similar to that reported from *Mytilicola* in *C. gigas* (Sparks, 1962). Encapsulation by host mussels may be related to the season (Tripp and Turner, 1978), the location in the host, and the overall parasite burden (Huehner and Etges, 1981).

The host (*Crassostrea virginica*) response to the trematode *Bucephalus* sp. is variable, from little or no response (Cheng and Burton, 1965; Hopkins, 1957) to an intense cellular response to *Bucephalus* sporocysts hyperparasitized with haplosporidians (Mackin and Loesch, 1955). However, hosts may respond only to moribund parasites, and the health of a parasite may be influenced by the environmental conditions in which it finds itself imprisoned by its host (Douglass, 1976). Thus, an apparent host immune response in certain individuals may be due to the fact that microenvironmental conditions have led (not immunologically) to the demise of the parasite. In an *in vitro* assay, hemocytes of *C. virginica* respond to dead cercariae (several species) by encapsulation, whereas they ignore live cercariae (Font, 1980).

D. Responses to Protozoan and Fungal Parasites

Most studies of protozoan parasites of bivalves deal with parasite morphology and life cycles; few studies have focused on host responses. In part, this is

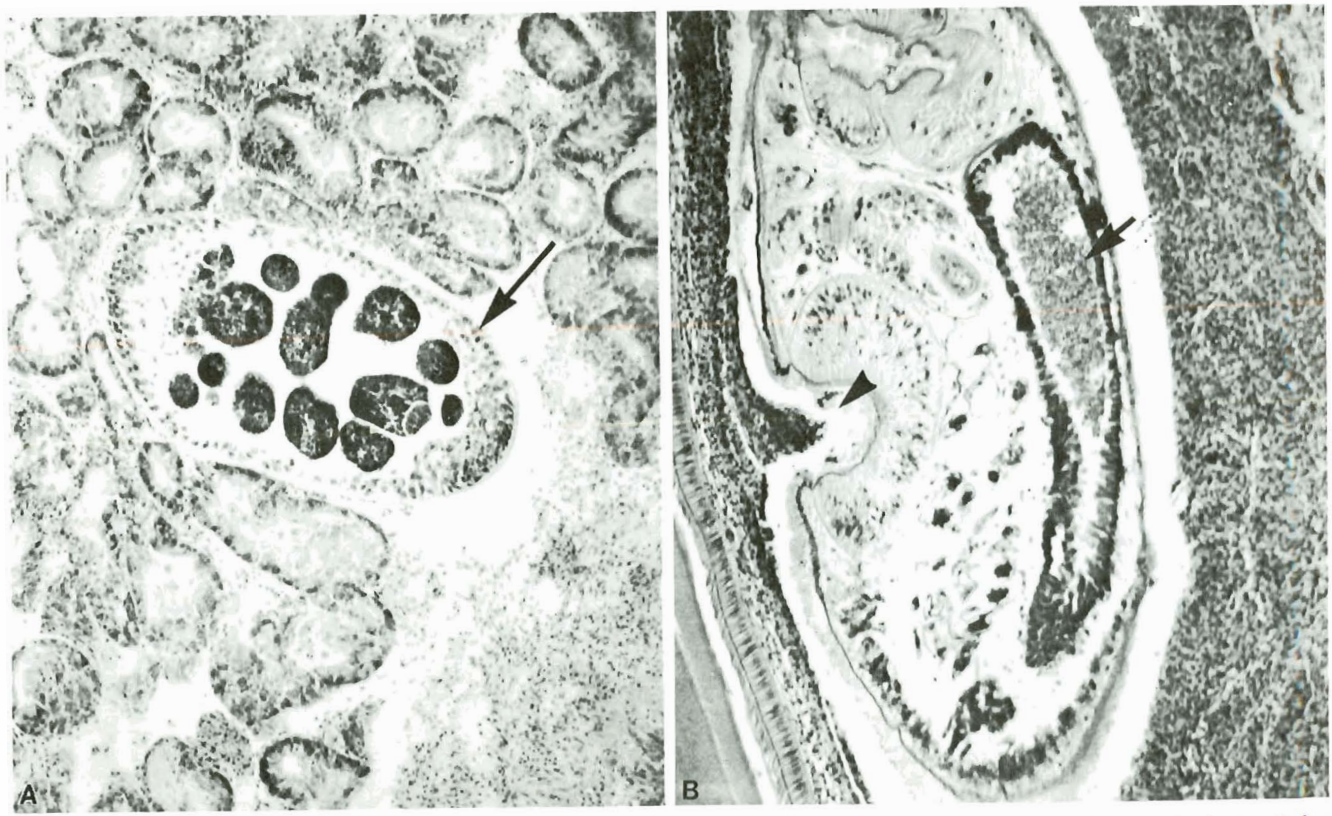


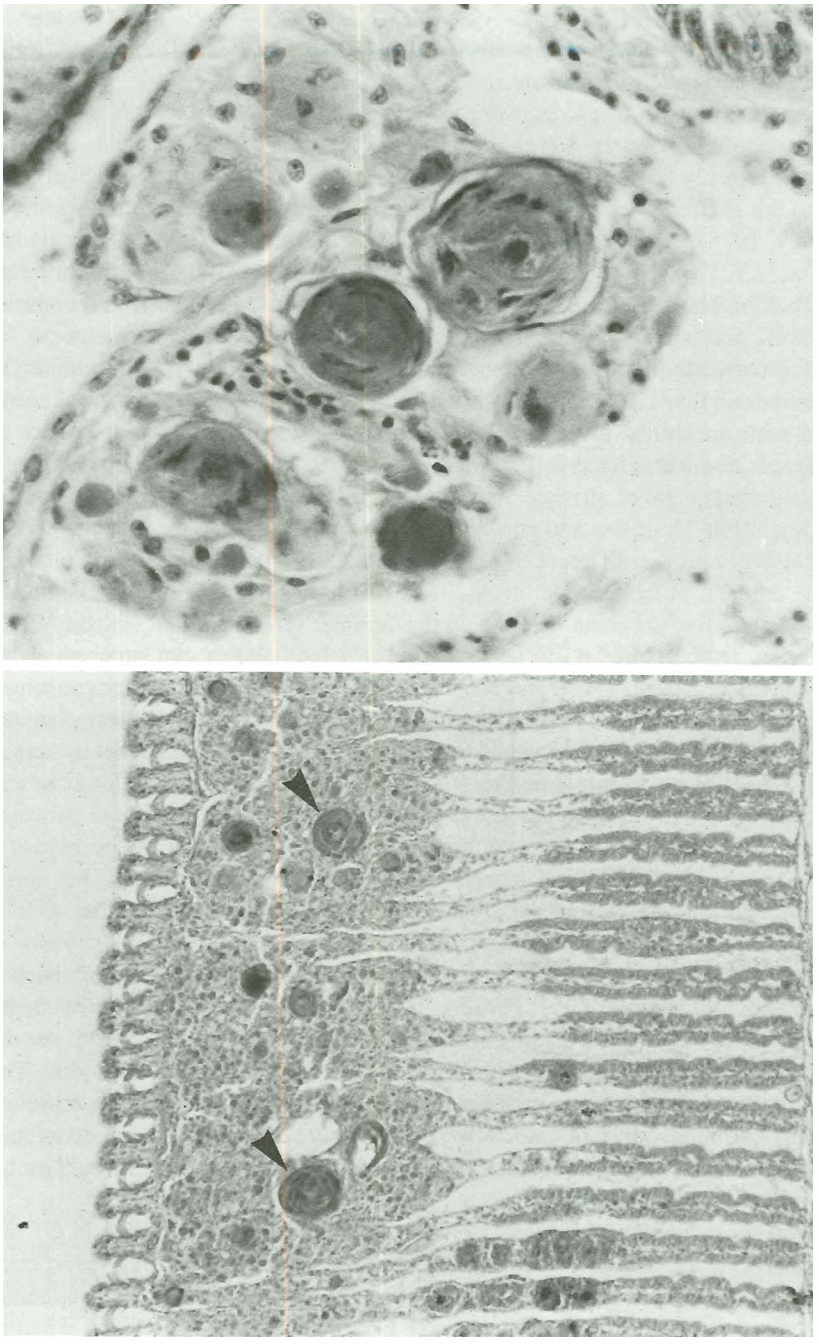
Fig. 5. (**A**) *Proctoeces maculatus* sporocyst (arrow) among *Mytilus edulis* digestive gland tubules. Notice the absence of a host cellular response to the sporocyst, in which several darkly stained germinal balls are to be seen. (**B**) Adult *P. maculatus* surrounded by numerous hemocytes within the pericardial cavity of *M. edulis*. Notice that the parasite is ingesting homocytes (arrowhead), some of which are located in the digestive tract (arrow) of the fluke. (Courtesy of M. R. Tripp.)

because of the general inefficacy of host immunity. By definition, parasites generally survive in their hosts.

As a result of massive *Crassostrea virginica* mortality caused by *Haplosporidium nelsoni* (previously *Minchinia nelsoni,* or MSX) in Chesapeake Bay (reviewed recently by Ford and Haskin, 1982), natural selection for resistance has led to the existence of resistant or susceptible populations in different locations (Farley, 1975). Efforts to transmit the infection in the laboratory consistently fail (Canzonier, 1974). Resistance and susceptibility are the extremes of a continuum which is probably under multigene control (S. E. Ford, 1982, personal communication) and appears to be nonspecific; susceptibility and resistance to *H. nelsoni* correlate precisely with susceptibility and resistance to *Labyrinthomyxa marina* (Valiulis, 1973). "Resistance to MSX-kill in native oysters is not correlated with an ability to prevent infection, but with restriction of parasites to localized, non-lethal lesions [Ford and Haskin, 1982]." Susceptible oysters may respond with a more marked leucocytosis than that seen in resistant oysters (Farley, 1968; Douglass and Haskin, 1976; Kern, 1976), but the evidence is not conclusive, and the differences may "be linked to greater parasite burden and tissue damage in susceptible stocks" (S. E. Ford, 1982, personal communication). Leukocyte numbers increase in the summer (Douglass and Haksin, 1976) and when temperatures reach near 20°C, "resistant oysters can suppress or rid themselves of MSX" (Ford and Haskin, 1982: p. 133). Hyalinocytes contribute a greater proportion of the leukocyte population in infected oysters, but the significance of this is unknown. If the hemocytic response in resistant oysters is really less, this could imply that resistance is humorally mediated (Douglass and Haskin, 1976). In resistant oysters, hemocytes phagocytose moribund parasites (Kern, 1976). No variability has been reported in infectivity of the parasite.

The gregarine *Nematopsis ostrearum* is phagocytosed in oysters, but some survive and multiply within the amebocytes (Prytherch, 1940). Another serious oyster pathogen, the fungus *Dermocystidum marinum,* also elicits leucocytosis in some oysters and is phagocytosed, but the pathogen survives and multiplies intracellularly (Machin, 1951; Perkins, 1976). Early in the infection, hosts begin encapsulating foci of the pathogen in connective tissues, but this fails. As the organism multiplies, the "capsule" disappears, and local host cells die. The infection spreads to tissues including epithelia, adductor and smooth muscles. Ciliates commonly live in association with bivalve ctenidia; *Boveria teredinidi* may enter the gill tissue of *Bankia gouldi,* where they elicit leucocytic encapsulation (Fig. 6; Hillman, 1979).

Haplosporidium nelsoni affects plasma levels of several enzymes in *C. virginicia* (Feng and Canzonier, 1970; Douglass and Haskin, 1976). However, even uninfected oysters have seasonal changes in enzyme titers. For example lysozyme tends to increase in the winter (Feng and Canzonier, 1970) whereas aspartate aminotransferase and alanine aminotransferase are at highest levels in



June in New Jersey (Douglass and Haskin, 1976). Although *H. nelsoni* depressed lysozyme titers in the summer in Chesapeake Bay oysters (Feng and Conzonier, 1970), the pathogen resulted in increased titers of the two aminotransferases and of phosphohexose isomerase in oysters with gill lesions (Douglass and Haskins, 1976). Increases probably reflect host efforts to maintain metabolic homeostasis rather than anti-*H. nelsoni* immunity. In the same study, an approximate two- to eightfold increase in lysozyme in lightly or heavily *Bucephalus*- infected oysters (Feng and Canzonier, 1970) may have reflected a host immune response. Total hemolymph protein may be depressed in oysters with systemic infections of *H. nelsoni,* but those with only gill lesions do not suffer hypoproteinemia (S. E. Ford, 1982, personal communication).

Reference to a humoral response to infection is usually interpreted to mean that the response is defensive. Thus, it is as well that Feng and Canzonier (1970) qualified their statement "that humoral responses indeed exist in oysters invaded by these parasites" by adding that "the functional significance of (such changes) relative to protection of the host . . . has yet to be determined [p. 497]." These same authors noted some hosts which carried "persistent high levels of infection," and concluded that this was evidence for "existence of unidentified host mechanism(s) which keep the infection in check [p. 508]." Such reasoning is dangerous, as it overlooks the advantages to the parasite of self-regulation.

Haplosporidium nelsoni infections may disappear in the winter. The cause may be nonimmunological; for example, the parasite, rich in mitochondria, may be unable to tolerate prolonged anaerobiosis in the host tissues (Ford and Haskin, 1982). Like other molluscs, bivalves do encapsulate unsuitable protists in their tissues (Hillman, 1979).

E. Responses to Foreign Particles and Macromolecules

Hemocytes of bivalves are varied with respect to morphology and to their individual complements of hydrolytic enzymes (Cheng, 1978, 1981; Huffman and Tripp, 1982). They are avidly phagocytic both *in vivo* and *in vitro*. Yonge (1925) and Takatsuki (1934) emphasized the nutritive roles of this behavior, noting that phagocytes migrate into gut lumina and onto body surfaces, where they engulf food particles before reentering the body and digesting the catch. This movement of amebocytes across epithelial boundaries has been observed repeatedly (Ransom, 1936, cited in Stauber, 1950; Bayne et al., 1979).

1. Clearance of Ink

Stauber (1950), inspired, like others, by Metchnikoff (1891), observed laden hemocytes emigrating across epithelia to cleanse oysters of injected ink particles. Stauber's paper (1950), clearly focusing attention on the defensive role of oyster hemocytes, was a landmark in molluscan immunobiology, although his study

involved only 10 *Crassostrea virginica*. Ink particles, injected into the heart, immediately formed emboli in the main arteries. Hemocytes subsequently accumulated, phagocytosed the ink, and moved off. The blockages were resolved by this emigration of ink-laden phagocytes and by circulatory pressure that forced the ink into the more distal and spacious sinuses. Some phagocytes moved directly into the sinuses, but many traversed the walls of the blocked arteries. This distribution of ink by the phagocytes contrasts to the situation in vertebrates, where colloidal carbon remains localized in organs of the reticuloendothelial system. By about 8 days postinjection (PI) in *C. virginica*, elimination began by the emigration of laden phagocytes; this occurred mainly in the digestive organs, but also in the palps and "oromyal chamber." Rarely was emigration observed in the gills, and it was absent in gonoducts and excretory tubules and through the pallial epithelium of the mantle, although carmine-laden phagocytes have been observed in epithelia of the excretory tubules and gonoducts, as well as in other organs (Takatsuki, 1934). Such differences could be due to seasonal (reproductive) factors, the use of different injection sites, or other reasons. The black color of feces and pseudofeces was gross evidence for the sanitary role of emigrating phagocytes. Although Stauber never confirmed that this material was due to laden cells, Feng (1965) found that injected proteins were present in fecal and pseudofecal amoebcytes 24 h PI. Table III summarizes Stauber's findings.

TABLE III

Time Sequence of Events Leading to Disposal of India Ink After Intracardial Injection into the Oyster[a,b]

Oyster number	Time after injection	Arterial occlusion	Presence of free india ink in blood vessels	Migrations of ink-laden phagocytes through arterial walls	Migrations of ink-laden phagocytes through epithelia
1	15 min	+	++++	−	
2	1 h	+	++++	−	
3	2 h	+	++++	−	
4	4 h	+	+++	−	
5	22 h	+	+	+	−
6	8 days	+	±	++	+
7	17 days	−	±	++	++
8	25 days	−	±	++	++
9	33 days	−	±	+	++++
10	42 days	−	±	−	++

[a] From Stauber (1950) with permission.

[b] The number of pluses is approximate and relative and is significant only when compared with others in the same vertical column.

Colloidal carbon, injected into the adductor muscle of the giant clam *Tridacna maxima,* spread rapidly throughout the body (Reade and Reade, 1976). By 10 min PI, the gills, inner mantle membranes, and muscles were uniformly black; in the kidney, heart, and digestive system, blackening took longer. Subsequently, all tissues paled sooner than the gut. Microscopically, events were much as described by Stauber (1950); however, early association of colloidal carbon with granulocytes was followed by phagocytosis by hyaline cells. The kidney was a major route of emigration. Reade and Reade (1976) state that *Tridacna* "do not appear to have a system of fixed phagocytic cells but rely on circulating hemocytes for particle clearance, which presumably includes invading microorganisms (p. 357)."

The beautifully illustrated work of Cuénot (1914) places Stauber's and the Reades' studies in nice perspective. Cuénot studied ink clearance in 17 bivalve species and concluded that most bivalves lack definite phagocytic organs, but that the digestive system is generally important. For example, in *Cardium, Donax, Mactra,* and *Scrobicularia,* clearance occurs primarily in fine arterioles of the digestive system. In *Solen marginatus,* the labial palps are uniquely important in phagocytosis. It appears that "nodules lymphoides," blackened by injected ink, occur on secondary arterioles and constitute the essential reticuloendothelial analogs.

2. Clearance of Eukaryotic Cells

Species differences exist not only in clearance systems but also in the fates of different injected agents. Yeast and various vertebrate erythrocytes (RBCs) injected into *Crassostrea virginica* immediately formed emboli, occulding many of the larger arteries (Tripp, 1958, 1960). Within 10 min PI, hemocytes had phagocytosed yeast or RBCs, from one to eight per hemocyte, with up to 20 in extreme cases. By 6 h PI, about 95% of the RBCs had been phagocytosed and the emboli were resolved, as were the ink-caused emboli (Stauber, 1950). By 48 h PI, yeast- and RBC-containing hemocytes were emigrating through epithelia of the gut and digestive diverticula; the pericardium, palps, gonoducts, and mantle epithelia were less often traversed.

3. Clearance of Bacteria

Most bacteria entering the body are rapidly clumped and phagocytosed, and are then killed by intracellular digestion; some damage may be inflicted on the bacteria in the plasma (Tripp, 1960). For most degradable material, disposal by diapedesis (transepithelial migration) is probably secondary to intracellular digestion (Yonge, 1926; Cheng and Rudo, 1976b). Growth of microorganisms from tissues taken at various times PI showed that oysters effectively destroyed vegetative bacteria and yeast, because "only a small proportion . . . could be recovered and only within 24–48 hours after injection" (Tripp, 1960: p. 280).

Bacterial spores, which were much more resistant to killing, were eventually eliminated by diapedesis. Tripp's (1960) conclusions regarding cell-mediated internal defense responses of bivalves (Table IV) still stand, although they have been extended.

Bacillus thuringiensis, Mycobacterium smegmatis, and a *Pseudomonas*-like organism injected intracardially into *Crassostrea virginica* were all rendered nonviable, but survival times varied widely (S. Y. Feng, 1966). The persistence of *M. smegmatis* for 5 days could have been due to the higher dose (9×10^9) relative to the doses of the *Pseudomonas*-like organism (1.2×10^7) and *B. thuringiensis* (2.5×10^7); phagocytosis and digestion of *M. smegmatis* were seen microscopically, and diapedesis of laden phagocytes occurred in the intestine.

Oyster hemocytes are attracted to metabolic products of certain live bacteria, both gram negative and gram positive (Cheng and Howland, 1979; Cheng and Rudo, 1976a; Howland and Cheng, 1982). The potentially pathogenic *Vibrio parahaemolyticus* does not stimulate positive chemotaxis of *C. virginica* hemocytes (Cheng and Howland, 1979). Hemocyte migration is altered by drugs that disrupt the cytoskeleton (Cheng and Howland, 1982).

It is not clear what role, if any, humoral antimicrobial factors, reported from oysters (Li, 1960), played in Tripp's and Feng's experiments. Some of the bacteria may have been susceptible to oyster lysozyme, although this is probably not normally present in high titers in the hemolymph (McDade and Tripp, 1967).

A single attempt to induce a bactericidal response in a bivalve was unsuccessful, although the procedure was identical to one which succeeded in a lobster (Weinheimer et al., 1969).

4. The Fates of Viruses

Leukocytes of *Crassostrea virginica* take up virus particles *in vitro* (Fries and Tripp, 1970). Bivalves can accumulate viruses from water and retain them in viable form for long periods, especially at low temperature (J. S. Feng, 1966), potentially serving as reservoirs and vectors of medically important species. Little is known about the mode and route of entry of these or of mollusc pathogens in general. *Crassostrea virginica* eliminate injected viruses (T2; Acton, 1970), and this action is very temperature dependent (*Staphylococcus aureus* Phage 80; J. S. Feng, 1966). Phages 80 were recoverable from shell liquor, feces, pseudofeces, and the water around the oysters (J. S. Feng, 1966), and T2 were recoverable from mantle, gill, muscles, and viscera (Acton, 1970); the mechanism(s) of transport to these sites is/are unknown. Rather than inactivating the phage, oyster plasma from naive or preinjected animals actually protected viability relative to that observed in 20 ‰ seawater, but there is no evidence for replication in oysters. Natural infections of bivalves with viruses have been reported (Comps and Masso, 1978; Elston, 1979).

TABLE IV

The Relative Importance of Various Processes in the Disposal of Particles Injected into the Oyster, *Crassostrea virginica*[a]

	Tripp (1960)			Tripp (1958)	Stauber (1950)
Method of removal	Bacterial spores	Yeast	Vegetative bacteria	RBC	Carbon
Phagocytosis and migration	++++	++++	+	++	++++
Phagocytosis and digestion	N.O.[b]	+	++++	++++	N.O.
Extracellular destruction	N.O.	N.O.	++	N.O.	N.O.

[a] Adapted from Tripp (1960) with permission.
[b] N.O., not observed.

Some of Acton's results (Fig. 7) have been claimed to show enhancement of secondary clearance (Acton, 1970; Acton and Evans, 1968; Acton et al., 1969; Weinheimer et al., 1969) by a cell- rather than a humorally mediated mechanism which is "not entirely specific." This claim remains to be independently substantiated. In view of the marked temperature dependence report by J. S. Feng (1966), it is regrettable that water temperatures in Acton's experiments fluctuated between 25 and 30°C. In neither the thesis (Acton, 1970) nor the publication (Acton and Evans, 1968) were any values other than means presented. The T2 bacterial phages used in this study were cleared slowly, requiring between 56 and 63 days.

5. The Fates of Proteins

The dinoflagellate *Gonyaulax excavata* produces complex toxins (White and Maranda, 1978) which accumulate in bivalve tissues and are responsible for lethal "paralytic shellfish poisoning" of human consumers. Tissue distribution of the toxins is uneven, with the highest concentrations in *Spisula solidissima* being in the mantle and gill (Blogoslawski and Stewart, 1978). In scallops (*Placopecten magellanicus*), the adductor muscle has detoxification properties (Shimizu and Yoshioka, 1981).

Bovine hemoglobin and diphtheria antitoxin are cleared from oyster plasma within a few hours (Feng, 1965); leukocytes are involved, becoming pink within 30 min of the hemoglobin injections. When human albumin, human gamma globulin (HGG), and *Limulus* hemocyanin are injected, clearance is accompanied by accumulation of protein (fluorescent labeled) in leukocytes of the perintestinal region. Some of these leukocytes quickly emigrate (they traverse epithelia within 15 min PI) and are found in feces and pseudofeces 24 h PI. Leukocytes also pinocytose HGG *in vitro* in a temperature-dependent fashion (Feng, 1965). Such trapping and internalization of foreign proteins are achieved

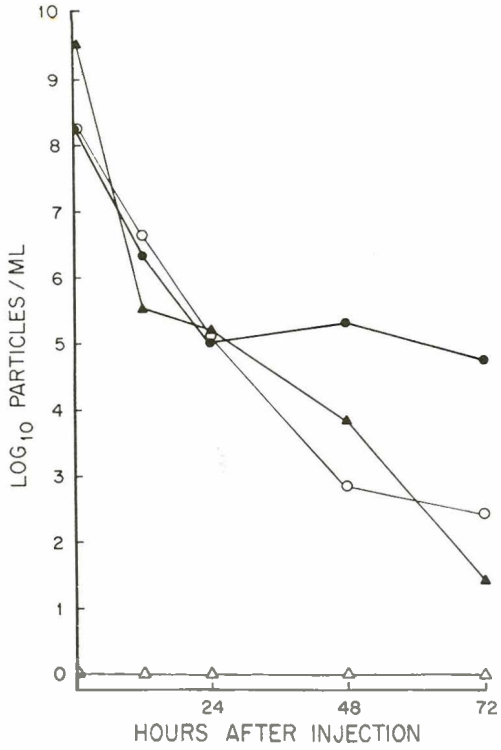


Fig. 7. Comparison of T2 bacteriophage clearance in the oyster with that in the lemon shark. Temperature ranged from 25 to 30°C during the course of these experiments. Each point on the graph represents the arithmetic mean of the number of plaque-forming units per milliliter of serum from four lemon sharks and six oysters. (●) Oyster, primary; (○) oyster, secondary; (▲) shark, primary; (△) shark, secondary.

using unkown mechanisms and receptors. However, with the lectin concanavalin A, it has been shown that *Crassostrea virginica* hemocytes will patch, cap, and internalize this glycoprotein in a manner closely resembling that seen in vertebrate immunocytes (Yoshino et al., 1979).

F. Responses to Tissue Implants

Orthotopic implantation, i.e., transfer to a topographically equivalent site, is technically very complex in molluscs (Hildemann et al., 1974) and has not been successfully accomplished. Although the efforts of Hildemann and colleagues to suture allogeneic mantle tissue orthotopically into *Pinctata margaritifera* (pearl oysters) encountered insurmountable problems, grafts often fused and began to

heal before necrosis and loss ensued. In my opinion, vasoconstriction of cut hemolymph channels in molluscan skin may preclude successful skin grafting.

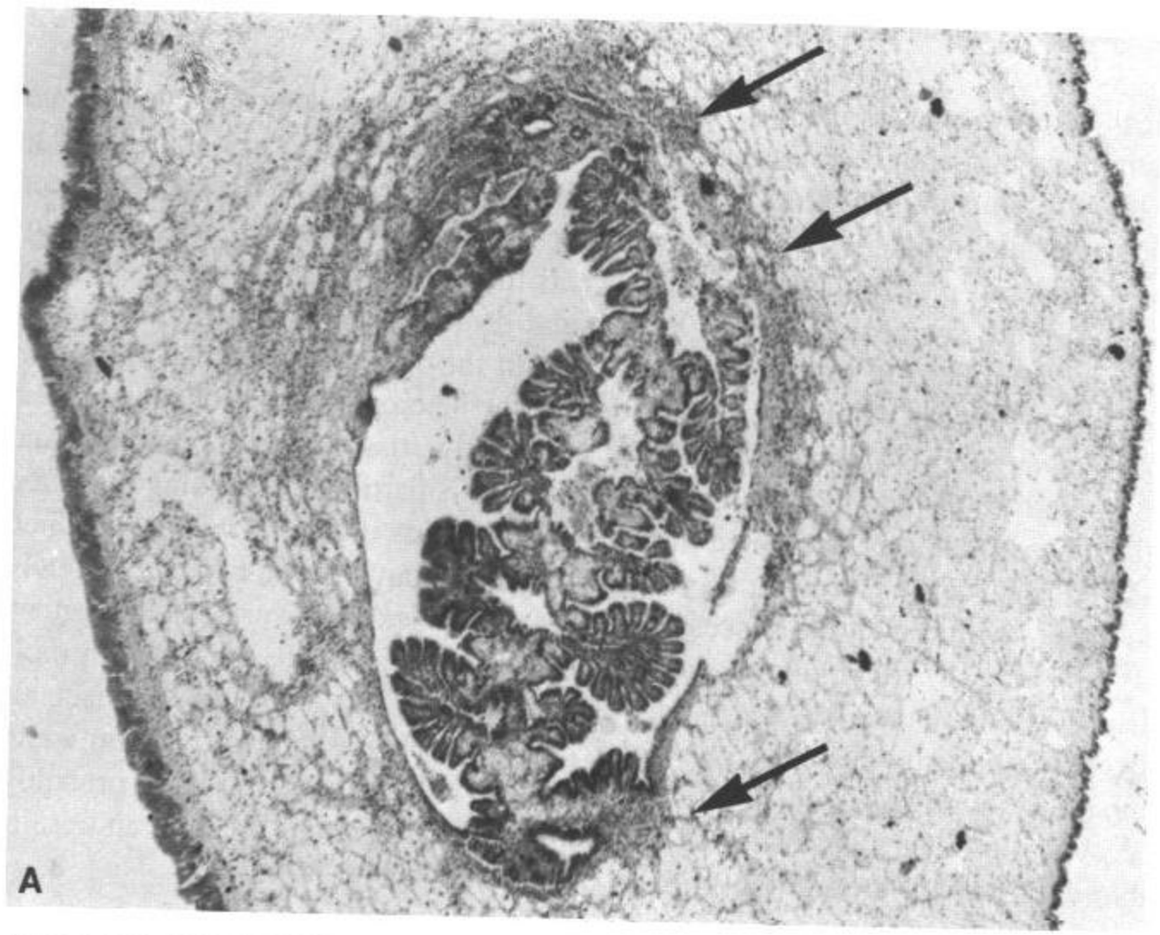
Heterotopic implantation (placing implants into abnormal tissues) has been done repeatedly in bivalves, in connection with pearl formation, and in immunological and parasitological studies. Pearl formation, reviewed extensively by Tsujii (1960; see also Machii, 1968), is not dealt with here. Allogeneic mantle fragments implanted into connective tissue in the visceral mass of *Crassostrea* elicited only weak cellular responses despite the heterotopic location (Canzonier, 1974; DesVoigne and Sparks, 1969); commonly, tissue fusion occurred, and even after 18 days implants appeared healthy. It is particularly striking that even gill tissue, shallowly implanted into the visceral mass of *C. virginica,* "retained its integrity for the entire period of observation (35 days), even when completely contained within a closed abscess without access to external surface [Canzonier, 1974]" (Fig. 8). Such allogeneic compatibility appears to be the norm in molluscs. Among other Metazoa, only the arthropods similarly fail to reject allogeneic implants. *Crassostrea virginica* responds by cellular infiltration when either moribund tissue or digestive gland tissue is implanted, the latter probably because it produces quantities of extracellular enzymes, thus creating an unsuitable environment around the implant (Canzonier, 1974).

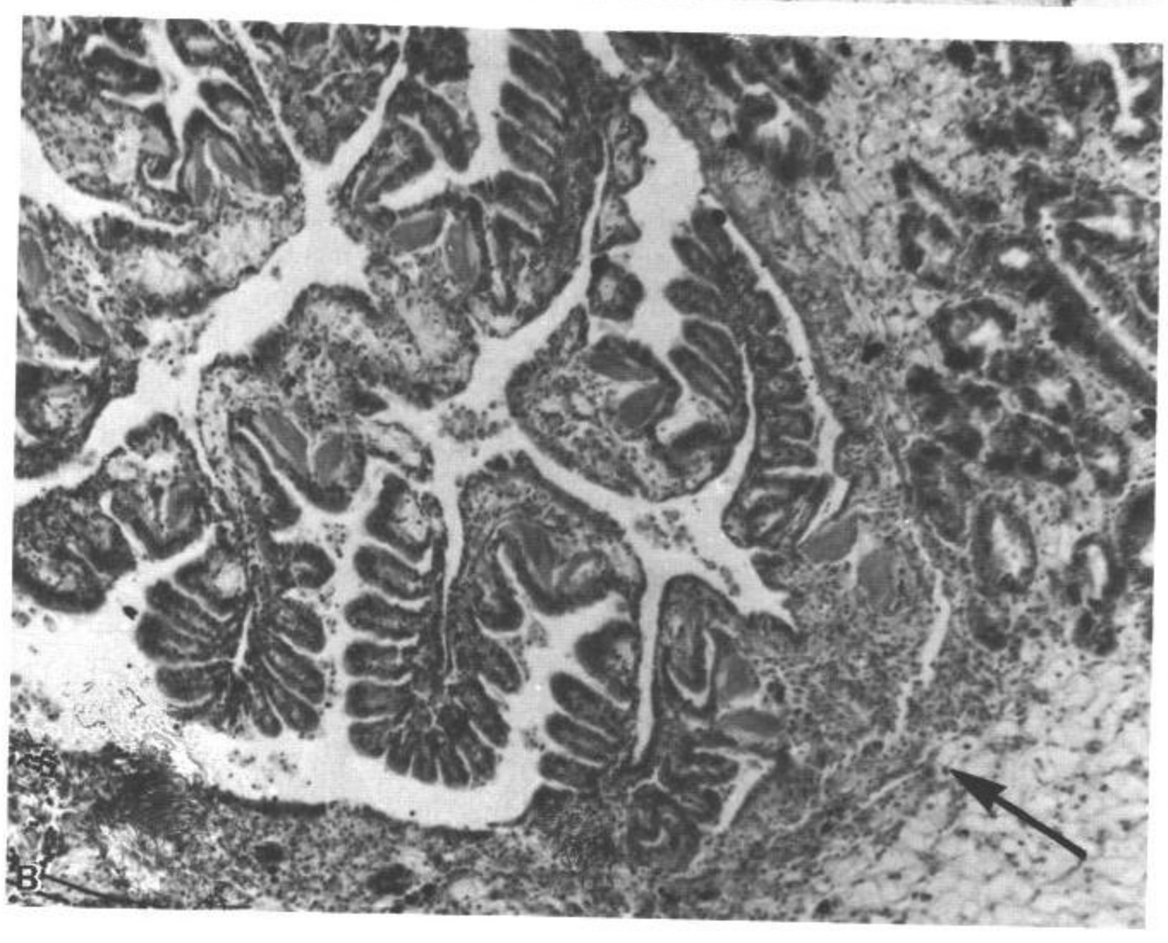
Xenogeneic implants made orthotopically elicit infiltration by hemocytes in *Mytilis californianus* (Bayne et al., 1979) (Table II). Hemocyte types respond differentially; basophilic cells accumulate first in tissues contacting the implant and then invade the implant (*Mya arenaria* mantle). After several days, acidophilic hemocytes predominate in and around the implant, the cellular components of which are phagocytosed as they die.

If a mussel which is responding to such an implant or has been sham-operated is reimplanted, the cellular response and implant destruction are more rapid; however, this appears not to be due merely to more numerous hemocytes, because inflammation around interfaces with second and third implants is more localized. The altered state of the recipient mussel is due to a qualitative change in hemocytes and/or to a subtle qualitative or quantitative change in plasma factors (Bayne et al., 1979).

G. Humoral Factors

Lectin-like agglutinins are common components of bivalve hemolymph. The potential roles of these molecules have often been discussed in an immunological context (Baldo et al., 1977; Hardy et al., 1978; Jenkin and Rowley, 1970; Vasta et al., 1982); for example, it is suggested that in *Tridacna,* they mediate the balanced symbiosis with unicellular dinoflagellates (Uhlenbruck and Steinhausen, 1977).


A


B

We lack evidence that antimicrobial (Li, 1960), antiviral (Prescott et al., 1976, cited by Tripp, 1970), and antitumor (Schmeer et al., 1966) substances isolated from bivalves are functional components of internal defense systems. Whereas hemolymph lysozyme may be important as an agent of internal defenses (Hardy et al., 1976; McDade and Tripp, 1967), the finding of much higher lysozyme levels in digestive tracts (McHenry et al., 1979) has led to the suggestion that lysozyme may serve primarily a digestive function in bivalves, with a secondary role in defense. In the clam *Mercenaria mercenaria,* hemolytic activity directed against rabbit erythrocytes can be enhanced (Anderson, 1981), but we lack evidence for its role in defense. It will be of interest to see if *in vivo* roles will be found for such hemolysins, and for oyster plasma components which are cytotoxic, mitogenic, and agglutinating for mammalian lymphocytes (Hardy et al., 1978).

IV. Internal Defenses of Gastropods

A. Responses to Surgical Damage

The skin and body wall constitute the first lines of defense against the entry of undesirable substances and objects into the body. Molluscs are as successful as any group in preventing hemorrhage and bodily invasion as a result of surface wounds. Their responses to cuts in the body wall are so effective as to cause great frustration to ambitious surgeons (Bayne et al., 1979; Hildemann et al., 1974), and they can be presumed to provide effective protection from other organisms. The immediate response is exudation of masses of mucus, with simultaneous muscular constriction of the wound. Nothing akin to fibrous clot formation occurs (cf. Sminia et al., 1973).

These processes and subsequent healing have been studied in nudibranchs (Kress, 1968), abalone (Armstrong et al., 1971), and *Lymnaea* (Sminia et al., 1973). The extent of hemolymph loss depends on both the extent of the wound and its location. Hemocytes, normally separate cells *in vivo,* are in small clumps in the lost fluid. Leukocytosis occurs after wounding; this consists of an increase in the number of circulating cells (Sminia et al., 1973) and is a phenomenon which has been exploited by persons who need numerous hemocytes for *in vitro* studies (Renwrantz and Cheng, 1977a, 1977b). Leukocytosis may also be stimulated by temperature changes (Pauley and Krassner, 1971) and parasitism (Stumpf and Gilbertson, 1980).

Fig. 8. Allogeneic ctenidia implanted heterotopically in a *Crassostrea virginica* visceral mass. The implant fuses with host tissue (arrows). Other than swelling of chitinous rods, there is little change in the composition of the tissue. (**A**) 25 days after implantation; (**B**) 35 days after transplantation. (From Canzonier, 1974, with permission.)

By 3 h, wounds may be plugged by amebocytes, which continue to accumulate (to 5 days in *Lymnaea*). When *Lymnaea* were preinjected with ink to "label" the phagocytes, wound plugs were black. The hemocytes are spherical at first; after about a day, they begin to flatten or elongate, a change which is typical in molluscan encapsulation responses. Most damaged cells and debris are engulfed and degraded by local phagocytes, normally amebocytes. Nervous tissue, however, is cleaned up by neuroglial cells (Sminia et al., 1973). Fibroblasts and local muscle cells form collagen (Sminia et al., 1973), and neighboring epithelia overgrow the amebocyte plug. As healing progresses, the plug becomes less distinct due to a loosening of the cell clump, the flattening of many of the amebocytes and the emigration of others, and continued formation of collagen between the amebocytes and in the neighboring undamaged tissue. Within about 2 months healing is complete; less scar tissue remains than is typical in healed vertebrate wounds.

The absence of tight or septate junctions between hemocytes in *Lymnaea* plugs, and the probable involvement of microfilaments in hemocyte shape changes, are similar to observations of *Biomphalaria* hemocytes during encapsulation responses (Loker et al., 1982).

Mutual adhesion of limpet (*Patella*) hemocytes has been investigated *in vitro* (Davies and Partridge, 1972; Jones et al., 1976; Partridge and Davies, 1974; see the review by Bayne, 1981a). Limpet hemocytes are 99% amebocytes and 1% "macrophages." After bleeding, the spherical amebocytes extend stout marginal spikes; the rate is dependent on temperature but independent of divalent cations. However, aggregation, which is cation dependent, occurs rapidly (in seconds), implying the triggering of a presynthesized system. Amebocytes and macrophages differ in their cation dependency for attachment and spreading on glass. Furthermore, amebocytes but not macrophages are inhibited by cytochalasin B; aggregates are not, however, dispersed with this drug.

B. Responses to Metazoan Parasites

This is the topic on which most literature exists in molluscan immuno-biology, due predominantly to the importance of gastropods as intermediate hosts of human and other vertebrate parasites, especially trematodes. I do not review the effects of parasites on their hosts; metabolic effects were reviewed by Becker (1980). For histopathology, see Pan (1965) and Schutte (1975); for data on the effects of parasites on mortality, duration of infection, and parasite fecundity, see Pan (1965), Anderson and May (1979), Loker (1979), Frandsen (1979), and Kuris and Warren (1980); for effects on host fecundity and gigantism, see Nassi (1979), Sluiters and Joose (1979), Wilson and Denison (1980), and Makanga (1981).

Numerous studies reviewed by Cheng (1968), Wright (1971), and Basch

(1976), and more recently (cf. Frandsen, 1979; Michelson and DuBois, 1978; Southgate, 1979; Sullivan and Richards, 1981) have dealt with differing susceptibilities of snails to specific trematodes and nematodes. Selection has shown that susceptibility–insusceptibility of the host is genetically determined (Newton, 1953; Richards, 1970), as is infectivity of at least trematode parasites (Richards, 1976). Thus, the outcome of a particular parasite–host encounter is governed by strain-specific genetic factors (compatibility) as well as by environmental (including age-related) factors (Yousif and Lammler, 1975). To quote Basch (1976): "in a compatible snail, the [parasite] develops without host–tissue reaction; in incompatible snails the [parasites] are rapidly surrounded by amebocytes . . . , and destroyed." Furthermore, in an individual snail exposed simultaneously to compatible and incompatible miracidia, encapsulation of the latter occurs in the immediate neighborhood of the healthy, unencapsulated compatible sporocysts (Kassim and Richards, 1979b).

1. Nematodes

Over 50 species of terrestrial and freshwater gastropods can serve as host for *Angiostrongylus cantonensis*. The host range has also been partially explored for *Daubaylia potomaca* (Chernin, 1962) and *D. helicophilus* (Poinar and Richards, 1979). Nematodes are encountered less frequently in gastropods than trematodes (Cappucci, 1978; Cheng, 1978; Chernin et al., 1960; Ogren, 1959). They enter via the gut or head–foot and may occur within the gut lumen, the lung, or in various internal tissues (Chitwood and Chitwood, 1937; Yousif and Lammler, 1977). Once within the host, nematodes appear, in general, to be relatively unaffected by host responses and to be quite unselective with respect to tissue location. Most do not kill the host, but *D. potomaca* kills planorbids such as *Biomphalaria glabrata,* in which it occurs in several organ systems (Chernin et al., 1960).

Within the host, nematodes may move around actively, feeding on host tissue. Eggs, moribund worms, and small larvae often become encapsulated, but this, however, rarely kills them. For example, in *Cepaea nemoralis,* larval *Muellerius capillaris* are surrounded by host hemocytes within hours, and although the hemocytes become flattened within 3 days, the capsule is later loosened and the larvae remain viable and grow (Sauerlander, 1979). In *Achatina fulica,* larval *Angiostrongylus* are encapsulated within 12 h (Sauerlander, 1976); by 3 days the hemocytes in the outer layers of the capsule have become flattened ("fibroblast-like"), followed by a thinning of the capsule wall and the formation of a cavity around the worm. This sequence of events also occurs when *A. cantonensis* is encapsulated in *B. glabrata* (Harris, 1975; Harris and Cheng, 1975a). *Marisa cornuarietis* responds to *A. cantonensis* in two ways (Yousif et al., 1979). Some focal encapsulations develop within 24 h PI but more slowly in denser tissues. The other response is a generalized proliferation of hemocytes (leukocytosis)

originating mostly in the posterior wall of the lung. These cells differ cytologically from hemocytes of uninfected snails and become involved in encapsulation by 40 days PI. Thirteen snail species studied by Trushin (1978) mounted similar defensive responses to protostrongylid larvae in the foot musculature; amebocytes, "histiocytes," and "fibroblasts" developed a connective tissue capsule around the nematodes. In some xerophilic snails, encapsulated larvae were killed.

Electron micrographs (Harris, 1975) and enzyme histochemistry (Harris and Cheng, 1975b) show that the main cell type in the hemocyte capsule is the granular amebocyte. During early days PI, pseudopodial processes "are directed roughly perpendicular to the nematode's cuticle," and the cell organelles "are for the most part restricted to the endoplasm near the nucleus . . . seldom within the pseudopodial extensions." This perinuclear location of organelles is also seen in hemocytes encapsulating trematodes (Loker et al., 1982). After the first week, the cytoplasmic extensions convert increasingly to concentric layers which give the appearance under the light microscope of a fibrous nodule, but no extracellular elements contribute to the capsule. The parasite, undamaged by the capsule despite the presence of acid and alkaline phosphatase, nonspecific esterase, and β-glucuronidase enzymes in the cells (Harris and Cheng, 1975b), grows and molts, remaining infective to rats which may ingest them.

The resistance of *A. cantonensis* to host lysosomes may be due to resistance of the cuticle to host enzymes (Cheng, 1974), but it is also possible that the hydrolases present in the encapsulating cells are not released and therefore remain "functionally separated from the nematode's surface" (Harris and Cheng, 1975b). This suggestion is supported by Harris's (1974) failure to find increased levels of enzyme activity in hemolymph from infected snails.

Antinematode inhibitors may play a role in snail resistance to nematodes (Ratanarat-Brockelman, 1975). Rhabditid nematodes parasitizing the lungs of pulmonate gastropods fail to mature until the snail or slug has died. *Rhabditis maupasi* can be grown axenically but can be inhibited from maturing if extracts of *Helix aspersa* are added to the medium (Fig. 9). Most inhibitory activity resides in hemocyanin-depleted plasma, in which the mean daily growth rate was 6% of that in controls. The effect is not species specific; *Neoaplectana glaseri*, an insect parasite, is also inhibited. The activity appears to require the simultaneous presence of a proteinaceous component and a smaller cofactor. The mechanism of action remains for investigation.

2. Gastropod–Trematode Interactions

> The major physiological mechanism determining specificity appears to involve the recognition capability of host cells vis-à-vis the surface of the penetrated parasite [Basch, 1975: p. 451.]"

a. Innate Responses. The mollusc-infective stages of trematodes are the miracidia. They are generally rather nonspecific in host location and attempt to

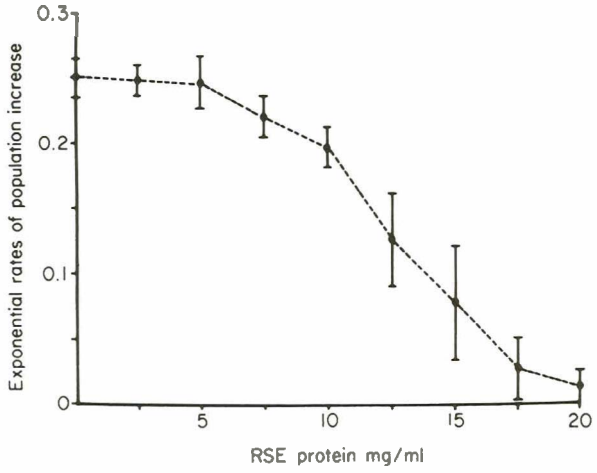


Fig. 9. Exponential rate of *Rhabditis maupasi* population increase to d 20 in nine groups of liquid cultures receiving 5 mg/ml rabbit liver extract but varying concentrations of raw extract of *Helix aspersa* (RSE). Vertical lines represent standard deviations. (From Ratanarat-Brockelman, 1975, with permission.)

penetrate quite nonselectively (Sudds, 1960, and others; cf. Frandsen, 1979). Snail skin may present an effective barrier to penetration by certain miracidia (Kinoti, 1971; Schutte, 1975) due to unsuitability of the parasite's penetration gland enzymes (Cheng, 1965) or to other factors. In general, miracidia may penetrate a wider variety of snails than that in which subsequent development will occur. Such development requires that the snail be a suitable host capable of supplying the parasite with its life needs, and that it be susceptible because it lacks resistance (Lie et al., 1977a). Resistance of a snail to a particular parasite is specific. By using radioactively labeled miracidia of one *Schistosoma mansoni* strain and unlabeled miracidia of another, encapsulation responses in doubly infected *Biomphalaria glabrata* were consistently against the incompatible strain even when miracidia entered simultaneously and the resulting sporocysts were in close proximity to one another in the snail (Kassim and Richards, 1979). Furthermore, loss of genetically based resistance to one strain of *S. mansoni* occurred with no change in resistance to four other trematodes (Lie et al., 1977a).

Compatibility and incompatibility, two extremes of a spectrum, become evident after penetration, during, and immediately after metamorphosis of the miracidium to the next (mother sporocyst) stage. This change takes minutes to hours, and if a sporocyst avoids encapsulation by host hemocytes for a day or so, it is likely to survive and asexually to produce daughter sporocysts which escape several days later. Having traveled in the circulation to other tissue locations, predominantly the digestive gland, the daughter sporocysts produce cercariae, again asexually. To propagate the life-cycle, the cercariae, which are produced

in large numbers over a period of several weeks, must escape from the snail. Although most of the research done and reviewed here has used the *B. glabrata–S. mansoni* system, there is no suggestion that their relationship is unusual. "Non-schistosomes such as *Fasciola,* with their lymnaeid hosts, also reveal a great variety of compatibility combinations (Boray, 1966; Kendall, 1970) and it is likely the same is true for all trematodes and their molluscan hosts" (Basch, 1976).

Encapsulation of trematodes differs in the severity, rate, and nature of the structural elements involved. Snail responses are seen as being any of the following:

1. Encapsulation with destruction (Fig. 10c)
2. Benign association of hemocytes with parasite surfaces
3. No cellular response (Fig. 10a)

Encapsulation with destruction occurs when miracidia penetrate a resistant snail (Cheng and Garrabrant, 1977; Kassim and Richards, 1979; Kinoti, 1971; Lie et al., 1980a; Loker et al., 1982; P. T. LoVerde, personal communication Newton, 1953; Schutte, 1975). This response has been well characterized histologically, ultrastructurally, and to some extent histochemically (Cheng and Garrabrant, 1977), and has been studied *in vitro* (Basch, 1979; Bayne et al., 1980a, 1980b; Benex and Jacobelli, 1980).

In brief, the rate and severity of the response vary with the degree of resistance; faster and more severe encapsulation is indicative of strong resistance. In highly resistant snails[3] as early as 3 h postexposure (PE), hemocytes contact the parasite surface. By 7.5 h, phagocytosis of microvilli and the underlying tegument begins. Within 24 h, the tegument is entirely destroyed, and host hemocytes lie in close apposition to the basement membrane which previously underlay the tegument; cells within the sporocyst show extensive pathological changes. Hemocytes begin to move beneath the basement membrane and to phagocytose the subtegumental components, which are digested intracellularly. By 48 h PE, capsular hemocytes are engorged with phagolysosomes, and only scattered remnants of the sporocyst remain. By 4 days, hemocytes have largely dissipated.

Although both granular and hyaline hemocytes occur in *B. glabrata* and other planorbids[4] (Cheng and Auld, 1977; Harris, 1975; Jeong and Heyneman, 1976; LoVerde et al., 1979), hyalinocytes are normally absent from capsules. Rarely, one sees cells with very electron-dense cytoplasm (Fig. 11, DC). Capsular gran-

[3]This particular account is based on a study (Loker et al., 1982) of the PR-1 strain *Schistosoma mansoni* in the 10-R2 (a highly resistant) strain of *B. glabrata.*

[4]Sminia and Barendsen (1980) hold that planorbids and lymnaeids all possess one hemocyte type, the amebocyte, and that different morphologies represent different stages in the life of the cell.

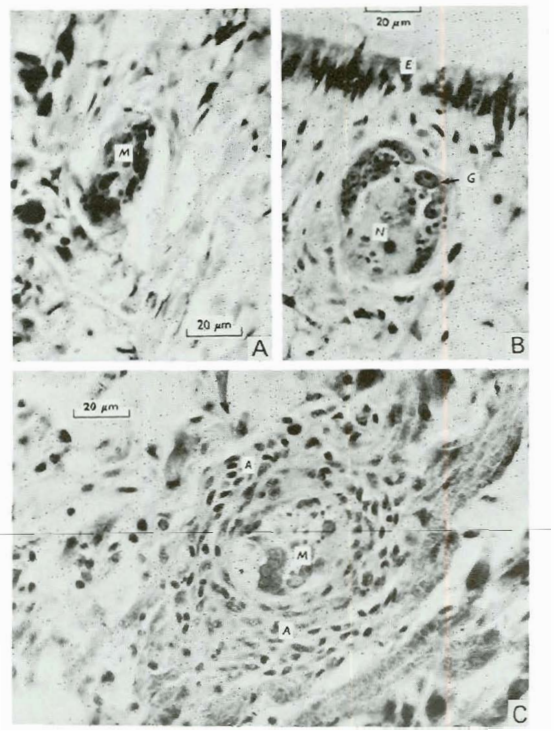


Fig. 10. (**A**) Degenerating miracidium of *Schistosoma mattheei* in dense tissue of the head-foot of *Bulinus africanus* approximately 17 h after penetration. (**B**) Normal sporocyst of *S. mattheei* in loose tissue of the head-foot of *B. africanus* approximately 17 h after penetration. (**C**) Amebocytic infiltration of *S. mattheei* miracidium in the head-foot of *B. truncatus* approximately 7 h after penetration. A, amebocyte; E, epithelium of head-foot; G, germinal cell of parasite; M, miracidium; N, neural mass of parasite. (From Kinoti, 1971, with permission.)

ulocytes are initially roughly spherical, but most flatten as the capsule grows to up to 15 cell layers and matures. Neighboring hemocytes interdigitate extensively; tight junctions and desmosomes are absent, and later the hemocytes resume their spherical shape as they dissipate. In hemocytes contacting the parasite, organelles are aggregated in the perinuclear region, leaving only a granular cytoplasm in the cell region closest to the sporocyst; only small primary phagosomes occur here. Extracellular fibrils, possibly collagen (cf. Sminia, 1974), may be transiently present in the outermost layers of the capsule (Krupa et al., 1977; Loker et al., 1982; Yoshino, 1976). Hemocytes which have become flattened or appear elongated have been termed *fibroblasts* (Pan, 1965; Schutte, 1975).

Although phagocytosis of the tegument may lead directly to sporocyst death, other possible mechanisms have not been ruled out. Extensive rough endo-

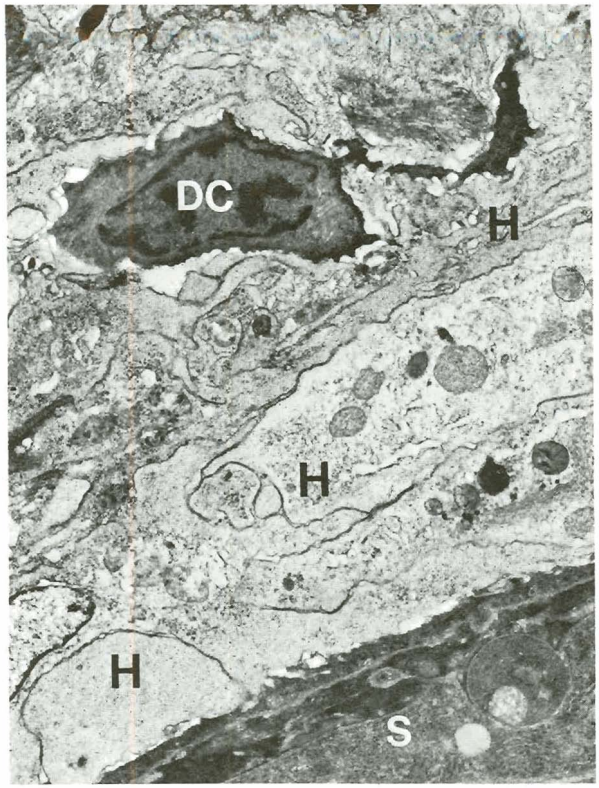


Fig. 11. Hemocytes of the *Biomphalaria glabrata* 10-R2 strain cocultivated 24 h with a mother sporocyst of the *Schistosoma mansoni* PR-1 strain. Most of the encapsulating hemocytes are ameboid granulocytes; a single dark cell is labeled DC. H, hemocytes; S, sporocyst. (Courtesy of E. S. Loker.)

plasmic reticulum, Golgi bodies, and nucleoli in capsular hemocytes suggest that they are synthesizing protein (Loker et al., 1982). Elevated acid phosphatase activity in these cells (Cheng and Garrabrant, 1977) may be associated with degradation of phagocytosed parasite material rather than offensive capacity, because evidence for release of packaged material from the granulocytes is lacking. *Biomphalaria* granulocytes have been found to contain acid and alkaline phosphatase, nonspecific esterase and β-glucuronidase (Harris and Cheng, 1975b), and peroxidase (Carter and Bogitsch, 1975).

Even in compatible infections, extensive cellular responses may be seen, such as when supernumerary miracidia (e.g., >10) simultaneously penetrate a snail (cf. Frandsen, 1979), or when daughter sporocysts (Kole, 1979) or cercariae (Pan, 1965; Schutte, 1975) err in their migrations and end up in ectopic sites. The resulting capsules are similar to those which form around mother sporocysts

in resistant snails (Loker, 1979). Cercariae, during their efforts to emigrate from the snail, must move from sporocysts in the digestive gland to the body surfaces of the head–foot or mantle. Hundreds may attempt this emigration daily, and significant numbers fail, ending up in unsuitable parts of the host. The apparent rapidity (hours?) of the encapsulation and elimination (Loker, 1979; Schutte, 1975) may result partly from the daily "resensitization" of the hosts by the numerous cercariae over long periods (Loker, 1979). In *B. salinarum,* "the presence of typical granulomata usually coincided with low cercarial shedding and a reduction in the density of infection [Schutte, 1975]," but as pointed out by the author, "whether the occurrence of granulomata suppressed parasite development or whether the lower parasite burden allowed the granulomata to develop, is not known." It is noteworthy that such tissue reactions appeared several weeks after patency (beginning of cercarial shedding). It is also noteworthy that cercariae have snail antigens on their surfaces (Jackson, 1976; Roder et al., 1977), but the possibility that these antigens serve a protective function against snail immunity remains unexplored and unlikely in view of the brief (minutes) and active nature of cercarial emigration.

A benign association of hemocytes with parasite surfaces occurs in compatible infections (Kinoti, 1971; Kole, 1979; Krupa et al., 1977; Loker, 1979; LoVerde, 1979; Meuleman, 1972; Meuleman et al., 1978; Pan, 1965; Popiel and James, 1979; Rondelaud and Barthe, 1980; Schutte, 1975; Yoshino, 1976). Such responses involve fewer hemocytes and occur more slowly than those in resistant snails, and do not lead to destructive encapsulation of healthy parasites. In fact, it appears possible that the sparse hemocytes in most such responses subserve the interests of the parasites, either providing nutrients, removing waste, or cleaning up locally damaged host tissues. In the prosobranch *Cerithidea californica,* for example, sporocysts of *Renicola buchanani* become coated with loosely aggregated hyalinocytes whose pseudopods soon interdigitate with the tegumental microvilli of the parasite (Yoshino, 1976). Later, the four- to eight-cell layer "capsule" is more dense, and the flattened hyalinocytes are closely juxtaposed to the parasite. In this compatible system, the structural evidence suggests that after an initial two-way, low-level aggression at the cellular level, a stable, cooperative relationship develops; the sporocyst, continuing development within the envelope of host cells, may have enslaved a subpopulation of host hemocytes.

A small number of normal host granulocytes is seen at the surfaces of developing *Schistosomatium douthitti* in *Lymnaea catascopium* (Loker, 1979) and *S. haemotobium* in *Bulinus guernei* (Krupa et al., 1977). Hemocyte glycocalices may be shifted from their normal location all over the cell to the side away from the parasite (Krupa and Lewis, 1977); the significance of this topographic heterogeneity remains to be discovered.

After the next larval generation has developed from a sporocyst, there remains

an empty sac composed of the parental body wall. Notable encapsulation may occur now (Frandsen, 1979; Lim, 1970; Loker, 1979); yet, at least in the *S. douthitti–L. catascopium* system, "many large collapsed and depleted mother sporocysts . . . persist for long periods without provoking effective host responses [Loker, 1979]." The most interesting immunological questions, concerning the failure of host cells to respond offensively, foreshadow those raised below and discussed later.

Some *B. glabrata* undergo "self-cure," in which an apparently established trematode infection is eliminated. In such snails, some sporocysts of *S. mansoni* may be lightly encapsulated by hemocytes which include hyalinocyte-like cells (Lie et al., 1980a), which are normally absent from typical resistance encapsulations. Other sporocysts degenerate in the absence of hemocytes, implying a possible role of humoral factors (Fig. 12). These results have also been taken to imply that living sporocysts may interfere with the normal functioning of the granulocytes (Lie et al., 1980a).

No cellular response occurs when (1) healthy miracidia penetrate the skin of compatible hosts, even if they die due to an unsuitable microenvironment (dense connective tissue/muscle) or to penetration by an excessive number of miracidia (Kinoti, 1971); in such cases, they rapidly disintegrate and disappear; (2) when

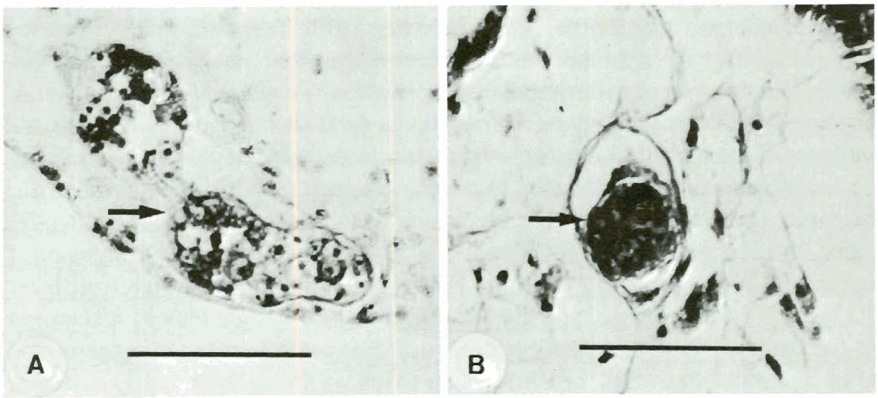


Fig. 12. (**A**) Degenerated, probably dead, small secondary sporocyst (arrow) in the space between the digestive gland and the epithelial tunica. Note the almost complete absence of amebocytes attached to the parasite. *Biomphalaria glabrata* 10-R2 snail exposed to 10 irradiated *Echinostoma liei* miracidia, reexposed 3 d later to 30 nonirradiated *S. mansoni* PR-1 miracidia and killed 51 d PE. (**B**) Cross section of degenerated small secondary sporocysts (arrow) in the hemolymph space of interfollicular connective tissue of the digestive gland. Note the amebocyte attached to the parasite and thickening of connective tissue supporting the hemolymph space occupied by the parasite. *Biomphalaria glabrata* 10-R2 snail exposed to 10 irradiated *E. paraensei* miracidia, reexposed 3 days later to 30 nonirradiated *S. mansoni* Lc-1 miracidia, and killed 49 days PE. Scale = 50 μm. (From Lie et al., 1980a, with permission. Copyright Liverpool School of Tropical Medicine.)

depleted sporocysts persist without provoking a response (Loker, 1979; Schutte, 1975); (3) in certain cases of self-cure (Lie and Heyneman, 1978a; Lie et al., 1980a); and (4) in host snails which have their innate resistance suppressed due to the presence of other trematodes (Cort et al., 1941; Fritsche and Gilbertson, 1981; Lie and Heyneman, 1977; Lie et al., 1976, 1977a, 1977b). Finally, some snails (*Bulimnea megasoma* and *Fossaria abrussa*) apparently fail to mount a tissue reaction to *Trichobilharzia elvae* which penetrate and die (Sudds, 1960).

b. Alterations of Natural Immune Status. Certain physid and planorbid snails, normally resistant to *Cotylurus flabelliformis* metacercariae, become susceptible if they already harbor infections of certain other trematodes (Cort et al., 1941; Fritsche and Gilbertson, 1981). The *C. flabelliformis* enter sporocysts and rediae of earlier residents and may be thereby "protected from any immune reactions of the abnormal host," the normal host being a lymnaeid snail.

As just mentioned, snail resistance to trematodes may be suppressed by other trematodes; for example, the survival of *Austrobihalrzia terrigalensis* in an Australian prosobranch only when the latter harbors other trematode infections may be due to suppression[5] of host defenses by other parasites (Walker, 1979). Most of the evidence for these sorts of interactions stems from extensive research by Lie and his colleagues (Lie and Heyneman, 1976b; Lie et al., 1976, 1977a, 1977b), and the situation is most clearly summarized in their own words (Lie et al., 1980a: p. 157, quoted here with permission; copyright Liverpool School of Tropical Medicine).

> Trematode larvae of *Echinostoma paraensei, E. liei, E. lindoense, Paryphostomum segregatum* and *Schistosoma mansoni* are each able to interfere with the natural resistance to trematode infection in the snail *Biomphalaria glabrata* (Lie et al., 1976; 1977a,b). Many snails with a strong natural resistance to *S. mansoni*, for example, become susceptible to the schistosome when infected first with *E. paraensei* sporocysts, whether derived from normal or irradiated miracidia. Interference with the natural resistance of the snail by irradiated echinostome sporocysts is temporary (Lie et al., 1977b), lasting only as long as the sporocysts live. After the irradiated sporocysts have died in the snails, the hosts usually regain their natural resistance to *S. mansoni*. In many instances, however, *S. mansoni* sporocysts that developed under the protection of the irradiated echinostome sporocysts survive after the death of the echinostomes. Our evidence suggests that as they grow older, *S. mansoni* sporocysts can develop their own ability to interfere with the defence mechanism of the snail (Lie et al., 1977b). In about 30% of these doubly-infected snails, however, regression of *S. mansoni* sporocysts occurs after (or sometimes before) disappearance of the protecting irradiated echinostome sporocysts. It also may take place before or after the formation of secondary *S. mansoni* sporocysts, or even after the production of cercariae. All these circumstances result in self-cure of the schistosome infection. In snails with self-cure the interference capacity developed by growing *S. mansoni* sporocysts presumably cannot overcome the snail's natural resistance regained after death of the protecting echinostome sporocysts.

[5]Lie et al. (1976) prefer to refer to this phenomenon as *interference* and to call the infected snails *compromised*.

Of several *Echinostoma* spp., *E. paraensei* is the strongest suppressor (Lie and Heyneman, 1979b); its effects vary with the strain of *B. glabrata* used and are dose dependent.

The opposite type of alteration also occurs. *Oncomelania hupensis formosana,* exposed first to incompatible and later to compatible strains of *S. japonica,* became resistant to the normally compatible strain (Lin et al., 1974). *B. glabrata* exposed to irradiated miracidia of *Ribeiroia marini* develop a heightened resistance to a homologous challenge (Sullivan et al., 1982). Furthermore, in *B. glabrata,* a relatively specific resistance to *Echinostoma lindoensi* can be induced by prior exposure of the snails to X-irradiated miracidia of the same species (Lie et al., 1975b; Lie and Heyneman, 1979a). Such "immunized" snails remain susceptible to *S. mansoni* and *Paryphostomum* sp. and become partially resistant to two other closely related *Echinostoma* spp., indicating partial specificity. In snails exposed twice to irradiated miracidia before the challenge dose of normal miracidia, resistance is further enhanced (Lie and Heyneman, 1976a); challenge miracidia are more rapidly encapsulated and destroyed, or may even be expelled through host epithelia after encapsulation. It appears that the hemocytes acquire a state of heightened activity reminiscent of that of activated macrophages. But there is a humoral component to the response: An agglutinin in the hemolymph increases in titer in infected snails and in snails with enhanced resistance (Jeong et al., 1981). To elicit this enhanced resistance, at least two requirements must be met: The snails must have a sufficient degree of preexisting resistance to ensure rapid destruction of the sensitizing parasites, and all sensitizing sporocysts must be completely destroyed (Lie and Heyneman, 1978b). Evidence for and against anamnesis in this response is discussed elsewhere (Bayne et al., 1980c).

It has now been found that *Echinostoma paraensei* elicits a higher degree of resistance to *E. lindoense* than to itself (Lie et al., 1982). This may imply that a "fixed specificity" (most effective against *E. lindoense,* and capable of enhancement) is stimulated quite nonspecifically, by either species. Responses with such properties—relatively nonspecific elicitation of specific effectors—is seen in other invertebrate systems, discussed by Lie et al. (1982).

In other host–parasite systems, similar protocols have either failed to induce resistance (cf. Loker, 1978a, 1978b) or imply that "resistance" due to "activated amebocytes" can be very nonspecifically induced (Kluhspies, 1979). Snails' ability to express *enhanced* resistance is partially destroyed by X irradiation immediately prior to a sensitizing infection with irradiated miracidia of *R. marini* (Sullivan and Richards, 1982). Efforts to destroy the *innate* resistance of *B. glabrata* to trematodes by gamma irradiation have shown that irradiation damage is not immediate. Michelson and DuBois (1981), whose paper implies that exposure to *S. mansoni* miracidia directly followed irradiation (to 5 krad),

found no reduction in resistance. Earlier, Faulk et al. (1973) reported that resistance of *B. glabrata* to *E. lindoensi* was reduced in a dose-dependent manner by up to 25 krad of gamma irradiation, but only if snails were held for more than 2 days between irradiation and exposure to miracidia, confirming an earlier report (Lim and Heyneman, 1972). Furthermore, a reduced encapsulation response to injured *S. mansoni* sporocysts was observed microscopically when snails were exposed to 15 krad in comparison with 5 krad (Lim, 1970). Because radiated snails regained their resistance if allowed to rest for 1 mo between irradiation and exposure to infection, a radiosensitive cell population was postulated to be important in snail resistance, and to be able to regenerate after sublethal radiation.

The surprising alteration of a susceptible to a resistant strain of *B. glabrata* by radiation (Michelson and DuBois, 1981) is probably not an immunological phenomenon. The dose (5 krad) is slightly over the LD_{50} reported in the same paper; it damaged surface epithelia and pathologically changed hemolymph composition. Probably the environment was unsuitable for sporocysts, which died and thus elicited encapsulation.

c. Mechanisms Operating in Recognition, Evasion of Recognition, Killing, Interference, and Enhanced Resistance. When encapsulation occurs in a resistant host, one can state that "recognition" has occurred and at least some elements of the granulocyte defensive repertoire have been triggered. Despite recent efforts, we still need to learn more in order to understand the mechanisms of recognition, evasion, killing, interference and enhanced resistance (Bayne, 1981b).

(i) RECOGNITION. Although immunoglobulin production is restricted to vertebrate lymphocytes, the non-self recognition capabilities of many invertebrates are finely tuned; a distinction is evident between closely related molecular species (see Section II). Thus, when endoparasites elicit no evident response in molluscan or other hosts, this may involve avoidance of recognition (Kinoti, 1971; Basch, 1975). Immune recognition, which is evident only when a defensive cell alters its steady state, must actually occur when a message is received at the cell surface. Such a putative message may be humoral or may be on a non-self or damaged-self surface. The questions raised here in relation to mechanisms of non-self recognition are among the most important in cellular and molecular biology. In gastropods, some recognition can occur in the absence of humoral factors, because some non-self particles can be phagocytosed by some species in plasma-free conditions (see Section IV,I). Hemocytes of *B. glabrata* may recognize *S. mansoni* antigens bound to sheep RBCs in plasma-free media (Golvan and Mougeot, 1973), and hemocytes from resistant snails can encapsulate and

then kill trematodes in culture media lacking snail-derived components (Bayne et al., 1980a). However, humoral factors are very important in other recognition events.

Agglutination of particles (e.g., RBCs) with appropriate sugars displayed on their surfaces occurs in the presence of lectins with two or more appropriate sugar-binding sites. Many molluscs contain such agglutinins in their body fluids (e.g., Gilbertson and Etges, 1967; Khalap et al., 1970; Michelson and DuBois, 1977; Stein and Basch, 1979; Tyler, 1946), and molecular characterization of some has been attempted (e.g., Hammarstrom and Kabat, 1969; Pauley, 1974). Although agglutination might be a fortuitous property of plasma molecules whose primary function is different, Prokop et al. (1968) coined the term *protectin* for these compounds. An agglutinin in the plasma of *B. glabrata* (Stanislawski et al., 1976) has binding sites for human blood group determinants which are present on *S. mansoni* miracidia and sporocysts (Stein and Basch, 1979; Yoshino et al., 1977). In a preliminary, unconfirmed report, Faulk et al. (1973) found that a trematode-binding component of *B. glabrata* plasma increased in response to infection. Furthermore, *B. glabrata* which are rejecting larval echinostomes have elevated titers of plasma agglutinin (Jeong et al., 1981). If such an agglutinin is cytophilic for *B. glabrata* hemocytes, it could explain the unconfirmed report (Golvan and Mougeot, 1973) that hemocytes of infected *B. glabrata* contain more binding activity for *S. mansoni* antigens. However, the putative mechanism by which such agglutinins mediate recognition is obscure (cf. Chorney and Cheng, 1980; Lackie, 1981); erythrocytes with sugar determinants which are recognized by the agglutinins of *B. glabrata* and *Helix* are not bound by these native agglutinins to the hemocytes of the respective snail (Stein and Basch, 1979). Nor do *B. glabrata* hemocytes attach to agglutinin-coated *S. mansoni in vitro* (Stein and Basch, 1979). These failures (like the failure of oyster agglutinin to enhance phagocytosis *in vitro;* Hardy et al., 1979b) may be due to peculiarities of the experimental situation, such as the presence of excess agglutinin-binding subunits which bind, and thus remove from reactivity, the agglutinin sites on the hemocytes. Just as susceptibility to trematodes is variable, so is determinant specificity of snail agglutinins. Thus, it remains an open question whether or not such lectin specificity determines susceptibility or resistance to particular trematode genotypes (Heyneman et al., 1971). An *in vivo* opsonic role for plasma agglutinins has now been clearly demonstrated in the reticuloendothelial functions of *Helix* (Renwrantz and Mohr, 1978); these and other relevant data are discussed in Section IV,I.

(ii) EVASION. Adult schistosomes in mammalian or avian blood vessels avoid immunologic attack by a variety of means, including the acquisition of host antigens which may function in disguise (cf. Damian, 1979). Larval schistosome surfaces do acquire agglutinin from their snail hosts (Stein and Basch,

1979) but apparently do not avoid recognition by the acquisition of factors from host hemolymph. If compatibility is due to the acquisition of host antigens by sporocytes in order to disguise themselves as snail "self," this would occur in susceptible plasma and might be expected to protect sporocysts from attack by resistant hemocytes. In an *in vitro* model of encapsulation, sporocyst fates are not altered by their preincubation in plasma from susceptible *B. glabrata* (Loker and Bayne, 1982). Furthermore, miracidia (Yoshino and Cheng, 1978) and sporocysts transformed from miracidia *in vitro* in the absence of snail factors (Basch and DiConza, unpublished abstract; Yoshino and Bayne, 1983) specifically bind antibodies produced in rabbits injected with snail plasma antigens and in rabbits injected with washed hemocytes (Yoshino, 1981b), implying that the schistosome genome codes for the synthesis of antigens which resemble those of the host snail. Although this implies that mimicry is crucial for evasion, it should be remembered that the interference phenomenon described earlier implies additional evasive or suppressive roles for secretory factors of the parasite. Quite possibly, snail immune responses are avoided by a combination of mimicry, acquired antigens, and interference.

(iii) KILLING. Although nematodes, trematodes, and cestodes all parasitize snails, trematodes are the only metazoans about which anything is known with respect to possible killing mechanisms. Plasma, even from resistant snails, never kills such parasites (Bayne et al., 1980a), even though it may contain parasite-binding lectins which may activate mammalian complement (Stein and Basch, 1979). Lysosomal enzymes (lysozyme, acid and alkaline phosphatase, β-glucuronidase, amylase, aminopeptidase, and lipase) occur in *B. glabrata* hemolymph (Michelson and DuBois, 1973; Rodrick and Cheng, 1974; Yoshino and Cheng, 1977), and activities may increase in response to trematode (Cheng et al., 1978a; Kassim and Richards, 1978a) and bacterial infections (Cheng and Butler, 1979; Cheng et al., 1977; Cheng et al., 1978b). However, despite damage to *Bacillus megaterium* by plasma from injected *B. glabrata* (Cheng, 1978), we lack evidence for defensive roles of plasma enzymes in metazoan infections (cf. the review by Bayne, 1981b). Degradative enzymes may reach damaging levels at the host–parasite interface, but this remains to be determined. Lysozyme levels in the peripheral circulation of resistant *B. glabrata* increased acutely after exposure to *S. mansoni* miracidia (Kassim and Richards, 1978a), but declined after only 3 h, whereas destruction of the sporocysts took 3–5 days. A similar but less dramatic elevation of lysozyme occurred in susceptible snails exposed to *S. mansoni;* therefore, plasma lysozyme per se may be unimportant in parasite destruction.

Evidence to date has failed to exclude possible killing roles for other cytotoxic mechanisms (cf. the review by Sanderson, 1981).

So-called miracidial immobilizing substances (MIS) from snails are of some

interest (Benex and Lamy, 1959; Hosaka and Berry, 1975; Lie et al., 1980b; Michelson, 1963, 1964). These substances slow or stop, over a period of up to 2 h, the miracidial cilia, and in some cases induce shedding of ciliated plates, a process which occurs when miracidia penetrate a snail. Titers in *B. glabrata* hemolymph reach up to 1 : 16, and are highest roughly 2–14 days PE (Lie et al., 1980b). Trematode species vary widely in the vigor with which they elicit MIS activity and in their reactivity to it (Hosaka and Berry, 1975; Lie et al., 1980b). Activity is due to several factors, occurs in various tissue extracts from even uninfected snails, and may be induced by a variety of abuses (Michelson, 1963), including exposure to miracidia (Lie et al., 1980b; Michelson, 1964). There is no evidence that MIS is a component of the snail's defensive armament. It is possible that responses to hemolymph MIS are normal metamorphic changes (miracidium–sporocyst). Also, pathological responses reported for miracidia in some tissue extracts may be due to released degradative enzymes and other nonimmunological factors, to parasite-induced toxins, and possibly to agglutinins (Michelson, 1963).

(iv) INTERFERENCE AND ENHANCEMENT OF RESISTANCE. No experimental system has been available to explore the possible mechanisms of these phenomena. *In vitro* systems are now available (Bayne et al., 1980a, 1980b; Loker and Bayne, 1982).

(v) THE FATES OF SUPERNUMERARY MIRACIDIA. Simultaneous exposure of a potential host snail to an excessive number of miracidia results in the success of only a proportion of those that penetrate. In one *B. glabrata–S. mansoni* host–parasite system, no more than eight sporocysts developed regardless of whether the snails were exposed to 10 or 25 miracidia, each of which (by individual testing) was capable of infecting the snail (Kassim and Richards, 1979b). This "saturation effect" is due to a transient refractoriness in the snail. By 2–4 days after primary infection, about 50% of a second miracidial invasion may survive, and by 4–8 days all successfully penetrating miracidia of a second invasion may survive. It is not clear how this refractoriness is mediated, or whether it is due to a host response or, in contrast, to factors released by the earliest arriving miracidia.

(vi) QUALITATIVE AND QUANTITATIVE RESPONSES OF HEMOCYTES TO HOST INFECTION. The hematopoietic tissues of gastropods vary as to location: In planorbids and lymnaeids, they are located where the kidney and the pericardial wall join in the posterior wall of the mantle cavity (Kinoti, 1971; Lie et al., 1975a; Pan, 1963; Rondelaud and Barthe, 1981). A low level of hematopoiesis may also occur in the peripheral circulation (Sminia, 1974).

Trematode and nematode infections, like some other insults, may cause in-

creased mitotic activity in these amebocyte-producing organs, as well as leukocytosis. Hemocyte counts are significantly elevated 3–5 days after *B. glabrata* are exposed to *S. mansoni* (Stumpf and Gilbertson, 1980) and to *E. lindoensi* (Jeong et al., 1980), and counts remain high until after larval destruction. In snails sensitized as a result of exposure to irradiated miracidia (Jeong et al., 1980), both leukocytosis and destruction of the sporocysts are more rapid after a second exposure to miracidia. In *Viviparus japonicus*, a prosobranch, only eosinophilic hemocytes increased in number during leukocytosis (Michelson, 1970); in *B. glabrata* at 20 h PE to *S. mansoni*, only the granulocytes increased, from 254 ± 131 to 650 ± 489 mm^{-3} (Stumpf and Gilbertson, 1980). Eosinophilic hemocytes also increased in *Marisa cornuarietis* 25 days PE with *Angiostronglyus cantonensis* (Yousif et al., 1979). In contrast, hemocyte numbers decline drastically in *B. glabrata* as a result of infection by *A. costaricensis*, to 20% of normal by 20 days PE (Stewart et al., 1981).

(vii) THE ROLE OF HYPERPARASITES IN SNAIL SUSCEPTIBILITY TO TREMATODES. Both amebae (Stibbs et al., 1979) and microspordians (Cort et al., 1960) may destroy trematode sporocysts. The killing of *S. mansoni* by amebae derived from *B. glabrata* tissues has been well documented *in vitro* (Owczarzak et al., 1979), but there is no evidence that these symbiotic protozoans are involved in snail resistance. On the contrary, microsporidians hyperparasitize trematodes *in vivo*, and have been suggested for biological control of the metazoan parasite (Canning and Basch, 1968).

C. Responses to Protozoans

Although members of all four major protozoan taxa enter into symbiotic association with gastropods (reviewed by (Malek and Cheng, 1974; Michelson, 1957), interactions with host immune systems have scarcely been studied. Sarcodines (*Hartmanella* spp.) occur intracellularly in the amebocytes of *Biomphalaria glabrata* and *Bulinus globosus*, and several other species are susceptible to infection (Richards, 1968). Affected amebocytes become surrounded by other fibroblastic cells, so that nodules are formed in several tissues.

Microsporidians (*Coccospora*) infect intestinal epithelia and adjoining tissues of *B. glabrata*. A possible host defensive response occurs when infected mantle epithelia sluff off mucus and infected cells into the extrapallial space (Richards and Sheffield, 1971). Abalones (*Haliotis ruber*) react to the apicomplexan *Perkinsus* by forming pustules composed of connective tissue fibers and leukocytes (Lester and Davis, 1981). The temperature dependence of the outcome of interactions between a potential pathogen and a molluscan immune response is illustrated nicely in this system: Pustules in abalone kept at 15°C contained predominantly dead parasites, whereas in those kept at 20°C, most were alive.

Ciliates *Tetrahymena limacis* and *T. rostrata* infect several pulmonate slugs and their eggs (Brooks, 1968). The renal cells of *Deroceras* (*Agriolimax*) *reticulatum* can be ruptured by *T. rostrata*, eliciting inflammation and a hypertrophic reaction from kidney amebocytes and cells in renal veins. The amebocytes flatten. Leukocytosis, proliferation of amebocytes in the epicardial tissues, and encapsulation of ciliates all represent typical defensive responses of the slug. The "granulomata" contain both rounded and flattened host cells, the innermost of which have phagocytosed debris. Tumor-like aggregations of hypertrophic amebocytes in the pericardial cavity may be products of pathologically hyperactive hematopoiesis by the epicardium. It is not clear whether the host cellular response is primarily to the ciliates themselves or to the tissue damage resulting from their activities. Host cells aggregate and encapsulate *T. rostrata* at their points of entry within 24 h of exposure of the slugs to the ciliates. "Necrotic, darkened masses of cellular debris" in the mantle cavity contain moribund amebocytes and ciliates and may be due to expulsion into the mantle cavity from ruptured pulmonary veins (Brooks, 1968).

D. Responses to Bacteria

1. Potential Pathogens

Due in part to the notable molluscan capacity to clear particulates from their hemolymph (Bayne and Kime, 1970; Cuénot, 1914; Reade and Reade, 1972; Renwrantz et al., 1981; Tripp, 1961a; van der Knaap et al., 1981b), no bacterial pathogens are yet available for biological control of molluscs despite repeated efforts to isolate such agents (Bayne, 1977; Cole et al., 1977; Dean et al., 1970; Dias, 1955; Ducklow et al., 1979; Michelson, 1957, 1961; Pan, 1956). Claims that *Bacillus pinottii* was pathogenic for *B. glabrata* (Diaz, 1955; Filho and Diaz, 1953) were later thrown into doubt (Tripp, 1961b). A gram-negative bacterium reported to cause disease in *Biomphalaria* spp. and *Physopsis* sp. was never identified (Berry, 1949). However, a *Mycobacterium* sp. (Michelson, 1961; Pan, 1956; Tripp, 1961a), a spirochete (Cole et al., 1977), and *Aeromonas liquefasciens* (Dean et al., 1970) have been associated with pathology in planorbid snails and *Achatina fulica*. The first two bacteria become localized in tubercles (Michelson, 1961) or nodules (Cole et al., 1977), which are aggregates of amebocytes, the outermost of which are flattened. Infected snails may survive for months. In the disease associated with *A. liquefasciens* (*hydrophila?*) (Dean et al., 1970), "leukodermic lesions" develop on the *Achatina* body surface. Death of infected snails appears to result only when infected specimens are subjected to additional stress, in conformity with higher internal bacterial densities found in *B. glabrata* subjected to stress (Ducklow et al., 1979).

2. Clearance of Bacteria from the Circulation

Bacterial clearance in a snail was noted first by Kowalevsky (1894), who discovered that bacteria injected in *Helix pomatia* collected in phagocytes located in the connective tissues of the kidney and foot, and above all in cells around the pulmonary vessels. In *B. glabrata* injected with *B. pinottii,* bacteria could not be found in tissue sections 1 h PI, nor could they be cultured from snail tissues after 48 h (Tripp, 1961b). A nonpathogenic bacterium isolated from *H. pomatia* is cleared rapidly (90% in 2 h) if grown *in vitro,* washed, and injected (Bayne and Kime, 1970). The same result or better occurs when *Serratia marscescens* (Bayne, 1973a, 1974, 1977) and a variety of other bacteria are injected into *Helix* (Bayne, 1977), when *Gaffkya homari, Micrococcus aquivivus,* a *Pseudomonas* sp., and a gram-negative rod are injected into *Aplysia californica* (Pauley et al., 1971b), and when *Staphylococcus saprohyticus* and *Escherichia coli* are injected into *Lymnaea stagnalis* (van der Knaap et al., 1981b). Repeated injections of 10^8 living bacteria into *Helix* lead to similar clearance kinetics, regardless of whether the second injection was made 1.7 h after the first or 2 wk later (Bayne and Kime, 1970). In contrast, *A. californica* receiving bacteria twice within a 48-h period cleared the second injection more rapidly (Pauley et al., 1971b), and clearance of *E. coli* in *L. stagnalis* might have been accelerated if snails had been preinjected with *E. coli* 4 days previously (Sminia, 1980); however, clearance of RBCs in *Helix* slowed when secondary injections followed primaries within several hours (Renwrantz et al., 1981).

Bacterial injections cause an acute drop in the number of circulating hemocytes (Bayne and Kime, 1970; Pauley et al., 1971b; Sminia et al., 1979b; van der Knaap et al., 1981b) (Fig. 13), a phenomenon which is dose dependent following RBC injections in *Helix* (Renwrantz et al., 1981).

After injections of large numbers of bacteria, clearance kinetics are such that the circulating hemocytes would not be capable, on their own, of effecting the observed clearance, and, as with ink (Cuénot, 1914) and RBCs (Renwrantz et al., 1981), they do not in fact do so (Bayne, 1974; van der Knaap et al., 1981). Hemocytes to which foreign particles have become attached may promptly leave the circulation, or the particles themselves may be removed initially by agglutination or by being trapped by noncirculating cells. In an effort to understand these early events, radioactively labeled *S. marscescens* were injected into *Helix* and the localization of ^{14}C was followed (Bayne, 1973, 1974). Isotope accumulated in most of the tissues with good supplies of hemolymph, but particularly in the digestive gland, corroborating the results of independent research (Reade, 1968). Bacterial phagocytosis in *L. stagnalis* is preceded by bacterial clumping. By 4 h PI, *E. coli* and *S. saprophyticus* are absent from the plasma, but amebocytes containing bacteria are present in both sinuses and in connective

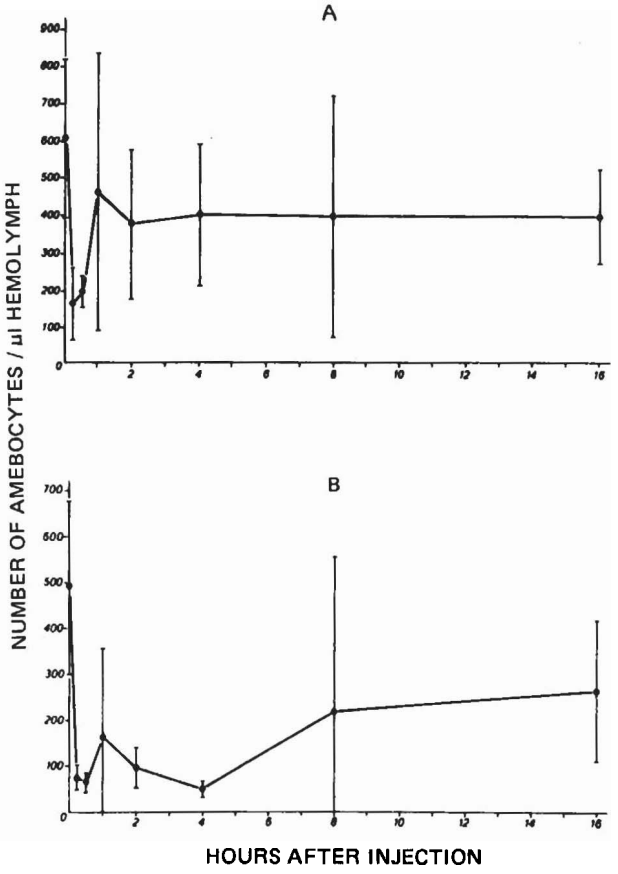


Fig. 13. Number of circulating hemocytes in *Lymnaea stagnalis* during clearance of (**A**) *Staphylococcus saprophyticus* (injected dose: 8.6 × 10^7 living organisms) and (**B**) *Escherichia coli* (injected dose: 5.0 × 10^7 living organisms). (From van der Knaap et al., 1981, with permission.)

tissues. Bacteria-laden amebocytes and fixed phagocytes (Sminia, 1980) become more numerous in the connective tissue over the next several hours, when phagosome–lysosome fusion occurs. Evidence for diapedesis of laden phagocytes is lacking (van der Knaap et al., 1981b). In the isotope experiments (Bayne, 1974), body burdens of ^{14}C declined by only 20–25% over 15 days, indicating that the products of bacterial digestion (Tripp, 1961a) are probably of nutritional value (cf. Cheng and Rudo, 1976b). Lysis of at least *S. marscescens* does not occur in hemolymph (Bayne, 1974; Johnson and Chapman, 1970; Pauley et al., 1971b). Agglutination, in contrast, may be important.

Agglutinins may be synthesized and secreted by snail hemocytes (Sminia,

1980) and albumen glands (Renwrantz and Mohr, 1978). Although it is unclear whether *Limax flavus* uses its sialic acid–specific lectin in internal defense (Miller, 1982), *Lymnaea stagnalis* plasma agglutinates *E. coli* and *S. saprophyticus*, both of which are readily cleared *in vivo* (van der Knaap, 1980). *Helix pomatia* contains agglutinins for *E. coli* (Uhlenbruck et al., 1966), *Pseudomonas aeruginosa*, and *S. marscescens* (Bayne, 1977), all of which can be cleared, but not for *Aeromonas formicans*, which escapes clearance (Bayne, 1982). Similarly, *A. californica* contains agglutinins for four bacterial species which are cleared efficiently, but not for *S. marscescens*, which is not well cleared (Pauley et al., 1971b). And *Viviparus malleatus* plasma agglutinates *Staphylococcus aureus*, which is chemotactic to the snail's hemocytes (Schmid, 1975). Agglutinin titers fall when bacteria are injected (as seen for RBC agglutinins in *Helix;* Renwrantz et al., 1981), and return to normal levels in 4–8 h in a temperature-dependent manner (Pauley et al., 1971a). The implied importance of agglutinins in the defensive responses has led to unsuccessful efforts to increase titers by preinjection of bacteria (Pauley et al., 1971b); only in oysters exposed to *Vibrio anguillarum* in the water have elevated titers of an opsonic factor been induced (Hardy et al., 1977). The suspicion that plasma agglutinins may serve opsonic functions *in vivo* in gastropods (Anderson and Good, 1976; Boyden, 1966; Cheng et al., 1969; Pauley et al., 1971b; Sminia et al., 1979) has received *in vivo* support (Renwrantz and Mohr, 1978; see Section IV,J).

Innate antibacterial factors are absent from snail plasma (Bayne, 1977; Cheng, 1969; Cooper-Willis, 1979; Johnson and Chapman, 1970; Pauley et al., 1971b; van der Knaap, 1980; van der Knaap et al., 1981b). However, lysozyme and other lysosomal enzymes can be released into snail plasma (Cheng et al., 1977, 1978a, 1978b; Cooper-Willis, 1979; Kassim and Richards, 1978a; Michelson and DuBois, 1973), but van der Knaap et al (1981b) argue that due to pH and cation requirements (Kassim and Richards, 1978b), lysozyme may be inactive when free in snail plasma. Only in abalones (three *Haliotis* spp.) has an induced bactericidal response been reported (Cushing et al., 1971); no effort has been made to confirm this report, and characterization of the active factor(s) was not attempted. It has been suggested (Cooper-Willis, 1979) that plasma enzymes may serve to alter the surface properties of bacteria so that their apparent foreignness is enhanced, with apparent opsonic effects.

3. Altered Snail Immunity to Bacteria

Whereas no natural microbial pathogens are available for gastropods, injections of *P. aeruginosa* (Bayne, 1980a) and *A. formicans* (Bayne, 1982) will kill *Helix* at doses of $>10^8$/g and $>10^6$/g, respectively. Repeated injections with sublethal doses of *Pseudomonas* (10^7 viable cells per gram) causes *Helix* to be able to survive 10^8 viable cells per gram (Bayne, 1980a); live vaccine works more effectively than heat-killed vaccine, and protection lasts for at least 30

days. In *Lymnaea stagnalis,* preinjections of *E. coli* result in enhanced clearance rates and more rapid recovery from injections made 4 days later (van der Knaap et al., 1983a). Enhanced immunity may result from some quantitative or qualitative change in the population of phagocytes, or from an increase in some humoral factor(s) which serves to facilitate cell-mediated clearance/killing. Viable counts of bacteria in the digestive gland decrease more rapidly in immune than in naive snails (Bayne 1980b), but the mechanism(s) by which immune snails acquire elevated protection remains unclear.

Clearance of bacteria from the body may be facilitated if phagocytes respond chemotactically to bacteria. The hemocytes of *V. malleatus* are attracted to *S. aureus* (Schmid, 1975). A humoral plasma component, probably an agglutinin, is necessary. Because *N*-acetyl-D-glucosamine eliminated the chemotactic response and decreased agglutinin titers against *S. aureus* and rabbit RBCs, it may be a determinant recognized by the agglutinin. Chemotactic behavior is not easily demonstrated for hemocytes of *Biomphalaria* (personal observations).

E. Responses to Fungi

Although fungi have been associated with pathology in both egg (Cowper, 1946) and adult (Gorokhov, 1978; Malek, 1952; Pan, 1956) mortality in freshwater snails, no studies have been done on responses to these pathogens. Hemocyte phagocytosis of yeast occurs *in vivo* (Renwrantz et al., 1981; Tripp, 1961a) and *in vitro* (Prowse and Tait, 1969; Sminia et al., 1979b). Opsonization has been found to be necessary for phagocytosis of fixed yeast cells (Anderson and Good, 1976; Prowse and Tait, 1969), whereas the uptake of unfixed yeast seems to be opsonin independent (Anderson and Good, 1976; Renwrantz et al., 1981). Heat-killing and autoclaving alter yeast fates *in vivo* (Tripp, 1961a). After phagocytosis in *Biomphalaria glabrata,* some fungi are degraded intracellularly; others are carried to the exterior by diapedesis of laden amebocytes (Tripp, 1961a). Whereas many pathogens avoid tissues of the central nervous system, a fungal symbiont reported in *B. glabrata* (Pan, 1956) is particularly associated with the nervous system. In infected nerves and ganglia, amebocytes form nodules, replacing the neurons and phagocytosing the organism. A fungal pathogen of the nudibranch *Tritonia diomeda* is encapsulated by amebocytes in the body wall and destroyed (McLean and Porter, 1982).

F. Responses to Viruses

Virus-like particles have been briefly reported in a gastropod, the terrestrial slug, *Agriolimax reticulatus* (David et al., 1977). Unlike other foreign agents, injected viruses (T_1-phage) persist in gastropod hemolymph (*Achatina*) over several weeks (Nelstrop et al., 1968). Secondary injections appear to be cleared

even more slowly than primaries. The availability of a cell line from *B. glabrata*[6] should facilitate research in gastropod virology.

G. Responses to Other Particulates

In the most broadly based study of *in vivo* clearance by gastropods, Cuénot (1914) traced the fates of Chinese ink and carmine in 40 species of prosobranch, opisthobranch, and pulmonate. Injected ink is quickly localized in specific tissues, such as the "sac papillaire" of *Trochus*, rapidly clearing the plasma. This acute response is a physical (agglutinating) phenomenon and is followed by phagocytosis, in which both free hemocytes and fixed phagocytes are active. Diapedesis is slow and feeble. In basommatophorans, fine carmine accumulates in spongy tissue of the lung floor, and fixed phagocytes are less obvious than in other gastropods. In most Monotocardia, phagocytes are localized in the kidney and gills. In opisthobranchs, Cuénot (1914) found "un veritable organe phagocytaire defini, volumineux, qui est abondamment vascularisé par l'aorte," but there has not yet been any follow-up to this discovery. Cuénot's paper should be consulted directly for its richness of detail with respect to the species he studied, as he describes much specific variation and summarizes the several earlier studies in which *in vivo* phagocytosis was reported in gastropods.

More modern studies have added detail to Cuénot's account. In *Littorina scabra* injected with ink into the pedal sinuses, ink-laden phagocytes undergo diapedesis mainly through epithelia of the foot, alimentary tract, and gills, and via the kidney (Cheng et al., 1969), eliminating essentially all injected ink within 6 days. Charcoal particles of unspecified size, injected into the body wall tissues of *Lehmania poirieri*, a pulmonate slug, are encapsulated en masse (Arcadi, 1968), a fate resembling that of carmine particles injected intramuscularly (IM) into *Aplysia californica* (Pauley and Krassner, 1972). After IM injections, some ink and some carmine are widely dispersed within the *Aplysia* body but most carmine is effectively confined to the injection site, as reported also for *B. glabrata* (Tripp, 1961a). By 8 h PI, carmine nodules in *Aplysia* are evident within capsules of elongated hemocytes. Nodules grow for several days by the immigration of carmine-laden phagocytes, a process which may involve chemotaxis. These cells die, depositing carmine at the periphery of the nodule (Fig. 14). Maximum capsule thickness ($\leq$10 cells thick) is reached by 10 days PI. Muscles and collagen become intermingled with the encapsulating hemocytes.

Whereas Cuénot (1914) first demonstrated species differences in handling injected ink and carmine, Tripp (1961a) showed clearly that the fates of particles injected into one species (*B. glabrata*) are dependent largely on the size and

[6]This cell line is available from the American Type Culture Collection, 12301 ParkLawn Drive, Rockville, MD 20852, under the designation ATCC CRL 1494.

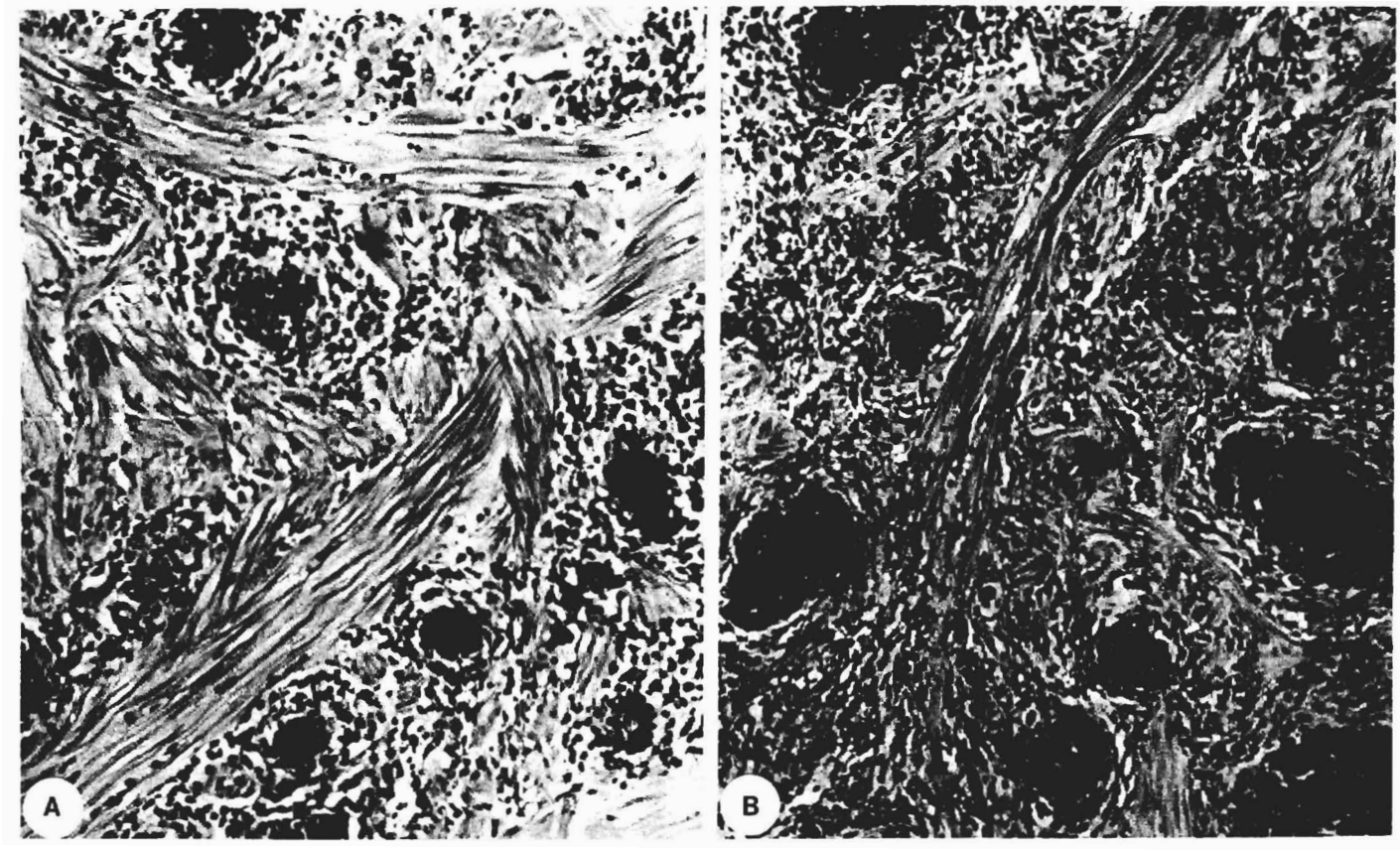


Fig. 14. *Aplysia californica;* nodules composed of carmine and phagocytes (**A**) 4 days PI; (**B**) 10 days PI. The nodules have enlarged during this time and there is heavy infiltration of hemocytes throughout the surrounding musculature. (From Pauley and Krassner, 1972, with permission.)

nature (chemical and physical) of the particle. Yeast, a gram-positive coccus, chicken RBCs, carmine, willow pollen, and polystyrene spheres, some pretreated in various ways, were injected. Particles smaller than hemocytes were rapidly phagocytosed and either (1) disposed of by diapedesis (yeast and bacteria), (2) degraded intracellularly (yeast, bacteria, RBCs), or (3) retained in nodules (carmine, pollen, polystyrene). Whereas phagocytosis was obvious in minutes, diapedesis occurred over at least 20 days.

Depending on the size and nature of the injected particles and molecules, different cells are phagocytic in pulmonates (*Cepaea:* Wolburg-Buchholz, 1972, 1973; *Lymnaea:* Sminia, 1980; van der Knaap, 1980). In general, larger particles (ϕ = 20 nm–8μm) are engulfed by amebocytes and fixed (reticulum) phagocytes, whereas colloidal material and proteins (ϕ <20 nm) accumulate in "globular cells" (Wolburg-Buchholz, 1972, 1973) or "pore cells" (Curtis and Cowden, 1978; Sminia, 1980; van der Knaap, 1980), as indicated in Table V and Fig. 15. In *Lymnaea stagnalis,* the connective tissue of the heart/kidney region is rich in all three cell types (Sminia, 1980). The amebocytes contain peroxidase, nonspecific esterases, and acid phosphatase, and efficiently degrade biotic materials. The pore cells, in contrast, degrade the endocytosed proteins slowly; ferritin and hemoglobin are still present after 12 weeks. Peroxidase is absent from both pore cells and reticulum cells. Lysosomal enzymes increase in activity in phagocytically stimulated cells, but only peroxidase and nonspecific esterase are demonstrable in the phagocyte Golgi apparatus, implying their synthesis in the

TABLE V

Phagocytic Capacity of the Three Types of Phagocytes in *Lymnaea stagnalis*[a]

Injected materials	Amebocytes	Reticulum cells	Pore cells
Abiotic particles			
India ink	+++[b]	+++	−[b]
Trypan blue	+	+	+++
Colloidal gold Ø < 20 nm	++[b]	++	+
Ø > 20 nm	+++	+++	−
Proteins			
Ferritin	+[b]	+	+++
Hemoglobin	+	+	+++
Peroxidase	+	+	+++
Cellular material			
Yeast cells	+++	+++	−
Bacteria	+++	+++	−
RBC	+++	+++	−

[a] Reprinted with permission from Sminia (1980). © 1980 Pergamon Press, Ltd.
[b] +++: High ingestion; ++: moderate ingestion; +: low ingestion; −: none.

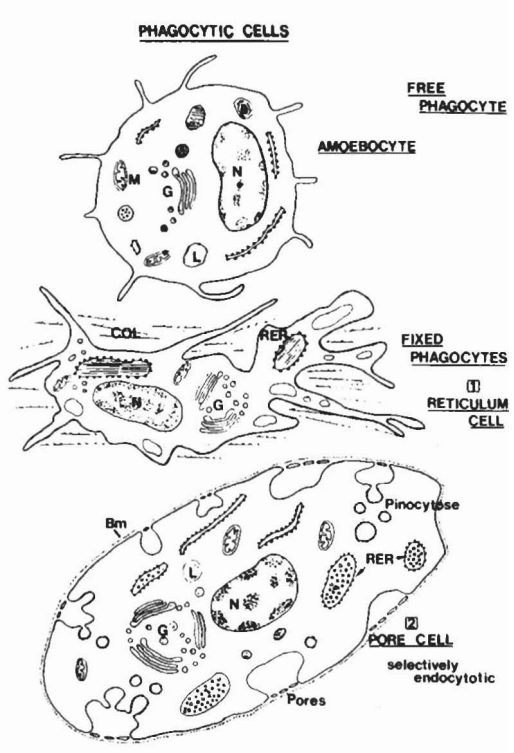


Fig. 15. Schematic drawing of the phagocytic cells of *L. stagnalis*. Amebocytes are mobile, whereas reticulum cells and pore cells are fixed. Pore cells, in contrast to other cells, are selectively endocytotic. For further details, see Table V. (Reprinted with permission from Sminia (1980). © 1980 Pergamon Press, Ltd.)

cell. The size of the particles entering pore cells is restricted by the need to pass through the basement membrane and holes in a sieve-like cell surface.

Thorotrast, a radiopaque colloidal substance, is cleared from the body of *Bullia* (a prosobranch) only slowly (4–6 weeks, Brown and Brown, 1965). The major route appears to be similar to that in *Lymnaea* injected with colloidal ink: Laden hemocytes in the heart region migrate into the pericardium and thence via the renopericardial duct into the kidney, finally to be voided in the mantle cavity. The ctenidia may also be sites of suicidal diapedesis. These routes differ from those used by *B. glabrata* hemocytes (Tripp, 1961a), possibly due to the nature of the endocytosed material. It was therefore of interest to note that in *Helix aspersa*, a terrestrial pulmonate, both localization (>2 days) and body clearance (>12 days) of thorotrast were slow, and the chief migratory route for laden phagocytes was neither the heart-kidney nor the mantle but the reproductive system (Brown, 1967)! The gut may have been an additional migratory route.

In a report related to the specificity of recognition, Muscatine et al. (1975) found that elysioid sacoglossans selectively accept one of two types of chloroplast from *Caulerpa sertularioides* for an intimate mutual symbiosis, but the selection mechanism has not been investigated.

H. Responses to Tissue Implants

Allogeneic recognition is a property of even the lowest metazoan phyla (Hildemann and Johnson, 1979; Hildemann et al., 1977) so it might be expected that molluscs would readily demonstrate such a capability. Yet it has not proven possible to demonstrate unambiguously allogeneic recognition in molluscs. This property must be evidenced by a recipient-mediated attack on implant tissue from another individual of the same species, when reimplanted self-tissue or tissue from an inbred relative is not attacked but grows in place.

In *Biomphalaria glabrata,* implanted allogeneic tissue, although washed for 4 h in distilled water (!), elicited no fibroblast response and only a "transient amoebocytic infiltration, the latter in response to damaged and necrotic cells [Tripp, 1961a]." By 20 days the epithelium of the intact implant fuses with that of the recipient. A similar fate awaits allogeneic implants in *Lymnaea stagnalis* (Bayne et al., 1980c; Sminia et al., 1964; van der Knaap, 1980). In contrast, formalin-fixed foot tissue is encapsulated in *B. glabrata* in a manner resembling that seen in incompatible *Schistosoma mansoni–B. glabrata* infections, although the fixed tissue is not readily degraded (Tripp, 1961a). Xenogeneic tissue is similarly encapsulated, atrophies, and is invaded by host cells and removed in *B. glabrata* (Tripp, 1961a) and in *Lymnaea stagnalis* (Sminia et al., 1974). Immature allogeneic reproductive tracts implanted into mature specimens of *Agriolimax reticulatus* undergo maturation rather than destruction (Runham and Hunter, 1970). Furthermore, embryos implanted into the hemocoel of adult *Bulinus truncatus* and *B. globlosus* are not rejected (Brisson, 1971); such embryos may become partially encapsulated, and development is massively disturbed, but growth continues even when embryos of a pigmented strain are implanted into albino adults. Even the destruction of xenogeneic implants (buccal masses, *Helix* into *Agriolimax,* and *Biomphalaria* into *Lymnaea:* Musser and Harding, respectively, personal communications) is slow, taking 3 weeks or more at normal temperatures. These results conflict with those of Cheng and Galloway (1970), who observed destruction of both xenogeneic and allogeneic implants in *Helisoma duryi normale.* Such a fate should be expected for cubes of digestive gland tissue placed into the cephalopedal sinus, for reasons of bacteriological infection, abundant lytic enzymes, heterotopic location, and the extensive damage to the implant necessitated by preparing cubes from a large tissue mass. Under such circumstances, a host cellular response to the necrotic allograft should not be interpreted as indicative of immunologic recognition.

I. Hemocyte Phagocytosis and Opsonins

Hemocytes of *Helix aspersa* (Prowse and Tait, 1969), *Otala lactea* (Anderson and Good, 1976), *Biomphalaria glabrata* (Abdul-Salam and Michelson, 1980a, 1980b; Faulk et al., 1973; Jeong and Heyneman, 1976), *Lymnaea stagnalis* (Sminia et al., 1979b), and *Aplysia californica* (Pauley et al., 1971a) all have phagocytic capabilities *in vitro*. Phagocytic efficiency is influenced by many variables, including temperature, pH and integrity of anaerobic glycolysis (Abdul-Salam and Michelson, 1980b), the size, (Sminia et al., 1979a; Tripp, 1961a) and nature of the particles (Anderson and Good, 1976; Sminia et al., 1979a; Tripp, 1961a), whether the snail carries a patent trematode infection (Abdul-Salam and Michelson, 1980a), the presence of lectins (concanavalin A) bound to the phagocyte (van der Knaap, 1980; Schoenberg and Cheng, 1982), the integrity of the "cell coat" or glycocalyx (Sminia et al., 1981; van der Knaap, 1980), and the presence of homologous factors.

The dependence of particle clearance rates on surface properties is evident with human RBCs in *Helix* (Renwrantz et al., 1981); A-type cells are cleared twice as fast as B-RBCs and four times as fast as rabbit RBCs; also, rough and smooth strains of *Azotobacter vinelandei* are cleared at different rates.

Agglutinins, because of their lectin-like properties (binding sugars), may function opsonically in gastropods. Opsonic activity, due to factors which coat foreign particles and thereby enhance their phagocytosis, has been claimed for *Helix* (Prowse and Tait, 1969; Renwrantz and Mohr, 1978), *A. californica* (Pauley et al., 1971b), *Otala lactea* (Anderson and Good, 1976), and *L. stagnalis* (Sminia et al., 1979b; van der Knaap et al., 1983b). In order to prove opsonic activity in the plasma, it is not sufficient to demonstrate, as some authors have, increased *in vitro* phagocytosis in plasma-containing media relative to saline; it is essential to demonstrate increased phagocytosis in a physiological plasma-free medium when particles, preincubated in plasma and then washed, are offered to phagocytes.

1. Critical Appraisal of Claimed Opsonic Activity in Gastropod Plasma and of Direct Hemocyte Recognition

Snail phagocytes have no absolute need for plasma factors in phagocytosis (Abdul-Salam and Michelson, 1980a, 1980b; Jeong and Heyneman, 1976; Sminia et al., 1979b, 1981). Certain particles may bind to phagocytes as a result of nonspecific properties, such as hydrophobicity or charge, and the nature of the cell coat may influence much of this nonspecfic binding. On balance, it appears that snail phagocytes have some integral receptors in the plasma membrane, but that the efficacy of their recognition function can be enhanced by plasma components (Sminia et al., 1979b, 1981). Cytoadherence of certain particles (e.g., RBCs) to phagocytes may require opsonin, whereas other particles (e.g., yeast)

may bind directly to recognition sites on snail phagocytes (Renwrantz et al., 1981).

As we have seen, agglutinins occur in gastropod plasma. Such agglutinins are commonly considered to be lectins, i.e., carbohydrate-binding proteins or glycoproteins. Often, lectin molecules contain multiple sugar-binding sites. Such lectins would cross-link and therefore precipitate macromolecules, and will agglutinate (by bridging) particles which carry on their surfaces the sugars bound (recognized) by the multivalent lectin. But is opsonic activity due to agglutinating lectins of snail plasma?

In *Octala lactea,* agglutinating activity has not been found in the plasma, but an agglutinin for sheep RBCs (SRBCs) can be prepared from the albumen glands, and an opsonic property is claimed for it (Anderson and Good, 1976). However, the claim has not been substantiated because the agglutinin was neither purified nor absorbed out, nor were phagocytosis runs done in extract-free medium with soaked, rinsed RBCs. In experiments with *Aplysia* (Pauley et al., 1971b), phagocytosis was allowed to occur in the presence of plasma, precluding conclusions about opsonic activity. Interpretation of results on phagocytosis of formalized sheep RBC and yeast by *H. aspersa* hemocytes is complicated by contradictions in the paper (Prowse and Tait, 1969). The authors state in Material and Methods that opsonized particles were "washed 3 times to remove all traces of excess serum," but in their Results section, phagocytosis is described as being carried out in 50% serum (i.e., plasma) in saline. However, their data do appear to show that plasma in which sheep RBCs have been soaked is unsupportive (? suppressive) of SRBC phagocytosis, and that yeast phagocytosis is depressed in yeast-adsorbed plasma.

In contrast, the studies of Sminia et al. (1979b) and van der Knaap (1980) prove that *L. stagnalis* plasma contains opsonins for yeast and formalized SRBCs, and those of Renwrantz et al. (1981) show that *H. pomatia* plasma contains opsonins for erythrocytes. *Lymnaea* opsonic activity was demonstrated with *in vitro* phagocytosis experiments; both yeast and SRBCs were more avidly phagocytosed if they had been presoaked in 50% snail plasma than in saline alone (Sminia et al., 1979b). *Helix* opsonic activity was demonstrated *in vivo;* a secondary injection of human A_1 or B-RBCs given 12–19 h after a primary injection of $1–2 \times 10^9$ homologous RBCs was subject to slower clearance, possibly due to the partial exhaustion of opsonin. This slowing was eliminated if the RBCs of the secondary injection were presoaked in *H. pomatia* plasma and rinsed. This implies that they were opsonized (Renwrantz and Mohr, 1978). The opsonin is not specific; secondary clearance of B-type human RBCs is slowed if the primary dose was A-RBCs or even human serum albumin (Renwrantz et al., 1981). Unlike RBC clearance rates, those of yeast are unaltered by prior injections. Yeast binding to clearance cells is inhibited by *N*-acetyl-D-glucosamine (GNac) and *N*-acetyl-D-galactosamine (GalNac), but not by fucose. These results

appear compatible with the theses that yeast opsonin is present in superabundance and is not depleted by the primary inoculum, or that it is replenished within 3 h, but the authors consider that yeast clearance is opsonin independent. Such a difference from RBCs might reflect the cellulose-rich nature of the yeast cell wall, in contrast to the lipoprotein-rich cell membrane of RBCs (Sminia et al., 1979b). The agglutinin and the opsonin may be the same molecule; first, purified albumen gland agglutinin is opsonic (Renwrantz and Mohr, 1978), and second, both opsonization and agglutination are inhibited by GNac and GalNac (Renwrantz et al., 1981). Fucose does not influence the rate of primary clearance of RBCs (Harm and Renwrantz, 1980)(Fig. 16).

It has been suggested that hemocyanin may function opsonically in molluscs (Stuart, 1968; van der Knaap, 1980). In light of this, it is interesting that clearance of yeast in *Helix* was *slowed* when *Helix* hemocyanin was coupled to the yeast surface by glutaraldehyde (Renwrantz et al., 1981), a result that is concordant with those of Crichton and Lafferty (1975) (see Section II).

RBC opsonization is evidently not dependent simply on the lectin properties of the opsonin. Binding of RBCs to hemocytes can be accomplished by certain exotic lectins (e.g., derived from plants or sponges), as well as by homologous lectins (i.e., derived from the snail; Renwrantz and Cheng, 1977b). However, phagocytosis is enhanced only by the homologous lectin. Thus, normal opsonin–hemocyte bridging involves something more than the carbohydrate–lectin bridge on the hemocyte surface. Either (1) binding of a particle by opsonin molecules may cause conformational changes in the opsonin which result in hemocyte recognition and phagocytosis, or (2) bridging of opsonized particles and hemocytes and subsequent phagocytosis may result from a suprathreshhold concentration of the opsonins on the particle, i.e., a concentration of opsonins exceeding that which exists humorally.

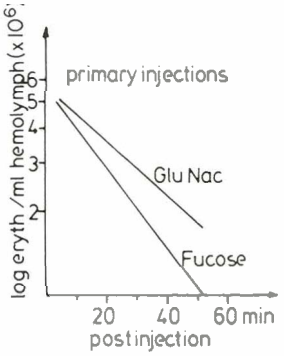


Fig. 16. Clearance rates of human RBCs in *Helix pomatia*. When injected with fucose in the medium, the rate is the same as when injected with saline only. In contrast, *N*-acetylglucosamine slows the clearance rate, presumably by competitively inhibiting the opsonin. (From Harm and Renwrantz, 1980, with permission.)

Although hemocyte populations in snails are morphologically conformist, existing at most as three types (see Sminia, 1981), mounting evidence implies that the macrophage-like granulocyte class contains subpopulations which differ in their surface chemistry. Anti-A from the *H. pomatia* albumen gland agglutinates a subpopulation of *H. pomatia* living hemocytes (Renwrantz and Cheng, 1977a). Subpopulations of glutaraldehyde-fixed phagocytes are distinguished (agglutinated) by anti-A of *H. pomatia* or *Cepaea nemoralis* or *C. hortensis,* and by phytohemagglutinin. Furthermore, subpopulations of *Helix* living hemocytes in saline recognized (rosetted) RBCs of mouse, rabbit, rat, and sheep (Renwrantz and Cheng, 1977b). None recognized human (ABO) or guinea pig RBCs, unless an agglutinin was present to bridge the human RBCs and the hemocytes. Thus, some snail hemocytes have integral binding sites for determinants on some foreign surfaces (see also van der Knaap et al., 1981a). Other foreign surfaces must be bound by subagglutinating quantities of multivalent lectins. Whereas sugars are exposed on hemocyte surfaces, lectins with appropriate recognition sites do not suffice to opsonize merely on the basis of their sugar-binding properties.

Abiotic particles such as colloidal iron and thorium dioxide, cleared *in vivo,* can be seen in association with the glycoprotein cell coat (glycocalyx) of *Helix* and *Cepaea* hemocytes (Wolburg-Buchholz, 1973), but nothing is known about the mechanisms of recognition. These will probably be found to involve electrostatic and hydrophobic interactions. Both free and fixed (i.e., tissue-resident) phagocytes of *Cepaea* and *Helix* display acidic groups, "presumably predominant carboxyl groups of an acid polysaccharide (Wolburg-Buchholz, 1973]"; similar cell coats cover the hemocytes of *Bulinus* (Krupa and Lewis, 1977) and *Lymnaea* (Sminia et al., 1981).

Helix pomatia hemocytes display mannose and/or glucose, fucose, galactose, and galactogen and/or *N*-acetylneuraminic acid, because these carbohydrates inhibit RBC–hemocyte bridging in the presence of RBCs presoaked in lectins of the appropriate specificity (Renwrantz and Cheng, 1977a). Because the cell coat (glycocalyx) is generally rich in sugars (Krupa et al., 1977; van der Knaap, 1980; Wolburg-Buchholz, 1973), it is interesting that pronase treatment, which removes the glycocalyx from *Lymnaea* hemocytes (van der Knaap, 1980), resulted in the display of more sugars on *H. pomatia* hemocytes (Renwrantz and Cheng, 1977a), implying that these sugars are constituents of the glycolipids and/or that pseudocryptoantigens are exposed when proteins of the cell coat are attacked by pronase. Concanavalin A receptors on the hemocytes of *L. stagnalis* are sensitive to both trypsin and pronase (Sminia et al., 1981), whereas those on *B. glabrata* hemocytes are pronase sensitive but trypsin resistant (Yoshino, 1981a). Once bound, concanavalin A is rapidly patched, capped, and internalized by *B. glabrata* hemocytes (Yoshino, 1981a, 1981b).

We have used the words *opsonin* and *opsonization* loosely. In the work of Renwrantz and colleagues, opsonic activity is claimed when a retardation of

secondary clearance is ameliorated by presoaking particles in plasma or albumen gland agglutinin. Actual clearance occurs in the first 2–3 h PI. However, it is also shown (Renwrantz et al., 1981) that during this time phagocytosis is not an important part of clearance. Instead, RBCs adhere to cells lining sinuses in the digestive gland, kidney, and head–foot. Hemocytes with phagocytosed RBCs (Renwrantz, 1979) or yeast (Renwrantz et al., 1981) are at maximum numbers in the circulation 80 h (RBCs) or 40–50 h (yeast) PI. So-called opsonic activity therefore is a manifestation of binding of RBCs/yeast to the sinus walls, not of phagocytosis per se.

Furthermore, we have discussed agglutinins as though their specificity was constant for each species of snail. In fact, hemagglutinin specificity varies even from individual to individual within single strains of *B. glabrata* (Michelson and DuBois, 1977) and within populations of *L. stagnalis* (van der Knaap, 1980). The significance of this is not known; it may dictate differences of individual susceptibility to parasites and pathogens. Such diversity of putative mediators of immune recognition may be an evolutionary strategy to ensure survival of part of a population in species of short-lived invertebrates which are dependent predominantly for their survival on innate immune mechanisms.

J. Other Humoral Factors in Immunity

Soluble factors previously mentioned in this chapter include a nematode growth inhibitor, agglutinins/opsonins, miracidial immobilizing substance, and lysosomal enzymes, but for neither of the last two are there data which prove roles in defense. Lysins for human erythrocytes occur in all tested strains of *Biomphalaria glabrata* plasma (Michelson and DuBois, 1977). Titers up to 1 : 16 were detected when tests were done at 4°C, but were lower at physiological temperatures (27°C). No data exist on the characterization of the plasma components responsible for this activity, or on its possible defensive functions, although it is clear that *Serratia marscescens* are not lysed when injected into either *Aplysia* (Pauley et al., 1971b) or *Helix* (Bayne, 1974). The Sephadex-binding hemagglutinin from *B. glabrata* made human A-RBC prone to lysis by complement in human serum (Stein and Basch, 1979). For a protease inhibitor in *Helix pomatia* plasma (Renwrantz, 1979), little more is known than its occurrence and molecular weight (approximately 3×10^3).

V. Internal Defenses of Cephalopods

A. Responses to Parasites

Cephalopod kidneys are the home of dicyemid Mesozoa. Other parasites, such as monogenetic trematodes and cestodes, of course, occur in cephalopods. Near-

ly 30 species of squid, octopods, and cuttlefish are known to be infected with larval and adult digenetic trematodes (Overstreet and Hochberg, 1975). As early as 1761, Linnaeus described *Fasciola barbata* from *Loligo vulgaris* (cited and discussed by Overstreet and Hochberg, 1975). The hectocotylus arms of male cephalopods, detached during copulation and left in the female mantle cavity, were long thought to be exotic parasites of the female; in view of their detachment and the general vulnerability of cephalopod arms, it is to be expected that cephalopods have an effective healing capacity.

B. Responses to Wounding and Noxious Agents

Octopus vulgaris from which an arm is cut will regenerate a new arm. Lange (1920) made a detailed study of wound healing and regeneration in *Octopus, Eledone,* and *Sepia.* Cephalopod blood does not clot. Within minutes, the edges of a wound curl inward, and bleeding is evidently prevented by local vasoconstriction (cf. Browning, 1979). After a few hours, blood seeps out and the amebocytes aggregate to form a protective blastema. Phagocytosis of damaged and degenerating muscle and nerve cells (and, it must be assumed, of microbes) occurs. The epithelial cells at the wound perimeter change from columnar to pavement (i.e., they flatten) and move out over the blastema, covering it within 1–2 days. The blastema cells later construct connective tissue as the arm bud grows and new suckers form.

Inflammatory responses occur in *Sepia, Octopus,* and *Eledone* injected with tar (Jullien et al., 1956). Tissue proteolysis at the injection site results from leukocyte disruption. A scab forms and is separated from the surrounding tissue by a groove where amebocytes undergo transformation to a flattened form, ''encapsulating'' the region. Cuttlefish injected with crystals of the carcinogen 1,2,5,6-dibenzanthracene rapidly develop circular or oval ''tumors'' around the site (Jacquemain et al., 1947). Cells undergo autolysis and become disorganized. The surface epithelium is lost, and the lesions break off in bits. Notably, these lesions fail to elicit an organized cellular reaction; a feeble, diffuse infiltration of undifferentiated or dedifferentiated ''histiocytes'' occurs. In the liver of *Sepia,* small crystals of an insect toxin (''cantharidine'') or formalinized cotton swabs induce leukocytic infiltration (Jullien et al., 1956), which is very noticeable within 24 h. Some amebocytes infiltrate the damaged tissue and die, whereas others encapsulate the region, becoming ''fibroblast-like,'' a change which the authors consider irreversible.

C. Responses to Injected Substances and Particles, Including Bacteria

The general contention that cephalopods represent a more highly specialized physiological condition than all other molluscs is supported by studies of their

clearance systems. Injected particles are rapidly cleared from the blood in highly localized sites (Fig. 17). Recall that cephalopod circulatory systems are more nearly "closed" than those of other molluscs. The capillary beds (or "exchange vessels"; Browning, 1979) of the gills, accessory branchial hearts, optic glands, salivary glands, and "white body" clearly serve reticuloendothelial functions, and diffuse connective tissues are not involved (Bayne, 1973b; Cazal and Bogoraze, 1943; Cuénot, 1914; Stuart, 1968). Within 1 min of the injection of ink into cuttlefish, the gills are blackened, implying effective removal during its first passage through the gill (Cuénot, 1914). Large quantities of ink may be lethal, causing ctenidial emboli and asphyxia (Kowalevsky, 1894; cited by Cuénot). In *Octopus* and *Loligo,* ink clearance occurs only in the gill and in the appendix of the branchial hearts (Fig. 18). Clearance, presumably by agglutination to the walls of the vessel, precedes phagocytosis; this begins within 1 h, and by 5 h PI no trace of ink is present in the blood (Cuénot, 1914). In the gills, phagocytosis is uniquely a function of fixed cells in the finest vessels (Cuénot, 1914). The blood leukocytes do not accumulate ink and may not be phagocytic for ink, "une exception interessante," although those of *Eledone* can phagocytose human RBCs *in vitro* (Stuart, 1968). Although the appendix of the branchial heart is phagocytic in *Octopus* and *Loligo,* it is not in *Sepia* and *Sepiola,* a difference which correlates with differences in structural details (Cuénot, 1914). In *Eledone cirrosa,* ink also accumulates in the posterior salivary glands and the white body (Stuart, 1968), with other tissues being quite free of ink.

Unlike ink (carbon), which remains confined in blood vessels, ferritin (an electron-dense, 500,000 molecular weight, iron-containing protein (ϕ = 110 Å), rapidly (1–2 min) leaks through the basement membrane into the extravascular spaces of *Octopus* (Browning, 1979; Froesch and Mangold, 1976). Unfortunately, tissues selected for ultrastructural study from ferritin- and ink-injected *Octopus* exclude most components of the reticuloendothelial system (Browning, 1979).

Injected bacteria (anthrax and tuberculosis bacteria: Kowalevsky, 1894; *Serratia marscescens:* Bayne, 1973b) are also efficiently cleared by *Octopus;* phagocytosis occurs in small connective tissue cells of the branchial hearts and gills (Kowalevsky, 1894). By ligaturing the efferent branchial vein of *Octopus dofleini* in two places, I was able to isolate a blood "pool" and inject *S. marscescens* (Bayne, 1973b). Bacteria were not eliminated, implying that hemocytes alone are not responsible for the clearance seen in intact animals; for this, the blood must circulate. Thus, although leukocytes are probably capable of phagocytosis (observed *in vitro* with *Eledone* cells and human RBCs; Stuart, 1968), actual *in vivo* clearance of bacteria must occur in specific tissues, as suggested by Kowalevsky (1894).

Eledone plasma enhances *in vitro* phagocytosis of RBCs by leukocytes, al-

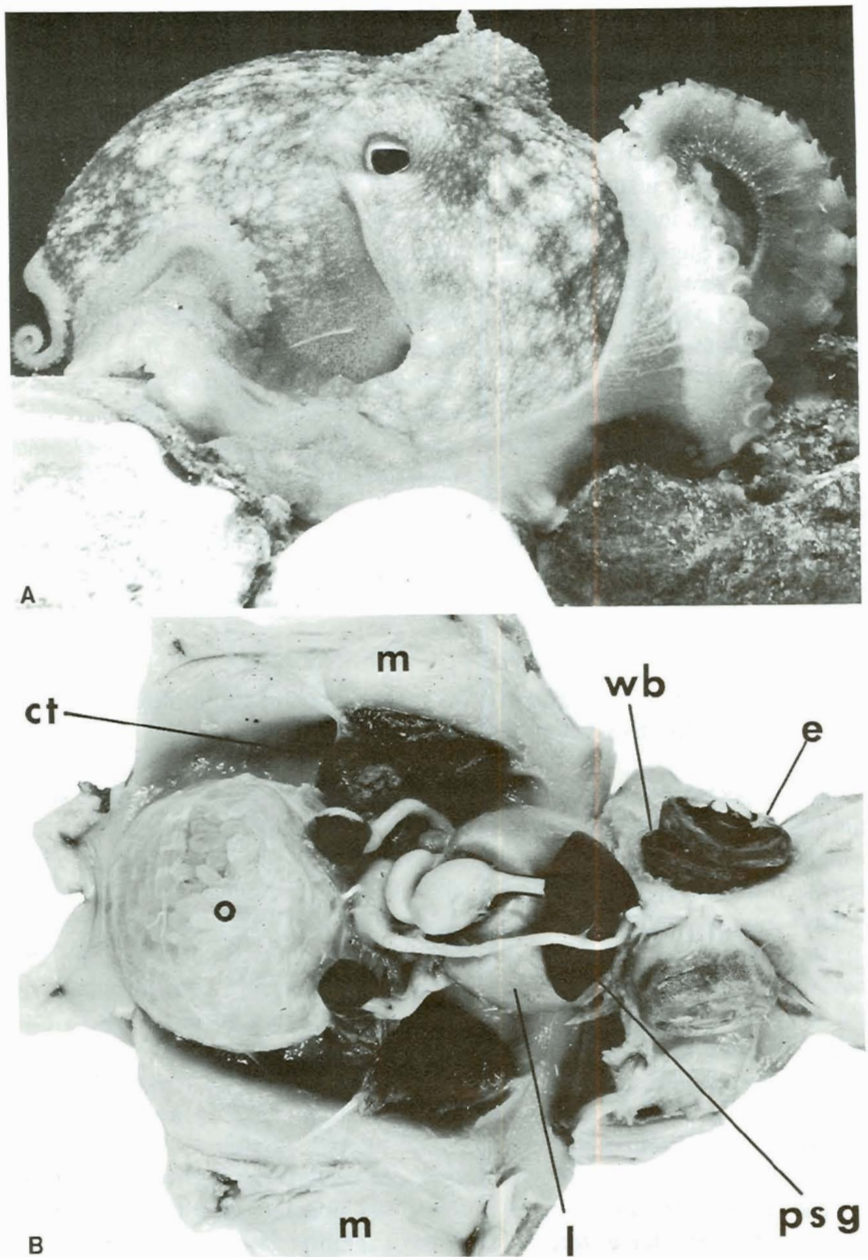


Fig. 17. *Eledone cirrosa*, the Lesser Octopus (A), dissected to show the organs, all of which are pale colored normally, which have been blackened due to accumulation of injected ink (B). e, eye; wb, white body; psg, posterior salivary gland; l, liver; ct, right ctenidium; m, mantle; o, ovary. (From Stuart, 1968, with permission.)

though no agglutinin for human and fish RBCs is present (Stuart, 1968). *Staphylococcus aureus* and *Salmonella typhi* were similarly not agglutinated; however, coating by plasma factors may occur because rabbit anti-*Eledone* plasma caused agglutination of *S. typhi* and other particles after they were soaked in *Eledone* plasma and rinsed three times (except for the *S. typhi,* rinsed once). Because a rabbit, injected with its own RBCs after they had been soaked in *Eledone* plasma and rinsed four times, produced antibodies to *Eledone* hemocyanin, Stuart suggested that "the haemocyanin might act both as a respiratory pigment and as an opsonin for foreign material."

Neither antibacterial activity nor enhanced secondary clearance of *S. marscescens* was found to occur in *O. dofleini* (Bayne, 1973b).

On the basis of obscure reasoning, Froesch (1979) concluded that "the optic gland hormone, which is known to control sexual maturation, feeding and death in octopus, appears to be involved in a defence mechanism against non-*Octopus* proteins." He had injected a variety of foreign proteins into *O. vulgaris*. Ferritin was shown to accumulate in stellate cells around optic gland capillaries but, surprisingly, was still present in blood 3 days PI; injected rabbit immunoglobulin G remained at nearly the initial concentration for at least 6 days. However, these octopi were subjected to traumatic surgery and were probably not well.

VI. General Characteristics of Molluscan Immunologic Responses Compared with Those of Other Phyla

Many aspects of molluscan immunobiology resemble equivalent processes in most other phyla. One example is the recognition of non-self, in some cases with a high degree of discrimination (Sections II and IV,I). In molluscs, internal defense functions appear to be more cell dependent than in many arthropods and vertebrates; i.e., molluscs lack inducible antibacterial factors (present in insects, crustaceans, and vertebrates) and inducible opsonin (vertebrate antibody). Immunity appears to be more reliant upon innate components such as circulating and sedentary phagocytes, and some humoral facilitators of phagocytosis (opsonins) and cell-mediated cytotoxicity against parasites.

Molluscs resemble arthropods in their evident failure to reject allografts, which studies of other metazoan phyla show that they all do. However, molluscs are distinctly different from crustacea and insects, as well as vertebrates, in lacking a strong adaptive molluscan response to bacteria. Furthermore, arthropod defenses include melanization in capsules and nodules, which is not seen in molluscs. Yet another difference from most arthropods and vertebrates is the absence of a strong molluscan mechanism for extracellular clotting of hemolymph.

Many of these differences may be related to a stronger dependence of molluscs

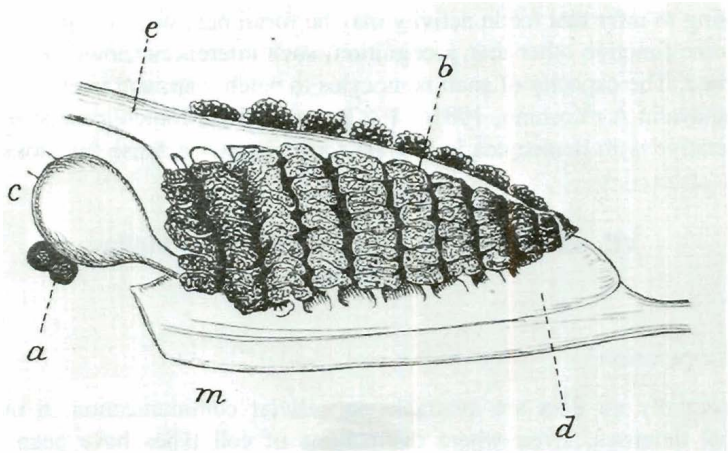


Fig. 18. Gill and branchial heart of *Octopus vulgaris* 7 days after injection of ink into the animal. a, appendix of the branchial heart blackened by absorbed ink; b, lateral fold of the gill, also blackened by absorbed ink; c, branchial heart; d, subbranchial gland; e, efferent branchial vessel; m, attachment of the gill to the body wall. Drawn from a fresh specimen. (From Cuénot, 1914, with permission.)

upon mucociliary mechanisms as first lines of defense. In vertebrates, many otherwise vulnerable epithelia depend upon mucociliary mechanisms for protection against potential pathogenic microbial colonization. Arthropods, lacking such mechanisms, possess effective clotting mechanims to localize microbes which may threaten to enter the hemocoel through breaks in the normally invulnerable exoskeleton. The molluscan body is covered by cleansing sheets of mucus, which it may produce in abundance when irritated. The importance of mucociliary mechanisms of defense has not yet been established, but it is probably far greater in molluscs than in vertebrates and arthropods generally.

The probable reliance of molluscs on lectins as mediators of specific recognition may be a feature shared with all nonvertebrate organisms, including invertebrates, plants, prokaryotes, and viruses. For example, recognition functions have been demonstrated for lectins in the *Rhizobium*–host plant association (Paau et al., 1981), in sponges (Bretting and Konigsmann, 1979), in reticuloendothelial functions of the mammalian liver (Hubbard et al., 1979), in the nematode-trapping mechanism of a fungus (Nordbring-Hertz and Mattiasson, 1979), in the T-lymphocyte cells of the human immune network (Koszinowski and Kramer, 1981), and possibly in the sperm–egg interactions of tunicates (Rosati and DeSantis, 1980).

Lectin distribution may occasionally appear anomalous, for example, in gastropod egg capsules. However, in this case, lectins may be restricting the spread of bacteria which might enter one egg in a batch. Therefore, although it is

tempting to infer that lectin activity may be fortuitous, with the molecules serving some function other than recognition, such inferences should not be readily accepted. The capacity of snail hemocytes to patch, cap, and internalize at least concanavalin A (Yoshino, 1981c, 1982) supports the contention that lectins are cooperative with hemocytes in at least some internal defense functions.

VII. Challenges for Future Research in Molluscan Immunobiology

A. Cooperation

Essentially no data are available on cellular communication in molluscan internal defenses. Even where distinctions of cell types have been possible (hyalinocytes and granulocytes), little is known of distinct functions. Communication may be important to such responses as inflammation and encapsulation, and may involve chemotactic factors. This absence of data is at least partially due to the generally restricted diversity in the morphologies of molluscan hemocytes. More needs to be known about possible functional subpopulations of molluscan hemocytes, and about possible humoral mediators of cell responses, analogs of vertebrate lymphokines.

B. Allogeneic Recognition

Whereas even poriferans and cnidarians react antagonistically with specificity and memory against allogeneic tissues, molluscs have failed to show any propensity for such activities. In this they resemble some arthropods (Lackie, 1981). Because nonself recognition with a fine discriminatory capacity is a property of molluscs, their evident failure to reject allografts is an anomaly. Is there an absence of a histocompatibility type of system (H-system; Hildemann et al., 1981), or do the allografts so far examined somehow suppress attack?

C. Altered Immune Status

At least in gastropods, both enhanced resistance and suppression of resistance have been demonstrated (Section IV,B). The operative mechanisms are completely unknown, and they will remain thus until we begin to understand immunologic recognition in molluscs. More model systems are needed in which to study such alterations. Marine molluscs and their parasites should provide suitable models. It is particularly probable that humoral mediators are important in these phenomena.

The suggestion (LoVerde et al., 1982) that an inbred strain of snail may react

against "self" may be taken to imply the possibility of autoimmune disease in molluscs.

D. Immunologic Memory

This, too, occurs in nearly all phyla so far appropriately examined. Yet, conclusive evidence is still needed for the Mollusca. In part, this gap is due to apparent failure of allogeneic rejection responses, which have provided convenient models for such research in other taxa. However, allogeneic rejection is not essential to answering questions dealing with immunologic memory (Bayne et al., 1980c). Other models need to be utilized to answer the question: Does immunologic memory occur in molluscs? If the answer is affirmative, as can be anticipated, then it behooves us to seek understanding of the responsible cells and molecules, and of underlying mechanisms.

E. Immunologic Recognition

Mechanisms of immunologic recognition provide perhaps the most fundamental questions in this area of concern. Brought into focus largely by Lackie (1981), Table VI outlines one possible scheme for the basis of immunologic recognition in molluscs and other invertebrates.

Recognition systems based on carbohydrate–lectin interactions are being discovered with increasing frequency and in diverse phyla. Even though it now appears that individual molluscs may have several different plasma lectins (Vretblad et al., 1979) and lectins may be associated with hemocyte surfaces (Vasta et al., 1982), this cannot satisfy all the needs of internal defense systems. Nonspecific recognition may be possible because of physicochemical properties, but specific recognition does exist (Section IV,B) and must entail an additional system of molecular recognizers. The finding (Yoshino, 1983) of anti-Thy-1 reactive determinants on the hemocyte of a snail is tantalizing, because partial molecular homology exists between Thy-1 and immunoglobulin. Does the lack of immunoglobulin in molluscs (and all nonchordates) mean that these animals are unable to recognize specifically antigenic determinants composed only of amino acids? If so, can it be shown that parasites and pathogens have exploited this in ways which are denied them in vertebrates, including man?

VIII. Summary

This chapter focuses on the cellular and humoral defense reactions which have been shown to occur both *in vivo* and *in vitro* in response to parasites and pathogens, to physical damage, and to soluble and particulate agents used by researchers to probe molluscan natural internal defenses. Omitted are the fields

TABLE VI

Possible Bases of Immunologic Recognition in Molluscs and Other Invertebrates

Recognizer Cell (RC)

The properties of the potential RC include:
1. A particular hydrophobic value
2. A particular surface charge
3. Lectins protruding from the cell surface
4. Possibly also receptors for humoral lectins

Recognition Mechanisms

I. Healthy self fails to be recognized because it
   A. Has hydrophobic and charge values which fall within compatible ranges which do not cause RC attraction by physicochemical means
   B. Lacks available sugars for which the putative lectins have recognition sites
II. Nonself and wounded self are recognized either
   A. Nonspecifically because they have hydrophobic or charge values which result in attraction of RCs due to physicochemical forces; the resulting close apposition stimulates the RC due to movements of components of the plasma membrane and/or the underlying cytoskeleton, or
   B. Specifically, because
      (i) Lectins on the RC bind to sugars on the surface, or
      (ii) Lectins in the fluid bind sugars on the surface and stimulate RC because
         (a) They locally exceed a critical concentration threshhold
         (b) There is altered molecular configuration (cf. Chorney and Cheng, 1980)
         (c) There is alteration of the surface properties so that they now lie outside the compatible range of hydrophobicity or charge

of general pathology caused by exotic agents such as ionizing radiation (Mix, 1972), toxicology of environmental pollutants (e.g., phenol, Fries and Tripp, 1970; benzpyrene, Anderson, 1978), and the metabolic effects of parasites on their molluscan hosts (Cheng, 1965).

Molluscs are capable of diverse, subtle, and complex responses to damage and to the invasion of their bodies by foreign agents. From a descriptive phase, the field of molluscan immunobiology is maturing into an experimental one. Studies of mechanisms of immune-type recognition and mechanisms of cell-mediated immune responses are beginning to provide an understanding of how molluscs maintain health in environments rich in potential parasites and pathogens. The dependence of effector cells on humoral and cell-bound recognition factors, and the dynamic interplay between hosts and parasites, imply complex immune systems with subtle effector mechanisms. The field is a rich one for studies on comparative immunobiology, with possible spin-offs for the control of pest and intermediate host populations and for the better management of desirable molluscan populations such as in aquaculture.

Acknowledgments

It is a pleasure to thank Drs. Eric S. Loker, K. J. Lie, L. Renwrantz, T. P. Yoshino, and Susan E. Ford for their constructive comments on the manuscript. They and others too numerous to mention have contributed to my enthusiasm for the subject, I thank them all.

References

Abdul-Salam, J. M., and Michelson, E. H. (1980a). *Biomphalaria glabrata* amoebocytes: Effect of *Schistosoma mansoni* infection on *in vitro* phagocytosis. *J. Invertebr. Pathol.* **35,** 241–248.

Abdul-Salam, J. M., and Michelson, E. H. (1980b). *Biomphalaria glabrata* amoebocytes: Assay for factors influencing *in vitro* phagocytosis. *J. Invertebr. Pathol.* **36,** 52–59.

Acton, R. T. (1970). Immunobiological and Immunochemical Studies of the Oyster *Crassostrea virginica*. Ph.D. Thesis, University of Alabama, Birmingham, Alabama.

Acton, R. T., and Evans, E. E. (1968). Bacteriophage clearance in the oyster *Crassostrea virginica*. *J. Bacteriol.* **96,** 1260–1266.

Acton, R. T., Evans, E. E., and Bennett, J. C. (1969). Immunobiological capabilities of the oyster *Crassostrea virginica*. *Comp. Biochem. Physiol.* **29,** 149–160.

Anderson, G. L. (1975). The effects of intertidal height and the parasitic crustacean *Fabia subquadrata* Dana on the nutrition and reproductive capacity of the California Sea Mussel, *Mytilus californianus* Conrad. *Veliger* **17,** 299–306.

Anderson, R. M., and May, R. M. (1979). Prevalence of schistosome infections within molluscan populations: observed patterns and theoretical predictions. *Parasitology* **79,** 63–94.

Anderson, R. S. (1978). Metabolism of an environmental carcinogen by invertebrate animals. *In* "Animal Models of Comparative and Developmental Aspects of Immunity and Disease" (M. E. Gerschwin and E. L. Cooper, eds.), pp. 25–36. Pergamon, Oxford, New York.

Anderson, R. S. (1981). Inducible hemolytic activity in *Mercenaria mercenaria* hemolymph. *Dev. Comp. Immunol.* **5,** 575–585.

Anderson, R. S., and Good, R. A. (1976). Opsonic involvement in phagocytosis by mollusk hemocytes. *J. Invertebr. Pathol.* **27,** 57–64.

Arcadi, J. A. (1968). Tissue response to the injection of charcoal into the pulmonate gastropod *Lehmania poirieri*. *J. Invertebr. Pathol.* **11,** 59–62.

Armstrong, D. A., Armstrong, J. L., Krassner, S. M., and Pauley, G. B. (1971). Experimental wound repair in the Black Abalone, *Haliotis cracherodii*. *J. Invertebr. Pathol.* **17,** 216–227.

Baker, R. A. (1976a). Tissue damage and leucocytic infiltration following attachment of the mite *Unionicola intermedia* to the gills of the bivalve mollusc *Anodonta anatina*. *J. Invertebr. Pathol.* **27,** 371–376.

Baker, R. A. (1976b). Cellular responses of the freshwater mussel *Anodonta anatina* produced by the mite *Unionicola intermedia*. *Parasitology* **73,** 33. (Abstract).

Baldo, B. A., Uhlenbruck, G., and Steinhausen, G. (1977). Invertebrate anti-galactans. A comparative study of agglutinins from the clam *Tridacna maxima*, the marine sponge *Axinella polypoides* and the anemone *Cerianthus membranaceus*. *Comp. Biochem. Physiol. A* **56,** 343–351.

Bang, F. B. (1961). Reaction to injury in the oyster (*Crassostrea virginica*). *Biol. Bull.* **121,** 57–68.

Basch, P. F. (1975). An interpretation of snail-trematode infection rates: specificity based on concordance of compatible phenotypes. *Int. J. Parasitol.* **5,** 449–452.

Basch, P. F. (1976). Parasitological Review. Intermediate Host Specificity in *Schistosoma mansoni*. *Exp. Parasitol.* **39,** 150–169.

Basch, P. F. (1979). *Biomphalaria* hemocyte migration and parasite encapsulation in implanted flat glass tubes. *J. Invertebr. Pathol.* **34,** 99–101.

Bayne, C. J. (1973a). Molluscan internal defense mechanism: The fate of C^{14}-labelled bacteria in the land snail *Helix pomatia* (L.). *J. Comp. Physiol.* **86,** 17–25.

Bayne, C. J. (1973b). Internal defense mechanisms of *Octopus dofleini*. *Malacological Review* **6,** 13–17.

Bayne, C. J. (1974). On the immediate fate of bacteria in the land snail *Helix*. *In* "Contemporary Topics in Immunobiology, Invertebrate Immunology" (E. L. Cooper, ed.), Vol. 4, pp. 37–45. Plenum, New York/London.

Bayne, C. J. (1977). Molluscan immunobiology: The elevation of responses. *In* "Developmental Immunobiology" (J. B. Solomon and J. D. Horton, eds.), pp. 67–74. Elsevier/North Holland, Amsterdam.

Bayne, C. J. (1980a). Molluscan immunity: Induction of elevated immunity in the land snail (*Helix*) by injections of bacteria (*Pseudomonas aeruginosa*) *Dev. Comp. Immunol.* **4,** 43–54.

Bayne, C. J. (1980b). Molluscan immunity: interactions between the immunogenic bacterium *Pseudomonas aeruginosa* and the internal defense system of the snail *Helix pomatia*. *Dev. Comp. Immunol.* **4,** 215–222.

Bayne, C. J. (1980c). Humoral factors in molluscan parasite immunity. *In* "Aspects of Developmental and Comparative Immunology" (J. B. Solomon, ed.), Vol. 1, pp. 113–124. Pergamon, Oxford and New York.

Bayne, C. J. (1981a). Gastropod cells *in vitro*. *In* "Advances in Cell Culture". (K. Maramorosch ed.), Vol. 1, pp. 297–334. Academic Press, New York.

Bayne, C. J. (1982a). Recognition and killing of metazoan parasites, particularly in molluscan hosts. *In* "Developmental Immunology: Clinical Problems and Aging" (E. L. Cooper and M. A. Brazier, eds.), pp. 109–114. Academic Press, New York.

Bayne, C. J. (1982b). Molluscan immunobiology: Isolation of an *Aeromonas formicans* which escapes the internal defense system of *Helix pomatia*. *Dev. Comp. Immunol.* **6,** 675–682.

Bayne, C. J., and Kime, J. B. (1970). *In vivo* removal of bacteria from the hemolymph of the land snail *Helix pomatia* (Pulmonata: Stylommatophora). *Malacological Review* **3,** 103–113.

Bayne, C. J., Moore, M. N., Carefoot, T. H., and Thompson, R. J. (1979). Hemolymph functions in *Mytilus californianus:* The cytochemistry of hemocytes and their responses to foreign implants and hemolymph factors in phagocytosis. *J. Invertebr. Pathol.* **34,** 1–20.

Bayne, C. J., Buckley, P. M., and DeWan, P. C. (1980a). Macrophage-like hemocytes of resistant *Biomphalaria glabrata* are cytotoxic for sporocysts of *Schistosoma mansoni in vitro*. *J. Parasitol.* **66,** 413–419.

Bayne, C. J., Buckley, P. M., and DeWan, P. C. (1980b). *Schistosoma mansoni:* Cytotoxicity of hemocytes from susceptible snail hosts for sporocysts in plasma from resistant *Biomphalaria glabrata*. *Exp. Parasitol.* **50,** 409–416.

Bayne, C. J., Sminia, T., and van der Knaap, W. P. W. (1980c). Immunological memory: Status of molluscan studies. *In* "Phylogeny of Immunological Memory" (M. J. Manning, ed.), pp. 57–64. Elsevier/North Holland Biomedical Press, Amsterdam/New York/Oxford.

Becker, W. (1980). Metabolic interrelationship of parasitic trematodes and Mollusca, especially *Schistosoma mansoni* in *Biomphalaria glabrata*. *Z. Parasitenkd.* **63,** 101–111.

Benex, J., and Jacobelli, G. (1980). Exploration de l'immunité cellulaire chez les mollusques hôtes des schistosomes. Premiers résultats relatifs à *Biomphalaria glabrata*. *Bull. Soc. Pathol. Exot.* **73,** 206–213.

Benex, J., and Lamy, L. (1959). Immobilisation des miracidiums de *Schistosoma mansoni* par des extraits de planorbes. *Bull. Soc. Path. Exot.* **5,** 188–193.

Berry, E. G. (1949). A recently observed snail disease. *Ann. Rep. Am. Malacol. Un. News Bull.* pp. 10–11.

Blogoslawski, W. J., and Stewart, M. E. (1978). Paralytic shellfish poison in *Spisula solidissima:* Anatomical location and ozone detoxification. *Mar. Biol.* **45,** 261–264.

Boray, J. A. (1966). Studies on the relative susceptibility of some lymnaeids to infection with *Fasciola hepatica* and *Fasciola gigantica* and on the adaptation of *Fasciola* species. *Ann. Trop. Med. Parasitol.* **60,** 114–124.

Boyden, S. V. (1966). Natural antibodies and the immune response. *Adv. Immunol.* **5,** 1–28.

Bretting, H., and Konigsmann, K. (1979). Investigations on the lectin-producing cells in the sponge *Axinella polypoides* (Schmidt). *Cell Tiss. Res.* **201,** 487–497.

Brisson, P. (1971). Embryologie expérimentale—Développement d'embryons avancés de *Bulinus* (Gastéropodes Pulmonés) implantés chez des sujets matures. *C. R. Acad. Sci. Paris* **272,** 3199–3201.

Britton, J. C., Barcellona, W. J., Hagan, J., and LaGrone, M. L. (1980). Ctenidial autotomy in *Corbicula fluminea* in response to massive granulomas. *Science* **212,** 551–553.

Brooks, W. M. (1968). Tetrahymenid ciliates as parasites of the gray garden slug. *Hilgardia* **38,** 205–276.

Brown, A. C. (1967). Elimination of foreign particles by the snail, *Helix aspersa. Nature* **213,** 1154–1155.

Brown, A. C., and Brown, R. J. (1965). The fate of thorium dioxide injected into the pedal sinus of *Bullia* (Gastropoda: Prosobranchiata). *J. Exp. Biol.* **42,** 509–519.

Browning, J. (1979). *Octopus* microvasculature: Permeability to ferritin and carbon. *Tissue & Cell* **2,** 371–383.

Bubel, A., Moore, M. N., and Lowe, D. (1977). Cellular responses to shell damage in *Mytilus edulis* L. *J. Exp. Mar. Biol. Ecol.* **30,** 1–27.

Buss, L. W., and Jackson, J. B. C. (1979). Competitive networks: Nontransitive competitive relationships in cryptic coral reef environments. *Am. Nat.* **113,** 223–234.

Canning, E. U., and Basch, P. F. (1968). *Perezia helminthorum* sp. nov., a microsporidian hyperparasite of trematode larvae from Malaysian snails. *Parasitology* **58,** 341–347.

Canzonier, W. J. (1974). Tissue grafts in the American oyster, *Crassostrea virginica. Proc. Nat. Shellfish. Assoc.* **64,** 92–101.

Cappucci, D. T., Jr. (1978). Nematodes in the alimentary canal of terrestrial slugs. *Veliger* **21,** 306–307.

Carter, O. S., and Bogitsh, B. J. (1975). Histologic and cytochemical observations of the effects of *Schistosoma mansoni* on *Biomphalaria glabrata. Ann. N.Y. Acad. Sci.* **266,** 380–393.

Cazal, P., and Bogaraze, D. (1943). *Bull. Inst. Océanogr. Monaco.* No. 842.

Cheng, T. C. (1965). Histochemical observations on changes in the lipid composition of the American oyster, *Crassostrea virginica* (Gmelin), parasitized by the trematode *Bucephalus* sp. *J. Invertebr. Pathol.* **7,** 398–407.

Cheng, T. C. (1968). The compatibility and incompatibility concept as related to trematodes and molluscs. *Pac. Sci.* **22,** 141–160.

Cheng, T. C. (1969). An electrophoretic analysis of hemolymph proteins of *Helisoma duryi normale* experimentally challenged with bacteria. *J. Invertebr. Pathol.* **14,** 60–81.

Cheng, T. C. (1974). Electron microscope studies on reactions in molluscs to helminths. *Proc. Int. Congress Parasitol. 3rd,* Munich, Germany **3,** (G7), 1707–1708.

Cheng, T. C. (1976). Identification of proliferative lesions in mollusks. *Mar. Fish. Rev.* **38,** 5–6.

Cheng, T. C. (1978). The role of lysosomal hydrolases in molluscan cellular response to immunologic challenge. *Comp. Pathol.* **4,** 59–71.

Cheng, T. C. (1981). Bivalves. *In* "Invertebrate Blood Cells" (N. A. Ratcliffe and A. F. Rowley, eds.), pp. 641. Academic Press, New York.

Cheng, T. C., and Auld, K. R. (1977). Hemocytes of the pulmonate gastropod *Biomphalaria glabrata. J. Invertebr. Pathol.* **30,** 119–122.

Cheng, T. C., and Burton, R. W. (1965). Relationships between *Bucephalus* sp. and *Crassostrea virginica:* Histopathology and sites of infection. *Chesapeake Sci.* **6,** 3–16.

Cheng, T. C., and Butler, M. S. (1979). Experimentally induced elevations in acid phosphatase activity in hemolymph of *Biomphalaria glabrata* (Mollusca). *J. Invertebr. Pathol.* **34,** 119–124.

Cheng, T. C., and Howland, K. H. (1979). Chemotactic attraction between hemocytes of the oyster, *Crassostrea virginica,* and bacteria. *J. Invertebr. Pathol.* **33,** 204–210.

Cheng, T. C., and Galloway, P. C. (1970). Transplantation immunity in mollusks: The histoincompatibility of *Helisoma duryi normale* with allografts and xenografts. *J. Invertebr. Pathol.* **15,** 177–192.

Cheng, T. C., and Garrabrant, T. A. (1977). Acid phosphatase in granulocytic capsules formed in strains of *Biomphalaria glabrata* totally and partially resistant to *Schistosoma mansoni. Int. J. Parasitol.* **7,** 467–472.

Cheng, T. C., and Howland, K. H. (1982). Effects of colchicine and cytochalasin B on chemotaxis of oyster (*Crassostrea virginica*) hemocytes. *J. Invertebr. Pathol.* **40,** 150–152.

Cheng, T. C., and Rifkin, E. (1970). Cellular reactions in marine molluscs in response to helminth parasitism. *In* "A Symposium on Diseases of Fishes and Shellfishes" (S. F. Snieszko, ed.), pp. 443–496. *Am. Fish. Soc. Spec. Pub.* **5,** Washington, D.C.

Cheng, T. C., and Rudo, B. M. (1976a). Chemotactic attraction of *Crassostrea virginica* hemolymph cells to *Staphylococcus lactus. J. Invertebr. Pathol.* **27,** 137–139.

Cheng, T. C., and Rudo, B. M. (1976b). Distribution of glycogen resulting from degradation of ^{14}C-labelled bacteria in the American oyster, *Crassostrea virginica. J. Invertebr. Pathol.* **27,** 259–262.

Cheng, T. C., Thakur, A. S., and Rifkin, E. (1969). Phagocytosis as an internal defense mechanism in the Mollusca: With an experimental study of the role of leucocytes in the removal of ink particles in *Littorina scabra* Linn. *In* "Symposium on Mollusca", pp. 547–566. *Mar. Biol. Assoc. India,* Bangalore.

Cheng, T. C., Chorney, M. J., and Yoshino, T. P. (1977). Lysozymelike activity in the hemolymph of *Biomphalaria glabrata* challenged with bacteria. *J. Invertebr. Pathol.* **29,** 170–174.

Cheng, T. C., Lie, K. J., Heyneman, D., and Richards, C. S. (1978a). Elevation of aminopeptidase activity in *Biomphalaria glabrata* (Mollusca) parasitized by *Echinostoma lindoense* (Trematoda). *J. Invertebr. Pathol.* **31,** 57–62.

Cheng, T. C., Guida, V. G., and Gerhart, P. L. (1978b). Aminopeptidase and lysozyme activity levels and serum protein concentrations in *Biomphalaria glabrata* (Mollusca) challenged with bacteria. *J. Invertebr. Pathol.* **32,** 297–302.

Chernin, E. (1962). The unusual life-history of *Daubaylia potomaca* (Nematoda: Cephalobidae) in *Australorbis glabratus* and in certain other fresh-water snails. *Parasitology* **52,** 459–481.

Chernin, E., Michelson, E. H., and Augustine, D. L. (1960). *Daubaylia potomaca,* a nematode parasite of *Helisoma trivolvis,* transmissible to *Australorbis glabratus. J. Parasitol.* **46,** 599–605.

Chitwood, B. G., and Chitwood, M. B. (1937). Snails as hosts and carriers of nematodes and Nematomorpha. *Nautilus* **50,** 130–135.

Chorney, M. H., and Cheng, T. C. (1980). Discrimination of self and non-self in invertebrates. In "Contemporary Topics in Immunobiology" (J. J. Marchalonis and N. Cohen, eds.), pp. 37–54. Plenum, New York/London.

Cole, R. M., Richards, C. S., and Popkin, T. J. (1977). Novel bacterium infecting an African snail. *J. Bacteriol.* **132,** 950–956.

Colwell, R. R., and Sparks, A. K. (1967). Properties of *Pseudomonas enalia,* a marine bacterium pathogenic for the invertebrate *Crassostrea gigas* (Thunberg). *App. Microbiol.* **15,** 980–986.

Comps, M., and Masso, R.-M. (1978). Studies using fluorescent microscopy to investigate the

viruses of the portugese oyster. *Int. Colloq. Invertebr. Pathol.* **9,** 138. Ann. Mtg. Soc. Invertebr. Pathol., Prague. (Abstract).

Comps, M., and Deltreil, J.-P. (1979). Pathologie des invertebrés. Un microorganisme de type rickettsien chez l'Huitre portugaise *Crassostrea angulata* Lmk. *C. R. Acad. Sc. Paris* **289,** 169–171.

Comps, M., Bonami, J.-R., Vago, C., and Campillo, A. (1976). Pathologie des invertebrés. Une virose de l'huitre portugaise (*Crassostrea angulata,* Lmk). *C. R. Acad. Sci. Paris, D* **282,** 1991–1993.

Comps, M., Bonami, J.-R., and Vago, C. (1977). Pathologie des invertebrés. Mise en evidence d'une infection rickettsienne chez les Huitres. *C. R. Acad. Sci. Paris, D* **285,** 427–429.

Cooper, K. R., Brown, R. S., and Chang, P. W. (1982a). The course and mortality of a hematopoietic neoplasm in the soft-shell clam, *Mya arenaria. J. Invert. Pathol.* **39,** 149–157.

Cooper, K. R., Brown, R. S., and Chang, P. W. (1982b). Accuracy of blood cytological screening techniques for the diagnosis of a possible hematopoietic neoplasm in the bivalve mollusc, *Mya arenaria. J. Invertebr. Pathol.* **39,** 281–289.

Cooper-Willis, C. A. (1979). Changes in the acid phosphatase levels in the haemocytes and haemolymph of *Patella vulgata* after challenge with bacteria. *Comp. Biochem. Physiol. A* **63,** 627–631.

Cort, W. W., Oliver, L., and Bracknett, S. (1941). The relation of physid and planorbid snails in the life cycle of the strigeoid trematode *Cotylurus flabelliformis* (Faust, 1917). *J. Parasitol.* **27,** 437–448.

Cort, W. W., Hussey, K. L., and Ameel, D. J. (1960). Studies on a microsporidian hyperparasite of strigeoid trematodes. 1. Prevalence and effect on the parasitized larval trematodes. *J. Parasitol.* **46,** 317–326.

Cowper, S. (1946). Some notes on the maintenance and breeding of schistosome vectors in Great Britain, with special reference to *Planorbis guadaloupensis* Sowerby. *Ann. Trop. Med. Parasitol.* **40,** 163–170.

Crichton, R., and Lafferty, K. J. (1975). The discriminatory capacity of phagocytic cells in the chiton (*Liolophura gaimardi*). *In* "Immunologic Phylogeny" *Adv. Exp. Med. Biol.* **64,** (W. H. Hildemann and A. A. Benedict, eds.), pp. 89–98. Plenum, New York.

Crichton, R., Killby, V. A. A., and Lafferty, K. J. (1973). The distribution and morphology of phagocytic cells in the chiton *Liolophura gaimardi. Aust. J. Exp. Biol. Med. Sci.* **51,** 357–372.

Cuénot, L. (1914). Les organes phagocytaires des mollusques. *Arch. Zool. Exp. Gen.* **54,** 267–305.

Curtis, S. K., and Cowden, R. R. (1978). Responsiveness of the slug (*Limax maximus*) to injections of fluorescein- and rhodamine-conjugated immunogens. *Dev. Comp. Immunol.* **4,** 727–733.

Cushing, J. E., Evans, E. E., and Evans, M. L. (1971). Induced bactericidal responses of abalones. *J. Invertebr. Pathol.* **17,** 446–448.

Dales, R. P. (1979). Defence of invertebrates against bacterial infection. *J. R. Soc. Med.* **72,** 688–696.

Damian, R. T. (1979). Molecular mimicry in biological adaptation. *In* "Host-parasite Interfaces" (B. B. Nichol, ed.), pp. 103–126. Academic Press, New York.

Davies, P. S., and Partridge, T. (1972). Limpet haemocytes. Studies on aggregation and spike formation, Vol. 1. *J. Cell Sci.* **11,** 757–769.

David, W. A. L., Taylor, C. E., and Atkey, P. T. (1977). Nonoccluded virus-like particles in the mollusc *Agriolimax reticulatus* (Stylommatophora: Limacinae). *J. Invertebr. Pathol.* **29,** 242–243.

Dean, W. W., Mead, A. R., and Northey, W. T. (1970). *Aeromonas liquefaciens* in the Giant African Snail, *Achatina fulica. J. Invertebr. Pathol.* **16,** 346–354.

DesVoigne, D. M., and Sparks, A. K. (1968). The process of wound healing in the Pacific oyster *Crassostrea gigas* (Thunberg). *J. Invertebr. Pathol.* **12,** 53–65.

DesVoigne, D. M., and Sparks, A. K. (1969). The reaction of the Pacific oyster, *Crassostrea gigas,* to homologous tissue implants. *J. Invertebr. Pathol.* **14,** 293–300.

Diaz, E. (1955). Isolamento e selecai de microorganismos de Planorbideos utilizaveis em ensaios de luta biologica contra estes Invertebrados. *O. Hospital* **47,** 9–16.

Douglass, W. R. (1976). Host response to infection with *Bucephalus* in *Crassostrea virginica. Proc. Nat. Shellfish. Assoc.* **65,** 1. (Abstract).

Douglass, W. R., and Haskin, H. H. (1976). Oyster-MSX interactions; Alterations in hemolymph enzyme activities in *Crassostrea virginica* during the course of *Michinia nelsoni* disease development. *J. Invertebr. Pathol.* **27,** 317–323.

Ducklow, H. W., Boyle, P. J., Maugel, P. W., Strong, C., and Mitchell, R. (1979). Bacterial flora of the schistosome vector snail *Biomphalaria glabrata. Appl. Environ. Microbiol.* **38,** 667–672.

Dupouy, J., Martinez, J. C., and Gallien, M. L. (1973). Action de *Proctoeces maculatus* (Looss 1901) (Trematoda, Fellodistomatidae) sur de le developpement des gonades chez *Mytilus galloprovincialis* Lmk. *C. R. Acad. Sci. Paris, D* **277,** 1889–1892.

Elston, R. (1979). Viruslike particles associated with lesions in larval pacific oysters (*Crassostrea gigas*). *J. Invertebr. Pathol.* **33,** 71–74.

Eng, L. L. (1976). A note on the occurrence of a symbiotic oligochaete, *Chaetogaster limnaei,* in the mantle cavity of the Asiatic Clam, *Corbicula manilensis. Veliger* **19,** 208.

Farley, C. A. (1975). Epizootic and enzootic aspects of *Minchinia nelsoni* (Haplosporida) disease in Maryland oysters. *J. Protozool.* **22,** 418–427.

Faulk, W. P., Lim, H. K., Jeong, K. H., Heyneman, D., and Price, D. (1973). An approach to the study of immunity in invertebrates. *In* "Non-specific factors influencing host resistance" (W. Braun and J. Ungar, eds.), pp. 24–32. Karger, Basel, Switzerland.

Feng, J. S. (1966). The fate of a virus, *Staphylococcus aureus* Phage 80, injected into the oyster, *Crassostrea virginica. J. Invertebr. Pathol.* **8,** 496–504.

Feng, S. Y. (1958). Observations on the distribution and elimination of spores of *Nematopsis ostrearum* in oysters. *Proc. Nat. Shellfish. Assoc.* **48,** 162–173.

Feng, S. Y. (1965). Pinocytosis of proteins by oyster leucocytes. *Biol. Bull.* **129,** 95–105.

Feng, S. Y. (1966). Experimental bacterial infections in the oyster *Crassostrea virginica. J. Invertebr. Pathol.* **8,** 505–511.

Feng, S. Y., and Canzonier, W. J. (1970). Humoral responses in the American oyster (*Crassostrea virginica*) infected with *Bucephalus* sp. and *Minchinia nelsoni. In* "A Symposium on Diseases of Fishes and Shellfishes" (S. F. Snieszko, ed.), pp. 497–510. *Am. Fish. Soc., Spec. Pub.* **5,** Washington, D.C.

Filho, O. C., and Diaz, E. (1953). Correspondence. *Trans. R. Soc. Trop. Med. Hyg.* **47,** 581–582.

Font, W. W. (1980). Effects of hemolymph of the American oyster, *Crassostrea virginica,* on marine cercariae. *J. Invertebr. Pathol.* **36,** 41–47.

Ford, S. E., and Haskin, H. H. (1982). History and epizootiology of *Haplosporidium nelsoni* (MSX), an oyster pathogen in Delaware Bay, 1957–1980. *J. Invertebr. Pathol.* **40,** 118–141.

Francis, L. (1973). Intraspecific aggression and its effect on the distribution of *Anthopleura elegantissima* and some related sea anemones. *Biol. Bull.* **144,** 73–92.

Frandsen, F. (1979). Discussion of the relationships between *Schistosoma* and their intermediate hosts; assessment of the degree of host-parasite compatibility and evaluation of schistosome taxonomy. *Z. Parasitenkd.* **58,** 275–296.

Fries, C. R., and Tripp, M. R. (1970). Uptake of viral particles by oyster leukocytes *in vitro. J. Invertebr. Pathol.* **15,** 136–137.

Fritsche, T. C., and Gilbertson, D. E. (1981). Development of *Cotylurus flabelliformis* metacer-

cariae in planorbid and lymnaeid snails and effects on intermediate host mortality. 56th Ann. Mtg. Am. Soc. Parasitol. (Abstract).

Froesch, D. (1979). Antigen-induced secretion in the optic gland of *Octopus vulgaris*. *Proc. R. Soc. Lond. B* **205,** 309–384.

Froesch, D., and Mangold, K. (1976). Uptake of ferritin by the cephalopod optic gland. *Cell Tiss. Res.* **170,** 549–551.

Gilbertson, D. E., and Etges, F. J. (1967). Haemagglutinins in the haemolymph of planorbid snails. *Ann. Trop. Med. Parasitol.* **61,** 144–147.

Golvan, M. Y. J., and Mougeot, G. (1973). Mis en évidence des propriétés cytoadhérentes des cellules de l'hémolymphe de *Biomphalaria glabrata* parasité par *Schistosoma mansoni*. *C. R. Acad. Sci. Paris D* **276,** 1229–1231.

Gorokhov, V. V. (1978). The pathology of molluscs caused by microorganisms. *Int. Colloq. Invertebr. Pathol.* **11,** 45. Ann. Mtg. Soc. Invertebr. Pathol., Prague. (Abstract.)

Gutierrez, M. (1977). Datos sobre parasitologia e histopatologia del ostion, *Crassostrea angulata* Lmk. de la costa sudatlantica de Espana. *Invest. Pesq.* **41**(3), 543–567.

Guiterrez, P. M., and Pascual, E. (1976). Sur une maladie épidemique parasitaire de la glande digestive de *Crassostrea angulata* avec mortalité anormale le long de la côte sud-atlantique d'Espagne. *Haliotis* **5,** 81–84.

Hammarstrom, S., and Kabat, E. A. (1969). Purification and characterization of a blood group A reactive hemagglutinin from the snail *Helix pomatia* and a study of its combining site. *Biochemistry* **8,** 2686–2705.

Hardy, S. W., Fletcher, T. C., and Gerrie, L. M. (1976). Factors in haemolymph of the mussel, *Mytilus edulis* L., of possible significance as defence mechanisms. *Trans. Biochem. Soc.* **4,** 473–475.

Hardy, S. W., Grant, P. T., and Fletcher, T. C. (1977a). A haemagglutinin in the tissue fluid of the Pacific oyster, *Crassostrea gigas,* with specificity for sialic acid residues in glycoproteins. *Experientia* **33,** 767–769.

Hardy, S. W., Fletcher, T. C., and Olafsen, J. A. (1977b). Aspects of cellular and humoral defense mechanisms in the Pacific oyster, *Crassostrea gigas*. *In* "Developmental Immunobiology" (J. B. Solomon and J. D. Horton, eds.), pp. 59–66. Elsevier/North Holland, Amsterdam.

Hardy, S. W., Thomson, A. W., and Fletcher, T. C. (1978). Effect of haemolymph and agglutinins from the Pacific oyster (*Crassostrea gigas*) on cultured human and rabbit lymphocytes. *Comp. Biochem. Physiol A* **60,** 473–477.

Harm, H., and Renwrantz, L. (1980). The inhibition of serum opsonins by a carbohydrate and the opsonizing effect of purified agglutinin on the clearance of nonself particles from the circulation of *Helix pomatia*. *J. Invertebr. Pathol.* **36,** 64–70.

Harris, K. R. (1974). "Studies on encapsulation in *Biomphalaria glabrata*." Ph.D. dissertation, Lehigh University, Bethelehem, Pennsylvania.

Harris, K. R. (1975). The fine structure of encapsulation in *Biomphalaria glabrata*. *Ann. N.Y. Acad. Sci.* **266,** 446–464.

Harris, K. R., and Cheng, T. C. (1975a). The encapsulation process in *Biomphalaria glabrata* experimentally infected with the metastrongylid *Angiostrongylus cantonensis:* light microscopy. *Int. J. Parasitol.* **5,** 521–528.

Harris, K. R., and Cheng, T. C. (1975b). The encapsulation process in *Biomphalaria glabrata* experimentally infected with metastrongylid *Angiostrongylus cantonensis:* enzyme histochemistry. *J. Invertebr. Pathol.* **26,** 367–374.

Harshbarger, J. C. (1976). Descriptions of polyps and epidermal papillomas in three bivalve mollusk species. *Mar. Fish. Rev.* **38,** 25–29.

Harshbarger, J. C., and Chang, S. C. (1976). Chlamydiae (with Phages), Mycoplasmas, and Rickettsiae in Chesapeake Bay bivalves. *Science* **196,** 666–668.

Heyneman, D., Faulk, W. P., and Fundenberg, H. H. (1971). *Echinostoma lindoense:* Larval antigens from the snail intermediate host, *Biomphalaria glabrata. Exp. Parasitol.* **29,** 480–492.

Hildemann, W. H., and Johnson, I. S. (1979). Immunocompetence in the lowest metazoan phylum: Transplantation immunity in sponges. *Science* **204,** 420–422.

Hildemann, W. H., Dix, T. G., and Collins, J. D. (1974). Tissue transplantation in diverse marine invertebrates. *In* "Contemporary Topics in Immunobiology, Invertebrate Immunology" (E. L. Cooper, ed.), Vol. 4, pp. 141–150. Plenum, New York/London.

Hildemann, W. H., Raison, R. L., Cheung, G., Hull, C. J., Akaka, L., and Okamoto, J. (1977). Immunological specificity and memory in a scleractinian coral. *Nature* **270,** 219–223.

Hildemann, W. H., Clark, E. A., and Raison, R. L. (1981). "Comprehensive immunogenetics." Elsevier, New York.

Hillman, R. E. (1963). An observation of the occurrence of mitosis in regenerating mantle epithelium of the eastern oyster, *Crassostrea virginica. Chesapeake Sci.* **4,** 172–174.

Hillman, R. E. (1979). Encystment of the ciliate *Boveria teredinidi* in the tissues of the molluscan woodborer *Bankia gouldi* in Barnegat Bay, New Jersey. *J. Invertebr. Pathol.* **34,** 78–83.

Hopkins, S. H. (1957). Our present knowledge of the oyster parasite "*Bucephalus*". *Proc. Nat. Shellfish. Assoc.* **47,** 58–61.

Hosaka, Y., and Berry, E. G. (1975). Schistosome miracidial immobilization caused by tissue extracts prepared from various species or strains of snails. *Jap. J. Parasitol.* **24,** 318–331.

Howland, K. H., and Cheng, T. C. (1982). Identification of bacterial chemoattractants for oyste (*Crassostrea virginica*) hemocytes. *J. Invertebr. Pathol.* **39,** 123–132.

Hubbard, A. L., Wilson, G., Ashwell, G., and Stukenbrok, H. (1979). An electron microscope autoradiographic study of the carbohydrate recognition systems in rat liver. *J. Cell Biol.* **83,** 47–64.

Huehner, M. K., and Etges, F. J. (1981). Encapsulation of *Aspidogaster conchicola* (Trematoda: Aspidogastrea) by Unionid mussels. *J. Invertebr. Pathol.* **37,** 123–128.

Jackson, T. F. H. G. (1976). Intermediate host antigens associated with the cercariae of *Schistosoma haematobium. J. Helminthol.* **50,** 45–47.

Jacquemain, R., Jullien, A., and Noel, R. (1947). Sur l'action de certains corps cancerigenes chez les Cephalopodes. *C. R. Acad. Sci. Paris.* **225,** 441–443.

Jenkin, C. R., and Rowley, D. (1970). Immunity in invertebrates. The purification of a haemagglutinin to rat and rabbit erythrocytes from the hemolymph of the Murray mussel (*Velesunio ambiguus*). *Aust. J. Exp. Biol. Med. Sci.* **48,** 129–137.

Jeong, K. H., and Heyneman, D. (1976). Leukocytes of *Biomphalaria glabrata:* morphology and behavior of granulocytic cells *in vitro. J. Invertebr. Pathol.* **28,** 357–362.

Jeong, K. H., Lie, K. H., and Heyneman, D. (1980). Leucocytosis in *Biomphalaria glabrata* sensitized and resensitized to *Echinostoma lindoense. J. Invertebr. Pathol.* **35,** 9–13.

Jeong, K. H., Sussman, S., Rosen, S. D., Lie, K. H., and Heyneman, D. (1981). Distribution and variation of hemagglutinating activity in the hemolymph *Biomphalaria glabrata. J. Invertebr. Pathol.* **38,** 256–263.

Johnson, P. T., and Chapman, F. A. (1970). Comparative studies on the *in vitro* response of bacteria to invertebrate body fluids. 2. *Aplysia californica* (sea hare) and *Ciona intestinalis* (tunicate). *J. Invertebr. Pathol.* **16,** 259–267.

Jones, J. B. (1981). A new microsporidium from the oyster *Ostrea lutaria* in New Zealand. *J. Invertebr. Pathol.* **38,** 67–70.

Jones, G. E., Gillett, R., and Partridge, T. (1976). Rapid modification of the morphology of cell contact sites during the aggregation of limpet haemocytes. *J. Cell Sci.* **22,** 21–33.

Jullien, A., Cardot, J., Ripplinger, J., and Claudey, D. (1956). Réactions inflammatoires provo-

quées expérimentalement dans le foie des Cephalopodes decapodes. *C. R. Acad. Sci. Paris* **243,** 456–458.

Kassim, O. O., and Richards, C. S. (1978a). *Schistosoma mansoni:* Lysozyme activity in *Biomphalaria glabrata* during infection with two strains. *Exp. Parasitol.* **46,** 213–217.

Kassim, O. O., and Richards, C. S. (1978b). *Biomphalaria glabrata:* Lysozyme activities in the hemolymph, digestive gland, and headfoot of the intermediate host of *Schistosoma mansoni*. *Exp. Parasitol.* **46,** 218–224.

Kassim, O. O., and Richards, C. S. (1979). Host reactions in *Biomphalaria glabrata* to *Schistosoma mansoni* miracidia, involving variations in parasite strains, numbers and sequence of exposures. *Int. J. Parasitol.* **9,** 565–570.

Katkansky, S. C., Sparks, A. K., and Chew, K. K. (1967). Distribution and effects of the endoparasitic copepod, *Mytilicola orientalis,* on the Pacific oyster, *Crassostrea gigas,* on the Pacific Coast. *Proc. Nat. Shellfish. Assoc.* **57,** 50–58.

Kendall, S. B. (1970). Relationships between the species of *Fasciola* and their molluscan hosts. *Adv. Parasitol.* **8,** 251–258.

Khalap, S., Thompson, T. E., and Gold, E. R. (1970). Haemagglutination and haemagglutination-inhibition reactions of extracts from snails and sponges. *Vox Sang.* **18,** 501–526.

Killby, V. A., Crichton, R., and Lafferty, K. J. (1973). Fine structure of the phagocytic cells in the chiton, *Liolophura gaimardi. Aust. J. Exp. Biol. Med. Sci.* **51,** 373–391.

Kinoti, G. K. (1971). Observations on the infection of bulinid snails with *Schistosoma mattheei. Parasitology* **62,** 161–170.

Kluhspies, G. (1979). Aquired resistance of *Lymnaea stagnalis* against miracidia-invasion. *Zentralbl. Bakteriol. Hyg., Abt. 1. Abt. Ref.* **263,** 205 (Abstract).

Kole, M. (1979). Electron microscopical observations on defense mechanisms against daughter sporocysts in a prosobranch gastropod. *Zentralbl. Bakteriol. Hyg., Abt. 1. Abt. Ref.* **263,** 206. (Abstract).

Koszinowsky, U. H., and Kramer, M. (1981). Selective inhibition of T suppressor-cell function by a monosaccharide. *Nature* **289,** 181–184.

Kowalevsky, A. (1894). Etudes expérimentales sur les glandes lymphatiques des invertebrés (communication preliminaire). Mélanges biologiques. *Bull. Acad. Imp. Sci. St. Petersbourg* **13,** 437.

Kress, A. (1968). Untersuchungen zur Histologie, Autotomie und Regeneration dreier Doto-Arten *Doto coronata, D. pinatifida, D. fragilis* (Gastropoda: Opisthobranchiata). *Rev. Suisse Zool.* **75,** 235–303.

Krupa, P. L., and Lewis, L. M. (1977). Differential surface coat staining of snail hemocytes interacting with trematode parasites. *Biol. Bull.* **153,** 433. (Abstract).

Krupa, P. L., Lewis, L. M., and Del Vecchio, P. (1977). *Schistosoma haematobium* in *Bulinus guernei:* electron microscopy of hemocyte-sporocyst interactions. *J. Invertebr. Pathol.* **30,** 35–45.

Kuris, A. M., and Warren, J. (1980). Echinostome cercarial penetration and metacercarial encystment as mortality factors for a second intermediate host, *Biomphalaria glabrata. J. Parasitol.* **66,** 630–635.

Lackie, A. M. (1981). Immune recognition in insects. *Dev. Comp. Immunol.* **5,** 191–204.

Lange, M. M. (1920). On the regeneration and finer structure of the arms of the cephalopods. *J. Exp. Zool.* **31,** 1–58.

Lester, R. J. G., and Davis, G. H. G. (1981). A new *Perkinsus* species (Apicomplexa, Perkinsea) from the Abalone *Haliotis ruber. J. Invertebr. Pathol.* **37,** 181–187.

Li, C. P. (1960). Antimicrobial activity of certain marine fauna. *Proc. Soc. Exp. Biol. Med.* **104,** 366–368.

Li, M. F., and Clyburne, S. (1979). Mortalities of Blue Mussel (*Mytilus edulis*) in Prince Edward Island. *J. Invertebr. Pathol.* **33,** 108–110.

Lie, K. J., and Heyneman, D. (1976a). Studies on resistance in snails. 3. Tissue reactions to *Echinostoma lindoense* sporocysts in sensitized and resensitized *Biomphalaria glabrata*. *J. Parasitol.* **62,** 51–58.

Lie, K. J., and Heyneman, D. (1976b). Studies on resistance in snails. 6. Escape of *Echinostoma lindoense* sporocysts from encapsulation in the snail heart and subsequent loss of the host's ability to resist infection by the same parasite. *J. Parasitol.* **62,** 298–302.

Lie, K. J., and Heyneman, D. (1977). *Schistosoma mansoni, Echinostoma lindoense,* and *Paryphostomum segregatum:* Interference by trematode larvae with acquired resistance in snails, *Biomphalaria glabrata*. *Exp. Parasitol.* **42,** 343–347.

Lie, K. J., and Heyneman, D. (1978a). Destruction of *Schistosoma mansoni* in snails with self-cure. *Int. Cong. Parasitology, 4th,* August, 1978, Warsaw. Sect. E. p. 68–69. (Abstract).

Lie, K. J., and Heyneman, D. (1978b). Inducible resistance to trematode infections in snails. Fourth International Congress of Parasitology, August, 1978, Warsaw. Sect. E. p. 124. (Abstract).

Lie, K. J., and Heyneman, D. (1979a). Acquired resistance to echinostomes in four *Biomphalaria glabrata* strains. *Int. J. Parasitol.* **9,** 533–537.

Lie, K. J., and Heyneman, D. (1979b). Specificity of natural resistance to trematode infections of *Biomphalaria glabrata*. *Int. J. Parasitol.* **9,** 529–531.

Lie, K. J., Heyneman, D., and Yau, P. (1975a). The origin of amebocytes in *Biomphalaria glabrata*. *J. Parasitol.* **61,** 574–576.

Lie, K. J., Heyneman, D., and Lim, H. K. (1975b). Studies on resistance in snails: Specific resistance induced by irradiated miracidia of *Echinostoma lindoense* in *Biomphalaria glabrata* snails. *Int. J. Parasitol.* **5,** 627–631.

Lie, K. J., Heyneman, D., and Jeong, K. H. (1976). Studies on resistance in snails. 7. Evidence of interference with the defense reaction in *Biomphalaria glabrata* by trematode larvae. *J. Parasitol.* **62,** 608–615.

Lie, K. J., Heyneman, D., and Richards, C. S. (1977a). *Schistosoma mansoni:* Temporary reduction of natural resistance in *Biomphalaria glabrata* induced by irradiated miracidia of *Echinostoma paraensei*. *Exp. Parasitol.* **43,** 54–62.

Lie, K. J., Heyneman, D., and Richards, C. S. (1977b). Studies on resistance in snails: interference by nonirradiated echinostome larvae with natural resistance to *Schistosoma mansoni* in *Biomphalaria glabrata*. *J. Invertebr. Pathol.* **29,** 118–125.

Lie, K. J., Jeong, K. H., and Heyneman, D. (1980a). Tissue reactions induced by *Schistosoma mansoni* in *Biomphalaria glabrata*. *Ann. Trop. Med. Parasitol.* **74,** 157–166.

Lie, K. J., Jeong, K. H., and Heyneman, D. (1980b). Inducement of miracidia-immobilizing substance in the hemolymph of *Biomphalaria glabrata*. *Int. J. Parasitol.* **10,** 183–188.

Lie, K. J., Jeong, K. H., and Heyneman, D. (1982). Further characterization of acquired resistance in *Biomphalaria glabrata*. *J. Parasitol.* **68,** 529–531.

Lim, H. K. (1970). Ph.D. thesis. Univ. California, San Francisco.

Lim, H. K., and Heyneman, D. (1972). Intramolluscan inter-trematode antagonism: A review of factors influencing the host-parasite system and its possible role in biological control. *Adv. Parasitol.* **10,** 191–268.

Lin, J. H., Fang, W. S., Huang, R. T., and Chen, S. W. (1974). Trematode control by dual infection with suitable and unsuitable miracidia in the same snail. *Taiwan J. Vet. Med. Anim. Husb.* **25,** 55–62.

Loker, E. S. (1978a). Normal development of *Schistosomatium douthitti* in the snail *Lymnaea catascopium*. *J. Parasitol.* **64,** 977–985.

Loker, E. S. (1978b). *Schistosomatium douthitti:* Exposure of *Lymnaea catascopium* to irradiated miracidia. *Exp. Parasitol.* **46,** 134–140.

Loker, E. S. (1979). Pathology and host responses induced by *Schistosomatium douthitti* in the freshwater snail *Lymnaea catascopium*. *J. Invertebr. Pathol.* **33,** 265–273.

Loker, E. S., and Bayne, C. J. (1982). *In vitro* encounters between *Schistosoma mansoni* primary sporocysts and hemolymph components of susceptible and resistant strains of *Biomphalaria glabrata*. *Am. J. Trop. Med. Hyg.* **31,** 999–1005.

Loker, E. S., Bayne, C. J., Buckley, P. M., and Kruse, K. T. (1982). Ultrastructure of encapsulation of *Schistosoma mansoni* mother sporocysts by hemocytes of juveniles of the 10-R2 strain of *Biomphalaria glabrata*. *J. Parasitol.* **68,** 84–94.

LoVerde, P. T. (1979). Parasite interaction between *Bulinus tropicus* and *Schistosoma haematobium:* Fine structure. Zentralbl. Bakteriol. Hyg., Abt. 1. Abt. Ref. **263,** 207. (Abstract).

LoVerde, P. T., Sieber, P., and Schepis, M. J. (1979). Hemocytes of the planorbid snails, *Bulinus* and *Biomphalaria:* Light and electron microscopy. *Trans. Am. Microsc. Soc.* **98,** 148. (Abstract).

LoVerde, P. T., Gherson, J., and Richards, C. S. (1982). Reaction against self in an invertebrate? *J. Invertebr. Pathol.* **39,** 247–249.

Machii, A. (1968). Histological studies on the pearl-sac formation. *Bull. Nat. Pearl Res. Lab.* (Jpn.) **13,** 1489–1539.

Mackin, J. G., and Loesch, H. (1955). A haplosporidian hyperparasite of oysters. *Proc. Nat. Shellfish. Assoc.* **45,** 182–183.

Mackin, J. G., and Schlicht, F. G. (1976). A proteomyxan amoeba stage in the development of *Labyrinthomyxa patuxent* (Hogue) Mackin and Schlicht, with remarks on the relation of the proteomyxids to the neoplastic diseases of oysters and clams. *Mar. Fish. Rev.* **38,** 16–18.

Makanga, B. (1981). The effect of varying the number of *Schistosoma mansoni* miracidia on the reproduction and survival of *Biomphalaria pfeifferi*. *J. Invertebr. Pathol.* **37,** 7–10.

Malek, E. A. (1952). "Morphology, bionomics and host-parasite relations of Planorbidae (Mollusca, Pulmonata)." Ph.D. Thesis. Univ. of Michigan, Ann Arbor.

Malek, E. A., and Cheng, T. C. (1974). "Medical and economic malacology," 398 pp. Academic Press, New York/London.

McDade, J. E., and Tripp, M. R. (1967). Lysozyme in oyster mantle mucus. *J. Invertebr. Pathol.* **9,** 581–582.

McHenry, J. G., Birkbeck, T. H., and Allen, J. A. (1979). The occurrence of lysozyme in marine bivalves. *Comp. Biochem. Physiol. B***63,** 25–28.

McLean, N., and Porter, D. (1982). The yellowspot disease of *Tritonia diomedea* Bergh, 1894 (Mollusca: Gastropoda: Nudibranchia): Encapsulation of the thraustocytriaceaus parasite by host amoebocytes. *J. Parasitol.* **68,** 243–252.

Metchnikoff, E. (1891). "Lectures on the comparative pathology of inflammation". Dover Publications, New York.

Meuleman, E. A. (1972). Host-parasite interrelationships between the freshwater pulmonate *Biomphalaria pfeifferi* and the trematode *Schistosoma mansoni*. *Neth. J. Zool.* **22,** 355–427.

Meuleman, E. A., Lyaruu, D. M., Khan, M. A., Holzmann, P. H., and Sminia, T. (1978). Ultrastructural changes in the body wall of *Schistosoma mansoni* during the transformation of the miracidium into the mother sporocyst in the snail host *Biomphalaria pfeifferi*. *Z. Parasitenkd.* **56,** 227–242.

Michelson, E. H. (1957). Studies on the biological control of schistotome-bearing snails. Predators and parasites of fresh-water mollusca: A review of the literature. *Parasitology* **47,** 413–426.

Michelson, E. H. (1961). An Acid-fast pathogen of fresh-water snails. *Am. J. Trop. Med. Hyg.* **10,** 423–433.

Michelson, E. H. (1963). Development and specificity of miracidial immobilizing substances in extracts of the snail *Australorbis glabratus* exposed to various agents. *Ann. N.Y. Acad. Sci.* **113,** 486–491.

Michelson, E. H. (1964). Miracidia-immobilizing substances in extracts prepared from snails infected with *Schistosoma mansoni*. *Am. J. Trop. Med. Hyg.* **13,** 36–42.

Michelson, E. H., and DuBois, L. (1973). Increased alkaline phosphatase in the tissues and hemolymph of the snail *Biomphalaria glabrata* infected with *Schistosoma mansoni*. *Comp. Biochem. Physiol. B* **44,** 763–767.

Michelson, E. H., and DuBois, L. (1977). Agglutinins and lysins in the molluscan family Planorbidae: A survey of hemolymph, egg-masses, and albumen-gland extracts. *Biol. Bull.* **153,** 219–227.

Michelson, E. H., and DuBois, L. (1978). Susceptibility of Bahian populations of *Biomphalaria glabrata* to an allopatric strain of *Schistosoma mansoni*. *Am. J. Trop. Med. Hyg.* **27,** 782–786.

Michelson, E. H., and DuBois, L. (1981). Resistance to schistosome infection in *Biomphalaria glabrata* induced by gamma radiation. *J. Invertebr. Pathol.* **38,** 39–44.

Miller, R. L. (1982). A sialic-acid specific lectin from the slug *Limax flavus*. *J. Invertebr. Pathol.* **39,** 210–214.

Mix, M. C. (1972). Chronic histopathological degeneration of selected tissues in the Pacific oyster, *Crassostrea gigas,* following acute g-irradiation and its relation to mitotic inhibition. *Radiat. Res.* **49,** 176–189.

Morton, B. (1977). The occurrence of inflammatory granulomas in the ctenidial marsupium of *Corbicula fluminea* (Mollusca:Bivalvia): A consequence of larval incubation. *J. Invertebr. Pathol.* **30,** 5–14.

Muscatine, L., Pool. R. R., and Trench, R. K. (1975). Symbiosis of algae and invertebrates: Aspects of the symbiont surface and the host-symbiont interface. *Trans. Am. Micros. Soc.* **94,** 450–469.

Nassi, H. (1979). Coincidence entre le blocage précoce de la ponte de *Biomphalaria glabrata* (Gasteropoda:Pulmonata) et la localisation cérébrale des jeunes rédies mères de *Ribeiroia marini guadeloupensis* (Trematoda:Cathaemasiidae). *C. R. Acad. Sci. Paris, D* **289,** 165–168.

Nelstrop, A. E., Taylor, G., and Collard, P. (1968). Studies on phagocytosis. 3. Antigen clearance studies in invertebrates and poikilothermic vertebrates. *Immunology* **14,** 347–356.

Newton, W. L. (1953). The inheritance of susceptibility to infection with *Schistosoma mansoni* in *Australorbis glabratus*. *Exp. Parasitol.* **2,** 242–257.

Nordbring-Hertz, B., and Mattiasson, B. (1979). Action of nematode-trapping fungus shows lectin-mediated host-microorganism interaction. *Nature* **281,** 477–479.

Ogren, R. E. (1959). The nematode *Cosmocercoides dukae* as a parasite of the slug. *Proc. Penn. Acad. Sci.* **33,** 236–241.

Oprandy, J. J., Chang, P. W., Pronovost, A. D., Cooper, K. R., Brown, R. S., and Yates, V. J. (1981). Isolation of a viral agent causing hematopoietic neoplasia in the soft-shell clam, *Mya arenaria*. *J. Invertebr. Pathol.* **39,** 45–51.

Overstreet, R. M., and Hochbert, F. G., Jr. (1975). Digenetic trematodes in cephalopods. *J. Mar. Biol. Assoc. U. K.* **55,** 893–910.

Owczarzak, A., Stibbs, H. H., and Bayne, C. J. (1979). The destruction of *Schistosoma mansoni* mother sporocysts *in vitro* by amoebae isolated from *Biomphalaria glabrata:* An ultrastructural study. *J. Invertebr. Pathol.* **35,** 26–33.

Paau, A. S., Leps, W. T., and Brill, W. J. (1981). Agglutinin from alfalfa necessary for binding and nodulation by *Rhizobium meliloti*. *Science* **213,** 1513–1514.

Pan, C.-T. (1956). Studies on the biological control of schistosome-bearing snails: a preliminary report on pathogenic microorganisms found in *Australorbis glabratus*. *J. Parasitol.* **42,** 33. (Abstract).

Pan, C.-T. (1963). Generalized and focal tissue responses in the snail, *Australorbis glabratus,* infected with *Schistosoma mansoni*. *Ann. N.Y. Acad. Sci.* **113,** 475–485.

Pan, C.-T. (1965). Studies on the host-parasite relationship between *Schistosoma mansoni* and the snail *Australorbis glabratus*. *Am. J. Trop. Med. Hyg.* **14,** 931–976.

Partridge, T., and Davies, P. S. (1974). Limpet haemocytes. 2. The role of spikes in locomotion and spreading. *J. Cell Sci.* **14,** 319–330.

Pauley, G. B. (1974). Physicochemical properties of the natural agglutinins of some mollusks and crustaceans. *Ann. N.Y. Acad. Sci.* **234,** 145–160.

Pauley, G. B., and Krassner, S. M. (1971). The effect of temperature on the number of circulating hemocytes in the California sea hare, *Aplysia californica. Calif. Fish Game* **57,** 308–309.

Pauley, G. B., and Krassner, S. M. (1972). Cellular defense reactions to particulate materials in the California sea hare, *Aplysia californica. J. Invertebr. Pathol.* **19,** 18–27.

Pauley, G. B., and Sparks, A. K. (1967). Observations on experimental wound repair in the adductor muscle and the leydig cells of the oyster *Crassostrea gigas. J. Invertebr. Pathol.* **9,** 298–309.

Pauley, G. B., Granger, G. A., and Krassner, S. M. (1971a). Characterization of a natural agglutinin present in the hemolymph of the California sea hare, *Aplysia californica. J. Invertebr. Pathol.* **18,** 207–218.

Pauley, G. B., Krassner, S. M., and Chapman, F. A. (1971b). Bacterial clearance in the California sea hare, *Aplysia californica. J. Invertebr. Pathol.* **18,** 227–239.

Payne, W. L., Gerding, T. A., Dent, T. G., Bier, J. W., and Jackson, G. J. (1980). Survey of the U.S. Atlantic coast surf claim *Spisula solidissima,* and clam products for anisakine nematodes and hyperparasitic protozoa. *J. Parasitol.* **66,** 150–153.

Poinar, G.-O., and Richards, C. S. (1979). *Daubaylia helicophilus* n.sp. (Daubayliidae: Nematoda) a parasite of the snail, *Gyraulus spirillus* from Taiwan. *Ann. Parasitol.* **54,** 615–619.

Popiel, I., and James, B. L., (1979). The host-parasite interface between marine molluscs and larval digenea. *Zentralbl. Bakteriol. Hyg. Abt. 1. Abt. Ref.* **263,** 203. (Abstract).

Prokop, O., Uhlenbruck, G., and Kohler, W. (1968). A new source of antibody-like substances having anti-blood group specificity. *Vox Sang.* **14,** 321–333.

Prowse, R. H., and Tait, N. N. (1969). *In vitro* phagocytosis by amoebocytes from the haemolymph of *Helix aspersa* (Muller). 1. Evidence for opsonic factor(s) in serum. *Immunology* **17,** 437–443.

Ratanarat-Brockelman, C. (1975). Inhibition of *Rhabditis maupasi* (Rhabditidae: Nematoda) maturation and reproduction by factors from the snail host, *Helix aspersa. J. Invertebr. Pathol.* **25,** 229–237.

Reade, P. C. (1968). Phagocytosis in invertebrates. *Aust. J. Exp. Biol. Med. Sci.* **46,** 219–229.

Reade, P., and Reade, E. (1972). Phagocytosis in Invertebrates. 2. The clearance of carbon particles by the clam, *Tridacna maxima. J. Reticulendothel. Soc.* **12,** 349–360.

Reade, P., and Reade, E. (1976). Phagocytosis in invertebrates: studies on the hemocytes of the clam *Tridacna maxima. J. Invertebr. Pathol.* **28,** 281–290.

Renwrantz, L. R. (1979). An investigation of molecules and cells in the hemolymph of *Helix pomatia* with special reference to immunobiologically active components. *Zool. Jahrb. Physiol.* **83,** 283–333. (In German).

Renwrantz, L. R., and Cheng, T. C. (1977a). Identification of agglutinin receptors on hemocytes of *Helix pomatia. J. Invertebr. Pathol.* **29,** 88–96.

Renwrantz, L. R., and Cheng, T. C. (1977b). Agglutinin-mediated attachment of erythrocytes to hemocytes of *Helix pomatia. J. Invertebr. Pathol.* **29,** 97–100.

Renwrantz, L., and Mohr, W. (1978). Opsonizing effect of serum and albumin gland extracts on the elimination of human erythrocytes from the circulation of *Helix pomatia. J Invertebr. Pathol.* **31,** 164–170.

Renwrantz, L., Schancke, W., Harm, H., Erl, H., Liebsch, H., and Gercken, J. (1981). Discriminative ability and function of the immunobiological recognition system of the snail *Helix pomatia. J. Comp. Physiol.* **141,** 477–488.

Richards, C. S. (1968). Two new species of *Hartmanella* amoebae infecting freshwater mollusks. *J. Protozool.* **15,** 651–656.

Richards, C. S. (1970). Genetics of a molluscan vector of *Schistosomiasis. Nature* **227,** 806–810.

Richards, C. S. (1976). Variations in infectivity for *Biomphalaria glabrata* in strains of *Schistosoma mansoni* from the same geographical area. *Bull. W. H. O.* **54,** 706–707.

Richards, C. S., and Sheffield, H. G. (1971). Unique host relations and ultrastructure of a new microsporidian of the genus *Coccospora* infecting *Biomphalaria glabrata*. *Proc. 4th Int. Colloq. Insect Path.* 439–452.

Roder, J. C., Bourns, T. K. R., and Singhal, S. K. (1977). *Trichobilharzia ocellata:* Cercariae masked by antigens of the snail, *Lymnaea stagnalis*. *Exp. Parasitol.* **41,** 206–212.

Rodrick, G. E., and Cheng, T. C. (1974). Activities of selected hemolymph enzymes in *Biomphalaria glabrata* (Mollusca). *J. Invertebr. Pathol.* **24,** 374–375.

Rondelaud, D., and Barthe, D. (1980). Etude descriptive d'une réaction amibocytaire chez *Lymnaea truncatula* Muller infestée par *Fasciola hepatica* L. *Z. Parasitenkd.* **61,** 187–196.

Rondelaud, D., and Barthe, D. (1981). The development of the amoebocyte-producing organ in *Lymnaea truncatula* Müller infected by *Fasciola hepatica* L. *Z. Parasitenkd.* **65,** 331–341.

Rosati, F., and De Santis, R. (1980). Role of the surface carbohydrates in sperm-egg interaction in *Ciona intestinalis*. *Nature* **3,** 762–764.

Ruddell, C. L. (1971a). Elucidation of the nature and function of the granular oyster amebocytes through histochemical studies of normal and traumatized oyster tissues. *Histochemie* **26,** 98–112.

Ruddell, C. L. (1971b). The fine structure of oyster agranular amebocytes from regenerating mantle wounds in the Pacific oyster, *Crassostrea gigas*. *J. Invertebr. Pathol.* **18,** 260–268.

Runham, N. W., and Hunter, P. J. (1970). "Terrestrial slugs." Hutchinson University Press, London.

Sanderson, C. J. (1981). The mechanism of lymphocyte-mediated cytotoxicity. *Biol. Rev.* **56,** 153–197.

Sauerlander, R. (1976). Histologische Veranderungen bei experimentell mit *Angiostrongylus vasorum* odor *Antiostrongylus cantonensis* (Nematoda) infizierten Achatschnecken (*Achatina fulica*). *Z. Parasitenkd.* **49,** 263–280.

Sauerlander, R. (1979). *Cepaea nemoralis* (Helicidae, Stylommatophora) als experimenteller Zwischenwirt fur *Muellerius capillaris* (Protostrongylidae, Nematoda). *Z. Parasitenkd.* **59,** 53–66.

Schmeer, M. R., Horton, D., and Tanimura, A. (1966). Mercenene, a tumor inhibitor from *Mercenaria:* Purification and characterization studies. *Life Sci.* **5,** 1169–1178.

Schmid, L. S. (1975). Chemotaxis of hemocytes from the snail *Viviparus malleatus*. *J. Invertebr. Pathol.* **25,** 125–132.

Schoenberg, D. A., and Cheng, T. C. (1982). Concanavalin A-mediated phagocytosis of yeast by *Biomphalaria glabrata* hemocytes *in vitro:* effects of temperature and lectin concentration. *J. Invertebr. Pathol.* **39,** 314–322.

Schutte, C. H. J. (1975). Studies on the South African strain of *Schistosoma mansoni:* Notes on certain host-parasite relationships between intra-molluscan larvae and intermediate host, Part 3. *S. Afr. J. Sci.* **71,** 8–20.

Shimizu, Y., and Yoshioka, M. (1981). Transformation of paralytic shellfish toxins as demonstrated in scallop homogenates. *Science* **212,** 547–549.

Sinderman, S. F. (1977). Disease diagnosis and control in North American marine aquaculture. *Dev. Aquacult. Fish. Sci.* **6,** Elsevier, Amsterdam.

Sluiters, J. F., and Joose, J. (1979). The effect of a *Trichobilharzia ocellata*-infection on the female reproductive activity of *Lymnaea stagnalis* and its endocrine control system. *Zentralbl. Bakteriol. Hyg. Abt. 1. Abt. Ref.* **263,** 201. (Abstract).

Sminia, T. (1974). Haematopoiesis in the freshwater snail *Lymnaea stagnalis* studied by electron microscopy and autoradiography. *Cell Tiss. Res.* **150,** 443–454.

Sminia, T. (1980). Phagocytic cells in Mollusca. *In* "Aspects of Developmental and Comparative Immunology. I." (J. B. Solomon, ed.), pp. 125–132. Pergamon, Oxford/New York.

Sminia, T. (1981). Gastropods. *In* "Invertebrate Blood Cells" (N. A. Ratcliffe and A. F. Rowley, eds.), pp. 191–232. Academic Press, New York.

Sminia, T., and Barendsen, L. (1980). A comparative morphological and enzyme histochemical study on blood cells of the freshwater snails *Lymnaea stagnalis, Biomphalaria glabrata,* and *Bulinus truncatus*. *J. Morphol.* **165,** 31–39.

Sminia, T., Pietersma, K., and Scheerboom, J. E. M. (1973). Histological and ultrastructural observations on wound healing in the freshwater pulmonate *Lymnaea stagnalis*. *Z. Zellforsch.* **141,** 561–573.

Sminia, T., Borghart-Reinders, E., and van de Linde, A. W. (1974). Encapsulation of foreign materials experimentally introduced into the freshwater snail *Lymnaea stagnalis*. *Cell Tiss. Res.* **153,** 307–326.

Sminia, T., van der Knaap, W. P. W., and Kroese, F. G. M. (1979a). Fixed phagocytes in the freshwater snail *Lymnaea stagnalis*. *Cell Tiss. Res.* **196,** 545–548.

Sminia, T., van der Knaap, W. P. W., and Edelenbosch, P. (1979b). The role of serum factors in phagocytosis of foreign particles by blood cells of the freshwater snail (*Lymnaea stagnalis*. *Dev. Comp. Immunol.* **3,** 37–44.

Sminia, T., Winsemius, A. A., and van der Knaap, W. P. W. (1981). Recognition of foreignness by blood cells of the freshwater snail *Lymnaea stagnalis,* with special reference to the role and the structure of the cell coat. *J. Invertebr. Pathol.* **38,** 175–183.

Southgate, V. E. (1979). Host-parasite relationships between schistosomes and their intermediate hosts. *Zentralbl. Bakteriol. Hyg., Abt. 1. Abt. Ref.* **263,** 195. (Abstract).

Sparks, A. K. (1962). Metaplasia of the gut of the oyster *Crassostrea gigas* (Thunberg) caused by infection with the copepod *Mytilicola orientalis* Mori. *J. Insect Pathol.* **4,** 57–62.

Sparks, A. K. (1972). "Invertebrate pathology: noncommunicable diseases." Academic Press, New York/London.

Sprague, V. (1971). Diseases of oysters. *Ann. Rev. Microbiol.* **25,** 211–230.

Stanislawski, E., Renwrantz, L., and Becker, W. (1976). Soluble blood group reactive substances in the hemolymph of *Biomphalaria glabrata* (Mollusca). *J. Invertebr. Pathol.* **28,** 301–308.

Stauber, L. A. (1950). The fate of India ink injected intracardially into the oyster, *Ostrea virginica* Gmelin. *Biol. Bull.* **98,** 227–241.

Stauber, L. A. (1961). Immunity in invertebrates, with special reference to the oyster. *Proc. Nat. Shellfish. Assoc.* **50,** 7–20.

Stein, P. C., and Basch, P. F. (1979). Purification and binding properties of hemagglutinin from *Biomphalaria glabrata*. *J. Invertebr. Pathol.* **33,** 10–18.

Stewart, G. L., Ubelaker, J. E., and Curtis, D. (1981). Pathophysiologic alterations in *Biomphalaria glabrata* infected with *Angiostrongylus costaricensis*. *Proc. Ann. Mtg. Am. Soc. Parasitol., 56th* Montreal. (Abstract, p. 38).

Stibbs, H. H., Owczarzak, A., Bayne, C. J., and DeWan, P. (1979). Schistosome sporocyst-killing amoebae isolated from *Biomphalaria glabrata*. *J. Invertebr. Pathol.* **33,** 159–170.

Stuart, A. E. (1968). The reticulo-endothelial apparatus of the lesser octopus, *Eledone cirrosa*. *J. Pathol. Bacteriol* **96,** 401–412.

Stumpf, J. L., and Gilbertson, D. E. (1980). Differential leukocytic responses of *Biomphalaria glabrata* to infection with *Schistosoma mansoni*. *J. Invertebr. Pathol.* **35,** 217–218.

Sudds, R. H. (1960). Observations of schistosome miracidial behavior in the presence of normal and abnormal snail hosts and subsequent tissue studies of these hosts. *J. Elisha Mitchell Sci. Soc.* **76,** 121–133.

Sullivan, J. T., and Richards, C. S. (1981). *Schistosoma mansoni,* NIH-Sm-PR-2 strain, in susceptible and nonsusceptible stocks of *Biomphalaria glabrata:* comparative histology. *J. Parasitol.* **67,** 702–708.

Sullivan, J. T., and Richards, C. S. (1982). Increased susceptibility to infection with *Ribeiroia marini* in *Biomphalaria glabrata* exposed to x-rays. *J. Invertebr. Pathol.* **40,** 303–304.

Sullivan, J. T., Richards, C. S., Lie, K. J., and Heyneman, D. (1982). *Ribeiroia marini* irradiated miracidia and induction of acquired resistance in *Biomphalaria glabrata*. *Exper. Parasitol.* **53,** 17–25.

Takatsuki, S. (1934). On the nature and functions of the amoebocytes of *O. edulis*. *Am. J. Microsc. Sci.* **76,** 379–431.

Tripp, M. R. (1958). Disposal by the oyster of intracardially injected red blood cells of vertebrates. *Proc. Nat. Shellfish. Assoc.* **48,** 143–147.

Tripp, M. R. (1960). Mechanisms of removal of injected microorganisms from the American oyster, *Crassostrea virginica* (Gmelin). *Biol. Bull.* **119,** 283–282.

Tripp, M. R. (1961a). The fate of foreign materials experimentally introduced into the snail *Australorbis glabratus*. *J. Parasitol.* **47,** 745–751.

Tripp, M. R. (1961b). Is *Bacillus pinottii* pathogenic in *Australorbis glabratus? J. Parasitol.* **47,** 464.

Tripp, M. R. (1963). Cellular responses of mollusks. *Ann. N.Y. Acad. Sci.* **113,** 467–474.

Tripp, M. R. (1970). Defense mechanisms of mollusks. *J. Reticuloendothel. Soc.* **7,** 173–182.

Tripp, M. R., and Turner, R. M. (1978). Effects of the trematode *Proctoeces maculatus* on the mussel *Mytilus edulis*. *In* "Comparative Immunobiology: Invertebrate Models for Biomedical Research" (L. A. Bulla and T. C. Cheng, eds.), Vol. 4, pp. 73–84. Plenum, New York/London.

Trushin, I. N. (1978). Pathological changes in land snails caused by Protostrongylid larvae. *Int. Colloq. Inverterb. Pathol.* **11,** 114. Ann. Mtg. Soc. Inverterb. Pathol., Prague. (Abstract).

Tsujii, T. (1960). Studies on the mechanisms of shell- and pearl-formation in Mollusca. *J. Fac. Fish. Prefect. Univ. Mie* **5,** 1–70.

Tyler, A. (1946). Natural heteroagglutinins in the body-fluids and seminal fluids of various invertebrates. *Biol. Bull.* **90,** 213–219.

Uhlenbruck, G., and Steinhausen, G. (1977). Tridacnins: Symbiosis-profit or defense purpose? *Dev. Comp. Immunol.* **1,** 183–192.

Uhlenbruck, G., Prokop, O., and Haferland, W. (1966). Agglutination von *E coli* durch ein Agglutinin aux *Helix pomatia*. *Zentralbl. Bakteriol. Hyg. Abt. 1. Abt. Ref.* **199,** 271–272.

Valiulis, G. A. (1973). Comparison of the resistance to *Labyrinthomyxa marina* with resistance to *Minchinia nelsoni* in *Crassostrea virginica*. Ph.D. Thesis. Rutgers University, New Brunswick, New Jersey.

van der Knaap, W. P. W. (1980). Recognition of foreignness in the internal defence system of the freshwater gastropod *Lymnaea stagnalis*. *In* "Aspects of Developmental and Comparative Immunology". (J. B. Solomon, ed.), Vol. 1, pp. 91–98. Pergamon, Oxford, England.

van der Knaap, W. P. W., Boerrigter-Barendsen, L. H., van den Hoeven, D. S. P., and Sminia, T. (1981a). Immunocytochemical demonstration of a humoral defence factor in blood cells (amoebocytes) of the pond snail, *Lymnaea stagnalis*. *Cell Tiss. Res.* **219,** 291–296.

van der Knaap, W. P. W., Sminia, T., Kroese, F. G. M., and Dikkeboom, R. (1981b). Elimination of bacteria from the circulation of the pond snail *Lymnaea stagnalis*. *Dev. Comp. Immunol.* **5,** 21–32.

van der Knaap, W. P. W., Tensen, C. P., Kroese, F. G. M., and Boerrigter-Barendsen, L. H. (1983a). Adaptive defense reactions against bacteria in the pond snail *Lymnaea stagnalis*. *Dev. Comp. Immunol.* (In press.)

van der Knaap, W. P. W., Doderer, A., Boerrigter-Barendsen, L. H., and Sminia, T. (1983b). Some problems of an agglutinin in the haemolymph of the pond snail *Lymnaea stagnalis*. *Biol. Bull.* (In press.)

Vasta, G. R., Sullivan, J. T., Cheng, T. C., Marchalonis, J. J., and Warr, G. W. (1982). A cell-associated lectin of the oyster hemocyte. *J. Invert. Pathol.* **40,** 367–377.

Vretblad, P., Hjorth, R., and Lass, T. (1979). The isolectins of *Helix pomatia:* Separation by isoelectric focusing and preliminary characterization. *Biochim. Biophys. Acta* **579,** 52–61.

Walker, J. C. (1979). *Austrobilharzia terrigalensis:* A schistosome dominant in interspecific interactions in the molluscan host. *J. Parasitol.* **9,** 137–140.

Weinheimer, P. F., Acton, R. T., and Evans, E. E. (1969). Attempt to induce a bactericidal response in the oyster. *J. Bacteriol.* **97,** 462–463.

White, A. W., and Maranda, L. (1978). Paralytic toxins in the dinoflagellate *Gonyaulax excavata* and in shellfish. *J. Fish. Res. Board Can.* **87,** 397–402.

Wilson, R. A., and Denison, J. (1980). The parasitic castration and gigantism of *Lymnaea truncatula* infected with the larval stages of *Fasciola hepatica*. *Z. Parasitenkd.* **61,** 109–119.

Wolburg-Buchholz, K. (1972). Blasenzellen im Bindgewebe des Schlundrings von *Cepaea nemoralis* L. (Gastropoda, Stylommatophora). 2. Aufnahme und Speicherung von Ferritin. *Z. Zellforsch.* **130,** 262–278.

Wolburg-Buchholz, K. (1973). Surface coat and endocytosis of specialized connective tissue cells in pulmonates. *Z. Zellforsch.* **136,** 139–146.

Wolf, P. H. (1977). An unidentified protistan parasite in the ova of the blacklipped oyster, *Crassostrea echinata,* from northern Australia. *J. Invertebr. Pathol.* **29,** 244–246.

Wright, C. A. (1971). "Flukes and Snails." The science of biology series; 4. Unwin Univ. Books, London.

Yonge, C. M. (1926). Structure and physiology of the organs of feeding and digestion in *Ostrea edulis*. *J. Mar. Biol. Assoc. U. K.* **14,** 295–388.

Yoshino, T. P. (1976). Encapsulation response of the marine prosobranch *Cerithidea californica* to natural infections of *Renicola buchanani* sporocysts (Trematoda: Renicolidae). *Int. J. Parasitol.* **6,** 423–431.

Yoshino, T. P. (1981a). Concanavalin A-induced receptor redistribution on *Biomphalaria glabrata* hemocytes: Characterization of capping and patching responses. *J. Invertebr. Pathol.* **38,** 102–112.

Yoshino, T. P. (1981b). Snail hemocyte-like antigens associated with larval schistosome surface membranes. *Ann. Mtg. Am. Soc. Parasitol., 56th,* Montreal. (Abstract 98).

Yoshino, T. P. (1981c). Comparison of concanavalin-A-reactive determinants on hemocytes of two *Biomphalaria glabrata* snail stocks: receptor binding and redistribution. *Dev. Comp. Immunol.* **5,** 229–240.

Yoshino, T. P. (1982). Lectin-induced modulation of snail hemocyte surface determinants: clearance of con-A-receptor complexes. *Dev. Comp. Immunol.* **6,** 451–462.

Yoshino, T. P. (1983). Lectins and antibodies as molecular probes of molluscan hemocyte surface membranes. *Dev. Comp. Immunol.* (In press.)

Yoshino, T. P., and Bayne, C. J. (1983). Mimicry of snail host antigens by miracidia and primary sporocysts of *Schistosoma mansoni*. *Parasite Immunol.* **5** (In press.)

Yoshino, T. P., and Cheng, T. C. (1977). Aminopeptidase activity in the hemolymph and body tissues of the pulmonate gastropod *Biomphalaria glabrata*. *J. Invertebr. Pathol.* **30,** 76–79.

Yoshino, T. P., and Cheng, T. C. (1978). Snail host-like antigens associated with the surface membranes of *Schistosoma mansoni* miracidia. *J. Parasitol.* **64,** 752–754.

Yoshino, T. P., Cheng, T. C., and Renwrantz, L. R. (1977). Lectin and human blood group determinants of *Schistosoma mansoni:* Alteration following *in vitro* transformation of miracidium to mother sporocyst. *J. Parasitol.* **63,** 818–824.

Yoshino, T. P., Renwrantz, L. R., and Cheng, T. C. (1979). Binding and redistribution of surface membrane receptors for Concanavalin A on oyster hemocytes. *J. Exp. Zool.* **207,** 439–450.

Yousif, F., and Lammler, G. (1975). The suitability of several aquatic snails as intermediate hosts for *Angiostrongylus cantonensis*. *Z. Parasitendk.* **47,** 203–210.

Yousif, F., and Lammler, G. (1977). The mode of infection with and the distribution of *Angiostrongylus cantonensis* larvae in the experimental intermediate host *Biomphalaria glabrata*. *Z. Parasitenkd.* **53,** 247–250.

Yousif, F., Blahser, S., and Lammler, G. (1979). The cellular responses in *Marisa cornuarietis* experimentally infected with *Angiostrongylus cantonensis*. *Zentralbl. Bakteriol. Hyg., Abt. 1. Abt. Ref.* **263,** 210–211. (Abstract).