The Compatibility and Incompatibility Concept as Related to Trematodes and Molluscs[1]

THOMAS C. CHENG[2]

IN THIS PAPER I propose to bring together significant facts already known to have some bearing on the mechanisms which govern or influence host compatibility or incompatibility during parasitism, to add information based on my own recent research, and to present some speculations.

From the broad viewpoint, host compatibility may be defined as the expression of the physical and physiological (including chemical) states of the host and parasite which enable the parasite to invade and carry out its life processes, including perpetuation of the species. Incompatibility refers to those factors which completely or partially prevent the establishment and normal development of the parasite. Both of these are highly complex phenomena influenced to one degree or another by a series of separate but commonly interrelated factors.

As working models from which we can seek evidence to support or reject various working hypotheses, we have chosen to examine the relationship between molluscs and digenetic trematodes, since this category of association, unlike a number of others, is an example of parasitism whether the definition of "parasitism" is couched in nutritional, pathological, ecological, or immunological terms. Molluscs, except in a very few unusual instances, serve as intermediate hosts for the Digenea, but this fact does not render them less appropriate as experimental tools. In fact, inasmuch as it is generally accepted that molluscs were the original hosts during the evolutionary adaptation to parasitism of the progenitors of modern day digenetic trematodes, one might expect to find the occurrence of certain adaptive mechanisms more firmly entrenched in them than in vertebrate definitive hosts. Furthermore, it is easier to simulate experimentally the natural environmental conditions of molluscs than those of vertebrates in captivity, and, with relative ease, one can test basic premises on a number of species from a variety of habitats, ranging from marine to fresh water, and from various attitudes.

It has long been known that compatibility and incompatibility need not be "all or none" phenomena, since both interspecific and intraspecific (or strain) differences do occur, as is indicated by the rate of parasite development, infectivity of the cercariae or metacercariae, the number of progeny produced by delayed polyembryony, etc. In fact, an understanding of the factors governing compatibility and incompatibility in turn most probably will provide answers for why these manifestations occur.

Since the initial host-parasite contact, the invasion process, the establishment of the parasite within the host, and the escape process are distinct aspects of a successful parasitic relationship (see the review by Cheng, 1967), one would expect factors correlated with all of these phases to contribute to some degree in regulating compatibility and/or incompatibility, and, indeed, available evidence indicates that this is so. In the following paragraphs are briefly reviewed those facts which support this concept. Space does not permit the citation of all the relevant literature; therefore, only selected studies are cited as examples.

INITIAL HOST-PARASITE CONTACT

Recently, Timon-David (1965) has again raised the question of the importance of host-

[1] The research reported in this paper was supported by grants from the American Cancer Society and the Office of Research Administration, University of Hawaii. This paper was originally presented at a symposium sponsored by the Division of Invertebrate Zoology, American Society of Zoologists, entitled "The Parasites of Invertebrates," held on December 30, 1966 in Washington, D.C. Manuscript received December 13, 1966.

[2] Department of Zoology, University of Hawaii, Honolulu, Hawaii 96822.

attraction in governing host-specificity in mollusc-trematode relationships. This topic, which has been critically reviewed by myself (Cheng, 1967), among others, is still a controversial one. The controversy is not whether host-attraction does occur since, in my opinion, the studies of Faust and Meleney (1924), Faust (1934), Faust and Hoffman (1934), Barlow (1925), Tubangui and Pasco (1933), Mathias (1925), Kloetzel (1958, 1960), Kawashima et al. (1961*a*), Campbell (1961), Davenport et al. (1962), Etges and Decker (1963), and MacInnis (1965) have demonstrated rather conclusively that attraction between miracidia and molluscs does occur. This is a subtle phenomenon, however, which is operative only within very restricted distances and can be observed only with the application of quantitative techniques. The controversy is over the question whether chemotaxis is in any way related to host-specificity and hence influences compatibility. Although the studies of Faust and Meleney (1924), Barlow (1925), Neuhaus (1953), and Etges and Decker (1963) suggest that miracidial attraction is host-specific, the results of Sudds (1960), Kawashima et al. (1961*a*), and Barbosa (1965) suggest that attraction of miracidia to a specific mollusc need not be correlated with subsequent compatibility.

Experiments carried out in our laboratory have revealed that the miracidia of *Fasciola gigantica* are stimulated by the plasma and tissue extracts of laboratory-raised *Galba ollula,* the natural host in Hawaii. These reactions, however, are not specifically elicited by *G. ollula,* since similar reactions were observed when miracidia were exposed to the plasma and tissue extracts of two other species of freshwater gastropods, *Tarebia granifera mauiensis* and *Helisoma duryi normale.*

In the first series of experiments, *F. gigantica* miracidia, between 15 and 25 minutes post-hatching, were placed in shallow Petri dishes (50 mm in diameter, 15 mm high) in which had been placed specific dilutions of the plasma or tissue extracts of *H. duryi normale, T. granifera mauiensis,* or *G. ollula.* Each dish was placed over a grid marked off in 2.54-mm squares. Using a $\frac{1}{10}$-second-interval stop-watch and observing under a dissection microscope illuminated by indirect lighting, the swimming speed of a single miracidium was timed, recorded as seconds/2.54 mm, and later expressed as mm/sec (Tables 1 and 2). As controls, the swimming velocities of miracidia of similar age placed in distilled water were determined.

The six test media consisted of 1:10, 1:50, and 1:100 dilutions of molluscan plasma and similar dilutions of tissue extracts. Blood was collected from the molluscs' body sinuses by gently cracking the shell of each snail, without injuring the soft tissues, and permitting the blood to drain to the lower edge of an inclined Stender dish from whence it was rapidly collected with a hypodermic needle and syringe. The cellular components of whole-blood samples were removed by centrifugation. The tissue extracts were prepared by homogenizing the soft tissues of each snail in 1 cc of distilled water after the tissues had been completely desanguinated and perfused with running distilled water for 15 minutes. After homogenization in an ice bath, the homogenates were centrifuged and the aqueous extracts collected were considered the "concentrated" extracts.

All snails used were laboratory-raised and known to be parasite-free. The concentrated plasma and tissue extracts of each species were pooled and the desired dilutions were made from the pooled samples. All observations were made at 22 $\pm$ 1° C.

From the data presented in Tables 1 and 2, it is evident that all three dilutions of the plasma and tissue extracts of *G. ollula* (the natural host), *H. duryi normale,* and *T. granifera* stimulated *F. gigantica* miracidia to increase their swimming velocities. In addition, the miracidial swimming pattern was conspicuously altered. Miracidia in distilled water usually swam linearly, rotating along their longitudinal axes. Divergences from such a course were gradual rather than abrupt. When placed in plasma or extracts, their swimming behavior became erratic. They turned abruptly and frequently.

In the second series of experiments, 10 miracidia, 10–15 minutes post-hatching, were placed in small Petri dishes (60 mm in diameter, 13 mm high) which contained 10 cc of distilled water. In the center of each dish was placed an agar block of three mm^3 which had been presoaked in concentrated plasma or tissue extracts of *G. ollula, H. duryi normale,* or *T. granifera*

TABLE 1

Comparisons of the Swimming Velocities of *Fasciola gigantica* Miracidia in Distilled Water (Controls) and in Three Dilutions of Molluscan Plasma (The Student *t* Test Was Employed to Determine Significances)

	Distilled water (controls)		1:10 plasma				1:50 plasma				1:100 plasma			
Molluscan species	No. of trials	Velocity (mm/sec)	No. of trials	Velocity (mm/sec)	P	Signif.	No. of trials	Velocity (mm/sec)	P	Signif.	No. of trials	Velocity (mm/sec)	P	Signif.
G. ollula[1]	40	0.79	25	1.65	> 0.001	+	30	1.88	0.01	+	20	1.91	0.01	+
H. duryi normale[2]	101	1.96	81	2.70	> 0.001	+	81	2.80	> 0.001	+	118	2.85	> 0.001	+
T. granifera[3]	60	2.30	60	3.5	< 0.01	+	60	3.5	< 0.01	+	60	3.5	< 0.01	+

1 The miracidia used were 25 minutes post-hatching.
2 The miracidia used were 18–20 minutes post-hatching.
3 The miracidia used were 15 minutes post-hatching.

TABLE 2

Comparisons of the Swimming Velocities of *Fasciola gigantica* Miracidia in Distilled Water (Controls) and in Three Dilutions of Molluscan Aqueous Tissue Extracts (The Student *t* Test Was Used to Determine Significances)

	Distilled water (controls)		1:10 extract				1:50 extract				1:100 extract			
Molluscan species	No. of trials	Velocity (mm/sec)	No. of trials	Velocity (mm/sec)	P	Signif.	No. of trials	Velocity (mm/sec)	P	Signif.	No. of trials	Velocity (mm/sec)	P	Signif.
G. ollula[1]	40	0.79	20	1.69	> 0.001	+	20	0.99	> 0.001	+	20	1.26	> 0.0001	+
H. duryi normale[2]	101	1.96	80	0.94	< 0.001	+	80	0.81	< 0.001	+	80	1.25	< 0.001	+
T. granifera[3]	60	2.30	60	3.30	> 0.01	+	60	3.33	> 0.01	+	60	3.10	0.05	+

1 The miracidia used were 25 minutes post-hatching.
2 The miracidia used were 18–20 minutes post-hatching.
3 The miracidia used were 15 minutes post-hatching.

mauiensis for 10 hours. Although exact behavioral patterns exhibited by miracidia in the proximity of an agar block were determined, as well as the number of miracidia in each concentric zone at various time intervals, these results will not be reported at this time. Only the total number of contacts made by the miracidia per 5 minutes during the initial 10-minute period are reported (Table 3). The same number of miracidia placed in distilled water with untreated agar blocks served as controls. All observations were made at 22 ± 1° C.

From the data presented in Table 3, it is apparent that significantly more contacts were made between miracidia and plasma- and tissue extract-soaked agar blocks than with control blocks. The exception was in the case of *T. granifera* tissue extract-soaked blocks.

It may be concluded then that the miracidia of *F. gigantica* respond to a velocity-increasing stimulant and an attractant in the plasma and tissue extracts of certain species of molluscs, and that these factors are not host-specific and need not indicate successful subsequent penetration and development. It is noted that the velocity-increasing stimulant and the attractant may be the same factor.

As has been pointed out in an earlier review (Cheng, 1967), the effectiveness of the "host factor" (a term introduced by Davenport (1955) to designate the stimulatory material of host origin) in guiding miracidia to their molluscan host is doubtful under certain circumstances. For example, Etges and Decker (1963) have pointed out that the naturally-occurring negative geotaxis and postive phototaxis of *Schistosoma mansoni* miracidia most probably eclipse the chemotactic effect of the "host factor." Even between these taxes, Chernin and Dunavan (1962) have demonstrated that the negative geotaxis is a more powerful determinant of miracidial behavior than is positive phototaxis. Thus, it is only under those conditions where naturally-occurring taxes guide the miracidia to the immediate proximity of the mollusc that the influence of the "host factor," which is operative only within short distances, is effective (Fig. 1).

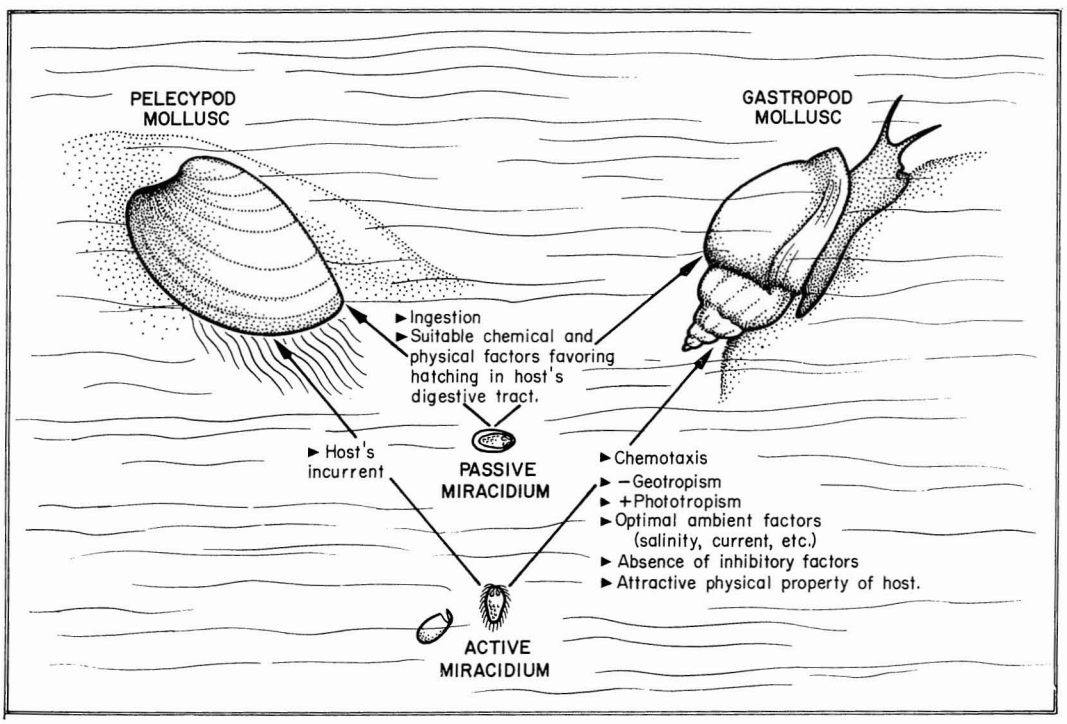

FIG. 1. Diagrammatic drawing illustrating factors which can govern or influence miracidium-mollusc contact during the pre-invasion phase. The terms "tropism" and "taxis" are used interchangeably.

TABLE 3

Comparisons of the Number of Contacts Between *Fasciola gigantica* Miracidia and Nonpresoaked Agar Blocks (Controls) and Blocks Presoaked in Concentrated Plasma and Tissue Extracts of Three Species of Snails (The Student *t* Test Was Employed to Determine Significances)

	CONTROL BLOCKS		PLASMA-SOAKED BLOCKS				TISSUE EXTRACT-SOAKED BLOCKS			
MOLLUSCAN SPECIES	NO. OF TRIALS	MEAN NO. OF CONTACTS (5 MIN.)	NO. OF TRIALS	MEAN NO. OF CONTACTS (5 MIN.)	P	SIG-NIF.	NO. OF TRIALS	MEAN NO. OF CONTACTS (5 MIN.)	P	SIG-NIF.
G. ollula	40	1.55	60	6.35	< 0.0001	+	30	4.30	< 0.01	+
H. duryi normale	117	1.65	117	6.58	< 0.0001	+	97	5.40	< 0.0001	+
T. granifera	60	0.9	60	4.80	> 0.001	+	60	0.4	> 0.5	−

It should also be mentioned that it is highly doubtful if chemotactic forces are influential during the pre-invasion relationship between miracidia and pelecypod molluscs, since the ability of this group of molluscs to effect incurrents, through either their siphons or shell edges, undoubtedly results in the passive intake of miracidia via the currents (Fig. 1).

Besides innate taxes, the nature of the environment is known to determine whether attraction between mollusc and miracidium can be effective (Fig. 1). An excellent example of this has been contributed by Kawashima et al. (1961*b*). Earlier, these investigators (1961*a*) had demonstrated that, although the miracidium of *Paragonimus ohirai* is attracted to three species of brackish water snails of the genus *Assiminea* (*A. parasitologica, A. japonica,* and *A. latericea miyazakii*), only one of the three, *A. parasitologica,* is a compatible host. *A. latericea miyazakii* is an incompatible host, while *A. japonica* can be infected experimentally but the level of infection is consistently low. Thus, it would appear that in nature some other factor or factors must be operative to bring about the selection of *A. parasitologica* by miracidia. It was subsequently shown, in a study of the locomotive speed and survival of *P. ohirai* miracidia in various concentrations of NaCl, that the lower the salt concentration is the more active the miracidia become, with the optimum salinity being 0.25% NaCl or less. Concurrent studies on the ecology of the three species of snails revealed that the optimum salinity for *A. parasitologica* is 0.25%, that for *A. latericea miyazakii* is 0.4%, and that for *A. japonica* is 0.6%. These findings demonstrate that an environmental factor, salinity in this case, can serve as a mechanism determining host selection. Thus, these investigators have demonstrated that the influence of the molluscs' attractants can be masked by an ambient factor and have also releaved further evidence that attraction of miracidia to mollusc need not mean successful subsequent development.

Substances of host origin need not always enhance establishment of the parasite. Some may be inhibitory. For example, Cheng et al. (1966*a*) have demonstrated that the plasma of seven species of marine pelecypods, *Mercenaria mercenaria, Mya arenaria, Crassostrea gigas, C. virginica, Tapes philippinarum, Mytilus edulis,* and *Modiolus demissus,* will stimulate the cercariae of *Himasthla quissetensis* to encyst; as the result of the rapidity of this process in the instances of *C. gigas* and *C. virginica,* the cercariae are prevented from penetrating these oysters, especially *C. gigas,* and hence these pelecypods may be considered incompatible hosts (Cheng et al., 1966*b*). These findings demonstrate how in some instances a factor of host origin operative during the pre-invasion phase of host-parasite relationship could determine the incompatibility of the host.

It should be pointed out that the tissue extracts of the same seven species of pelecypods have a negative effect on *H. quissetensis* cercariae, reducing their life spans significantly. However, the fact that the cercariae are stimulated to encyst in *M. mercenaria, M. arenaria, T. philippinarum, M. edulis,* and *M. demissus* before the tissue component(s) could cause their death is believed to be responsible for the compatibility of those pelecypods as second intermediate hosts. This is because the cyst wall serves as a protective layer against continued contact with the tissue component(s).

Wright (1959) has suggested that the "host factor" may be in the form of species-specific substances incorporated in the body-surface mucus of molluscs; Kawashima et al. (1961*a*) have demonstrated that *Paragonimus ohirai* miracidia are attracted to amino acid mixtures placed in cellophane bags; and MacInnis (1965) has found that butyric acid, galactose, L-cysteine HCl, and even 1.0 mM HCl will stimulate "contact with return" of over 80% of *S. mansoni* miracidia in an artificial test system. However, no direct evidence is yet available to indicate the nature of "host factors." Moreover, it should be pointed out that although organic molecules, possibly amino acids, fatty acids, and sugars, are the attractants, there is also some evidence which indicate that the pH or some other physical property of the host may be responsible, at least in part, for the attraction (Kawashima et al., 1961*a*) (Fig. 1). This most probably explains the attraction of *S. mansoni* miracidia for dilute HCl, as demonstrated by MacInnis.

In the case of those species of trematodes which do not include a free-swimming mira-

cidial stage, successful host-parasite contact is dependent upon the ingestion of the egg by the mollusc, followed by hatching within the latter's alimentary tract (Fig. 1). Although some information is available pertaining to the hatching process of eggs in water (Standen, 1951; Rowan, 1956, 1957), surprisingly little information is available on the factor or factors which influence hatching of trematode eggs in molluscs. It is generally believed that the mollusc's digestive juices in some manner stimulate hatching (Krull and Mapes, 1952; Timon-David, 1965; and others), although the exact mechanisms have not been studied. Nevertheless, it is apparent that the biochemistry and physical properties of the mollusc's digestive tract could serve as determinants of compatibility, as manifested by hatching, or of incompatibility, as manifested by nonhatching (Fig. 1).

THE INVASION PROCESS

Because of obvious medical implications, considerable information is available about factors governing the penetration of cercariae into mammals and other vertebrate hosts. On the other hand, little is known about miracidial penetration into molluscs, either from the exterior or through the gut wall. Nevertheless, this barrier could be a factor determining compatibility or incompatibility.

Certain aspects of the processes involved during the successful penetration of *Lymnaea* (= *Limnaea*) *truncatula* and *L. auricularia* by *Fasciola hepatica* and *F. gigantica,* respectively, have been studied by Dawes (1959, 1960*a, b, c*). According to him, the miracidium first becomes attached to the molluscan host's integument by suctorial action resulting from application of the "cup" formed by the inversion of the anterior papilla assisted by mucus. This is followed by the secretion of cytolytic enzymes from ". . . the gut and the unicellular pharyngeal 'glands' into the 'cup.' " The subsequent enzymatic activity results in the lysis of the host's epithelial and subepithelial tissues. Only after the host's integument has been perforated does the parasite enter, but not before it has sloughed its ciliated epidermis. For this reason, Dawes considers the penetrating form to be a sporocyst and not a miracidium.

The question may be asked whether the miracidial cytolytic enzymes must be chemically specific for the integument of specific species of molluscs. If this is the case, the compatibility of enzyme to substrate could serve as a factor governing successful penetration, hence host-compatibility (Fig. 2). Indeed, Lie (1963) believes that the prevention of penetration of certain echinostome miracidia by unnatural snails is responsible for incompatibility.

In addition to the miracidium's cytolytic enzymes, Dawes (1960*c*) has expressed the view that the shedding of the ciliated epidermis by the miracidium, thus transforming it to a sporocyst, is a prerequisite for successful infection of the snail. This hypothesis has been challenged by Lengy (1962), who has demonstrated that *Schistosoma bovis* miracidia do not shed their ciliated epidermis prior to penetrating. Similarly, Maldonado and Metienzo (1947) demonstrated earlier that *S. mansoni* miracidia do not shed their plates until after successful penetration; and Heyneman (1966) has successfully initiated the infection of *Lymnaea rubiginosa* with *Echinostoma audyi* miracidia and of *Indoplanorbis exustus* with *Echinostoma malayanum* miracidia by inoculating these miracidia through the mantle via a minute hole drilled in the molluscs' shells, thus suggesting that the shedding of the miracidial epidermal plates is not a prerequisite to successful infection, at least in these species. It remains true, nevertheless, that certain species of fasciolid miracidia may shed their plates prior to penetration. Campbell and Todd

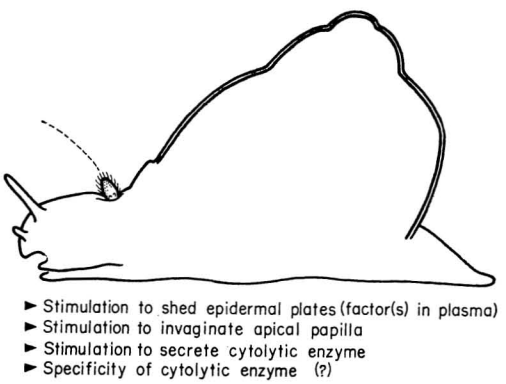

FIG. 2. Diagrammatic drawing illustrating factors which may govern or influence successful penetration of mollusc by miracidium.

(1955), for example, have reported that *Fascioloides magna* miracidia shed their ciliated plates on the exterior after a brief contact with the molluscan host's tissues, and Barlow (1925) has found the transformation of *F. buski* miracidia into sporocysts when bathed in "snail tissue juices." I observed that *F. gigantica* miracidia shed their plates when placed in concentrated and 1:10 dilutions of the plasma of *Galba ollula,* the natural host (Fig. 3), and in similar concentrations of the plasma of *Helisoma duryi normale,* an incompatible host, but not in the plasma of *Tarebia granifera mauiensis* and *Littorina pintado,* both of which are also incompatible hosts. Similar phenomena were not observed when miracidia were placed in tissue extracts of all four species of snails, nor were they observed in greater dilutions of *G. ollula* and *H. duryi normale* plasma. Thus, this phenomenon is apparently not related to host compatibility. Rather it suggests that miracidia which possess the innate ability to shed their epidermal plates prior to penetration can be stimulated to do so by some factor(s). Nevertheless, it would appear that the occurrence of the stimulatory factor(s) in the natural hosts could influence compatibility during this phase of host-parasite relationship, especially if Dawes' contention is true among fasciolid trematodes (Fig. 2).

No information is yet available relative to the nature of the stimulatory factor(s); however, the fact that *F. gigantica* miracidia did not shed their plates when placed in extracts of desanguinated and aqueously perfused snails indicates that the factor is present in plasma rather than in tissue fluids.

It is also significant that *F. gigantica* miracidia were stimulated to shed their plates only in concentrated plasma and in a 1:10 dilution of plasma. This may be interpreted to mean that in nature the stimulatory effect would occur only when miracidia become intimately associated with or are in actual contact with the snail, since plasma, seeping from the wound resulting from the parasite's lytic enzymes, would be rapidly diluted as it diffuses though the aqueous medium.

In addition to the shedding of epidermal plates, invagination of the apical papilla as well as the secretion of some substance, perhaps the lytic enzyme, were also noticed in *F. gigantica* miracidia exposed to plasma from *G. ollula* and *H. duryi normale* (Fig. 4). Thus it would appear that the formation of the apical "cup" as well as secretion are stimulated not by physical contact but by some factor(s) present in the mollusc's plasma (Fig. 2).

ESTABLISHMENT OF THE PARASITE

Successful establishment of germinal sacs (sporocysts, rediae, or both) within the mollusc implies that the form which has invaded the host will reach a suitable site, overcome the host's internal defense mechanisms, be the target of host-elaborated growth and development-stimulating factors, be able to obtain its required nutrients, and at the same time not kill its host (Fig. 5). These requirements are considered separately.

Reaching a Suitable Site

Although tissue specificity still remains one of the unsolved problems in parasitology, it is a well-documented phenomenon. For example, for a large number of species among the Digenea the molluscan host's hepatopancreas or gonads appear to be the preferred sites of normal larval development. This does not mean that aberrant parasites cannot develop in ectopic sites. Indeed, such exceptions to the rule are known. Investigations into the nature and development of larvae which grow at ectopic sites not only can provide insights into the physico-chemical requirements of these parasites but also can reveal some of the factors which inhibit or prevent the parasite from reaching its normal developmental site. A series of such studies is summarized to illustrate this point.

Among the Plagiorchioidea, the mother sporocysts of certain species are known to be attached to the external surface of their molluscan hosts' alimentary tracts (Cort et al., 1954; Rankin, 1944; Leigh, 1946; Schell, 1961, 1962*a;* Cheng, 1961*a, b;* and others). Surrounding each of the daughter sporocysts arising from these mother sporocysts is a so-called paletot. The question is: what is the origin of this paletot? According to Cort and Olivier (1943), Cort and Ameel (1944), and Cort et al. (1954), the paletot is formed from the

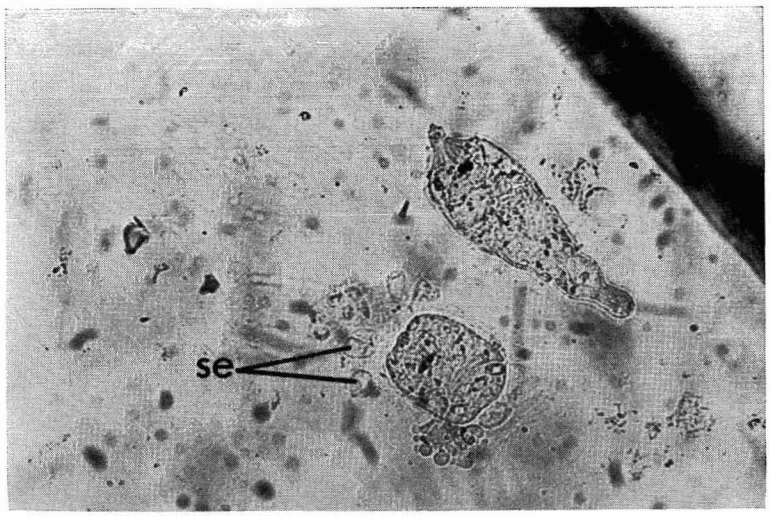

FIG. 3. Photomicrograph showing *Fasciola gigantica* miracidium which has shed its epidermal plates after exposure to a 1:10 dilution of *Galba ollula* plasma; 1 minute after exposure. *se,* Shed epidermal plates.

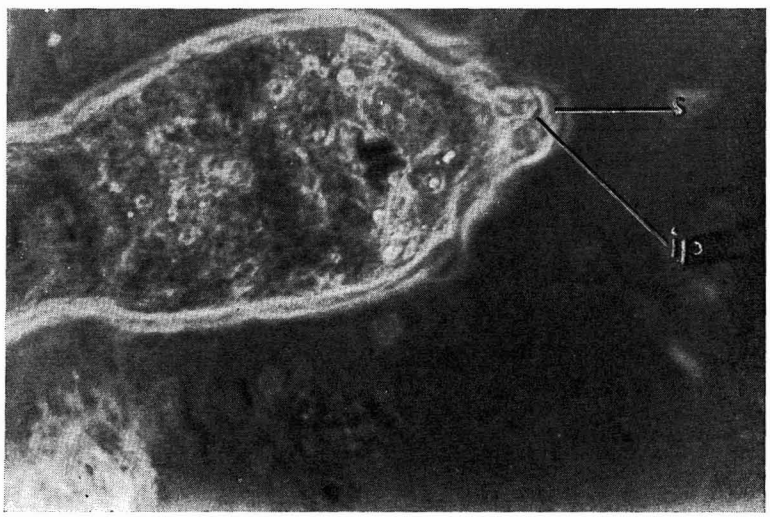

FIG. 4. Phase-contrast photomicrograph showing invagination of apical papilla of *Fasciola gigantica* miracidium and secretion of lytic enzyme(?) after exposure to concentrated *Galba ollula* plasma. *ip,* Invaginated papilla; *s,* secreted material.

multiplication of the cells of the mother sporocyst wall which invaginates and surrounds each daughter sporocyst. More recently Schell (1961, 1962*a*), who studied the sporocysts of *Haplometrana intestinalis* and *Glypthelmins quieta,* has expressed the opinion that the paletot is not of parasite origin but represents an enveloping membrane which has resulted from the proliferation of the basement membrane surrounding the snail's gut, thus preventing further invasion by the mother sporocyst beyond that space delimited by the intestinal epithelium on one side and the basement membrane on the other. If Schell's observations are correct, the reason why mother sporocysts of *H. intestinalis* and *G. quieta* are found abutting upon their molluscan

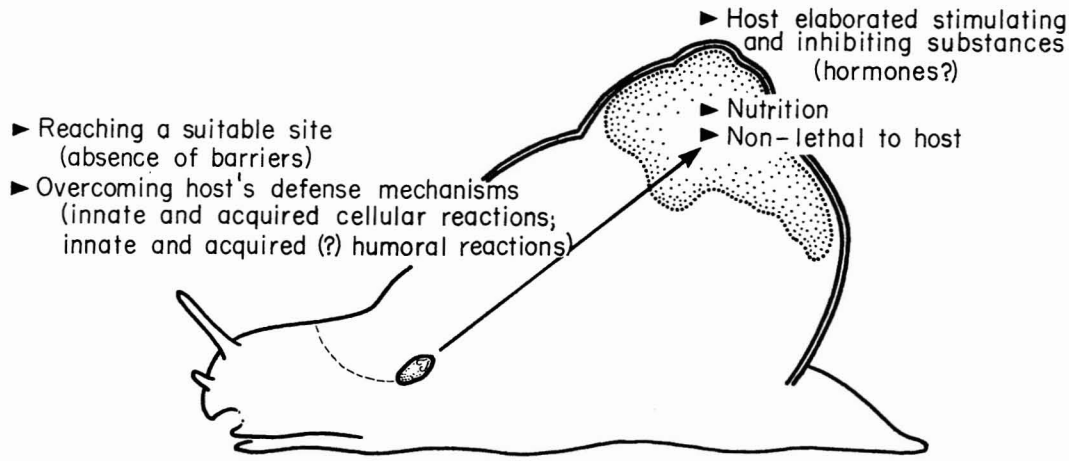

FIG. 5. Diagrammatic drawing illustrating factors which can govern or influence the successful establishment of trematode larvae in a mollusc.

hosts' alimentary tracts rather than between the hepatopancreatic lobules—as is the case with certain other plagiorchioid trematodes (Mattes, 1936; Maldonado, 1945; Cort et al., 1952)—is that the migration of these sporocysts becomes restricted by their hosts' basement membranes. Although the mother sporocysts of both *H. intestinalis* and *G. quieta* become successfully established at this site and produce daughter sporocysts, the migration of the mother sporocysts does become restricted. Schell (1962*a*) has stated: "In following the development of *G. quieta* it became evident that the thin basement membrane beneath the intestinal epithelium of the host snail plays an important role in protecting the snail from invasion by the parasite." Is it possible, then, that certain species of trematodes, which could not undergo normal development at the site restricted by the basement membrane, would in this way be prevented from becoming established? Although examples of this, as far as I have been able to determine, have not yet been described among mollusc-larval trematode relationships, the condition known as schistosome dermatitis caused by avian schistosome cercariae in human skin is an example of the inability of these carcariae to successfully penetrate the depth of the abnormal host's skin: being unable to survive in the surfacial areas of the skin, they die. In this connection, Lewert and Lee (1954), Lewert (1958), and Lewert and Mandlowitz (1963) have demonstrated that the physical and chemical natures of the basement membrane and ground substance do determine whether the entrance of invasive forms of helminths will be successful.

The evidence presented indicates that barriers, especially in the form of basement membrane and perhaps ground substances, situated in the path of invading trematode larvae could prevent them from reaching a satisfactory site for further development and thus serve as determinants of host incompatibilty.

Host's Defense Mechanisms

What is known about the nature of internal defense mechanisms in molluscs, both cellular and humoral, has been reviewed in recent years by Stauber (1961), Cheng and Sanders (1962), Tripp (1963), and Cheng (1967). The role of molluscan leucocytes (amoebocytes or phagocytes) and fibrous elements in innate immunity by causing the encapsulation of trematode larvae, is well established. The results of Newton (1952, 1954), Brooks (1953), Sudds (1960), and others strongly indicate that encapsulation generally occurs around larvae in unnatural hosts. However, slight and restricted encapsulation may also occur around larvae in their natural hosts (Cheng and Cooperman, 1964; Probert and Erasmus, 1965; Schell, 1961, 1962*a, b;* Pan, 1965), but these extremely light capsules usually inflict little or no damage upon

the parasites. On the other hand, the extensive capsules which surround parasites in unnatural hosts usually result in destruction of the parasites. The chemical basis for this destruction remains undetermined; nevertheless, it may be generalized that encapsulation by leucocytes and/or fibers resulting in death is by far the most effective form of innate defense mechanism in molluscs against incompatible trematode larvae.

Although supposedly innate humoral factors in molluscs have been reported by various workers (see the review by Cheng, 1967), their effectiveness as defense mechanisms against invading trematode larvae is unknown or uncertain. Recently, however, Heyneman (1966) has successfully demonstrated in transplantation studies that the inability of *Echinostoma malayanum* to become established in *Lymnaea rubiginosa* and of *E. audyi* in *Indoplanorbis exustus* is due to "physiological rejection within snail tissues distinct from the factors responsible for failure of miracidia to attach to or penetrate the body wall of the nonadapted host." Although it would be tempting to interpret Heyneman's findings to indicate the occurrence of an innate humoral factor, the destruction of *E. malayanum* and *E. audyi* larvae could very well have resulted from encapsulation. Unfortunately, follow-up histological studies which would confirm this conclusion are not available.

Two other examples of possible occurrence of innate humoral immunity which prevents the establishment of larval trematodes have been reported. Benex and Lamy (1959) showed that tissue extracts from the planorbid snail *Planorbis corneus* will immobilize *S. mansoni* miracidia, and these French workers suggest that species of snails which are refractory to *S. mansoni* infection may possess "immune-like" immobilizing substances. Sudds (1960) has shown that when *Trichobilharzia elvae* miracidia penetrate two abnormal hosts, *Bulimnaea megasoma* and *Fossaria abrussa,* the parasites die and begin to degenerate within 1.5–6 days, without any indication of a host tissue reaction. Again, it would be tempting to interpret these findings as indications of the presence of innate humoral immunity but, under the conditions of the experiments, other possible explanations cannot be completely ruled out.

Occasionally suggestions have appeared in the literature (Sogandares-Bernal, 1965) that snails at different ages present different degrees of susceptibility to infection by trematode larvae. Most, if not all, of these reports have resulted from either field studies (where the ages of snails have been estimated by their sizes) or qualitative assays of infectivity. Whether such age-correlated resistance is due to some innate humoral factor or even to a cellular factor remains unknown.

The only evidence of acquired cellular immunity I have been able to find is that presented by Barbosa and Coelho (1956). They demonstrated that, although *Biomphalaria glabrata* previously "cured" of *Schistosoma mansoni* infection can be reinfected, some tissue reaction involving leucocytes and fibrous elements is evoked in reinfected snails, a phenomenon not found in initial infections. This finding could mean that some type of incomplete acquired immunity exists in *B. glabrata* after the initial infection and is manifested during reinfection as cellular response.

The belief held by some workers that acquired humoral immunity can occur in molluscs stems primarily from the reports of Winfield (1932) and Nolf and Cort (1933). These investigators reported that the presence of *Cotylurus flabelliformis* sporocysts in varieties of *Lymnaea stagnalis* prevents almost all of the cercariae of this trematode from successfully penetrating and encysting as metacercariae. Later, Cort et al. (1945) repeated these studies and reported that the same phenomenon occurs in *Stagnicola emarginata angulata* parasitized by *C. flabelliformis* sporocysts. They noted that the few cercariae which did succeed in penetrating were inhibited from developing into metacercariae unless they entered sporocysts and were thus presumably protected from the host's antibodies. On the basis of these reports, Culbertson (1941) concluded that ". . . it is clear that snails acquire an immunity after infection by trematodes. . . ." Several later authors, especially Michelson (1963) and Cheng (1967), have cautioned that this generalization is unwarranted since, as of this date, the ability of molluscs to produce antibodies has not been conclusively demonstrated.

The results of two other studies suggest the

occurrence of acquired humoral immunity. Chowaniec (1961) reported that only a small proportion of snails already harboring *Fasciola hepatica* could be infected with the same parasite, while most of the control snails could be readily infected. In the second study, Lie et al. (1966) demonstrated that only 5% of *Lymnaea rubiginosa* infected with one species of echinostome could be superinfected with a second, while 89% of uninfected control snails could be infected. In neither of these studies, however, were antibodies demonstrated. It is of interest that, in the case of *L. rubiginosa,* Lie et al. stated another possible explanation: young invading sporocysts of the second species are killed and ingested by the rediae of the first species.

The most convincing evidence of acquired humoral immunity in molluscs is that contributed by Michelson (1963, 1964), who demonstrated that *Schistosoma mansoni* miracidia-immobilizing substances occur in the tissue extracts of *Biomphalaria glabrata* infected with this trematode. Although Michelson found that his controls (extracts of other species of uninfected snails, extracts of snails infected with an acid-fast bacillus, an echinostome metacercaria, the nematode *Daubaylia potomaca,* snails inoculated with bovine albumin, *S. mansoni* eggs, and polystyrene spheres, uninfected *B. glabrata,* and water) all gave positive results, in no instance did the percentage of immobilization reach the level observed in extracts of *S. mansoni*-infected snails. Michelson cautiously states: "Although the suggestion that the immobilizing phenomenon might be associated with an antigen-antibody interaction is an appealing one, data are lacking to substantiate this hypothesis. The possibility that the immobilizing substance(s) might be related either to parasite-produced toxins or to products resulting from alternations in the snail's metabolism cannot be excluded."

It may be concluded, then, that innate cellular immunity appears to be the most efficient mechanism by which trematodes are prevented from developing in incompatible molluscs, although acquired cellular immunity may occur. The role of humoral factors, either innate or acquired, remains uncertain.

Influence of Host-Elaborated Growth- and Development-Stimulating or -Inhibiting Substances

This vast area of host-parasite relationships has hardly been touched. From what is known about the metabolic interaction between larval trematodes and molluscs, it is inconceivable that compatible hosts do not influence in some manner the growth and differentiation processes of their parasites and thus enhance their normal sequence of development, or, conversely, that incompatible hosts do not in some manner inhibit the normal developmental sequences of their parasites.

Meade and Pratt (1966) have reported that, when rediae of *Metagonimoides oregonensis* are experimentally transplanted from naturally infected *Oxytrema silicula,* in which the gonads had been destroyed, to young uninfected snails with healthy gonads, a certain number will survive but that differences are apparent between the transplanted rediae, their progeny, and those in naturally infected snails. They noted that the transplanted rediae more than doubled their natural size, "mucus and debris" were included in their caeca, and the enclosed metacercariae were no longer distinguishable. Burns and Pratt (1953) had shown earlier that the rediae of *M. oregonensis* give rise to both cercariae and metacercariae within their brood chambers and that no daughter rediae occur. Furthermore, although some metacercariae, released from transplanted rediae into the body cavities of acceptor snails, survived for up to 6 weeks, none of these were infective when fed to a known compatible experimental definitive host, the golden hamster. These uninfective metacercariae also exhibited some behavioral and morphological peculiarities. They displayed greater activity, their eyespots disappeared, and the prominent Y-shaped excretory vesicle which normally appears black was often enlarged and possessed fewer granules. Meade and Pratt are of the opinion that these differences in the transplanted rediae and metacercariae had resulted from the influence of their new host's gonadal hormone(s). The same hormone(s) presumably was present at a very much lower level or not present at all in the original castrated hosts. Whether this conclusion is justified must await more direct evidence.

From the study cited above, it would appear that some host factor(s), perhaps hormones, in *O. silicula* with healthy gonads does disrupt the normal development of *M. oregonensis* rediae and metacercariae during the later phase of the relationship. The significance of this finding to our discussion is that it is an example of a host-elaborated substance which "inhibits" normal development and thus promotes incompatibility. The phenomenon reported by Meade and Pratt, however, does not appear to hold true in all transplanted mollusc-larval trematode associations. Chernin (1966), for example, has reported successful transplants of *S. mansoni* mother and daughter sporocysts from *B. glabrata* to acceptor snails of the same species which were followed by normal cercarial formation. Perhaps this difference can be explained by the fact that *M. oregonensis* includes a redial stage while *S. mansoni* includes sporocyst stages. It is well known that rediae inflict significantly more damage upon molluscs than do sporocysts. Thus, perhaps the donor *B. glabrata* is never completely castrated and *S. mansoni,* as the result of a long relationship with *B. glabrata* and exposure to its hormones, is not adversely affected by hormones during the latter phases of its development, as is *M. oregonensis.*

Another example of possible host-stimulated developmental alterations among larval trematodes has been reported by James (1964) and discussed by Cable (1965). James reported that the intramolluscan life cycle stages of the gymnophallid trematode *Parvatrema homoeotecnum* include a "primary germinal sac" with adult features, including an oral sucker, ventral sucker, pharynx, and bifid caeca, and a "daughter germinal sac" which is unique in that, in addition to the adult features found on the "primary germinal sac," it also possesses a bifurcate tail. The "daughter germinal sacs" increase in size and lose their tails while still within the "primary germinal sac." Further development does not occur until they rupture out of primary sacs. Daughter sacs then continue to develop in one of three possible ways: (1) most produce cercariae and metacercariae; (2) a few produce a second generation of "daughter germinal sacs"; and (3) very occasionally, cercariae, metacercariae, and second-generation "daughter germinal sacs" are produced in the same "daughter germinal sac." These larval stages are found in the haemocoelic spaces of the hepatopancreas and gonad of *Littorina saxatilis tenebrosa.* According to James, the "primary germinal sac" could be interpreted to be a metacercaria; while according to Cable, the "daughter germinal sac" could be considered a cercaria. Thus, the usual sequence of larval stages is reversed in *P. homoeotecnum.* Since the usual life history pattern among related gymnophallids includes two molluscan intermediate hosts, both being marine pelecypods (see Stunkard and Uzmann, 1958), Cable has given the following as one possible explanation for this variation: "It may be significant that metacercariae of other gymnophalline species live in loose, even superficial association with their hosts whereas the species that James (1964) described invades the snail to the extent commonly seen in molluscs serving as the first intermediate host of trematodes in general. As a result, that species probably gets a double exposure of the most intimate sort to the tissues and body fluid of mollusks." This, of course, implies that the tissues and body fluids of molluscs may have influenced that unusual developmental sequence. Although in this instance the presumably host-stimulated developmental alterations do not affect the parasite deleteriously, it is conceivable that such changes could in certain instances deter or inhibit delayed polyembryony and thus render the host incompatible.

Nutrient Requirements

Available evidence indicates that trematode parasites utilize carbohydrates as their primary energy source. They acquire their carbohydrates in the form of glucose resulting from the degradation of the host's stored glycogen or, if there is no stored glycogen in the vicinity of their natural habitat, they utilize the mollusc's blood sugars (Cheng, 1963*b*). In addition to sugars, these larvae apparently utilize free amino acids from the mollusc's hemolymph and perhaps even from the surrounding host cells which are lysed or ruptured mechanically.

Lipids, in the form of short-chain fatty acids, are also taken up by germinal sacs, but are primarily stored rather than utilized in cercariae and in certain species in the walls of

germinal sacs. The current belief that these stored fatty acids are not utilized in energy production while within the mollusc's hepatopancreas stems from von Brand's (1951) belief that this environment is essentially anaerobic and hence is not conducive to lipid metabolism. The non-occurrence of aerobic metabolism in sporocysts and in rediae appears to be confirmed by the electron microscopic studies of Bils and Martin (1966), which revealed the absence of mitochondria in sporocyst and redial walls. On the other hand, mitochondria have been demonstrated in the wall of *Parorchis acanthus* rediae (Rees, 1966), and in the walls of *Philophthalmus gralli* rediae (Cheng and Hamamoto, unpublished data).

Cheng and Snyder (1962*a*, *b*; 1963), by employing histochemistry, arrived at the conclusion that glucose and fatty acids are taken up by sporocysts through their body walls. Electron microscope studies by Bils and Martin (1966) tend to corroborate this with the finding of conspicuous microvilli along the outer surfaces of sporocysts. In the case of rediae, Cheng (1962, 1963*c*) found that the ingestion of the host's cells is the primary method of nutrient acquisition, although some absorption may occur through the body wall also. This, again, appears to be corroborated by the finding of microvilli on redial walls by Bils and Martin (1966), Rees (1966), and Cheng and Hamamoto (unpubl.). For a detailed account of nutrient acquisition and contents of intramolluscan larval trematodes, see the reviews by Cheng (1963*a*, 1967).

Relative to the relationship between nutrition and compatibility and incompatibility, it may be asked whether the introduction of germinal sacs to some site within its natural molluscan host, or into a foreign host, where the physico-chemical nature of the host-parasite interphase prevents the uptake of nutrients could cause the parasite to fail to become established. For example, could the destruction of heavily encapsulated germinal sacs in incompatible hosts actually represent, at least in part, death due to starvation?

It is known that the rate of cercarial development is dependent upon the amount of food assimilated by the snail host and upon the number of larvae competing for the available nutrients in the snail, among other factors (Kendall, 1949). It would follow that, even if the host-parasite interphase is favorable for nutritional uptake but nutrients are not sufficiently available as the result of a poorly nourished host or because of competition between a large number of germinal sacs, normal development could not occur—and this would constitute incompatibility.

Lethality to Host

Surprisingly little information is available pertaining to the lethality of trematode larvae to molluscs. Some investigators (Rees, 1931; Kendall, 1964; and James, 1965) have suggested that during certain mollusc-trematode associations death of the host does not occur. Yet Schreiber and Schubert (1949) and Pan (1965), both working with *B. glabrata* parasitized with *S. mansoni,* have shown that a high incidence of mortality does occur. In fact, Schreiber and Schubert went as far as to quote a "half life" for parasitized snails. These known mortalities, however, cannot be considered to reflect incompatibility, since the parasites do develop normally and death, as Faust and Hoffman (1934), Schreiber and Schubert (1949), and Pan (1965) have pointed out, has resulted from the rapid multiplication of larvae and mass emergence of cercariae. From the available information, it would appear that the death of molluscs resulting from invasion by "pathogenic" trematodes is extremely rare. No indisputable examples have yet been reported, although Kendall (1950) has shown that *Fasciola hepatica* does inflict conspicuous deleterious effects on *Lymnaea stagnalis*, *L. palustris*, and *L. glabra*, but not on *L. auricularia.* The fact that mass mortality seldom occurs could be interpreted to mean that the defense mechanisms of molluscs are highly efficient.

It appears appropriate at this point to interject the following comment. Death of molluscs due to a pathogenic trematode infers extremely severe pathogenicity, a topic which has been reviewed recently (Cheng, 1967). Yet, despite known instances of drastic histopathological alterations in parasitized molluscs caused by trematode larvae, few proven cases of rapid and virulent deaths are known. This should serve as a warning to shellfisheries biologists and molluscan pathologists, who have been known to

draw unwarranted conclusions relative to the lethality of parasites, particularly helminths, based on histopathological studies.

ESCAPE PROCESS

Our present knowledge concerning the passive and/or active mechanisms employed by cercariae while escaping from their molluscan hosts is based primarily on studies carried out on the human-infecting species of schistosomes (see reviews by Probert and Erasmus, 1965; Cheng, 1967), although some information is available on *Fasciola hepatica* (Kendall and McCullough, 1951), *Neodiplostomum intermedium* (Pearson, 1961), and *Cercaria X* (Probert and Erasmus, 1965). All these studies have been concerned with the processes which make possible successful escape, and hence, in a manner of speaking, govern compatibility. On the other hand, if some factor or factors within the mollusc interfere with cercarial escape and thus prevent the parasites from continuing their normal course of development, this factor or factors may contribute to host-incompatibility. In seeking evidence for this hypothetical possibility, one should be cautious in distinguishing between consistent barriers, either structural or physiological, which prevent escape and in so doing endanger the perpetuation of the parasite species, and an occasional accidental arrest of the escape of a few cercariae. Examples of the latter include the report by Cheng and Cooperman (1964) that occasionally an escaping cercaria of *Glypthelmins pennsylvaniensis* may accidentally migrate into the foot musculature of its snail host, *Helisoma trivolvis*, and become encapsulated, and the report by Pan (1965) that some escaping *S. mansoni* cercariae do become trapped in the loose vascular connective tissue of *A. glabratus* and die. Yet, in the latter case the frequency of this event suggests that it is a normal occurrence. If all of the cercariae were thus trapped, one could cite this as an example of incompatibility due to prevention of escape. It is of interest to note that if a similar phenomenon is not found in other mollusc-trematode associations, one might consider *B. glabrata* as being partially incompatible with *S. mansoni* as far as escape is concerned. Indeed, available information indicating the incompatibility of different strains of *B. glabrata* with strains of *S. mansoni* does suggest that this relationship is not completely free of factors favoring incompatibility.

SUMMARY

Compatibility between miracidia and molluscs may be determined prior to actual contact. In some instances this may be governed by the occurrence of host-specific "host factors" which stimulate and attract the parasite to a compatible host only. There is sufficient evidence to indicate, however, that the attractant need not be host-specific and need not indicate subsequent successful establishment and growth. Furthermore, ambient environmental conditions as well as innate taxes, when such occur, are generally stronger determinants of miracidial migration and behavior than are chemotactic forces. Thus, it is only when the other factors serve to bring miracidia into the immediate vicinity of the mollusc that chemotaxis becomes an effective attractant. There is also evidence which suggests that materials extruded from molluscs may inhibit rather than serve to enhance host-parasite contact.

In the case of miracidia which do not hatch until the eggs are ingested by the mollusc, the physical and chemical factors present in the host's gut can serve as determinants of compatibility or incompatibility.

It is possible that, during the invasion process, both the specificity of the miracidium's lytic secretions and the specificity of substances which stimulate the miracidium to shed its epidermal plates, secrete lytic enzymes, and invaginate its apical papilla could in some instances determine the compatibility of the host, especially if these are prerequisites of successful penetration.

Subsequent to successful invasion, the parasite generally has to reach a suitable site within the mollusc for further development. If this migration is prevented by some tissue(s), such as a basement membrane, incompatibility results. If such a barrier is absent or is overcome, the parasite must still overcome the host's internal defense mechanisms (immunity). From the information available, cellular immunity, both innate and acquired, occurs in molluscs; and it is primarily by the formation of capsules

comprised of leucocytes and/or fibers that incompatible trematode larvae are destroyed.

Since some indirect evidence suggests that substances of host origin, perhaps hormones, can alter the normal developmental pattern of intramolluscan trematode larvae, it is proposed that the absence of growth- and development-stimulating substances or the presence of growth- and development-inhibiting substances may be factors responsible for incompatibility.

Relative to the nutritional requirements of trematode larvae, their availability in the mollusc, as well as deficiencies in nutriments resulting from competition between larvae, could influence the compatibility of the association. Furthermore, the physico-chemical nature of the host-parasite interphase could influence the successful or unsuccessful uptake of nutrients and hence govern compatibility or incompatibility.

Finally, in order that the relationship be considered a completely compatible one, the parasite cannot destroy its host prior to its successful escape or the escape of its germ cell-bearing progeny. Thus, factors of parasite origin which are lethal to the host and factors of host origin which prevent escape must also be considered as determinants of incompatibility.

REFERENCES

BARBOSA, F. S. 1965. Ecology of the larval parasitic stages of *Schistosoma mansoni.* Rev. Inst. Med. Trop. Sao Paulo 7:112–120.

BARBOSA, F. S., and M. V. COELHO. 1956. Pesquisa de immunodade adquirida homologa em *Australorbis glabratus,* nas infestacoes por *Schistosoma mansoni.* Rev. Brasil. Malariol. 8:49–56.

BARLOW, C. H. 1925. The life cycle of the human intestinal fluke *Fasciolopsis buski* (Lankester). Am. J. Hyg., Monogr. Ser. 4:1–98.

BENEX, J., and L. LAMY. 1959. Immobilisation des miracidiums de *Schistosoma mansoni* par des extraits de planorbes. Bull. Soc. Pathol. Exot. 52:188–193.

BILS, R. F., and W. E. MARTIN. 1966. Fine structure and development of the trematode integument. Trans. Am. Microscop. Soc. 85: 78–88.

BROOKS, C. P. 1953. A comparative study of *Schistosoma mansoni* in *Tropicorbis havenensis* and *Australorbis glabratus.* J. Parasitol. 39:159–163.

BURNS, W. C., and I. PRATT. 1953. The life cycle of *Metagonimoides oregonensis* Price (Trematoda: Heterophyidae). J. Parasitol. 39:60–67.

CABLE, R. M. 1965. "Thereby hangs a tail." J. Parasitol. 51:3–12.

CAMPBELL, W. C. 1961. Notes on the egg and miracidium of *Fascioloides magna* (Trematoda). Trans. Am. Microscop. Soc. 80:308–319.

CAMPBELL, W. C., and A. C. TODD. 1955. *In vitro* metamorphosis of the miracidium of *Fascioloides magna* (Bassi, 1875) Ward, 1917. Trans. Am. Microscop. Soc. 74:225–228.

CHENG, T. C. 1961*a*. Description, life history, and developmental pattern of *Glypthelmins pennsylvaniensis* n. sp. (Trematoda: Brachycoeliidae), new parasite of frogs. J. Parasitol. 47:469–477.

——— 1961*b*. Studies on the morphogenesis, development and germ cell cycle on the sporocysts and cercariae of *Glypthelmins pennsylvaniensis* Cheng, 1961 (Trematoda: Brachycoeliidae). Proc. Penna. Acad. Sci. 35:10–22.

——— 1962. The effects of parasitism by the larvae of *Echinoparyphium* Dietz (Trematoda: Echinostomatidae) on the structure and glycogen deposition in the hepatopancreas of *Helisoma trivolvis* (Say). Am. Zool. 2:513.

——— 1963*a*. Biochemical requirements of larval trematodes. Ann. N.Y. Acad. Sci. 113: 289–321.

——— 1963*b*. Histological and histochemical studies on the effects of parasitism of *Musculium partumeium* (Say) by the larvae of *Gorgodera amplicava* Looss. Proc. Helminth. Soc. Wash. 30:101–107.

——— 1963*c*. The effects of *Echinoparyphium* larvae on the structure of and glycogen deposition in the hepatopancreas of *Helisoma trivolvis* and glycogenesis in the parasite larvae. Malacologia 1:291–303.

——— 1967. Marine Molluscs as Hosts for Symbioses: With a Review of Known Parasites of Commercially Important Species. In: F. S. Russell, ed., Advances in Marine Biol-

ogy, Vol. V. Academic Press, London. 424 pp.

CHENG, T. C., and J. S. COOPERMAN. 1964. Studies on host-parasite relationships between larval trematodes and their hosts. V. The invasion of the reproductive system of *Helisoma trivolvis* by the sporocysts and cercariae of *Glypthelmins pennsylvaniensis*. Trans. Am. Microscop. Soc. 83:12–23.

CHENG, T. C., and S. T. HAMAMOTO. 1967. An electron microscopical study of the absorptive surfaces of the redia and cercaria of *Philophthalmus gralli* (Trematoda). Unpubl.

CHENG, T. C., and B. G. SANDERS. 1962. Internal defense mechanisms in molluscs and an electrophoretic analysis of a naturally occurring serum hemagglutinin in *Viviparus malleatus* Reeve. Proc. Penna. Acad. Sci. 36: 72–83.

CHENG, T. C., C. N. SHUSTER, JR., and A. H. ANDERSON. 1966*a*. Effects of plasma and tissue extracts of marine pelecypods on the cercaria of *Himasthla quissetensis*. Exptl. Parasitol. 19:9–14.

——— 1966*b*. A comparative study of the susceptibility and response of eight species of marine pelecypods to the trematode *Himasthla quissetensis*. Trans. Am. Microscop. Soc. 85:284–295.

CHENG, T. C., and R. W. SNYDER, JR. 1962*a*. Studies on host-parasite relationships between larval trematodes and their hosts. I. A review. II. The utilization of the host's glycogen by the intramolluscan larvae of *Glypthelmins pennsylvaniensis* Cheng, and associated phenomena. Trans. Am. Microscop. Soc. 81:209–228.

——— 1962*b*. Studies on host-parasite relationships between larval trematodes and their hosts. III. Certain aspects of lipid metabolism in *Helisoma trivolvis* (Say) infected with the larvae of *Glypthelmins pennsylvaniensis* Cheng and related phenomena. Trans. Am. Microscop. Soc. 81:327–331.

——— 1963. Studies on host-parasite relationships between larval trematodes and their hosts. IV. A histochemical determination of glucose and its role in the metabolism of molluscan host and parasite. Trans. Am. Microscop. Soc. 82:343–346.

CHERNIN, E. 1966. Transplantation of larval *Schistosoma mansoni* from infected to uninfected snails. J. Parasitol. 52:473–482.

CHERNIN, E., and C. A. DUNAVAN. 1962. The influence of host-parasite dispersion upon the capacity of *Schistosoma mansoni* miracidia to infect *Australorbis glabratus*. Am. J. Trop. Med. Hyg. 11:455–470.

CHOWANIEC, W. 1961. Influence of environment on the development of liver fluke, and the problem of superinvasion and reinvasion in the intermediate host. Acta Parasitol. Polon. 9:463–479.

CORT, W. W., and D. J. AMEEL. 1944. Further studies on the development of the sporocyst stages of plagiochiid trematodes. J. Parasitol. 30:37–50.

CORT, W. W., D. J. AMEEL, and A. VAN DER WOUDE. 1952. Development of the mother and daughter sporocysts of a snake plagiorchioid, *Lechriorchis primus* (Trematoda: Reniferidae). J. Parasitol. 38:187–202.

——— 1954. Germinal development in the sporocysts and rediae of the digenetic trematodes. Exptl. Parasitol. 3:185–225.

CORT, W. W., S. BRACKETT, L. OLIVIER, and L. O. NOLF. 1945. Influence of larval trematode infections in snails on their intermediate host relations to the strigeid trematode, *Cotylurus flabelliformis* (Faust, 1917). J. Parasitol. 31:61–78.

CORT, W. W., and L. OLIVIER. 1943. The development of the larval stages of *Plagiorchis muris* Tanabe, 1922, in the first intermediate host. J. Parasitol. 29:81–99.

CULBERTSON, J. T. 1941. Immunity Against Animal Parasites. Columbia Univ. Press, New York, N.Y.

DAVENPORT, D. 1955. Specificity and behavior in symbioses. Quart. Rev. Biol. 30:29–46.

DAVENPORT, D., C. A. WRIGHT, and D. CAUSLEY. 1962. Technique for the study of the behavior of motile microorganisms. Science 135:1059–1060.

DAWES, B. 1959. Penetration of the liver-fluke, *Fasciola hepatica* into the snail, *Limnaea truncatula*. Nature 184:1334–1335.

——— 1960*a*. Penetration of *Fasciola gigantica* Cobbold, 1856 into snail hosts. Nature 185:51–53.

——— 1960*b*. The penetration of *Fasciola hepatica* into *Limnaea truncatula*, and of *F.*

gigantica into *L. auricularia.* Trans. Roy. Soc. Trop. Med. Hyg. 54:9–10.

——— 1960*c*. A study of the miracidium of *Fasciola hepatica* and an account of the mode of penetration of the sporocyst into *Lymnaea truncatula.* In: Libro Homenaje al Dr. Eduardo Caballero y Caballero, pp. 95–111. Inst. Politecnico Nacional, Escuela Nac. de Cien. Biol., Mexico.

ETGES, F. J., and C. L. DECKER. 1963. Chemosensitivity of the miracidium of *Schistosoma mansoni* to *Australorbis glabratus* and other snails. J. Parasitol. 49:114–116.

FAUST, E. C. 1934. The reactions of the miracidia of *Schistosoma japonicum* and *S. haematobium* in the presence of their intermediate hosts. J. Parasitol. 10:199–204.

FAUST, E. C., and W. A. HOFFMAN. 1934. Studies on schistosomiasis mansoni in Puerto Rico. III. Biological studies. I. The extra mammalian phases of the life cycle. Puerto Rico J. Publ. Health Trop. Med. 10:1–49.

FAUST, E. C., and H. E. MELENEY. 1924. Studies on schistosomiasis japonica. Am. J. Hyg., Monogr. Ser. 3:1–339.

HEYNEMAN, D. 1966. Successful infection with larval echinostomes surgically implanted into the body cavity of the normal snail host. Exptl. Parasitol. 18:220–223.

JAMES, B. L. 1964. The life cycle of *Parvatrema homoeotecnum* sp. nov. (Trematoda: Digenea) and a review of the family Gymnophallidae Morozov, 1955. Parasitology 54: 1–41.

——— 1965. The effects of parasitism by larval Digenea on the digestive gland of the intertidal prosobranch, *Littorina saxatilis* (Olivi) subsp. *tenebrosa* (Montagu). Parasitology 55:93–115.

KAWASHIMA, K., I. TADA, and I. MIYAZAKI. 1961*a*. Host preference of miracidia of *Paragonimus ohirai* Miyazaki, 1939 among three species of snails of the genus *Assiminea.* Kyushu J. Med. Sci. 12:99–106.

——— 1961*b*. Ecological analysis on the mechanism of the host preference of miracidia of *Paragonimus ohirai* Miyazaki, 1939 in natural condition. Kyushu J. Med. Sci. 12:143–151.

KENDALL, S. B. 1949. Nutritional factors affecting the rate of development of *Fasciola hepatica* in *Limnaea truncatula.* J. Helminthol. 23:179–190.

——— 1950. Snail hosts of *Fasciola hepatica* in Britain. J. Helminthol. 24:63–74.

——— 1964. Some factors influencing the development and behaviour of trematodes in their molluscan hosts. In: A. E. R. Taylor, ed., Host-Parasite Relationships in Invertebrate Hosts, pp. 51–73. Second Symposium of the British Society for Parasitology. Blackwell Scientific Publications, Oxford.

KENDALL, S. B., and F. S. MCCULLOUGH. 1951. The emergence of the cercariae of *Fasciola hepatica* from the snail *Limnaea truncatula.* J. Helminthol. 25:77–92.

KLOETZEL, K. 1958. Observacoes sobre o tropismo de miracidio do *Schistosoma mansoni* pelo molusco *Australorbis glabratus.* Rev. Brasil. Biol. 18:223–232.

——— 1960. Novas observacoes sobre o tropismo de miracidio do *Schistosoma mansoni* pelo molusco *Australorbis glabratus.* Rev. Inst. Med. Trop. Sao Paulo 2:341–346.

KRULL, W. H., and C. R. MAPES. 1952. Studies on the biology of *Dicrocoelium dendriticum* (Rudolphi, 1819) Looss, 1899 (Trematoda: Dicrocoeliidae), including its relation to the intermediate host, *Cionella lubrica* (Miller). V. Notes on infections of *Dicrocoelium dendriticum* in *Cionella lubrica.* Cornell Vet. 42:339–351.

LEIGH, W. H. 1946. Experimental studies on the life cycle of *Glypthelmins quieta* (Stafford, 1900), a trematode of frogs. Am. Midl. Nat. 35:460–483.

LENGY, J. 1962. Studies on *Schistosoma bovis* (Sonsino, 1876) in Israel. I. Larval stages from egg to cercaria. Bull. Res. Council Israel, Sec. E: Exptl. Med. 10E:1–36.

LEWERT, R. M. 1958. Invasiveness of helminth larvae. Rice Inst. Pamphlet 45:97–113.

LEWERT, R. M., and C. L. LEE. 1954. Studies on the passage of helminth larvae through host tissues. I. Histochemical studies of extracellular changes caused by penetrating larvae. II. Enzymatic activity of larvae *in vitro* and *in vivo.* J. Infect. Dis. 95:13–51.

LEWERT, R. M., and S. MANDLOWITZ. 1963. Innate immunity to *Schistosoma mansoni* relative to the state of connective tissue. Ann. N.Y. Acad. Sci. 113:54–62.

LIE, K. J. 1963. The life history of *Echinostoma malayanum* Leiper, 1911. Trop. Geogr. Med. 15:17–24.

LIE, K. J., P. F. BASCH, and T. UMATHEVY. 1966. Studies on Echinostomatidae (Trematoda) in Malaya. XII. Antagonism between two species of echinostome trematodes in the same lymnaeid snail. J. Parasitol. 52:454–457.

MACINNIS, A. J. 1965. Responses of *Schistosoma mansoni* miracidia to chemical attractants. J. Parasitol. 51:731–746.

MALDONADO, J. F. 1945. The life history and biology of *Platynosomum fastosum* Kossak, 1910 (Trematoda: Dicrocoeliidae). Puerto Rico J. Publ. Health Trop. Med. 21:17–39.

MALDONADO, J. F., and J. A. MATIENZO. 1947. The development of *Schistosoma mansoni* in the snail intermediate host, *Australorbis glabratus*. Puerto Rico J. Publ. Health Trop. Med. 22:331–373.

MATHIAS, P. 1925. Recherches experimentales sur le cycle évolutif de quelques trématodes. Bull. Biol. France Belg. 59:1–123.

MATTES, O. 1936. Der Entwicklungsgang des Lanzettegel, *Dicrocoelium dendriticum*. Z. Parasitenk. 8:371–440.

MEADE, T. G., and I. PRATT. 1966. Changes in the redia and metacercaria of *Metagonimoides oregonensis* Price, 1931, transplanted from infected to uninfected snails. Proc. Helminthol. Soc. Wash. 33:35–37.

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.

——— 1964. Miracidia-immobilizing substances in extracts prepared from snails infected with *Schistosoma mansoni*. Am. J. Trop. Med. Hyg. 13:36–42.

NEUHAUS, W. 1953. Uber den chemischen Sinn der Miracidien von *Fasciola hepatica*. Z. Parasitenk. 15:476–490.

NEWTON, W. L. 1952. The comparative tissue reaction of two strains of *Australorbis glabratus* to infection with *Schistosoma mansoni*. J. Parasitol. 38:362–366.

——— 1954. Tissue response to *Schistosoma mansoni* in second generation snails from a cross between two strains of *Australorbis glabratus*. J. Parasitol. 40:1–4.

NOLF, L. O., and W. W. CORT. 1933. On immunity reactions of snails to the penetration of the cercariae of the strigeid trematode, *Cotylurus flabelliformis* (Faust). J. Parasitol. 20:38–48.

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.

PEARSON, J. C. 1961. Observations on the morphology and life cycle of *Neodiplostomum intermedium* (Trematoda: Diplostomatidae). Parasitology 51:133–172.

PROBERT, A. J. and D. A. ERASMUS. 1965. The migration of *Cercaria X* Baylis (Strigeida) within the molluscan intermediate host *Lymnaea stagnalis*. Parasitology 55:77–92.

RANKIN, J. S. 1944. A review of the trematode genus *Glypthelmins* Stafford, 1905, with an account of the life cycle of *G. quieta* (Stafford, 1900) Stafford, 1905. Trans. Am. Microscop. Soc. 63:30–43.

REES, F. G. 1931. Some observations and experiments on the biology of larval trematodes. Parasitology 23:428–440.

REES, G. 1966. Light and electron microscope studies of the redia of *Parorchis acanthus* Nicoll. Parasitology 56:589–602.

ROWAN, W. B. 1956. The mode of hatching of the egg of *Fasciola hepatica*. Exptl. Parasitol. 5:118–137.

——— 1957. The mode of hatching of the eggs of *Fasciola hepatica*. II. Colloidal nature of the viscous cushion. Exptl. Parasitol. 6: 131–142.

SCHELL, S. C. 1961. Development of the mother and daughter sporocysts of *Haplometrana intestinalis* Lucker, a plagiorchioid trematode of frogs. J. Parasitol. 47:493–500.

——— 1962*a*. Development of the sporocyst generations of *Glypthelmins quieta* (Stafford, 1900) (Trematoda: Plagiorchioidea), a parasite of frogs. J. Parasitol. 48:387–394.

——— 1962*b*. The life history of *Telorchis bonnerensis* Waitz (Trematoda: Reniferidae), a parasite of the long-toed salamander, *Ambystoma macrodactylum* Baird. Trans. Am. Microscop. Soc. 81: 137–146.

SCHREIBER, F. G., and M. SCHUBERT. 1949. Ex-

perimental infection of the snail *Australorbis glabratus* with the trematode *Schistosoma mansoni* and the production of cercariae. J. Parasitol. 35:364–366.

SOGANDARES-BERNAL, F. 1965. Studies on American paragonimiasis. I. Age immunity of the snail host. J. Parasitol. 51:958–960.

STANDEN, O. D. 1951. The effects of temperature, light and salinity upon the hatching of the ova of *Schistosoma mansoni.* Trans. Roy. Soc. Trop. Med. Hyg. 45:225–241.

STAUBER, L. A. 1961. Immunity in invertebrates, with special reference to the oyster. Proc. Natl. Shellfish Assoc. 50:7–20.

STUNKARD, H. W., and J. R. UZMANN. 1958. Studies on digenetic trematodes of the genera *Gymnophallus* and *Parvatrema.* Biol. Bull. 115:276–302.

SUDDS, R. H., JR. 1960. Observations of schistosome miracidial behavior in the presence of moral and abnormal snail hosts and subsequent tissue studies of these hosts. J. Elisha Mitchell Sci. Soc. 76:121–133.

TIMON-DAVID, J. 1965. Infestation expérimentale d'une hélicelle par huit espèces de trématodes digénétiques appartenant à quatre familles différentes. Ann. Parasit. Hum. Comp. 40:149–154.

TRIPP, M. R. 1963. Cellular responses of mollusks. Ann. N.Y. Acad. Sci. 113:467–474.

TUBANGUI, M. A., and A. M. PASCO. 1933. The life history of the human intestinal fluke, *Euparyphium ilocanum* (Garrison, 1908). Philippines J. Sci. 51:581–603.

WINFIELD, G. F. 1932. On immunity of snails infested with the sporocysts of the strigeid, *Cotylurus flabelliformis,* in the penetration of its cercariae. J. Parasitol. 19:130–133.

WRIGHT, C. A. 1959. The application of paper chromatography to a taxonomic study in the molluscan genus *Lymnaea.* J. Linn. Soc. (Zool.) 44:222–237.

VON BRAND, T. 1951. Chemical Physiology of Endoparasitic Animals. Academic Press, N.Y. 339 pp.