TAENIA TAENIAEFORMIS INFECTION IN THE RAT: ULTRASTRUCTURAL AND IMMUNOLOGICAL ASPECTS OF HOST-PARASITE INTERACTIONS

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A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Microbiology and Public Health

1980

ABSTRACT

TAENIA TAENIAEFORMIS INFECTION IN THE RAT:
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The *Taenia taeniaeformis*/rat system is a naturally occurring model of taeniid infections of greater medical and veterinary significance and is especially relevant to the field of immunoparasitology--the study of immunological aspects of host-parasite interactions. Investigations using this model furnish insight into the phenomenon of concomitant immunity and mechanisms by which parasites evade host defense mechanisms, and may lead to the development of vaccines effective against economically important taeniid parasites. Information derived from such studies could also have much broader applicability in other areas of immunobiology, for example, tumor immunology.

This investigation concerned ultrastructural and immunological aspects of host-parasite interactions between *T. taeniaeformis* and the rat, both *in situ* and *in vitro*.

The first 3 weeks of postoncospheral development in the liver were examined at the ultrastructural level to gain an understanding of the intimate relationships between host and

parasite during a phase of reorganization which is critical to the survival of the latter. From 1-7 days postinfection the larvae are invested with a coat of long, frequently branched, microvilli which greatly increase the surface area available for absorption, secretion and excretion and may serve as a physical barrier to host phagocytic cells. The tips of the microvilli break off, become segmented and distended, and are ultimately phagocytosed by host inflammatory cells. The microvillar fragments are likely to contribute to the early humoral host response directed against this organism and may also contain materials which are cytotoxic to cells which ingest them. Lipid inclusions, present within the tegument and subtegumental cell bodies from 3 days postinfection onwards, occasionally give the appearance of being ejected. Similar inclusions are frequently observed within surrounding host cells.

By 8 days postinfection, the morphology of the surface projections has changed drastically. These structures are now microtriches, each of which consists of 3 parts: a relatively broad base, a longer and more narrow electron-dense tip, and an extremely long, slender, faintly-staining "streamer." Hypotheses are presented pertaining to the fine structure and functions of the microtriches. Host cell processes interdigitate with the microtriches, lose their integrity, and contribute to a cellular "soup" within which the microtriches bathe. A possible role for proteolytic enzymes of parasite origin is presented.

Mature (5-6 month old) strobilocerci were incubated *in vitro* with various combinations of mixed rat peritoneal cells, antibody and complement to determine the extent of host-parasite interaction that would occur within 1 hour. Cell adherence and tegumental damage were examined by electron microscopy. Extensive tegumental damage and maximum cell adherence occurred in the presence of fresh serum, and exogenous antibody was not required. The predominant recognizable cell type adhering to the larval surface was the eosinophil. Finger-like pseudopodia were observed in the process of pinching microtriches from the parasite surface and probing shallow crevices near the tegumental free surface. The cells possessed numerous phagosomes which contained microtriches and tegumental cytoplasm. Mast cells frequently participated in the cellular reactions surrounding the larvae.

The immunogenicity and protective capabilities of early postoncospheral stages of *T. taeniaeformis* were investigated by inoculating rats intramuscularly with emulsions of complete Freund's adjuvant and homogenates of heavily-infected livers. Gel diffusion and immunoelectrophoresis techniques were used to show that antisera obtained from the inoculated rats contained antibodies which reacted with antigenic materials from mature strobiloceri. Immunized rats were resistant to homologous challenge, and postoncospheral stages both before and after the acquisition of microtriches appeared to be equally protective. The offspring of pregnant rats which had received inoculations of

homogenates containing 5 day old parasites were also resistant to challenge infection.

DEDICATED TO MY WIFE AND DAUGHTERS,

MY MOTHER AND FATHER, AND

OTHERS WHOM I LOVE

ACKNOWLEDGEMENTS

I would first like to thank my mentor, Jeffrey F. Williams, Ph.D., for accepting me into his laboratory and vastly expanding my knowledge of parasitology, immunology, and research techniques. I am extremely grateful for the countless hours he spent with me in the design and execution of experiments, analysis of data, and preparation of manuscripts. It was an honor for me to work side by side with the 1979 recipient of The American Society of Parasitologists' coveted Henry Baldwin Ward Medal, awarded for leadership in parasitological research. I am very proud of our accomplishments during these three years of association.

My sincere appreciation to my old friend, R. Wes Leid, Ph.D., for the original suggestion that I work with Dr. Williams, for permitting me the use of his own laboratory facilities, for helpful advice, and for giving me the opportunity to co-author a book chapter on "Zoonotic Onchocerciasis." Very special thanks to Mrs. Katie Leid for her warm friendship and sincere concern for my welfare and progress throughout this investigation.

I wish to acknowledge the comradeship and technical advice of Mr. H. Stuart Pankratz, who literally provided me

with a home in his electron microscopy laboratory and gave me unrestricted access to everything therein. Special thanks also to my dear friend, Dr. Betty Hamilton, for the outstanding diagrams; to Ms. Alma Shearer and Dr. George Conder for technical assistance with animal surgery, injections and dosing, the counting of parasite cysts, and immunological procedures; to Dr. Steve Heidemann for consultations regarding microtubules; and to Ms. Myrnice Ravitch for assistance with the statistical analyses.

My sincere gratitude to the United States Army Medical Department for affording me the opportunity to attain the Ph.D. degree, and for supporting me financially throughout that endeavor.

Finally, I wish to thank those people who sacrificed the most during these past three years: my wife, Linda, and my daughters, Barbara, Karen and Pamela. Without their love, patience, understanding, and encouragement I could never have achieved this goal.

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LITERATURE REVIEW

Hydatidosis and Cysticercosis

Cestodes (tapeworms) are parasitic flatworms (Phylum Platyhelminthes) of the Class Cestoidea. Several members of the family Taeniidae in the Order Cyclophyllidea are responsible for serious socioeconomic problems. These include *Echinococcus granulosus* and *E. multilocularis*, the larval stages of which cause hydatidosis (hydatid disease), and several species of *Taenia*, the larval stages of which cause cysticercosis.

The earliest known descriptions of adult and larval tapeworms date back over 2,300 years. According to Wardle and McLeod (1952), the beef tapeworm and some bladderworms (cysticerci) were well known to Hippocrates (460-377 B.C.), the "Father of Medicine", and to Aristotle (384-322 B.C.), the "Father of Zoology."

The most common intermediate host of *E. granulosus* is the sheep, but this parasite also causes one of the most serious larval cestode diseases in humans. The organism is widely distributed throughout the temperate and subtropical regions of the world, and the prevalence of hydatid disease is increasing in some areas (Matossian et al., 1977). *Echinococcus multilocularis* is less widespread than *E. granulosus* but is endemic in certain of the cooler regions of the world.

Rodents usually serve as intermediate hosts for this parasite, but human cases of alveolar hydatidosis also occur.

The *Taenia* species responsible for cysticercosis in food animals include *T. saginata* (cattle), *T. solium* (pigs), *T. hydatigena* (sheep), and *T. ovis* (sheep). Together these species impact severely on the food industry due to economic losses from condemnation of infected animal organs or carcasses and embargoes on the export of infected animals. Like hydatidosis, the prevalence of taeniasis-cysticercosis seems to be increasing in many countries (Abdussalam, 1976). Humans are obligate definitive hosts for *T. saginata* and *T. solium*, but the adult tapeworms only occasionally cause medical problems. On the other hand, cysticercosis in humans due to the larval stage of *T. solium* is very serious, as the eye and brain are common predilection sites. The subject of cysticercosis in humans and animals has been recently reviewed (Hird and Pullen, 1979).

It is clear that the measures which have been taken to control hydatidosis and cysticercosis, such as chemotherapy, public education, meat inspection, and proper waste disposal, have been inadequate. In recent years increased attention has been focused on the need to develop prophylactic and immunodiagnostic procedures for the prevention and detection, respectively, of cestode infections (Abdussalam, 1976; Flisser et al., 1979).

As has been recently stressed by Mitchell (1979) and Williams (1979), the *Taenia taeniaeformis*/mouse and *T. taeniaeformis*/rat systems are well suited to

immunoparasitological studies. These are naturally occurring host-parasite relationships in which the processes of tissue invasion, reorganization and growth of the organism, and stimulation of immune responses, are comparable to those which take place in taeniid infections of medical and veterinary importance.

The present investigation has focused upon certain ultrastructural and immunological features of *T. taeniaeformis* infection in the rat. Most of the literature review, therefore, has been devoted to the life cycle, development and immunology of this parasite.

Life Cycle of *Taenia taeniaeformis*

The larval form of *T. taeniaeformis* was recorded almost three centuries ago under the name *Vermis vesicularis muris* (Hartmann, 1695). Since then, this tapeworm has been given a variety of names, including *Hydatigera taeniaeformis* (Batsch, 1786), *Cysticercus fasciolaris* (Rudolphi, 1808), and *Taenia crassicollis* (Rudolphi, 1810). The latter terms were reserved for the larval stage but are no longer considered proper terminology (Noble and Noble, 1976).

The adult tapeworm has a cosmopolitan distribution and lives in the lumen of the small intestine of many members of the families Felidae and Mustelidae, including the domestic cat. The life cycle of this parasite is depicted in Figure 1. The adult worm consists of a holdfast organ (scolex) to assist in anchoring the parasite to the intestinal mucosa and a long, flattened, ribbon-like body (Wardle and McLeod,

Figure 1. Life cycle of *Taenia taeniaeformis*. Clockwise from top: domestic cat (definitive host), adult tapeworm shedding terminal proglottid, egg containing hexacanth embryo, rat (intermediate host), activated oncosphere, cysticercus, strobilocercus.

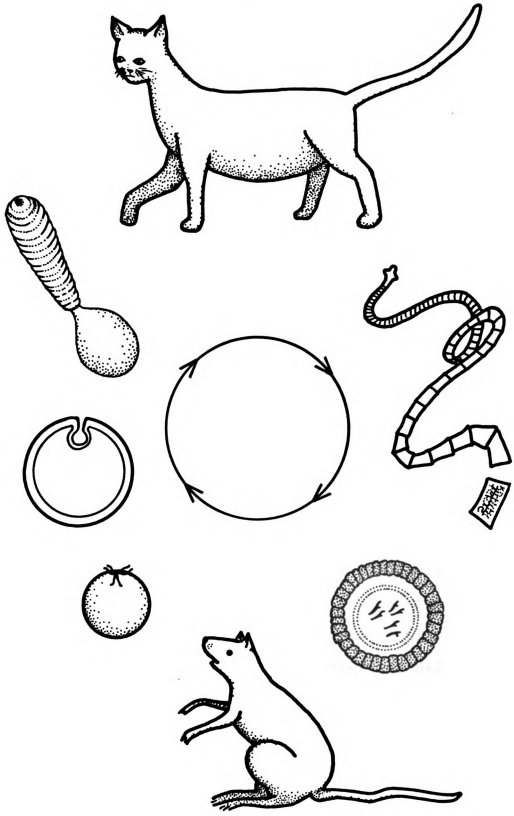

Figure 1

1952). The scolex has four protruding suckers (acetabula) and two concentric circles of hooks. The body has a succession of transverse, parallel grooves which divide it into segments (proglottids). The body, or strobila, is 5-6 mm wide and can attain a length of 600 mm (about 2 feet). The oldest proglottids are those located farthest from the scolex. Terminal proglottids become gravid, drop from the strobila, and exit the definitive host via the anus.

The *T. taeniaeformis* egg is spherical and 31-37 μm in diameter (Wardle and McLeod, 1952). With the light microscope, the embryophoric blocks give the appearance of radial striations, and the six hooks of the hexacanth embryo (oncosphere) can be seen. The development and ultrastructure of the egg and oncosphere have been described by Nieland (1968), and the egg surface has recently been examined by scanning electron microscopy (Jones et al., 1979).

Eggs must then be ingested by rats or mice. According to Banerjee and Singh (1969a) they hatch in the small intestine of rats within 15 min of inoculation *per os*, and the majority of oncospheres become attached to and start penetrating the tips of villi between 15 and 30 min postinfection (p.i.). In their experiments, most oncospheres had completed the intestinal migratory phase by 2 hours p.i. Turner and McKeever (1976), studying intestinal penetration of *T. taeniaeformis* in mice, observed that invasion of the mucosa was by cytolysis and was not the result of lysis of ground substance followed by a squeezing of the oncosphere between epithelial cells. Silverman and Maneely (1955) studied

penetration of intestinal mucosa by *Taenia* species other than *T. taeniaeformis* and speculated that polysaccharide substances secreted from "penetration glands" of the oncospheres might be immunogenic and stimulate resistance to reinfection.

After penetration, oncospheres migrate from the intestine to the liver, presumably via the mesenteric and portal veins. Perfusion of the liver of a rat 24 hours after *per os* infection yielded what Banerjee and Singh (1969a) concluded were squirming oncospheres. Larvae have been observed in liver sections examined by light microscopy as early as 21 hours p.i. (Bullock and Curtis, 1924).

Within the liver the larva develops from a solid morula of cells into a fluid filled, thin walled sphere (cysticercus), which becomes enclosed in a capsule of host origin. A single inverted scolex arises and eventually becomes everted. Early development of the rostellum and hooks and the chemical nature of the latter have been described by Crusz (1947, 1948). According to Rees (1951) the rostellum is armed with a double crown of 36 hooks, although the number of hooks actually varied from 26 to 52. The hooks become fully formed during larval development and no further growth occurs after the larva gains entrance to a definitive host (Wardle and McLeod, 1952).

Hutchison (1958) determined that larvae must attain an age of 60 days in mice before they are infective for cats, and this was later confirmed by Singh and Rao (1967b) using larvae from rats. By this age the larva has become a strobilocercus, a segmented worm-like form with a scolex at

one end and a relatively small bladder at the other. The musculature, excretory and nervous systems of fully developed strobilocerci were described by Rees (1951). Unless destruction of the larvae occurs, they continue to grow throughout the life of the intermediate host. By 1 year p.i., strobilocerci attain an average length of 24 cm in rats, and may reach a length of 60 cm or more in old rats (Bullock and Curtis, 1924).

Cats become infected following consumption of a mouse or rat liver containing larvae. According to Hutchison (1959), the terminal 20-70% of the strobilocercus, referred to as the pseudostrobila, is shed following entrance of the larva into the cat intestine. Most larvae establish themselves within the middle third of the small intestine, and growth begins immediately. Whereas Hutchison (1959) reported that egg production commences between the 16th and 18th day, Singh and Rao (1967b) could not detect eggs until 32 days p.i. Infection generally became patent after about 6 weeks.

Light Microscopic Observations on Early Postoncospheral Stages of *Taenia taeniaeformis* During *in situ* Development

Postoncospheral reorganization of *T. taeniaeformis* larvae has been studied at the light microscope level in a variety of naturally and experimentally infected rodents, but no comprehensive review has been published to date. In this section a chronological account of larval development will be presented to illustrate similarities and differences which exist in previously published reports.

It is important to bear in mind that there are marked differences in susceptibility to this parasite among various genera, species and strains of rodents (Dow and Jarrett, 1960; Olivier, 1962; Orihara, 1962; Turner and McKeever, 1976; Mitchell et al., 1980) and even within a given strain (Williams et al., 1980). It is also likely that strains of parasites differ in their effect upon host defense mechanisms. For these reasons, host-parasite relationships characterized in published reports are not always directly comparable.

The present study concerns only the first three weeks of experimental infection in the rat. Therefore, emphasis has been placed on published observations of 1 to 21 day old larvae in this host. Current terminology has been introduced wherever the original nomenclature was confusing or obsolete. To facilitate size comparisons, all reported dimensions have been consolidated in Table 1.

The earliest postoncospheral stage of *T. taeniaeformis* to be described *in situ* was at 21 hours p.i. (Bullock and Curtis, 1924). Lewert and Lee (1955) later described *T. taeniaeformis* larvae in rat liver at 24 hours p.i. The organisms still had oncospheral hooks and were located midway between the periphery and center of the liver lobule. They were sometimes surrounded by endothelial cells, indicating an intrasinusoidal location. Evans blue dye was inoculated into infected live rats; its retention in subsequently fixed tissue was interpreted as indicating the presence of water soluble glycoprotein. A 1 to 2 μm zone of amorphous Evans blue-staining material separated the parasites from

Table 1. Dimensions of early postoncospheral stages of *Taenia taeniaeformis* in the rat

No. of Days Postinfection	Dimensions (µm)	Reference
21 hours	15	Bullock and Curtis, 1924
1	16 x 10	Lewert and Lee, 1955
	15 x 10	Banerjee and Singh, 1969b
2	27 x 24	Singh and Rao, 1967a
	26 x 24	Banerjee and Singh, 1969b
3	27 - 32	Lewert and Lee, 1955
	37 x 33	Singh and Rao, 1967a
	42 x 36	Banerjee and Singh, 1969b
4	33 - 55	Lewert and Lee, 1955
	40 - 65	Orihara, 1962
	52 x 42	Singh and Rao, 1967a
	55 x 35	Banerjee and Singh, 1969b
	60 - 100	Cook, 1979
5	80	Lewert and Lee, 1955
7	450 x 346	Singh and Rao, 1967a
8	300 x 170	Lewert and Lee, 1955
10	200 - 460	Crusz, 1948
	750 - 950	Orihara, 1962
	485 x 385	Singh and Rao, 1967a
	455 x 300	Banerjee and Singh, 1969b
13	up to 500	Lewert and Lee, 1955
15	600 - 1000	Crusz, 1948
	2200 x 2100	Singh and Rao, 1967a
20	2400 x 2200	Singh and Rao, 1967a

adjacent hepatic cells, and sometimes the hepatic cells which bordered the larvae were also surrounded by this material. The authors used the Hotchkiss staining technique on fixed tissue to demonstrate that the 1 day old larvae were almost devoid of glycogen and that hepatic cells immediately adjacent to them contained less glycogen than hepatic cells located further from the parasites. The Hotchkiss technique utilizes a periodic acid leukofuchsin reagent. No leukocyte accumulations were observed in the vicinity of the larvae.

Singh and Rao (1967a) were unable to detect parasites in rat livers 24 hours p.i., and Banerjee and Singh (1969b) were unable to detect larvae prior to that time. Like Lewert and Lee (1955), these authors also located the oval 24 hour old larvae within hepatic sinusoids, midway between the periphery and the center of liver lobules. The organisms consisted of several cells, but embryonic hooks were not detected.

Singh and Rao (1967a) described the parasite at 2 days p.i. as a globular body, consisting of a few cells. In histopathological sections there was evidence of hemorrhage, with erythrocytes in the vicinity of some organisms. The liver showed mild hyperemia of vascular channels, and the cytoplasm of some hepatic cells showed vacuolations. The globular parasites described by Banerjee and Singh (1969b) also contained few cells and were present in a dilated hepatic sinusoid. In contrast, there was no evidence of hemorrhage,

extravasated erythrocytes, or pathological changes in hepatic cells in the vicinity of the parasites.

According to Lewert and Lee (1955), 3 day old larvae were solid masses of cells surrounded by an area of amorphous, Evans blue-retaining material, a few μm to 10-15 μm in thickness. Some of this material extended between the liver cords in the vicinity of the larva and was usually more extensive on one side of the parasite. The Hotchkiss staining technique revealed that the parasite contained intracellular glycogen and was enclosed in a deeply staining acellular "capsule" of glycoprotein (the parasite tegument). The Evans blue-retaining amorphous material between the liver cords was also Hotchkiss positive. Hepatic cells peripheral to the amorphous material were slightly compressed and devoid of glycogen.

Three day old larvae described by Singh and Rao (1967a) contained a greater number of nucleated cells than earlier stages. They were located in the liver parenchyma, and surrounding hepatic cells showed increased granularity of cytoplasm, acidophilia and/or vacuolations, and occasional condensation of nuclear material. Larvae were said to be surrounded by a hemorrhagic exudate. The findings of Banerjee and Singh (1969b) were at variance with this, however. They found no pathological alterations of hepatic parenchyma at this stage.

The 4 day old larvae described by Lewert and Lee (1955) were surrounded by a thin acellular "capsule" (tegument) that stained with both the Evans blue and Hotchkiss

techniques. At this stage there was an increase in glycogen content of the parasites and a decrease within surrounding hepatic cells. Orihara (1962) described developmental stages of *T. taeniaeformis* in Wistar rats. At 4 days p.i. the larvae were round or ellipsoid. A central bladder had formed in some of the parasites, and host reaction was "slight." Singh and Rao (1967a) stated that cell numbers in Day 4 larvae had increased and gave the appearance of being loosely scattered within a membranous covering (tegument). There was a clear zone between the parasites and the adjacent liver tissue. Parasites were surrounded by a hemorrhagic exudate with lytic and degenerative changes of neighboring hepatic cells. Extensive hemorrhagic tracts were observed, which they interpreted as evidence of larval migration.

Banerjee and Singh (1969b) also described 4 day old parasites as having loosely scattered nucleated cells enclosed by a thin membrane (tegument). The only pathological changes which they detected, however, were increased granularity of hepatic cells situated in close vicinity to the parasites and congestion of blood vessels. They stated categorically that no hemorrhagic tracts were observed. Four days p.i. was the earliest stage examined by Cook (1979). At this age the larvae were surrounded by a clear zone thought to represent the layer of microvilli previously described by Picone (1978). The circular parasites varied somewhat in appearance; some were a solid mass of cells, whereas others contained a definite central cavity (bladder).

A small focus of hepatic cell necrosis was seen adjacent to many parasites, and this was interpreted by the author as evidence of migration. These observations confirm the earlier report (Singh and Rao, 1967a) of evidence of larval migration as early as 4 days p.i. Although infiltration of some portal triads by eosinophils was observed, no inflammatory cells were seen within the necrotic area.

Bullock and Curtis (1924) described Day 5 larvae as either solid or vesicular, spherical, and often partly or completely separated from surrounding hepatocytes by a coarse fibrinous network within which inflammatory cells and degenerated hepatic cells were seen. According to Lewert and Lee (1955) larvae of this age consisted of a "hollow" structure containing scattered irregular glycogen masses, surrounded by a thin acellular "capsule" (tegument) which stained deeply with Evans blue and was Hotchkiss positive. Parasites were surrounded by glycogen free, slightly compressed hepatic cells and occasionally by degenerating hepatic cells, free nuclei, leukocytes and debris. Singh and Rao (1967a) reported that the hepatic cells in the vicinity of parasites at this stage showed extensive degenerative changes and some hyalinization. There was a slight increase in the number of infiltrating mononuclear cells in the periportal areas.

Bullock and Curtis (1924) reported that larvae were grossly visible as clear dots or vesicles on the liver surface by 6 days p.i., and by 7 days they were described as white specks by Singh and Rao (1967a). The latter authors

stated that the liver cytoarchitecture was disrupted and multiple hemorrhagic tracts were present. The hepatic cells in the vicinity of the tracts showed diffuse necrotic changes. When stained with hematoxylin and eosin, the nuclear chromatin of these cells appeared as scattered black granular detritus in the pinkish necrotic mass.

Cook (1979) found a distinct zone of hepatic necrosis on one side of Day 7 larvae which was taken to be a parasite migratory tract. He observed inflammatory cells, including large numbers of eosinophils, within this zone. However, no inflammatory reaction was seen around the parasite, which was no longer surrounded by a halo but was now in close contact with hepatic tissue. There was no evidence of degeneration or necrosis of hepatocytes adjacent to the "advancing" surface of the larva. There was marked eosinophil infiltration and a lymphoplasmacytoid response in portal areas. Serum levels of the hepatic cell enzymes alanine amino transferase and sorbitol dehydrogenase in *T. taeniaeformis*-infected rats were elevated at 6 and 7 days p.i., perhaps coinciding with or immediately following the most active period of hepatic migration and cell destruction.

Lewert and Lee (1955) described 8 day old larvae as thin "hollow" spheroids, surrounded by a 50-100 µm wide Evans blue-retaining area. This region stained diffusely by the Hotchkiss method. Basement membranes of adjacent hepatic sinusoids appeared to be altered to a greater extent than at earlier stages, which they interpreted as indicating that the glycoprotein was in a less polymerized state.

Sinusoidal cells contained more Hotchkiss positive granules.

In Crusz's (1948) report, 10 day old larvae were spherical to elongate. Investing the entire bladder was a delicate "cuticle" (tegument) with fine "hair-like processes" on the outer surface. This is the earliest reference to such structures on the surface of postoncospheral forms of *T. taeniaeformis*. Distinct cell boundaries could not be seen in the "subcuticula" (subtegument), which was described as irregularly disposed cytoplasm containing scattered, large, deeply staining, vesicular nuclei. Although true fibers had not yet formed, cells thought to be primordial muscle cells were seen just beneath the "cuticle." The bladders of some of the parasites contained cells and were lined with highly granular masses of cytoplasm.

The gourd-shaped 10 day old larvae observed by Orihara (1962) in Wistar rats were much larger than those described in other reports (Table 1). Beneath a thin, clear "cuticular" layer (tegument) was a germinal (subtegumental) zone several cells thick, arranged perpendicularly to the wall. A thickened portion (20 μm in thickness) of the germinal wall was referred to as the "scolex formation." The observation of a scolex anlagen in cysticerci of such a young age does not appear in other reports. Host reaction was said to be "slight", but cell detritus and histiocytes were observed in contact with the scolex region. Ten day old larvae in Gifu rats were also gourd-shaped and quite large. Germinal

cells 10-15 μm in length and arranged in several layers perpendicularly to the wall lay beneath a thin "cuticular" layer. A scolex anlage was also detected in this strain, and a necrotic cell mass consisting of polymorphonuclear leukocytes (mainly eosinophils) and immature granulation tissue was observed in contact with this structure.

The 10 day old parasites described by Singh and Rao (1967a) resembled small vesicles, with a fairly well defined wall and hair-like processes on the external border. Internal nucleated streaks connecting one end of the bladder to the other end were described. Early proliferating connective tissue, consisting mainly of spindle-shaped cells, was present between the hepatic cells surrounding the parasite. Extensive necrosis was seen around the previously mentioned tracts attributed to migrating larvae. Large quantities of granular detritus were interspersed throughout the necrotic mass.

Banerjee and Singh (1969b) commented on the differences between their measurements of 10 day old larvae and those of other authors and stated that

> Too much significance cannot be attached to the size of the larva correlating it with the age of infection, for it is realized that the growth of the individual larva cannot be uniform being dependent upon so many biological factors.

A zone of liver tissue extending 60-140 μm around the larvae showed pathological alterations. Most parasites were encircled by lymphocytes, macrophages, and fibroblasts. Giant cells of the Langhans type and eosinophils were occasionally observed. Ten day old larvae were reported by

Cook (1979) to be surrounded by a narrow fibroblastic zone, the start of a host capsule. The adjacent parasite migratory track was undergoing fibrosis, and portal triads contained large numbers of plasma cells and eosinophils.

By 12 days p.i., the surrounding proliferative reaction, which began around the 9th day, is well under way (Bullock and Curtis, 1924). The wide zone of cellular tissue between the larva and the hepatocytes consists of either loosely arranged or compact cells embedded in a fibrillar or granular matrix and constitutes the rudimentary host capsule. The cells vary in size, assume a variety of shapes, and most possess a single, large nucleus. Numerous mitotic figures are present.

Thirteen day old larvae were surrounded by a 100-200 µm area of altered liver containing compressed hepatic cells, fibroblast-like cells, and sometimes leukocytes (Lewert and Lee, 1955).

According to Crusz (1948), 15 day old "bladderworms" (cysticerci) were spherical and distended with fluid. Primordial muscle cells were observed, as were stellate "subcuticular" and "parenchyma" (subtegumental) cells which had cytoplasmic continuity between them. At 15 days p.i., the parasites observed by Singh and Rao (1967a) were also spherical and distended with fluid. The larva had a thick, fibrous "cuticle" (tegument) with cells lining its internal surface. The area around the parasites contained spindle-shaped cells and resembled a spindle cell sarcoma.

Lewert and Lee (1955) demonstrated that at 16 days p.i. parasites produce a collagenase-like enzyme which the authors speculated might serve to soften intercellular materials and basement membranes. Displaced cells would then more readily accommodate the rapidly growing parasites and might increase the rate of diffusion of nutrient materials to the larvae. The 16 day old larvae produced much greater quantities of this enzyme than 100 day old organisms. The authors speculated that the collagenase might be immunogenic and that immune responses directed toward young parasites might be directed against this enzyme. Cook (1979) reported that by 16 days p.i. the zone of fibroblastic reaction surrounding the parasite was sarcomatous in appearance with many mitotic figures and hyperchromatism of nuclei. This area had widened to form a host capsule infiltrated by lymphocytes, eosinophils and mast cells.

Singh and Rao (1967a) stated that a hemispherical scolex anlage, 0.5 μm in diameter, extended into the cavity of 20 day old larvae, and a rostellar rudiment was present. Parasites were surrounded by a thick zone of connective tissue.

At 22 days p.i. the larvae are surrounded by a host capsule approximately 300 μm in thickness, containing lymphocytes, eosinophils, mast cells and plasma cells (Cook, 1979). Prominent accumulations of plasma cells occur at the periphery of the capsule. A zone of eosinophils often occurred at the surface of the parasite, most frequently adjacent to the area of scolex formation. This eosinophil

layer had been observed earlier by Ansari and Williams (1976) to be as wide as 120 μm. The latter authors saw eosinophils within the zone of cellular infiltration surrounding the parasites as early as 11 days postinfection.

By this stage of development, the parasite cysts usually protrude from the surface of the liver in "blister-like elevations" (Bullock and Curtis, 1924). Some cysts lie entirely outside the liver, being connected to the latter by a slender fibrous "pedicle." Rarely, cysts are completely "buried" within the liver.

The following reports contain light microscopic observations of older *T. taeniaeformis* larvae within rat intermediate hosts: Bullock and Curtis (1924), Crusz (1948), Orihara (1962), Rees (1951), Singh and Rao (1967a), Cook (1979).

Conclusions which can be drawn from this brief review include the fact that larval development proceeds at different rates in different hosts, and even within a given host the rate of development is not uniform. Therefore, the dimensions or morphologic description of a given organism on a given day p.i. should not be interpreted as meaning that all larvae at that age are the same size or have the same appearance.

Investigations of this type can, however, reveal the stages of development and host-parasite interactions which occur during postoncospheral differentiation. These stages can be characterized as (1) cell reorganization, (2) cell multiplication, (3) cavity formation, (4) cytoplasmic

reorganization, and (5) scolex anlage formation (Gemmell, 1976). Pathologic changes in host tissues surrounding the developing parasite include a reduction in glycogen content of adjacent hepatocytes, destruction of host cells, an influx of inflammatory cells of variable composition, and the formation of a fibrous capsule. The rate and degree to which these changes occur obviously vary from one report to another. Since the degree of susceptibility is not defined in most cases, it is difficult to make direct comparisons. In host strains where many hepatic organisms fail to survive, pathologic changes may represent successful attack on the organisms, whereas in others, such as the Spartan rat (Cook, 1979), in which most organisms survive, the changes are more likely to represent normal stages in the development of the capsule around viable parasites.

Light Microscopic Observations on Early Postoncospheral Stages of *Taenia taeniaeformis* During *in vitro* Cultivation

Several investigators have had success cultivating *T. taeniaeformis in vitro*. Their observations are summarized here for comparison with *in situ* results. The report by Heath and Elsdon-Dew (1972) represents the first published account of *in vitro* cultivation of this parasite. Activated oncospheres appeared to require the presence of rat serum in the culture medium for vesiculation to occur, and immunofluorescence revealed that developing oncospheres rapidly acquired an unidentified constituent of rat serum on their surface. The authors speculated that the acquisition of a

host serum constituent *in vivo* might serve to protect the organism from recognition as foreign. Although the development of parasites *in vitro* followed a course similar to that reported previously *in vivo*, they noted that the larvae grown in culture were smaller at any given age.

Heath (1973) later reported an improved technique for the *in vitro* culture of taeniid larvae, including *T. taeniaeformis*. The medium he used contained fetal calf serum and rabbit erythrocytes, but no rat serum. He stated that the larvae developed into immature cysticerci at a rate comparable to that observed *in vivo*.

The report by Heath and Elsdon-Dew (1972) contains no micrographs and no precise dimensions are given of larvae at different stages. However, there is a figure showing diagrams of the parasites at various stages of growth, and approximate sizes can be calculated using the accompanying 50 µm scale marker. For example, newly hatched and activated oncospheres, with six protruding hooks and "penetration gland" drops on their surface, are depicted as being approximately 20 µm in diameter. Other dimensions are contained in Table 2. One day old larvae contained withdrawn oncospheral hooks and had numerous refractile secretory droplets on their surface. Two day old larvae still retained their hooks and consisted of more cells than earlier stages. A central cavity had started to develop in 3 day old larvae.

The report of Heath (1973) contains micrographs of some developmental stages. Rabbit erythrocytes in the culture medium were prevented from reaching the surface of 4 day old

Table 2. Dimensions of early postoncospheral stages of *Taenia taeniaeformis* cultured *in vitro*

No. of Days Postinfection	Dimensions (μm)	Reference
1	23 x 17	Heath and Elsdon-Dew, 1972
2	27	Heath and Elsdon-Dew, 1972
3	33	Heath and Elsdon-Dew, 1972
4	43 x 40	Heath and Elsdon-Dew, 1972
	60	Heath, 1973
5	70 x 65	Heath, 1973
5-6	53 x 50	Heath and Elsdon-Dew, 1972
7-8	63	Heath and Elsdon-Dew, 1972
9-10	87 x 83	Heath and Elsdon-Dew, 1972
20	1600-3200	Heath, 1973

parasites by what the author suspected was a layer of long microvilli. Microvilli are not discernible in the micrograph, but erythrocytes are no closer to the parasite surface than 27 μm. The report does not make it clear at what age this translucent barrier was first observed. There is a reference to an unpublished observation that long microvilli had been detected on 3 day old larvae of *T. pisiformis*, as well as on other non-taeniid cestodes. The 4 day old larva pictured does not appear to have a central cavity, but that shown by Heath and Elsdon-Dew (1972) definitely does. The latter authors depicted 5 and 6 day old larvae as having slender cytoplasmic processes traversing the central cavity. Heath (1973) described 5 day old parasites as having a definite central cavity and a surrounding translucent barrier to surface contact by erythrocytes.

In vitro cultivated 5 and 7 day old larvae were illustrated in a later paper by Heath and Pavloff (1975). They were surrounded by an "amorphous layer" which most erythrocytes were unable to penetrate; some lymphocytes had penetrated to varying degrees. The authors postulated that during the first 7 days of development an exterior microvillar layer prevented access of host lymphoid cells to the organism.

By days 7 and 8, parasites possessed fewer cytoplasmic processes stretching across the central cavity, and by days 9 and 10 they no longer contained internal cytoplasmic processes (Heath and Elsdon-Dew, 1972). The authors concluded that muscle systems had developed by this time,

because slow changes in larval shape occurred in culture. Squirming movements of 8-10 day old larvae were also reported by Picone (1978), who enzymatically liberated parasites from rat livers.

The micrograph of Day 11 larvae published by Heath (1973) shows them to be thin walled, round to oval cysts which vary greatly in size. The variation may be partly due to the fact that not all of the illustrated larvae lie in the same plane, and for this reason size calculations were not attempted. Also shown were Day 13 larvae, but sizes were not calculated for the same reason. Oval 22 day old larvae have developed muscle systems and scolex anlagen, and spiralling movements occur. Parasites older than 20 days are also pictured in the report by Heath.

Up to and including the fourth day, the parasites cultured *in vitro* are similar in size to the *in situ* dimensions which have been reported. From that point, the sizes depicted by Heath and Elsdon-Dew (1972) are much smaller than *in situ* larvae. On the other hand, the maximum sizes of the 20 day old larvae illustrated by Heath (1973) exceed the *in situ* dimensions reported by Singh and Rao (1967). The sequence of postoncospheral reorganization *in vitro* paralleled that observed *in vivo*.

Ultrastructure of Larval Stages of *Taenia taeniaeformis*

Bortoletti and Ferretti (1971) described 14, 30 and 100 day old *T. taeniaeformis* cysticerci from livers of experimentally infected mice. It is possible that the host-parasite

relationship illustrated in mice may be different from that observed in the rats used in the present investigation. However, this is the only published report on the ultrastructure of *T. taeniaeformis in situ*; therefore, the most significant observations have been summarized in this section.

At 14 days p.i. the globular fluid-filled larvae were no larger than 500 μm. The parasite membrane was in intimate contact with the surrounding host cells, described by the authors as damaged "phlogistic" (inflammatory) cells having granular cytoplasm, empty mitochondria, and thicker than normal nuclear chromatin. In some areas the plasma membrane seemed to be absent from host cell processes in close proximity to microthrix[1] tips. The external surface of the tegument was covered with a very dense and uniformly distributed layer of curved microtriches.

Microtriches were grouped in bundles and embedded in or surrounded by host cell cytoplasm. Each microthrix consisted of a base, 0.1 μm in diameter, and a longer, but more narrow, electron-dense tip. The authors said that these structures were covered with a double membrane continuous with the external plasma membrane of the tegument.

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[1]The term "microthrix" (plural "microtriches") was introduced by Rothman (1959). These structures, as described by Lumsden (1975), have a solid or densely fibrillar distal tip which is set off from the remainder of the shaft by a multilaminate base plate.

The authors' use of the term "double membrane" throughout their report is unfortunate and confusing. What they are referring to is a lipid bilayer which has a trilaminar appearance in electron micrographs of stained material. In fact, they refer to the same structure as a plasma membrane in some portions of their report and a double membrane in others. Transverse sections of the bases showed circular electron-dense formations slightly more than 10 nm from the external plasma membrane. Transverse sections of microthrix tips contained a very dense zone in the center which the authors described as "microtubules."

The tegument was 2-7 μm thick and contained mitochondria, vacuoles of varying sizes, and 0.10-0.15 μm rod-like formations, flattened in the center. The vacuoles and rod-like structures were also said to be surrounded by double membranes. The large (2-3 μm) vacuoles were irregular in shape and contained a homogeneous material which sometimes appeared "coagulated" and electron-dense. The rod-like formations were considered to be "deposits" of refuse material, rather than vesicles containing absorbed material.

Picone (1978) liberated young (1 to 10 day old) postoncospheral stages of *T. taeniaeformis* from rat livers and studied their ultrastructure. He pointed out that the rate of larval development was not uniform and that on any given day there was considerable morphologic heterogeneity. Electron micrographs were selected to illustrate the steps which occurred during the first 10 days of postoncospheral reorganization. Organisms from 1 to 8 days p.i. had

microvilli, rather than the microtriches observed by others on older larvae of *T. taeniaeformis* (Nieland and Weinbach, 1968; Bortoletti and Ferretti, 1971). The micrographs of *T. taeniaeformis* microvilli published by Picone are the first ultrastructural representation of these structures. Nine and 10 day old larvae, on the other hand, had true microtriches. Picone speculated that this abrupt change from microvilli to microtriches might be "related to the acquisition of invulnerability [to antibody] and/or changes in antigenicity."

At 24 hours p.i. the parasites were oval, 17 x 15 μm, and contained very few cells. No more than 5 nuclei were seen per thin section. The microvilli were 0.05 μm in width and up to 0.5 μm in length. Scant areas of muscle fibers were present within the cell mass. By 2 days p.i. there was a more dense microvillar layer, with lengths up to 1.54 μm. There were 8-16 nuclei per section, and subtegumental cells were surrounded by electron-transparent areas. Oncospheral hooks and muscle were present. Sections of 3 day old parasites showed cytoplasmic bridges, oncospheral hooks, and about 24 nuclei. Some cytoplasmic bridges contained lipid droplets. Microvilli were up to 2.0 μm long. By 4 days, the organisms were completely vesiculated, and subtegumental cells were separated by electron-transparent spaces. The following day they had a more prominent muscle layer than at previous stages, and by 6 and 7 days p.i. both longitudinal and circular muscle bundles were clearly established. The number of microvilli had

increased and some were branched. The tegument on day 8 was vacuolated, and large droplet-like areas, possibly lipid, were present on the inner aspects of the subtegumental cells. Microtriches abruptly appeared on Day 9. They had bases which were 0.13 μm wide and 0.85 μm long and electron-dense tips 1.15 μm in length. The tegument became thicker by day 10 and the microthrix border was denser than on 9 day old cysticerci.

Most of the 30 and 100 day old larvae described by Bortoletti and Ferretti (1971) had been removed from the liver cysts prior to fixation. Few ultrastructural differences were noted between the tegument of 14 day old larvae and the later stages. The basal portions of microtriches of 30 day old parasites were often observed to be in clusters of 2-4, enveloped by a single plasma membrane. No attempt was made to explain these formations. They could be the result of branching microtriches having a common base, and one such structure was illustrated in the report. They could also result from a section cut below the level of the outer tegumental membrane but containing several of the electron-dense microthrix inner rings which extend for a short distance beneath the outer membrane. The horseshoe-shaped inner rings depicted in several of the electron micrographs were probably due to the angle at which the microthrix bases were sectioned. The surface of the 30 day old parasite showed many protuberances, channels and invaginations. The mitochondria of the tegument were principally located in close proximity to the deep plasma

membrane. There were many large (2-3 μm) tegumental vacuoles, and these too were usually located near the basal plasma membrane. Rod-like formations were more numerous and appeared more electron-dense.

The tegument thickness of the 100 day old larvae varied from 3 to 5 μm, compared to 1-7 μm for 30 day old and 2-7 μm for 14 day old parasites. Electron micrographs of the 100 day old strobilocercus revealed subtle differences between the ultrastructure of the strobila (pseudostrobila) and that of the terminal bladder. For example, the tegumental mitochondria of the strobila are located randomly in the cytoplasm, whereas those of the bladder are located close to the basal plasma membrane. Small vacuoles are more numerous in the bladder tegument, whereas the electron-dense rod-like formations are more numerous in the tegument of the strobila. *In situ*, the 100 day old parasite is contained by the host capsule and immersed in a pulp of severely damaged host cells, cellular residue, and some apparently undamaged cells. Cell fragments were at times wedged between the segments of the strobila.

The fine structure of the bladder of 8 week old larvae from mice was studied by Nieland and Weinbach (1968). The tegument was densely packed with many small vesicles and mitochondria and had microtriches on its free surface. The subtegumental cells were highly branched; cytoplasmic extensions of these cells passed through a fibrillar zone and provided continuity with the tegumental cytoplasm. Two types of cytoplasm were observed in subtegumental cells;

one type was electron-dense, packed with rough endoplasmic reticulum and free ribosomes; the other was electron-lucent and contained abundant glycogen in alpha (rosette) form. Areas of each were often contiguous within the same cell. Nuclei were fenestrated with numerous pores and had prominent nucleoli. A prominent duct system was present, with ducts frequently connected to each other by thin cytoplasmic bridges. Globular-shaped projections extended into their lumina. The walls of the ducts were formed by cells containing electron-dense cytoplasm. Large osmiophilic amorphous inclusions resembling fat droplets were observed. Scanning electron microscopy was used by Jones et al. (1977) to illustrate the microtriches and excretory pores of the strobilocercus bladder, but the ages of the parasites were not given.

Verheyen et al. (1978) studied the topographical changes which occurred in larvae following mebendazole treatment of *T. taeniaeformis*-infected mice. Their report included scanning and transmission electron micrographs of the surface of normal 3 month old cysticerci. The morphology of the microtriches differed from one part of the larva to another. Those on the scolex had conical tips which had an anterior-posterior orientation. The microtriches of the pseudoproglottids and bladder had longer, more narrow tips, with those of the bladder being much longer than those of the pseudoproglottids. In their study of mebendazole effects, Borgers et al. (1975) included transmission electron micrographs of normal 1 year old strobilocerci. In addition to

the features already discussed, their micrographs clearly show the numerous microtubules which are present within the tegument and the subtegumental cells.

Taken together these reports provide an understanding of *in situ* larval morphology from 14 days to 1 year p.i. In general, the structure of *T. taeniaeformis* is like that of other cestodes, i.e., a syncytial tegument with digitiform projections extending from its free surface, and cytoplasmic extensions ("bridges") providing continuity between the tegumental cytoplasm and that of the subtegumental cell bodies. The work of Picone (1978) indicates that prior to 14 days p.i. very early postoncospheral stages have a structure quite different from later stages. Not only were they compact spherical masses of cells prior to vesiculation, but all stages prior to 9 days p.i. were coated with a microvillar border. These projections did not have the microthrix structure which had been described for older larvae. The early stages of development, which are so critical in the establishment and survival of the parasite, have not been examined ultrastructurally *in situ*. Thus, the nature of the interaction between the tegumental surface and host cells during this phase cannot be determined from the literature.

Ultrastructure of Larval Stages of Cestode Parasites Other Than *Taenia taeniaeformis*

Most studies on the ultrastructure of cestodes have involved adult worms, but a few investigators have examined

oncospheres and metacestodes. Observations which are relevant to the interpretation of developmental changes in *T. taeniaeformis* have been extracted from the latter reports and are reviewed in this section. Those reports which describe early postoncospheral stages are presented first, followed by a representative sample of studies on older larval stages recovered from mammalian tissues.

Other than the paper by Bortoletti and Ferretti (1971) on *T. taeniaeformis*, there are no ultrastructural descriptions of very early postoncospheral stages of taeniids in mammalian tissues. However, Collin (1970) described the ultrastructure of precysticercoids of *Hymenolepis citelli* which were recovered from *Tribolium confusum* beetles at 3 and 5 days p.i. The oval to spherical 3 day old organism was approximately 65 µm in diameter and contained numerous dividing cells and a central cavity. Subtegumental muscle was much less extensive than in the oncosphere. Fine microvillar projections extended from the parasite surface; these differed in appearance from the cytoplasmic projections of the oncosphere, which were much fewer, longer, and more randomly distributed. The structures were 1-4 µm long and 0.06 µm wide. Although the author did not comment on it, some of the electron micrographs show branching microvilli. Many host cells (hemocytes) were attached to the microvilli, and Collin felt that these frequently fused with the host cells. Five day old precysticercoids were elongate in shape and 100 x 300 µm. Microvilli were shorter and fewer in number than the 3 day stage. Host cells were still

attached but were fewer in number and were frequently undergoing cytolysis. Both circular and longitudinal muscle was evident beneath the tegument. Cell differentiation had advanced greatly by this time. The cells lining the central cavity were especially distinctive because they contained large amounts of alpha (rosette) glycogen granules and large oval osmiophilic bodies, which Collin described as lipid. Although the dimensions of these structures are not given, those shown were up to 1.0 x 0.7 μm.

The ultrastructure of 8 day old cysticercoids of *H. diminuta* which had been removed from *T. confusum* was studied by Ubelaker et al. (1970b). The microvilli which extended from the tegument were membrane-bound, branched, and sometimes had distended tips. This represents the first report of branching of cestode surface projections. The microvillus membrane was covered with a filamentous coating. The matrix contained slender filaments and small vesicles and was more dense than the underlying tegumental cytoplasm. Infoldings of the outer tegumental membrane were observed, and the authors suggested that some of the tegumental vesicles were pinocytotic.

In a separate report, Ubelaker et al. (1970a) described a possible defense function for the microvilli on cysticercoids of *H. diminuta*. They were distended at various levels along the shaft, and distentions appeared to pinch off the apical end. The authors speculated that the resulting vesicles contained secretory products which caused lysis of the insect hemocytes which contacted them.

The ultrastructure and histochemistry of mature cysticercoids of *Raillietina cesticillus* which had been removed from *Tribolium* beetles was studied by Baron (1971). The outermost layer of the parasite wall did not stain with any of the histological stains used and was referred to as the hyaline coat. Beneath this coat was a layer of about the same thickness (1.4 μm) which contained so many electron-dense globules that it was called the globular layer. They were also seen within the cytoplasmic projections of the subtegumental cells. Histochemical staining revealed that these globules (0.1-1.5 μm in diameter) consisted of protein rather than lipid. Baron speculated that this proteinaceous material originated in the deeper parts of the cyst wall, traveled up the cytoplasmic extensions, accumulated in the globular layer, and was passed out via the undulated tubules (20-50 nm in diameter) which arose from projections on the outer surface of the globular wall and traversed the hyaline coat. He further presumed the material to be a secretory substance which might be related to "an antibody-antigen reaction occurring between the host and the parasite" and suggested the possibility that the hyaline coat was "the result of an immune reaction with the insect host."

Baron (1968) reported on the ultrastructural features of cysticerci of *Taenia crassiceps* recovered from mice but did not comment on the age of the parasites. The microthrix bases in this organism were 0.5 x 0.08 μm, the tips 1.0 x 0.04 μm, and the limiting membrane was continuous with the tegument membrane. A dense region was detected beneath the

microthrix membrane which continued into the area of tegument just below the base. The tegument itself was about 1.5 μm thick and had a plasma membrane at its inner border. Numerous vacuoles, 0.03-0.15 μm in diameter, and mitochondria were present within the tegument. An outer layer of circular muscle and an inner layer of longitudinal muscle surrounded by a lightly-stained, fiber containing matrix were located immediately beneath the tegument. Subtegumental cells were connected to the tegument by cytoplasmic processes. Excretory tubules and flame cells were also seen. The latter contained about 100 flagella (cilia), transverse sections of which showed the typical 9 + 2 arrangement of microtubule doublets. In transverse sections of the ducts, the cytoplasm bordering the lumen had a nodular profile.

Ultrastructural characteristics of cysticerci of *Taenia saginata* were studied by Slais et al. (1971), but because the parasites were obtained from naturally infected cattle their ages were not known. The whip-like microtriches consisted of a 0.75 x 0.17 μm base and a 5-6 x 0.06 μm tip. The tips had a lightly stained superficial layer and a darker core which was less than 0.05 μm in diameter. The tegument ranged from 0.5 to 1.0 μm in depth and the subtegumental region was 8-10 μm thick. Subtegumental cells contained round, electron-dense "bodies" of various sizes, which the authors felt were lipid. Their numbers were greater in older parasites, but they were also present in relatively young larvae. Flame cells, ductules and ducts were described in detail.

The fine structure of racemose cysticerci of *Taenia solium* removed surgically from a human brain was described recently by Voge and Brown (1979). The tegument was covered with microtriches, although the authors referred to them as microvilli. The base was 0.1 μm in width and 1.2 μm in length; the tips were 0.06 μm in maximum width and 3.7 μm in maximum length. The bases of the microvilli were interconnected by numerous fine strands. When stained with ruthenium red, small knobs, possibly mucopolysaccharide, were seen on the microvillar surface.

Microtriches or microvilli are located in three distinct areas of the hydatid cyst: the interface between the germinal and laminated layers, the inner surface of the brood capsule, and the outer surface of the protoscolices. Their ultrastructure has been extensively studied (Morseth, 1967; Bortoletti and Ferretti, 1973; Lascano et al., 1975). It is important to realize that the hydatid cysts which were examined came from a variety of different hosts: sheep (Morseth, 1967), human (Bortoletti and Ferretti, 1973), and mice (Lascano et al., 1975) and this may have contributed to the variations in ultrastructural characteristics which were recorded.

According to Morseth (1967), the projections which arise from the basal surface of the germinal membrane and extend into the adjacent laminated layer are slender (about 0.13 μm wide), membrane-bound structures, approximately 0.5 μm in length, and lacking an electron-dense "spike." Bortoletti and Ferretti (1973) said they were 0.20-0.25 μm

wide, 0.6-0.8 μm long, and possessed a short, cone-shaped, electron-dense tip. Immediately beneath the tip they saw a small dense circular formation, the significance of which was not commented upon.

Throughout their report, Bortoletti and Ferretti (1973) use the term "double membrane", which is confusing. What they are referring to is a single plasma membrane which has a trilaminar appearance in stained thin sections. They did state, however, that a second membrane system was present within the microthrix base, which became covered and hidden by electron-dense material following staining of the sections. They believed that these densely stained membranes produced the dark ring which is commonly observed in transverse sections of cestode microthrix bases.

Lascano et al. (1975) were the first to describe complex saccular structures beyond the tips of the germinal membrane microtriches. The sacs were formed by a continuation of the microthrix limiting membrane. Beyond the sac was a constriction, followed by a short tube which apparently opened into the laminated layer of the hydatid cyst wall. The authors speculated that these sacs might be related to the synthesis of or communication with the laminated layer. The dense ring observed near the junction of base and tip was thought to be formed by a convergence of the dense bands present beneath the plasma membrane of the base.

The microtriches on the inner surface of the brood capsule wall were said by Bortoletti and Ferretti (1973) to be similar in appearance to those which they had observed on

the germinal membrane but were fewer in number and rare or totally absent at some sites.

Some of the protoscolex microtriches resembled those on the germinal membrane, but others differed markedly in that they had long electron-dense tips, up to 1.2 μm in length (Morseth, 1967; Bortoletti and Ferretti, 1973).

Subtegumental flame cells and ducts were illustrated and described for protoscolices of *Echinococcus granulosus* in the report by Morseth (1967), and for the germinal membrane by Lascano et al. (1975). The latter report also mentioned the presence of "abundant lipid droplets" within some germinal membrane subtegumental cells. The dimensions of one of the illustrated droplets were 3.9 x 3.5 μm.

The ultrastructure of *E. multilocularis* larvae from experimentally infected cotton rats was studied by Sakamoto and Sugimura (1969). As in *E. granulosus*, microtriches were found on the protoscolices, the inner surface of the brood capsule wall, and the surface of the germinal layer of the cyst wall in contact with the laminated layer. Subtegumental structures described in the report included flame cells, excretory ducts, and calcareous corpuscles.

In summary, the general structure of fully formed cestode larvae appears very similar in most species studied: a syncytial tegument covered by digitiform projections and connected to subtegumental cell bodies by cytoplasmic extensions. Whereas the young precysticercoids and cysticercoids possess microvilli, the surface projections of older larvae are always described as true microtriches.

Immunology of *Taenia taeniaeformis* in the Rat

Thorough reviews on the immunology of human and animal cestode infections have been published recently (Leid, 1977; Flisser et al., 1979; Williams, 1979). Only observations pertaining directly to larval *T. taeniaeformis* infections in rats will be presented here.

Taenia taeniaeformis infection in rats is a naturally occurring example of concomitant immunity, i.e., the relative or absolute resistance to the reestablishment of an homologous parasite in an already parasitized host (Mitchell, 1979). The immune mechanism(s) which destroy challenge organisms are incapable of eliminating the established parasites which stimulated those mechanisms. According to Mitchell (1979), concomitant immunity, also referred to as premunition and non-sterilizing immunity, is common in balanced host-parasite relationships.

The earliest immunological studies involving larval *T. taeniaeformis* infection were conducted in the 1930's by Miller and associates (Miller, 1931a, 1931b, 1932a, 1932b, 1935; Miller and Gardiner, 1932, 1934; Miller and Massie, 1932) and Campbell (1936, 1938a, 1938b, 1939). Although these investigators were the first to demonstrate conclusively the importance of antibody in resistance to helminth infection, the significance of their observations was not fully appreciated until a revived interest in this model system occurred in the 1970's.

The early studies had demonstrated that resistance could be passively transferred with immune serum and could be transferred from infected mothers to their offspring. It had also shown that rats could be immunized using dead parasites and parasite extracts. More recent investigations demonstrated that passive transfer of immunity could be achieved with serum obtained 14, 21 and 21 days after infection, and that the protective antibodies were associated with the IgG_{2a}-containing fractions of the 28 day serum (Leid and Williams, 1974a). As the infection progresses, the range of chromatographic fractions showing protective capacity is extended to all fractions containing IgG_2 or IgG_1 immunoglobulins (Musoke and Williams, 1975). None of the fractions enriched for IgM showed significant activity.

Skin sensitizing antibody or reagin, the physicochemical and biological characteristics of which were consistent with IgE, was detected in the serum 3-4 weeks after primary exposure to the parasite (Leid and Williams, 1974b). Peak titers were reached on day 32 and thereafter declined. Later studies led to the conclusion that reagins may play a role in resistance to reinfection by accelerating the process(es) by which invading organisms are killed in immune animals (Musoke et al., 1978). One mechanism proposed was that migrating oncospheres triggered the release of inflammatory mediators from IgE-sensitized tissue mast cells, leading to an increased microcirculation around the organisms and a more rapid translocation of protective antibodies across the vascular endothelium. Increased numbers of mast

cells have been observed in the small intestine of infected rats (Cook, 1979) and at the host-parasite interface in the liver (Lindsay and Williams, 1980). The release of histamine from peritoneal cells and lung fragments of infected rats following *in vitro* exposure to *T. taeniaeformis* antigen has been demonstrated (Leid, 1977).

Marked strain variations in susceptibility to first infection with *T. taeniaeformis* occur in both rats and mice (Dow and Jarrett, 1960; Olivier, 1962; Orihara, 1962; Turner and McKeever, 1976; Mitchell et al., 1980). Mitchell (1979) presented an attractive hypothesis to explain the variability within mouse strains. He stated that the development of protective antibody by the host is time-dependent, as is the development of protective mechanisms by the parasite. Therefore, the establishment of a parasite within a given host is "a race against time"; i.e., can the parasite develop protective mechanisms before the host develops antibodies lethal to the parasite? In resistant strains of mice, sufficiently high titers of protective antibody are attained relatively early in the infection before the parasite's protective mechanisms are fully functional. The reverse would be the case in susceptible strains; i.e., the parasite's protective mechanisms would become functional prior to host production of high titers of protective antibody.

Mitchell's hypothesis is especially relevant to the findings of Musoke and Williams (1975) relating to the passive transfer of immunity to *T. taeniaeformis* infection

in rats. A clear pattern of gradually acquired invulnerability of young parasites to immune serum was demonstrated both *in vivo* and *in vitro*. The effectiveness of the antibody begins to wane by 6 days p.i., and fewer than 50% of 7-8 day old parasites are killed by immune serum. The *in vitro* results, using larvae which had been liberated enzymatically from livers, indicate that the development of insusceptibility to antibody is derived from inherent changes on the part of the parasite. These results indicate that many developing parasites have acquired an effective defense mechanism as early as 6 days p.i.

Depletion of circulating complement in passively-immunized rats over the initial 5 day period of growth led to a very significant increase in parasite survival, suggesting that surviving larvae must develop a means of avoiding the combined effects of antibody and complement (Musoke and Williams, 1975). Subsequent studies demonstrated that the larvae are capable of causing local complement depletion at the host-parasite interface, that the fluid surrounding the parasites *in situ* inhibits complement-mediated lysis of red blood cells *in vitro*, and that anaphylatoxins are generated (Hammerberg et al., 1976; Hammerberg and Williams, 1978). This interaction with the complement system was shown to proceed in part via the alternate pathway.

The eosinophilic response of the rat to infection with *T. taeniaeformis* was studied by Ansari and Williams (1976). A slow rise in peripheral eosinophil counts occurred during the second week, and peaks of eosinophilia occurred

approximately 2.5 and 5 weeks p.i. Increased numbers of eosinophils were detected in the portal areas as early as 7 days p.i., and within the cellular infiltrate surrounding developing larvae by 11 days. By 22 days p.i., a wide zone of cells (up to 120 μm), consisting almost entirely of eosinophils, was directly apposed to the tegument in the vicinity of scolex formation and extended over approximately one-fourth of the parasite surface. Fewer eosinophils were present at 37 days p.i., and by 62 days this zone of eosinophils was completely gone. However, eosinophils were distributed throughout the surrounding host connective tissue capsule.

The authors speculated that the influx of eosinophils might be the result of antigen-antibody reactions occurring around the developing larva leading to the production of eosinophilotactic substances via the complement system (C3a, C5a, $\overline{C567}$) and/or via the release of eosinophil chemotactic factor of anaphylaxis (ECF-A) from reagin-sensitized mast cells. They further speculated that the eosinophilia might be the result of complement fixation by the parasite via the alternate pathway, with subsequent production of anaphylatoxins (Hammerberg et al., 1976).

Secondary peaks of peripheral eosinophilia were seen 3 to 7 days following challenge of infected rats, but no secondary eosinophilic responses were observed in the sections of liver or small intestine which were examined. Sharp peaks of peripheral eosinophilia were also seen 2-6 days following challenge of rats which had received immune serum or

fractions of immune serum containing IgG_{2a} 24 hours earlier (Ansari et al., 1976). The latter results indicate that the secondary eosinophilic responses were, at least in part, mediated by antibody. The protective IgG_{2a} antibodies are known to fix complement, and the anaphylatoxins thus generated may function as eosinophilotactic stimuli. It has been demonstrated that extracts of *T. taeniaeformis* larvae possess *in vitro* chemotactic activity for both human peripheral blood eosinophils and neutrophils (Goetzl and Austen, 1977).

As recently stated by Mitchell (1979), the *T. taeniaeformis*/rodent system is well suited to immunoparasitological studies, and this parasite may become something of a "type organism" in metazoan immunoparasitology. Reasons he cited include (1) host protective antibodies can be used to identify host functional antigens, (2) protection against first infection using vaccines consisting of parasite antigen preparations is readily achieved, (3) mechanisms of evasion of host-protective immunity can be dissected, and (4) strain variation in resistance to first infection can be exploited to study the mechanisms and genetics of host-protective immunity.

In addition, the concomitant immunity operable in *T. taeniaeformis* infection is similar to certain phenomena encountered in the field of tumor immunology (Larsh and Weatherly, 1975). Therefore, knowledge generated from the *T. taeniaeformis*/rat model could have broader applicability in other areas of immunobiology.

Interactions Between Taeniid Larvae and Host Cells *in vivo* and *in vitro*

Although many investigators have examined interactions between host cells and non-cestode parasites (for general review see Mitchell, 1979; for schistosome review see Capron et al., 1977), relatively few studies have focused on cestode-host cell associations (Larsh and Weatherly, 1975). Published reports concerning the interaction of taeniid larvae and host cells are reviewed here because a portion of the present investigation involved *in vitro* relationships between *T. taeniaeformis* strobilocerci and rat peritoneal cells.

There is very limited evidence for cell-mediated mechanisms operating *in vivo* in resistance against taeniid parasites. Blundell et al. (1969) were not successful in their attempt to transfer immunity to *Taenia hydatigena* in sheep using lymph node and spleen cells from immune donors. Kwa and Liew (1975) reported that a functional cell-mediated immunity (CMI) occurs in rats experimentally infected with *T. taeniaeformis*. They inoculated peritoneal cells from infected animals intravenously into normal recipients which were then immediately challenged. Optimal protection was about 50% when 6.25×10^6 cells were given. Fewer cells did not cause significant protection and a greater number of cells did not result in an increase in protection. While these results can be interpreted as indicating a role for CMI, it should be noted that the authors neglected to include a control group receiving peritoneal cells from non-infected

animals and based their statistical analyses on rats which received medium only.

Finally, Anderson and Griffin (1979) successfully transferred immunity and immunocompetence against *Taenia crassiceps* in rats using lymph node cells obtained from infected donors. Although they were unable to exclude the possibility of a contribution by antibodies produced by the adoptively transferred cells, they felt that this would be insignificant because they were unable to transfer resistance using sera from the donors.

None of these reports provides any insight into the nature of any cellular effector mechanisms, but some direct observations on host cell responses to cestode larvae *in vivo* were reported by Siebert et al. (1978a, 1978b, 1979). They demonstrated a biphasic host immune response in mice immunized with a subcutaneous inoculation of larvae of *T. crassiceps* and challenged 3 weeks later with an intraperitoneal inoculation of this parasite. Within the first 4 weeks of challenge, 85% of the implanted larvae showed evidence of tegumental damage in the absence of any adherent cells. This phase of the host response also occurred to a lesser degree in non-immunized mice. Vacuolization of the tegument, loss of the glycocalyx, and reduction in the numbers of mitochondria and microtriches were followed by loss of the tegument and death of the larvae. The tegumental damage was attributed to the effects of antibody, perhaps directed against digestive-absorptive enzymes on the tegument surface, followed by complement-mediated lysis. The death

of the larvae was thought to be due to impaired tegument function. Direct evidence for these processes is lacking.

The second phase of the host response in immunized mice usually occurs 4-8 weeks after the challenge inoculation, but in a few cases has been observed as soon as 1 week. In this phase, larvae unaffected by early immune damage are encapsulated by host cells and destroyed. The response selectively affects larvae which do not show evidence of early immune damage and does not occur in non-immunized mice. These observations will be reviewed in detail because of their relevance to certain aspects of the present investigation; it is an ultrastructural account of the interactions between peritoneal cells and a taeniid parasite.

Encapsulated larvae recovered 1, 4 and 7 weeks after implantation were selected by the authors as examples of early, intermediate and late stages of encapsulation. After one week of incubation in the peritoneal cavities of immunized animals, the parasites had no host cells in contact with their surfaces. Instead, a 20-40 μm clear space existed between the larva and the early capsule. The authors did not provide any explanation for this observation. The predominant cell type along the inner edge of the early capsule was the eosinophil. They were all damaged to some extent; some were completely disrupted. The middle and outer portions of the capsule consisted primarily of macrophages and lymphocytes, but there were also damaged and undamaged eosinophils. Fibroblasts rarely occurred, but occasional

epithelioid cells were present along the outer margin. No mast cells were observed in the early capsule. With the exception of large quantities of unidentified flocculent material near the microthrix tips, the microtriches and tegument were normal in appearance.

The microthrix border of larvae within intermediate capsules, however, was severely depleted and many of the remaining microtriches were shorter than normal. In most intermediate capsules, the eosinophils along the inner margin were closely applied to the parasite surface. Phagocytic vacuoles were abundant within these cells, some of which contained electron-dense material similar to that seen along the microthrix border. Mast cells were distributed throughout the capsule; the majority were degranulated. The authors stressed the point that it was only after mast cells were present that other cell types were normal in appearance and damage to the larval tegument became evident. They suggested that the release of mast cell granule contents may protect other cell types by binding or modifying potentially harmful substances secreted from the parasite. Lymphocytes, macrophages, and fibroblasts were also present. Loose aggregations of collagen were scattered throughout the capsule, particularly along the outer portions.

It was difficult to recognize the larvae within late capsules due to the severe damage which they had suffered. Macrophages and lymphocytes were the predominant cell types, although eosinophils and mast cells were seen occasionally and rarely, respectively. Numerous fibroblasts and large

amounts of collagen were present throughout the capsule.

There is evidence from several recent reports that host cells can adhere to and damage *T. taeniaeformis* larvae implanted in the peritoneal cavities of rats, an abnormal location for this parasite. Musoke and Williams (1976) demonstrated that 3.5-14 month old larvae of *T. taeniaeformis* could survive for at least 21 days when implanted into the peritoneal cavities of normal rats. Parasites were lying free in the cavities and normally active when removed. It was not established if host cells were adhering to their surfaces. However, when 5 month old larvae were implanted into rats which had been orally infected with *T. taeniaeformis* eggs 7-28 days previously, all implanted parasites were dead, fragmented and encapsulated in the omental tissues 21 days later. Since immune rat serum did not effectively transfer this response, the authors concluded that the reaction was probably mediated by cells. Larvae implanted into normal rats which had received foot pad inoculations of complete Freund's adjuvant on the day of implantation were also dead, fragmented, and encapsulated. It is possible that the adjuvant triggered a specific type of cellular defense mechanism directed against parasite antigens, but the effect may have been entirely non-specific.

Kwa and Liew (1978) concluded that their implantation experiments with 1 year old *T. taeniaeformis* larvae suggested a role for blocking antibody, but the data presented do not support that conclusion strongly. Using FITC-conjugated rabbit anti-rat IgG and fluorescence microscopy, they

demonstrated the presence of IgG on the surface of the strobilocerci. Not only did the authors refer to this material as "antibody", but they infer that it is "antibody lethal to newly established parasites." They did not demonstrate, however, that the fluorescent material was either protective antibody or even antibody.

When these larvae were implanted into the peritoneal cavities of normal rats or rats immunized with *T. taeniaeformis* "somatic antigens", they appeared healthy after 14 days; there were no adherent cells. On the other hand, trypsinized larvae invoked an intense cellular reaction in both normal and immunized rats. Those recovered from normal rats after 14 days were alive but had large numbers of adherent host cells, most of which were eosinophils, with small numbers of macrophages and neutrophils. Those recovered from the immunized rats were dead and completely encapsulated in fibrous tissue. The entire tegumental layer was destroyed. These results suggest that trypsin treatment made the larvae vulnerable to a host cellular reaction. It is possible that trypsin treatment removed "blocking antibody", but it could have also caused other surface alterations which made the larvae more susceptible to host cells.

Their experiments using 1 month old larvae gave different results, although the time period *in vivo* was not the same. None of the larvae recovered after 3 days showed damage or adherent host cells, but at 21 days all larvae were dead and encapsulated, regardless of treatment.

In the same paper, Kwa and Liew (1978) described *in vitro* interactions between 1 month old larvae of *T. taeniaeformis* and peritoneal cells harvested from rats immunized with "somatic antigens." Since the number of larvae per tube was not given, it was not possible to determine the cell:parasite ratio. The final cell concentration is also not clearly expressed but appears to have been 2.3×10^7 cells/ml.

Trypsinized larvae incubated for three days in the presence of cells, antiserum from immunized rats, and fresh normal rat serum had a few adherent cells on their surface. Neither phagocytic-like activity of the cells nor histological damage to the parasite were observed. Untrypsinized larvae incubated under the same conditions had no adherent host cells. Host cells were not detectable on the surface of trypsinized larvae which had been incubated in the absence of either antibody or complement. Although no conclusions were reached by the authors, the results suggest that host cells adhere only when the larvae have been trypsin-treated and that cells will adhere only in the presence of *both* antibody and complement.

Artificial Immunity to Larval *Taenia taeniaeformis* Infection

One of the goals of immunoparasitology is the development of safe, effective and inexpensive prophylactic agents (Mitchell, 1979). The *T. taeniaeformis*/rat and *T. taeniaeformis*/mouse model systems are naturally occurring host-parasite relationships which simulate those of

socioeconomically important cestode parasites, and are ideally suited for the development of prototype vaccines (Lloyd, 1979; Rajasekariah et al., 1980).

Successful immunization of rats against infection with *T. taeniaeformis* has been achieved through the use of a variety of somatic antigens, X-irradiated eggs, and the excretory/secretory/metabolic (E/S/M) antigens from different developmental stages (Miller, 1932a; Campbell, 1936; Dow et al., 1962; Rickard and Bell, 1971; Kwa and Liew, 1977; Ayuya and Williams, 1979). This subject has recently been extensively reviewed by Ayuya (1978). Therefore, only those reports pertinent to immunological aspects of the present investigation will be summarized in this section.

Concomitant immunity is likely to be directed against infective or very early forms of the parasite (Mitchell, 1979) and, for this reason, the search for antigens to be used in vaccines generally centers upon young developmental stages. With this in mind, the report of Rickard and Bell (1971) provides insight into the nature of the immune response to *T. taeniaeformis*. They placed about 20 activated oncospheres into filtration membrane chambers which were then implanted into the peritoneal cavities of rats. Up to 3 weeks of age, the rate of larval development was comparable to that reported for natural infections. The larvae induced an immunity to challenge infection which increased with the length of time of exposure.

Four important conclusions were reached: (1) the developing parasites released diffusible antigens capable

of stimulating immunity in the host, and direct contact between host cells and parasites was therefore not necessary; (2) effective immunizing antigens are produced early in the development of cestode larvae; (3) only small numbers of larvae are required to produce sufficient antigen to immunize the host effectively; and (4) parenteral administration of the antigens immunizes the host to subsequent oral challenge, and intestinal migration is not necessary.

Rajasekariah et al. (1980) have immunized mice successfully against *T. taeniaeformis* infection using homologous oncospheres or oncospheral products. They pointed out that the early developmental stages should contain a less complicated array of antigens than older larvae and should therefore provide a more suitable source of starting material for the purification and characterization of "host-protective" (functional) antigens.

Excretory/secretory/metabolic (E/S/M) antigens obtained from *in vitro* cultivation of oncospheres of *T. taeniaeformis* have been shown to protect both mice and calves from *T. taeniaeformis* and *T. saginata* infection, respectively (Lloyd, 1979). In addition, these same E/S/M products were highly effective when used to immunize pregnant heifers. Inoculations via either the intramuscular or intramammary route resulted in a passive transfer of immunity against *T. saginata* in newborn calves.

Earlier studies by Musoke et al. (1975) and Lloyd and Soulsby (1978) implicated colostral IgA in the natural passive transfer of resistance to *T. taeniaeformis* infection

from mother rats and mice to their young. The results of a later investigation suggested that these antibodies probably function at the intestinal level (Hammerberg et al., 1977).

It is clear from the limited immunological studies on the domesticated animal-taeniid systems that there are many analogous features between these and the *T. taeniaeformis* model. The protective antigens involved appear to be stable, effective via a variety of routes and in several different vehicles, and are not only shared between those species which affect ruminants but are also shared with *T. taeniaeformis*. The likelihood is that further purification and characterization of antigens in *T. taeniaeformis* will not only be important in the context of the experimental immunology of this host-parasite relationship but will also be relevant to the efforts being made to establish a practical prophylactic approach to taeniiasis in domestic livestock.

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ARTICLE 1

TAENIA TAENIAEFORMIS IN THE RAT: ULTRASTRUCTURE OF THE HOST-PARASITE INTERFACE ON DAYS 1-7 POSTINFECTION

P. G. Engelkirk and J. F. Williams

ABSTRACT

The host-parasite interface during the first week of postoncospheral reorganization of *Taenia taeniaeformis* has been examined at the ultrastructural level. The rate of development was not uniform, but by 4-5 days postinfection all organisms had changed from a microscopic, compact ball of relatively few cells to a vesiculated larva, the walls of which showed the arrangement of syncytial tegument, cytoplasmic bridges and subtegumental cell bodies typical of other cestodes. By 6 days the parasites were grossly visible as white spots on the liver surface.

During all developmental stages, microvilli, which were often branched, extended from the free tegumental surface and at 2 days postinfection they contributed over 70% of the total membrane surface area available for absorption and excretion. The results indicate that fragments of microvilli break off, become segmented and distended, and are ingested by host phagocytic cells, where they continue to swell, rupture and release their contents.

Microvillar segments were seen as far as 40 μm from the free tegumental surface. By 7 days the surface became bumpy and microvilli had unusual bases and electron-dense deposits on their surface. Although phagocytic host cells were observed within migratory tracks and surrounding organisms as early as 1 day postinfection, they caused no apparent damage to the parasite. Inclusions, probably lipid, were observed within the tegument and subtegumental cell bodies from 3 days postinfection onwards, and these

occasionally appeared to be in the process of ejection. Possible roles for microvillar fragments and lipid-like inclusions in the evasion of host defense mechanisms and the stimulation of early humoral responses are discussed.

INTRODUCTION

The *Taenia taeniaeformis* rat system is an important, naturally occurring model of concomitant immunity in parasite infections, in which protective resistance is likely to be directed against the invasive and/or early developmental stages of the organism (Mitchell, 1979). An improved understanding of postoncospheral reorganization *in situ* is necessary if experimental analysis of the immunology of this phase of taeniiasis is to be pursued. The extensive investigations of taeniid larval infection at the light microscopic level (e.g., Bullock and Curtis, 1924; Rausch, 1954; Silverman and Hulland, 1961; Sweatman and Henshall, 1962; Singh and Rao, 1967; Banerjee and Singh, 1969) provide limited insight into the events which take place at the host-parasite interface, yet the only ultrastructural account of the acute stage of cysticercosis in mammalian tissues is that by Bortoletti and Ferretti (1971) on 14 day old *T. taeniaeformis*.

In this paper we describe, in chronological sequence, the critical changes which occur in the host-parasite association during the first 7 days of postoncospheral development of *T. taeniaeformis*. The results define some important characteristics of the establishment of this parasite in

hepatic tissues and will serve as a foundation for future immunoelectron microscopic studies on the nature of initial evasive mechanisms. After the first week there is an abrupt transformation in both the larval surface and its relationships to the host; observations on these and subsequent events are presented elsewhere (Engelkirk and Williams, 1980).

MATERIALS AND METHODS

Animals

Twenty-eight day old female Spartan Spb[SD] rats purchased from Spartan Research Animals, Haslett, Michigan, were infected orally with *Taenia taeniaeformis* eggs and necropsied on days 1 through 7 postinfection. The doses given were graded to provide optimum numbers of organisms at early stages when parasites are most difficult to locate. The schedule was as follows: rats to be killed on day 1 received 100,000; those for days 2 and 3 received 25,000; those for days 4 and 5 were given 10,000; and animals left for days 6 and 7 received 1,000.

Parasite Maintenance

Parasites were maintained according to the method described by Leid and Williams (1974). The proglottids were placed into a petri dish containing distilled water and teased apart with a dissecting needle to release eggs. The suspension was filtered through gauze and centrifuged for 10 min at 150 g. The eggs were resuspended in

antibiotic-antimycotic solution (GIBCO, Grand Island, New York) containing penicillin, fungizone and streptomycin, and stored at 4°C. The number of eggs per ml was determined before dosing by counts on a series of 0.025 ml aliquots, and dilutions were made so that each rat received 0.5 ml.

Tissue Processing

Animals were killed by exposure to CO_2 vapor in a dry ice chamber. Livers were removed immediately and placed into ice-cold 4% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2. After several minutes the liver was sliced on a sheet of dental wax using an acetone-cleaned razor blade. Samples, approximately 2 mm^3, were then returned to cold 4% glutaraldehyde and stored 1-3 days at 4°C.

Postfixation, Dehydration and Embedding

Liver samples were washed twice in cold 0.1 M phosphate buffer, pH 7.2, and postfixed overnight at 4°C in 1% osmium tetroxide in the same buffer. Following 2 washes in buffer, they were dehydrated step-wise through increasing concentrations of ethanol up to 100%. Acetone was used as a transition solvent, and tissues were infiltrated with an epoxy-araldite mixture.

Microtomy and Electron Microscopy

Thick (3 μm) sections were cut using glass knives and an Ultratome III ultramicrotome (LKB-Produkter AB, Stockholm, Sweden). The sections were placed onto drops of filtered distilled water on a glass microscope slide, and evaporation

of the water was hastened by use of a hot plate. Following the addition of immersion oil and a coverslip, the sections were examined with a phase microscope at 400X. Once a parasite was located, the block face was trimmed and then thin sectioned with a diamond knife (E. I. DuPont de Nemours and Co., Wilmington, Delaware). Three-hundred mesh copper grids were used to collect the 50-90 nm sections, which were then stained for 10 min in 2% aqueous uranyl acetate and 5 min in 0.125% aqueous lead citrate. Grids were examined using a Model 300 transmission electron microscope (Philips Electronic Instruments, Inc., Southfield, Michigan).

Calculation of Surface Area

The lengths and widths of 10 parasites of each age were obtained using a light microscope and an ocular micrometer. The arithmetic mean of the 10 lengths and 10 widths was used as the average dimension (d), and the formula for the surface area of a sphere (πd^2) was used to provide an estimate of the tegumental free surface area. The surface area of a "typical" microvillus was estimated using the formula for the surface area of a cylinder, $2\pi r(r+h)$, with "r" being the length of the longest continuous microvillus observed at any given age.

OBSERVATIONS

Developing parasites of *T. taeniaeformis* do not reorganize and grow at a uniform rate during the first week of infection. The illustrations which follow best represent the changing

characteristics of the host-parasite interface over this period. Also included are electron micrographs which depict the most important morphological changes in parasites as they undergo reorganization and growth from a microscopic oncospheral cell mass to the cystic structure visible grossly by day 6.

One Day Postinfection

Day 1 parasites were located only after exhaustive searching and were often contained within sinusoidal lumina. They were always surrounded by a clear halo when examined by phase microscopy (Figure 1), but electron micrographs revealed that the halo contains numerous cytoplasmic projections (microvilli) extending from the parasite surface (Figure 2). The lengths of the microvilli are difficult to determine because the complete structure is rarely contained in a given thin section. Occasionally 2 microvilli appear to arise from a common point on the parasite surface.

At this early stage the organism consists of a compact mass of cells (Figure 2). Four nuclei, with prominent nucleoli, can be seen, and oncospheral hooks and muscle are still present. The cells are rich in ribosomes, endoplasmic reticulum, mitochondria and Golgi configurations. The outermost region of the parasite, 0.3 to 2.1 μm thick, is separated from the rest of the cellular mass by one or more membranes. In addition to microvilli, the area surrounding the parasite contains debris, some of which is membranous. Adjacent hepatocytes have incomplete ragged borders, and

FIGURE 1. *Taenia taeniaeformis* larva, 24 hours post-infection. The oval parasite contains an oncospheral hook and several prominent nuclei. It is surrounded by a characteristic clear halo, about 1.4 μm wide. To the left of the organism is an area of host cell destruction. This parasite and the adjacent region are further illustrated in Figs. 2 and 3. X 1450.

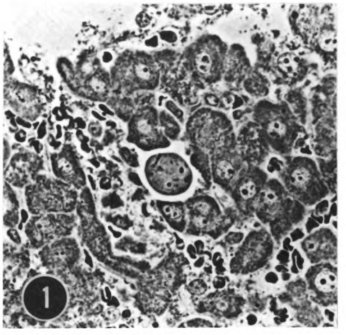


Figure 1

FIGURE 2. *Taenia taeniaeformis* larva, 24 hours post-infection. The parasite, 16.4 x 19.4 µm, has numerous microvilli, 70-80 nm in width, extending from its surface. The longest microvillus (arrow) which can be traced to the parasite surface is 3.4 µm in length. A nearby microvillus (multiple arrows), the complete length of which does not lie in this thin section, appears to be at least 5 µm long. Adjacent hepatocytes have incomplete borders and the area between these cells and the parasite contains amorphous and membranous debris. X 7250. Abbreviations: G, Golgi apparatus; H, oncospheral hook; M, mitochondrion; Mu, muscle; N, nucleolus; NP, nuclear pores.

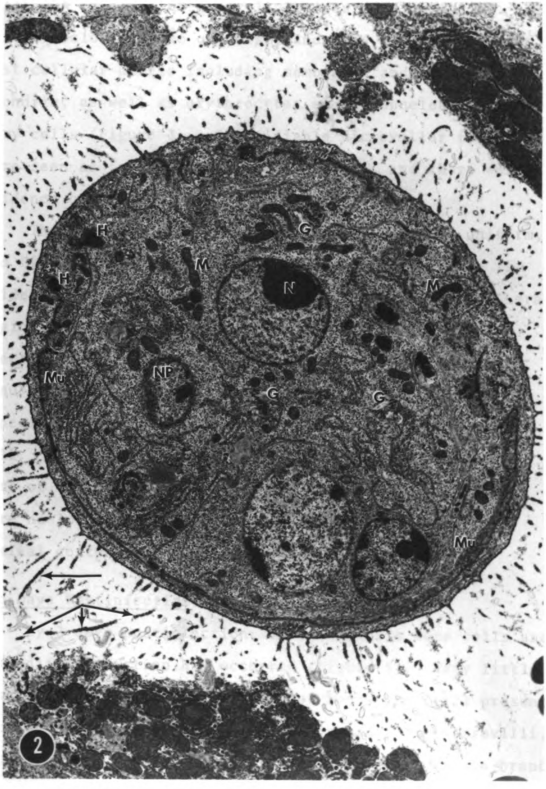


Figure 2

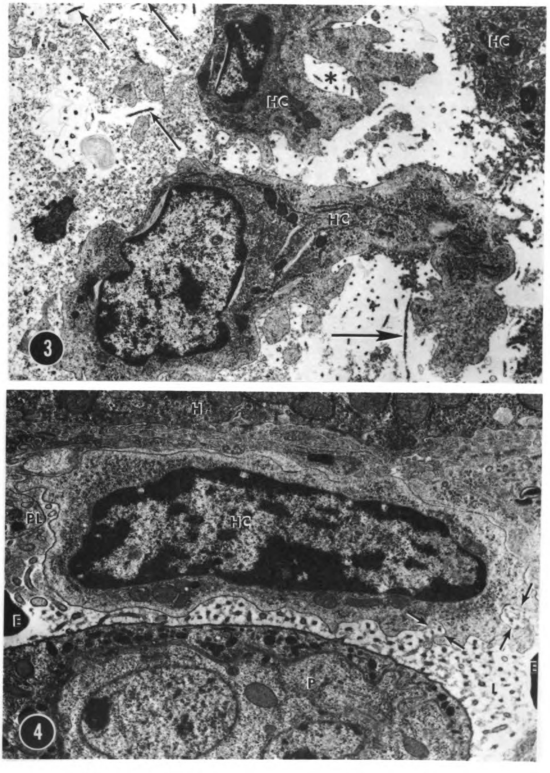
the debris probably represents material lost from damaged cells. A zone of intense cellular destruction was observed close to the organism, and this region contained host cellular debris including membranes and free mitochondria, as well as erythrocytes and mononuclear inflammatory cells (Figure 3). Identifiable microvillar fragments were seen as far as 33 μm from the larva, and there was evidence of phagocytosis of these structures.

The appearance of parasites on Day 1 was not uniform, and Figure 4 illustrates a situation where the larva is evidently confined with a hepatic sinusoid. The peripheral region of this parasite contains many round to oval electron-dense bodies. In addition to microvilli, the sinusoidal lumen is full of amorphous debris which is especially dense at the left end of the phagocytic host cell. The origin of this debris is not clear, although some of the nearby endothelial cells lining the sinusoid were no longer intact and may have contributed to the luminal contents.

Two Days Postinfection

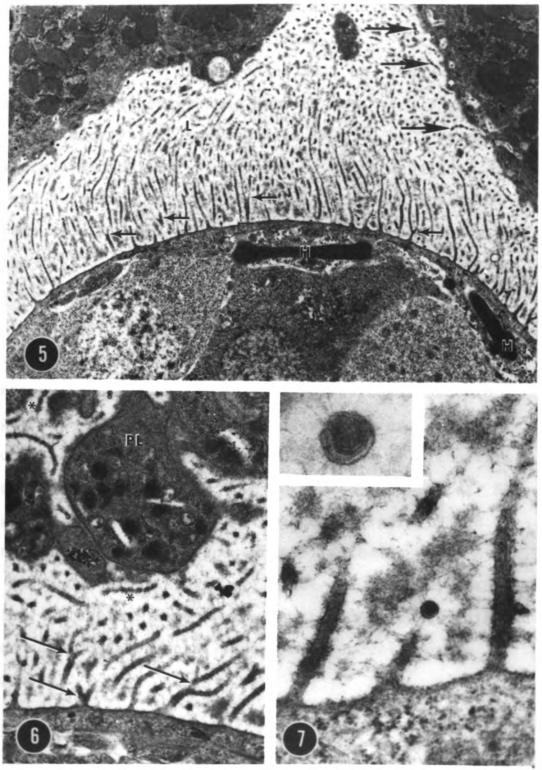
By 2 days postinfection, larvae contain more cells and some differentiation has occurred (Figure 5). Very little muscle is retained, but oncospheral hooks are still present. Extending from the larval surface are many long microvilli, some of which are branched (Figures 5 and 6), but the branch point does not occur at a consistent distance from the parasite surface. Although it is not possible to trace a

FIGURES 3-4. Host cellular response to *Taenia taeniaeformis* larvae, 24 hours postinfection. 3. Area of cellular destruction close to the organism shown in Fig. 2. Portions of phagocytic host cells (HC) can be seen, as well as amorphous and membranous debris. Many fragments of parasite microvilli (small arrows) are present, some of which are being engulfed by host cell processes (*). The longest piece of microvillus (large arrow) is 3.4 μm in length and 13.3 μm from the parasite surface. X 7250. 4. *Taenia taeniaeformis* larva and host cells. The peripheral region of the parasite (P) contains numerous electron-dense bodies, 0.1-0.3 μm in diameter, and is demarcated from the remainder of the cell mass by one or more membranes. The sinusoidal lumen (L) contains amorphous debris and parasite microvilli, some of which are surrounded by cytoplasmic processes (arrows) of a mononuclear host cell (HC). The hepatocyte (H) is apparently undamaged and contains numerous mitochondria, rough endoplasmic reticulum and glycogen rosettes. Many hepatocyte microvilli extend into the space of Disse. Portions of erythrocytes (E) and a platelet (PL) are also present. X 11,200.



Figures 3-4

FIGURES 5-7. *Taenia taeniaeformis* larvae, 2 days post-infection. 5. Parasite, 26.4 μm in diameter, within liver sinusoid. The peripheral region of the larva varies in thickness from 0.2-1.0 μm and is separated from the remainder of the cell mass by one or more membranes. Oncospheral hooks (H) are present. Long microvilli, 70-90 nm in width, extend from the surface. Some branching (small arrows) and beading (large arrows) of microvilli can be seen. The sinusoidal lumen (L) contains amorphous material, but surrounding host cells appear to be undamaged. Portions of parasite microvilli are found within the space of Disse. X 7250. 6. Microvilli and host platelets. Branching (arrows) microvilli, some of which have more than one branch point, can be seen. Segmentation or "beading" (*) is also illustrated. Clusters of platelets (PL) are present in close proximity to the larva. X 17,000. 7. Microvilli. Spine-like projections extend from the microvillar surface, and faint longitudinal lines can be seen within the shafts. Abundant amorphous material is present between the microvilli. X 55,000. Insert. In transverse section some microvilli appear to have concentric membranous rings. X 162,000.



Figures 5-7

single microvillus from origin to tip, lengths of up to 3.7 μm were measured. Their width is the same as on Day 1, but they have become more plentiful.

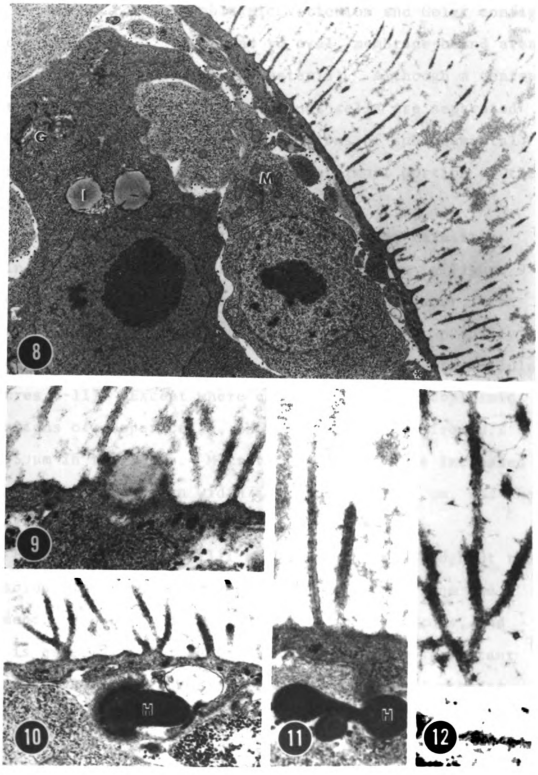
With an average dimension of 23.5 μm, 2 day old parasites have a tegumental free surface area of 1735 μm^2. The total microvillar surface area is 4730 μm^2, based upon an average microvillar width of 0.08 μm, a length of 3.7 μm, and a density of 2.9 per μm^2 of surface. The total surface area is thus 6465 μm^2. Microvilli contribute 73% of the total, thereby increasing the available surface area by almost four-fold.

Microvilli contain faint longitudinal striations and, in transverse sections, concentric membranous rings are sometimes seen around a moderately electron-dense core (Figure 7). The surface bears filamentous projections which may represent glycocalyx. Fragments of microvilli were seen more than 40 μm from the parasite surface. Some fragments appear segmented or "beaded" (Figures 5 and 6). Surrounding hepatocytes and endothelial cells were intact, but amorphous material was still present in the sinusoidal lumina. Platelets were common in the vicinity of this and other early developmental stages of *T. taeniaeformis* (Figure 6).

Three Days Postinfection

At this stage the total surface area is about seven-fold greater than on Day 1. There are many more cells, and cellular differentiation has progressed (Figure 8). All

FIGURES 8-12. *Taenia taeniaeformis* larvae, 3 and 4 days postinfection. 8. Three day old parasite, 32.5 µm in diameter, within liver sinusoid. Microvilli extend into the sinusoidal lumen, which also contains amorphous material. Cell differentiation has occurred; one cell contains prominent mitochondria (M), whereas the more electron-dense cell contains extensive rough endoplasmic reticulum, Golgi configurations (G), and 1.0-1.1 µm oval inclusions (I). Nuclei contain prominent nucleoli. Cells are surrounded by clear channels. A cytoplasmic extension of one of the subtegumental cells is continuous with the thin tegument (*). X 10,200. 9. Tegument of a 3 day old larva containing a round 0.5 x 0.3 µm inclusion, possibly being extruded. X 34,000. 10. Tegument of a 3 day old larva containing a section of an oncospheral hook (H) and a mass of glycogen rosettes. Branched microvilli extend from the parasite surface. X 21,500. 11. Tegument of a 3 day old larva illustrating an oncospheral hook (H) and longitudinal striations within microvilli. X 35,100. 12. Microvillus of a 4 day old larva showing 5 branch points. X 40,000.



Figures 8-12

cells contain numerous ribosomes and mitochondria. Some show extensive rough endoplasmic reticulum and Golgi configurations, and 0.8-1.7 µm, round to oval, membrane-bound areas containing homogeneous, opaque material. Although a central cavity has not yet formed, individual cells are separated by clear spaces. Cytoplasmic extensions of subtegumental cells are continuous with the thin outermost portion of the parasite (tegument).

Round inclusions sometimes appear at the parasite margin, possibly being extruded; the one shown in Figure 9 is considerably smaller than any of those at deeper locations. Extremely small electron-dense clusters, probably glycogen rosettes, are present both intra- and extracellularly (Figures 8-11). Except where continuous with cytoplasmic extensions of deeper cells, the tegument ranges from 0.1 to 0.5 µm in thickness. Microvillar density has increased slightly; they range in width from 0.06 to 0.09 µm, and the longest measurable segments were up to 3.4 µm.

Fragments of microvilli are distributed throughout the sinusoidal lumen and were seen as far as 35 µm from the tegument. "Beading" of microvillar segments occurs, and this is especially common in those fragments most distant from the parasite. Sometimes the "beads" seem distended. Microvillar branching is less frequent than at Day 2, but branched forms show multiple division points (Figure 10). Oncospheral hooks are still retained (Figures 10 and 11). The sinusoids contain intact microvilli, fragments of

microvilli, and unrecognizable debris, but adjacent endothelial cells, Kupffer cells or hepatocytes were intact.

Four Days Postinfection

The organisms were approximately 50 μm in diameter and the microvilli contributed over 40% of the surface area. Larvae were still located within sinusoidal lumina, and a central cavity had developed in some cases. Cell numbers had increased markedly and up to 35 cell nuclei could be seen per section. Subtegumental cells were separated from each other by clear channels and contained homogeneous round inclusions similar to those seen on Day 3. Cytoplasmic bridges were continuous with the tegument, the latter of which varied from 0.2-0.4 μm in thickness. Again, evidence of ejection of inclusions was seen. Glycogen-containing cells were present immediately beneath the tegument.

Microvilli were still plentiful (about one per μm^2 of tegumental free surface) and, although few branched, some were multiply branched (Figure 12). Some were as long as 2.9 μm, and pairs occasionally arose from a common point on the tegument. Longitudinal striations were visible within some microvilli.

Five Days Postinfection

The average dimension of Day 5 parasites was 108 μm. There was a very thin tegument, a zone of subtegumental cells, and a central bladder. The tegument ranged in thickness from 0.1 to 0.8 μm. Several spherical homogeneous inclusions were seen within the tegument, causing a bulging

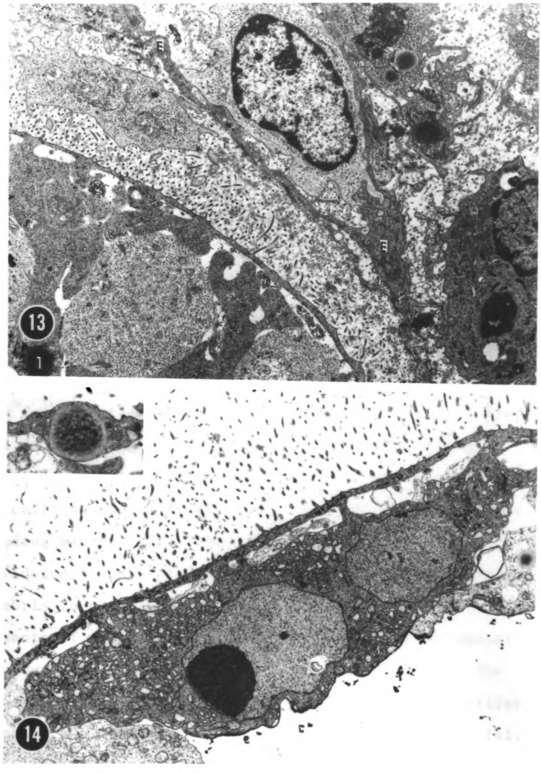
at these sites, and they were also present within subtegumental cells. The latter were surrounded by clear channels, some of which were continuous with the bladder. Glycogen rosettes were seen both within these channels and intracellularly. Small glycogen-containing cells lying directly beneath the tegument may represent precursors of muscle cells. The zone of subtegumental cells was 1-2 cells wide and varied from 8.8 to 16.9 μm in depth.

Surface microvilli are shorter than those at earlier days, the longest being 1.4 μm. Microvillar density had not changed, but branching was rarely observed. Surrounding sinusoids were congested with numerous pieces of microvilli, unstructured debris and many host phagocytic cells (Figure 13). Although the latter were present in the sinusoids surrounding the parasite, none appeared to be directly attacking it, and the tegument showed no evidence of damage. Free segments of microvilli were seen as far as 32 μm from the parasite. Other pieces appeared within host cell vacuoles or were in the process of being engulfed by pseudopodia. "Beaded" segments and distended "beads" were common. Some phagocytic cells contained opaque inclusions similar in size and appearance to those contained within the parasite. No damage to surrounding endothelial cells or hepatocytes was noted.

Six Days Postinfection

On Day 6, parasites are gourd- or pear-shaped and have an average dimension of 242 μm. There has been a 200-fold

FIGURES 13-14. *Taenia taeniaeformis* larvae, 5 and 6 days postinfection. 13. Five day old larva within a sinusoidal lumen. Endothelial cell (E) processes are clearly identifiable. Phagocytic host cells are enmeshed in fragments of parasite microvilli, some of which are "beaded" and swollen. Although the larval tegument is extremely thin, it appears undamaged. The electron-dense inclusion (I) at lower left is 1.5 x 1.2 µm. X 6450. 14. Six day old larva. At this stage of development the wall of the parasite is only one cell deep (7.3 µm at the thickest portion of the illustrated cell). The cell is connected to the tegument by delicate cytoplasmic bridges. X 6450. Insert. An opaque, mottled inclusion body, 1.4 µm in diameter, is contained within the tegument of a 6 day old larva, causing a bulging at this site. X 11,200.



Figures 13-14

increase in total surface area since Day 1, and the organisms are now barely visible on the liver surface as white spots. The narrow band of tegument is continuous with cytoplasmic extensions of underlying cells (Figure 14). It ranges from 0.2-0.5 μm in thickness and covers a subtegumental layer only one cell deep. At least three cell types are recognizable, including longitudinal and circular muscle lying immediately beneath the tegument.

Short microvilli extend from the parasite surface, and branched forms occur only rarely. Microvilli vary from 0.06-0.12 μm in width and are up to 2.1 μm long. Numerous fragments of microvilli and unstructured debris fill the adjacent area, which is no longer identifiable as a sinusoidal lumen. Free lengths of microvilli were present as far as 40 μm from the tegumental free surface. "Beading" of microvilli and swelling of individual beads was observed in those areas farthest from the parasites. A wide variety of host cells has accumulated in the vicinity of the parasite, including macrophages, fibroblasts and eosinophils. There is evidence of uptake of microvillar material and adherence of debris to phagocytic cell surfaces. Some phagocytized fragments of microvilli become greatly swollen and appear to rupture and assume "C"-shaped forms (Figure 15). The eosinophil shown in Figure 16 is surrounded by microvillar fragments and its surface bears fluffy amorphous material. Collagenous fibers (Figure 17), first seen in the vicinity on Day 6, were present between host cells and not apposed to the larval surface. Much of the surrounding electron-dense

FIGURE 15. Rat phagocytic cell, 6 days postinfection with *Taenia taeniaeformis*. Numerous fragments of parasite microvilli can be seen both extra- and intracellularly. Some of the fragments within phagosomes have swollen to the point where the membranes have broken, resulting in "C"-shaped structures. Some of the microvillar segments are bounded by two membranes (arrows). X 34,000. Abbreviations: E, extracellular environment; HC, host cell cytoplasm; P, host cell phagosomes.

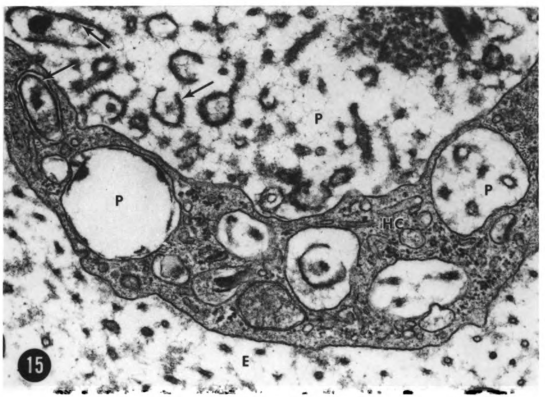
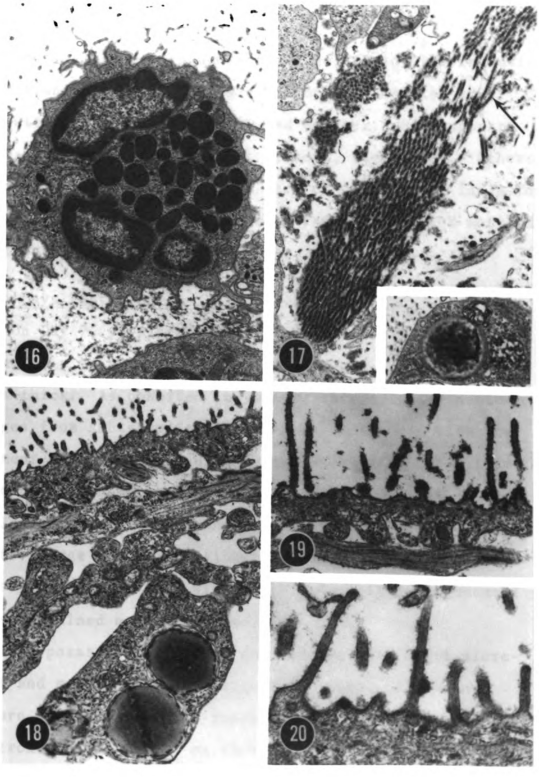


Figure 15

FIGURES 16-20. Host response and parasite surface at 6 and 7 days postinfection with *Taenia taeniaeformis*. 16. Eosinophil approximately 8 µm from the surface of a 6 day old larva. The cell is surrounded by microvillar fragments, and amorphous material can be seen adhering to its surface. X 11,100. 17. Collagenous fibers in the vicinity of a 6 day old parasite. Characteristic periodicity can be seen in some areas (arrow). X 14,000. Insert. Opaque, mottled inclusion, 1.6 µm in diameter, bounded by two membranes, located within the cytoplasm of a host phagocytic cell. X 11,600. 18. Seven day old larva, showing prominent muscle bands and subtegumental cell containing 2 opaque inclusions (the larger being 1.6 µm in diameter). The tegument appears more irregular and bumpy than on previous days. X 12,300. 19-20. Tegument of 7 day old larvae, showing microvilli with irregularly-shaped bases and adherent electron-dense deposits. Distal ends of some short microvilli are clearly membrane-bound. X 21,700 and 40,000.



Figures 16-20

amorphous material appeared to be tropocollagen which had not yet polymerized to the point where the characteristic periodicity was detectable.

Host cells contain numerous large, lipid-like inclusions, some of which closely resemble the opaque, mottled type seen within parasites on Day 6 and earlier. Some appear to be bounded by two membranes (Insert, Figure 17). Others appeared to be coalescing with each other. Larger inclusions (up to 5 μm) were more homogeneous centrally with a mottled, electron-dense border (not shown).

Seven Days Postinfection

On Day 7, parasites (average dimension 367 μm) consisted of a thin-walled bladder. The wall was one or two cells thick. As observed in younger larvae, cytoplasmic bridges from subtegumental cells were continuous with the tegument. The latter ranged in thickness from 0.3-1.1 μm and contained numerous membrane-bound vesicles. The circular and longitudinal muscle bundles are more developed than on Day 6. Large round inclusions are still present and, although none were seen within the tegument, subtegumental cells contained as many as four.

The parasite surface is now covered with short microvilli and many bumps, some with dense caps. A common feature of microvilli was the presence of deposits of electron-dense material on their surfaces (Figures 18-20), and some had odd-shaped bases not seen at earlier stages (Figures 19 and 20). Longitudinal striations were

occasionally seen within the shafts. Microvilli were 0.06-0.08 μm wide, and the longest measurable segment was 2.5 μm.

Seven day old larvae were surrounded by an intense host cellular response, of variable composition. At times there was a dense mass of cells which were predominantly macrophages and polymorphonuclear leukocytes, and many of these contained inclusions of the type illustrated previously. This area also contained fibroblasts, unstructured debris, and electron-dense material, probably tropocollagen. In other cases, there were concentric layers of cells, the majority of which appeared to be fibroblasts. In some areas tropocollagen was in contact with the microvilli.

DISCUSSION

These results extend our understanding of the host-parasite interface during the four initial postoncospheral phases of reorganization, cell multiplication, vesiculation, and cytoplasmic differentiation defined by Gemmell (1976). They provide evidence of a remarkable sequence of morphological changes in the parasite on days 1-7 postinfection which are associated with important effects upon adjacent host cells and also establish at least one means whereby parasite-derived materials are presented to and processed by the host defense system.

The occurrence of a clear area separating *T. taeniaeformis* from host cells over the first few days of infection has been recorded previously (Lewert and Lee, 1955; Singh and Rao, 1967; Banerjee and Singh, 1969; Cook, 1979). Although Lewert and Lee (1955) attempted to define the nature of

the amorphous halo histochemically, the possibility that a mass of cytoplasmic extensions from the organism might be present on these early stages was first raised by Heath (1973) and Heath and Pavloff (1975). Based upon observations of parasites during *in vitro* culture, they proposed that elongated microvilli were responsible for the barrier which prevented contact between host cells in the medium and the tegument of the developing parasites. Although microvilli were not seen, most cells appeared to come no closer than 20-30 μm. It is possible that microvilli also reach such lengths *in vivo*, but our observations suggest that they are shorter and that the tips become pinched off and ingested by surrounding host phagocytic cells. While the branched microvilli themselves may form a barrier to host cell contact, tip material could also serve in some defensive capacity, much in the same way that the distended apices of microvilli of cysticercoids of *Hymenolepis diminuta* are believed to be cytolytic for host hemocytes (Ubelaker et al., 1970a). Microvillar fragments may also contribute to the very rapid stimulation of humoral protective immunity (Leid and Williams, 1974; Kwa and Liew, 1978) by displaying membrane-bound surface antigens likely to be the targets of specific acquired resistance against secondary challenge organisms. The processes of microvillar fragmentation, segmentation, distention and phagocytosis are represented diagrammatically in Figure 21.

Whatever their functional role, the peculiar branching characteristic of microvilli of *T. taeniaeformis* is similar

FIGURE 21. Diagrammatic representation of microvillar fragmentation and segmentation ("beading") with subsequent distention and phagocytosis of individual "beads." Abbreviations: T, larval tegument; M, microvillus; HC, phagocytic host cell.

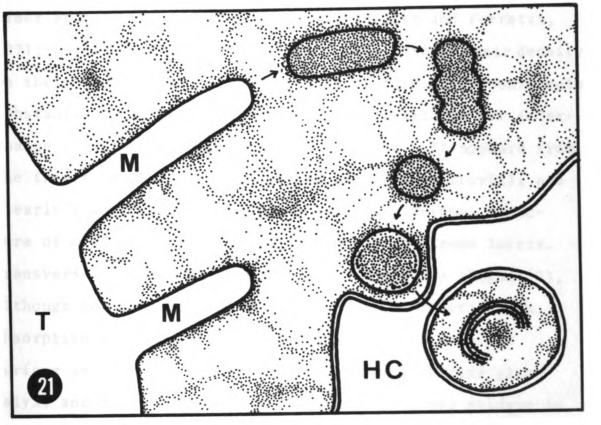


Figure 21

to that described for precysticercoids and cysticercoids of *H. diminuta* (Ubelaker et al., 1970a,b) and illustrated by Collin (1970) for precysticercoids of *H. citelli*. Therefore, it seems likely that this morphologic feature is common to postoncospheral stages of other cestodes as well. The digitiform projections at this stage are certainly microvilli, as distinct from the microtriches observed on older stages of *T. taeniaeformis* (Bortoletti and Ferretti, 1971; Engelkirk and Williams, 1980), and through their density on the tegument and their long, slender form, they contribute substantially to the total surface area available for interchange of nutrients and excretory products. It appears from the fact that distended "C"-shaped pieces of microvilli are clearly bounded by two membranes that the internal structure of microvilli may involve concentric membrane layers. Transverse sections also convey this impression (Figure 7), although how this relates to their possible functions in absorption and excretion is not clear. Their external surface is probably covered by a mucopolysaccharide glycocalyx, and the filamentous projections which are evident in our electron micrographs are likely to represent an artifactually distorted glycocalyx (Lee, 1966).

A considerable amount of nutrient absorption must take place over this early period because the organisms grow rapidly, presumably transporting substrates into the extremely thin tegumental layer and then to the subtegumental cell bodies via cytoplasmic bridges. Surprisingly, the reorganizing larvae retain their oncospheral hooks or fragments of

hooks during the first 3 days of this process. Hooks were seen in 1 day old larvae by Lewert and Lee (1955) and in 2 day old parasites *in vitro* by Heath (1973).

Oncospheres of *T. taeniaeformis* have an extensive muscle layer, which is probably involved in hook movement, penetration of host tissue and expulsion of "penetration gland" contents (Nieland, 1968). It is clear from Figure 2 that oncospheral muscle is still present on Day 1, and it seems very likely that the tissue damage which occurred near the organism was the result of migration through hepatic parenchyma. The oncospheral muscle disappears by 3 days postinfection, and new muscle bundles begin to develop beneath the tegument. It is unlikely that the scanty muscle present between days 2 and 5 would permit much migration. Although Singh and Rao (1967) and Cook (1979) saw evidence of larval tracks at 4 days postinfection, it is possible that they were observing inflammatory responses to earlier movement. The well developed muscle in Day 6-7 larvae may very well be used in a further migratory phase, suggested by the observation of Cook (1979) that peak serum levels of hepatocyte enzymes occur at 6-7 days postinfection. Our results and those of others (Singh and Rao, 1967; Cook, 1979) contrast with the belief of Heath (1973) and Heath and Pavloff (1975) that the larvae of *T. taeniaeformis* do not migrate *in vivo*.

Singh and Rao (1967) and Banerjee and Singh (1969) always found parasites within sinusoidal lumina, but our findings indicate that even the very early stages may migrate

away from the sinusoid. Although intact endothelial cells surrounded parasites on days 2-5, the destruction of host cells around larvae on days 6-7 made it impossible to determine their relationship to the sinusoidal system.

It is especially striking that the presence of phagocytes seemed related to the appearance of cellular debris and microvillar fragments, and inflammatory cells never contacted the developing parasite tegument directly. At no time during the investigation did the larvae appear damaged. The phagocytic cells were predominantly mononuclear, but some granulocytes, including eosinophils, participated. The presence of eosinophils in the cellular response by 6 days postinfection was earlier than previously reported, although Ansari and Williams (1976) saw them in portal triad areas after one week. The appearance of collagenous fibers as early as 6-7 days postinfection was unexpected. Platelets were frequently observed in close proximity to the developing organisms but were not present in sufficient numbers to ascribe any special role for them in the host response.

The opaque inclusions seen in both the subtegumental cells and tegument from 3 days postinfection onwards may consist of lipid. Lipid inclusions have been described in a variety of cestode larvae, including the coracidial sheath (embryophore) of *Diphyllobothrium dendriticum* (Gremmeltvedt, 1973) and *Spirometra mansoides* (Lumsden et al., 1974), subtegumental cells of *Diphyllobothrium latum* procercoids (Braten, 1968), cavity-lining cells of *Hymenolepis citelli* precysticercoids (Collin, 1970), and subtegumental cells of

cysticerci of *Taenia saginata* (Slais et al., 1971) and *Taenia pisiformis* (Shield et al., 1973).

Electron-dense "globules" have also been described in the cysticercoids of *Raillietina cesticillus* (Baron, 1971), but the results of histochemical tests indicated that they were proteinaceous rather than lipid. Baron suggested that they originated in subtegumental areas, traveled up cytoplasmic extensions, and were eventually passed out through the surface. He speculated that secretory globules might be immunogenic in the insect host. In our work many host cells, especially by 6 and 7 days postinfection, contained inclusions resembling those in the tegument and subtegument of the parasites. Some were bounded by two membranes, as if they had been ingested, whereas none of the parasite inclusions had this appearance.

The observations of Varute and More (1971) are also relevant to the issue of lipid droplet accumulation. They observed abnormal inclusions within hepatocytes up to 200-250 µm from *T. taeniaeformis* strobilocerci in the rat. Cytochemical tests indicated that they contained polysaccharide, lipid and lipofuscin, and it was concluded that abnormal lipid metabolism, with production of lipofuscin, was a feature of the host cell response to *T. taeniaeformis*. Dark brown pigment granules were also seen in non-neoplastic macrophages associated with *T. taeniaeformis*-induced sarcomas in rats (Osunkoya et al., 1972). In view of these findings, and the occurrence of multiple lipid-like inclusions within host phagocytic cells in the present study, it seems possible

that abnormal lipid metabolism occurs in inflammatory cells as well as hepatocytes in *T. taeniaeformis*-infected rats.

Quite apart from the implications of our findings in terms of postoncospheral reorganization in taeniid parasites in general, the establishment of a pattern of surface events in young *T. taeniaeformis* in which multiple elongate cytoplasmic extensions emerge immediately on arrival in the liver and are shed in profusion over the succeeding days has bearing on future immunological studies of this organism in particular. For example, the IgG_{2a} antibodies which are responsible for protection (Leid and Williams, 1974) might well be used as a probe for immunocytochemical localization of microvillar antigens (Sternberger, 1979). The emerging evidence for surface expression and release of complement-fixing factor as an evasive mechanism (Hammerberg et al., 1976; Hammerberg and Williams, 1978) could be supplemented by immunoelectron microscopical characterization of microvillar membranes and their shed fragments. The phenomenon of phagocytic uptake of membranous elements of these early stages may even be relevant to the form in which protective antigen could be most effectively administered in development of prophylactic procedures. The application of an array of immunological, biochemical, and ultrastructural techniques to questions such as these may enable us to visualize more completely the mechanisms of immune evasion which characterize cestodiasis in the rat and other hosts.

ACKNOWLEDGEMENTS

This work was supported by NIH Grant AI-10842. We are grateful to Mr. H. Stuart Pankratz for advice in matters pertaining to electron microscopy, to Dr. Betty Hamilton for preparing the microvillus diagram, and to Ms. Kate Baird for assistance in preparation of the manuscript. This is journal article number from the Michigan Agricultural Experiment Station.

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ARTICLE 2

TAENIA TAENIAEFORMIS IN THE RAT: ULTRASTRUCTURE OF THE HOST-PARASITE INTERFACE ON DAYS 8-22 POSTINFECTION

P. G. Engelkirk and J. F. Williams

ABSTRACT

The host-parasite interface of *Taenia taeniaeformis*-infected rats was examined at the ultrastructural level 8-22 days postinfection. During this phase of development the larvae are invested with a dense surface coat of complex microtriches which at first elongate and then shorten. Over the 8-14 day period the plasma membrane of the microthrix extends up to 11.5 μm beyond the distal end of the electron-dense tip, forming narrow, tubular "streamers." By 18 days postinfection the "streamers" have become withered. A variety of host cells interdigitate intimately with the microtriches without apparently harming them or the parasite tegument below. The host cells, on the other hand, become damaged, and their contents contribute to the amorphous material into which the microtriches project. By 22 days postinfection a spectrum of host cell-parasite relationships was observed, again without evidence of adverse effect upon the larval membrane. Many lipid inclusions were present within the parasites and surrounding inflammatory cells. Hypotheses are presented concerning microthrix structure and function, and the origin and role of lipid inclusions. A diagrammatic representation of microthrix structure is included, based upon our interpretation of high magnification electron micrographs at 14 and 22 days postinfection. The immunological implications of the interface characteristics over the period of study are discussed.

INTRODUCTION

Electron microscopic studies of larval cestodes in mammalian tissues provide evidence for host-parasite interaction via contact between complex digitiform surface projections (microtriches) and host cells and tissue fluids (e.g., Baron, 1968; Nieland and Weinbach, 1968; Bortoletti and Ferretti, 1971; Slais et al., 1971; Voge and Brown, 1979). In contrast, the few studies on early postoncospheral forms of cestodes have revealed that they become invested with a coat of simply structured microvilli (Collin, 1970; Ubelaker et al., 1970a,b; Engelkirk and Williams, 1980). The point at which microtriches appear and how their development affects the nature of the host-parasite interface have not been investigated for any larval taeniid parasite, although it is clear from the work of Bortoletti and Ferretti (1971) that 14 day old larvae of *Taenia taeniaeformis* show very different surface characteristics from those which we observed over the first week of growth *in vivo*.

In this study we describe the succession of changes which occur during the second two weeks of infection with *T. taeniaeformis*. The observations emphasize radical shifts in the relationship between the tegument and host over this period. Microtriches, abruptly appearing on day 8 and having a structure different from that described in previous reports, extend into the surrounding host cell mass and eventually contact incoming acute inflammatory cells without suffering any apparent harm.

MATERIALS AND METHODS

Animals

Seven 28 day old female Spb[SD] rats purchased from Spartan Research Animals, Haslett, Michigan, were infected orally with 1000 *Taenia taeniaeformis* eggs which had been processed as described previously (Engelkirk and Williams, 1980).

Tissue Processing, Microtomy and Electron Microscopy

One animal was killed on each of the following days postinfection by exposure to CO_2 vapor in a dry ice chamber: 8, 9, 10, 12, 14, 18, 22. Cysts (parasite plus host capsule) were carefully excised from the livers and fixed, postfixed, dehydrated and embedded intact. Tissue processing, microtomy and electron microscopy were accomplished as described previously (Engelkirk and Williams, 1980). Frozen sections of 8 day old larvae were stained with oil red O using standard procedures (Lillie, 1954).

OBSERVATIONS

The interrelationships between host and parasite during the second and third weeks of infection differ markedly from those recorded for the first 7 days after exposure. Light microscopic observations have revealed an increasing commitment of inflammatory cells, many of which become closely applied to the parasite surface (Bullock and Curtis, 1924; Ansari and Williams, 1976; Cook, 1979); however, at the ultrastructural level the intimacy of contact between host

cell and tegumental limiting membrane is more clearly evident, and the adverse consequences for the host become correspondingly more severe as the parasites grow and develop. Electron micrographs have been selected which exemplify these changes, with special emphasis on the zone of immediate contact between parasite tegumental projections and host cells, rather than on the trail of destruction left in the wake of migrating organisms.

Eight Days Postinfection

The most striking feature of 8 day old organisms was the presence of a dense surface coat of true microtriches (Figures 1-2), instead of the microvilli present on earlier stages (Engelkirk and Williams, 1980). Transitional forms were never seen, although fragments of shed microvilli were still present in the migratory tracks.

Each microthrix consists of three parts: a relatively broad base, a narrow, electron-dense tip, and a long, slender, lightly stained "streamer." Bases, tips and streamers are all bounded by a single membrane, which is continuous with the outer tegumental membrane. Tips appear to vary in length because the entire structure is only rarely contained in a given thin section. Lengths of up to 1.4 μm were measured, but portions of tips occur as far as 4.0 μm from the tegumental free surface. Small (about 21 nm), circular to oval, electron-dense structures are frequently present at the junctions of base and tip. The "streamers" appear to undulate, and their entire lengths are rarely, if ever, contained

FIGURE 1. *Taenia taeniaeformis* larva, 8 days post-infection. A densely packed border of "whip-like" microtriches extends from the tegument surface. Each microthrix consists of a relatively broad base, a narrow electron-dense tip, and a long, lightly stained "streamer." "Streamers" (*) extend at least as far as 5 µm (arrow) from the tegumental free surface. Host cell plasma membranes are indistinct or lacking in some areas, and cytoplasmic contents of these cells can be seen between microtriches. The parasite tegument (T) varies in thickness from 0.6 to 1.7 µm. Bundles of circular muscle (Mu) are located beneath the tegument. X 12,900.

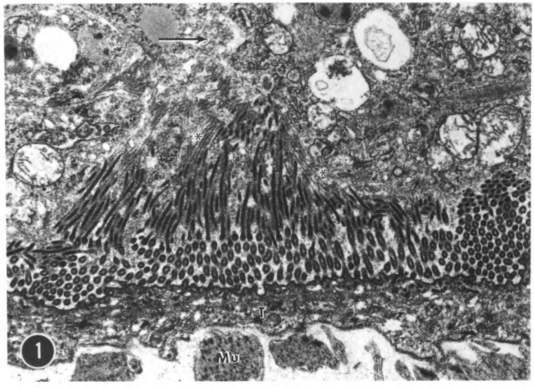
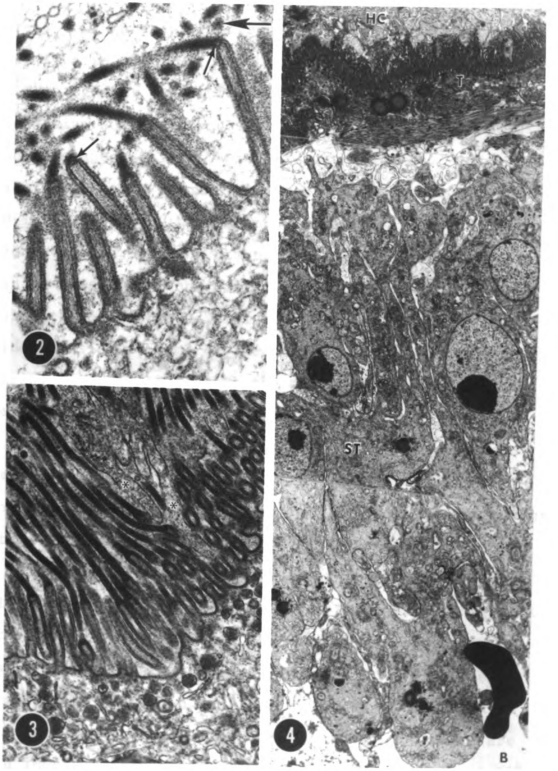


Figure 1

FIGURES 2-4. *Taenia taeniaeformis* larvae, 8 and 9 days postinfection. 2. Microthrix border of an 8 day old parasite. Microthrix bases are 0.09 to 0.11 µm in width and 0.5 to 0.8 µm in length. Each is bounded by a single plasma membrane, continuous with the outer membrane of the tegument. Longitudinal sections of bases reveal an electron-dense line on each side of the base, approximately 20 nm from the plasma membrane. These appear to run the entire length of the base. Four to 5 lighter longitudinal striations can be seen between the two electron-dense lines. At the junction of the base and tip, a small (21 nm) circular to oval, electron-dense structure is frequently observed (small arrows). The tips are 0.05-0.06 µm wide and in longitudinal section appear to contain two very dense lines, each of which is 20 nm from the plasma membrane. These lines, about 20 nm apart, run the entire length of the tips. A transverse section of a tip reveals a circular, electron-dense core 21 nm in diameter (large arrow). X 48,600. 3. Microthrix border of a 9 day old parasite. Microthrix bases are up to 0.8 µm in length, and tips are as long as 1.7 µm. Finger-like host cell processes (*) interdigitate with the microtriches. The tegument is rich in electron-dense microbodies and dumbbell-shaped vesicles. X 30,000. 4. Three part montage of electron micrographs of a 9 day old larva. Lipid inclusions are present in both tegumental and subtegumental cytoplasm. A densely packed muscle layer overlies subtegumental cells, which are oriented perpendicularly to the tegumental free surface. The total thickness of the wall from the tegumental free surface to the bladder (B) is approximately 40 µm. X 4350. Abbreviations: HC, host cell; T, parasite tegument; ST, subtegumental cell.



Figures 2-4

within the thin section. Streamers (20 nm wide) extend farther than 5 μm from the tegumental free surface in Figure 1, but in other electron micrographs they reach as far as 11.5 μm.

The tegument varies from 0.5 to 3.1 μm in thickness and contains numerous vacuoles, electron-dense microbodies, dumbbell-shaped vesicles, and glycogen rosettes. Also present within the tegumental cytoplasm are numerous large (1.0-1.2 μm) opaque, homogeneous inclusions. They correspond both in size and number with the many deeply staining areas observed in frozen sections of Day 8 larvae exposed to oil red O and will hereafter be referred to as lipid inclusions. Some of the larger vacuoles contain material which is similar to the matrix around the well developed subtegumental muscle bundles.

Lipid inclusions, resembling those of the tegument, occasionally occur in the perinuclear cytoplasm of the highly branched subtegumental cells. Cytoplasmic extensions in this region are surrounded by clear channels.

Macrophages and fibroblasts are the identifiable host cell types surrounding the parasite. Finger-like host cell processes interdigitate with clusters of microtriches, occasionally reaching the level of the junction of the microthrix base and tip. Host cell plasma membranes are indistinct or lacking in some areas, and microtriches are often immersed in a cytoplasmic "soup." Tropocollagen-like material was infrequently seen at the host-parasite interface.

The reactions around larvae were not always symmetrical. For example, both healthy and damaged inflammatory cells, cellular debris, tropocollagen, and segmented and distended pieces of parasite microvilli were present on one side of a Day 8 parasite, while at the opposite pole the parasite surface was in contact with apparently normal hepatocytes. Eosinophils, neutrophils, macrophages and fibroblasts participated in the reactions, and many cells, especially those which were deteriorating, contained large lipid accumulations, similar to those within the parasite tegument. Many free eosinophil granules were present in this region.

Nine Days Postinfection

The interdigitation of host cell processes and microtriches is especially pronounced on Day 9, apparently pushing the latter together into clumps. The microtriches themselves are more erect than on Day 8, and longitudinal sections of the bases are more common. Microthrix dimensions are similar, but tip lengths up to 1.7 µm occur (Figure 3). Sections of tips are found as far as 5.3 µm from the tegumental free surface. Electron-dense circles again appear at the base/tip junction in many microtriches. Occasionally two microtriches arise from a single wide base.

Membrane-bound, pit-like indentations are frequently situated between microthrix bases. The tegument is mildly convoluted and the cytoplasm contains lipid inclusions (0.7-1.4 µm diameter), as well as vesicles and microbodies (Figures 4-5).

FIGURE 5. Host-parasite interface at 9 days postinfection with *Taenia taeniaeformis*. An undulating tegument bears a densely-packed border of microtriches compressed into "sheafs" by host-cell processes. Microthrix "streamers" extend at least 10 μm (arrow) from the tegument, and "streamer"-like structures are detectable as far as 17 μm (double arrows). Although some cellular debris is present at the interface (lower left), other cells appear intact and undamaged. X 9700.

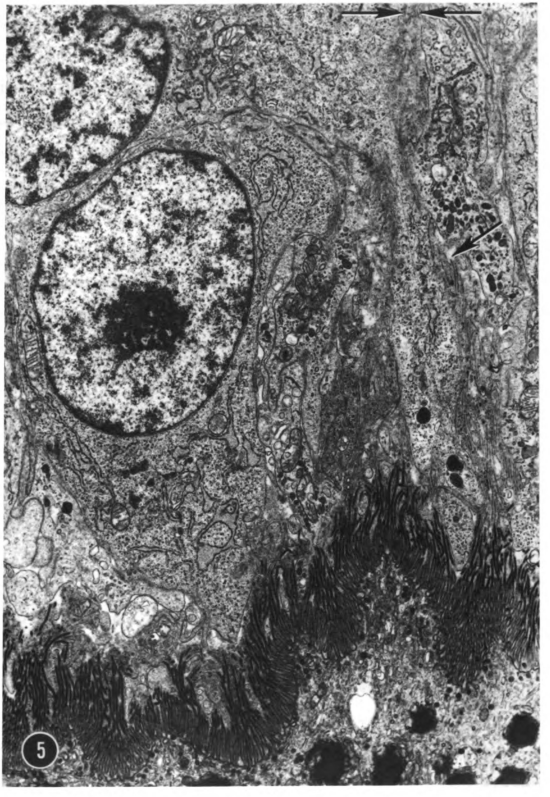


Figure 5

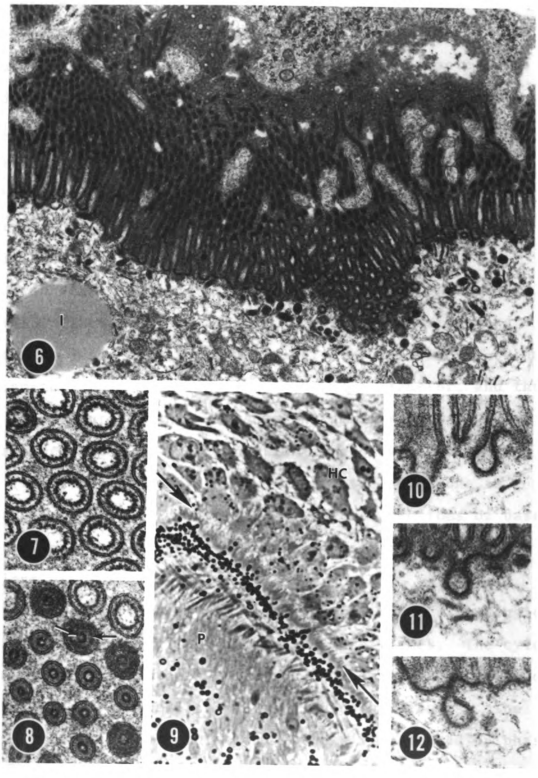
Circular and longitudinal muscles are more compact, and beneath the muscle layer the subtegumental cells are oriented perpendicularly to the tegument, giving a more structured appearance than at day 8. Cytoplasmic processes of the deepest cells can be traced to the muscle layer, and clear channels lie between the cells. Even at this depth, lipid inclusions occur which are identical to those within the tegument.

The parasites are still in contact with macrophages and fibroblasts, but host cell processes now extend all the way to the microthrix bases. There is no evidence of damage to the parasite membranes, and macrophages do not appear to be phagocytizing microtriches. Tips and "streamers" of microtriches lie between but do not penetrate host cell processes. Individual "streamers" cannot be followed for their entire length but extend at least 10 µm from the tegumental free surface (Figure 5).

Ten Days Postinfection

Few changes occur in the host-parasite interface between 9 and 10 days. Microthrix tips extend between host cell processes and appear to be immersed in amorphous debris (Figure 6) which is more electron-dense than host cell cytoplasm and bears a resemblance to the tropocollagen seen at earlier stages of development (Engelkirk and Williams, 1980). Collagenous fibers were not seen. Microtriches were never observed penetrating host cells and, although by this stage host cell processes extended all the way to the base of the

FIGURES 6-12. *Taenia taeniaeformis* larvae, 10 days postinfection. 6. Host-parasite interface. Dense tropocollagen-like material surrounds the microthrix tips and "streamers." Intact host-cell processes probe between microtriches. A lipid inclusion (I), 1.4 μm in diameter, is present within the tegumental cytoplasm, which also contains numerous electron-dense microbodies (0.07-0.20 μm) and dumbbell-shaped vesicles (0.10-0.18 μm in length). X 19,800. 7. Transverse sections of microthrix bases. The bases are bounded by a single plasma membrane, and the dense inner ring consists of 15-22 subunits. X 100,000. 8. Transverse sections of microthrix tips. Tips are also bounded by a single plasma membrane. A dense inner ring, 20-30 nm in diameter, surrounds a clear central zone. In some instances microtriches have been sectioned at what appears to be a transition zone; two dense inner rings are visible (arrows), the diameters of which correspond to those of the base and tip. X 102,500. 9. Phase micrograph illustrating numerous osmiophilic, round inclusions within the parasite (P) tegument and subtegumental area. Osmiophilic inclusions are also present within host cells (HC). The microthrix border is evident (arrows). X 2300. 10-12. Pit-like formations at the tegumental free surface. X 49,500.



Figures 6-12

microtriches, they never appeared to be damaging the parasite membrane.

Transverse sections of microthrix bases at 10 days (Figure 7) are round to oval, 0.09-0.13 μm in diameter, and bounded by a single plasma membrane. Occasionally larger and irregularly-shaped base sections occur. There is an inner electron-dense ring, generally 50-80 nm in diameter, which is not a membrane. This inner ring appears to be comprised of 15-22 roughly circular subunits, each approximately 7 nm in diameter.

Transverse sections of microthrix tips are shown in Figure 8. These too are bounded by a single membrane and contain an inner electron-dense ring. The tips are 50-60 nm in diameter; the inner ring is 20-30 nm in diameter, and there is a clear central zone. In some instances sections appear to have been made at the transition zone between bases and tips (Figure 8). These show two rings; the outer one is comparable to the circle seen in transverse sections of bases, and the inner one resembles the circle seen in transverse sections of tips.

The free surface of the tegument is limited by a single membrane continuous with that of the microtriches. The cytoplasm is rich in vacuoles, electron-dense microbodies, lipid inclusions and dumbbell-shaped membranous structures. The intensity of lipid accumulations is best illustrated by phase microscopy (Figure 9). Many pit-like formations occur at the tegumental surface near the proximal ends of microtriches

(Figures 10-12), and similar appearing, "fuzzy" vesicles are scattered throughout the tegumental cytoplasm.

Immediately beneath the tegument are well developed bundles of circular and longitudinal muscle. Many small electron-dense particles, probably glycogen, are closely associated with the muscle bundles, which are surrounded by a finely granular and fibrillar matrix.

Twelve Days Postinfection

By 12 days, elongated microthrix tips extend as far as 10 μm from the tegumental free surface (Figure 13). There appears to be an increased quantity of amorphous "soup" surrounding the microtriches. Host cell processes are still seen extending between "sheafs" of microtriches, in some cases to the tegumental free surface. These processes are often not clearly bound by membrane.

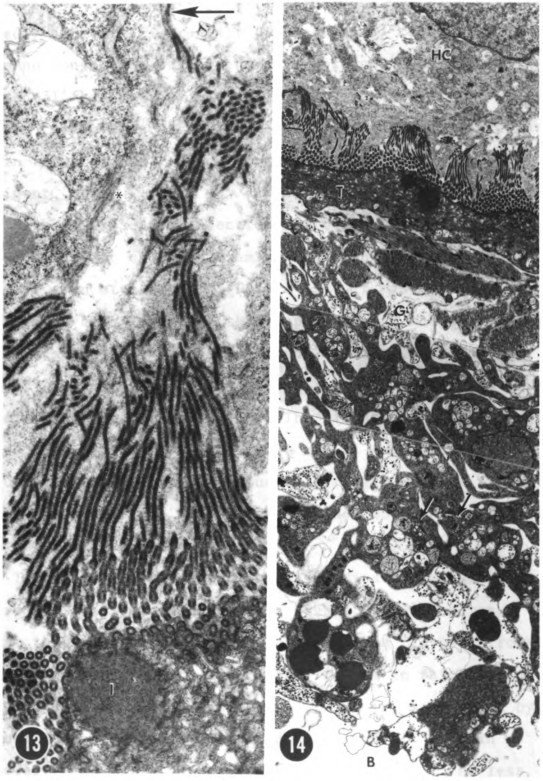
The tegument contains many lipid inclusions (0.9-2.5 μm), often in close proximity to the free surface membrane, which bulges outward in these areas (Figure 13). Tegument thickness varies from 1.6 to 8.3 μm.

Subtegumental cells do not have the structured vertical arrangement of the Day 9 larvae but have a greater number of cytoplasmic extensions and contain many more vacuoles than were seen in earlier stages. Mitotic figures were commonly seen in the nuclei of host cells surrounding the parasite.

Fourteen Days Postinfection

Between 12 and 14 days no major changes in the host-parasite interface or general organization of the larva

FIGURES 13-14. *Taenia taeniaeformis* larvae, 12 and 14 days postinfection. 13. Microtriches extending from the tegumental surface of a 12 day old larva. Electron-dense tips reach as far as 10 µm (arrow) from the tegumental free surface. Tips and "streamers" (*) are immersed in amorphous cellular debris. A lipid inclusion (I) bulges near the tegumental free surface. X 16,800. 14. Three part montage of electron micrographs of a 14 day old larva. Host cell (HC) processes extend between "sheafs" of microtriches. Areas of amorphous material resembling host cell cytoplasm can be seen near the tips of some microtriches. Two electron-dense, oval, lipid inclusions, 0.8 and 1.2 µm in diameter, are present in the distal cytoplasm of the tegument (T). Beneath the tegument are well developed longitudinal and circular muscle bundles, membrane-bound clusters of glycogen (G) rosettes, and numerous cytoplasmic extensions of subtegumental cells. Cytoplasmic vacuoles of various size and density contain loosely or densely packed globules. Golgi configurations are abundant (arrows). Cytoplasm near the central bladder (B) contains electron-dense lipid inclusions up to 1.9 µm in diameter. The total thickness of the wall from the tegumental free surface to the bladder is approximately 30 µm. X 4850.



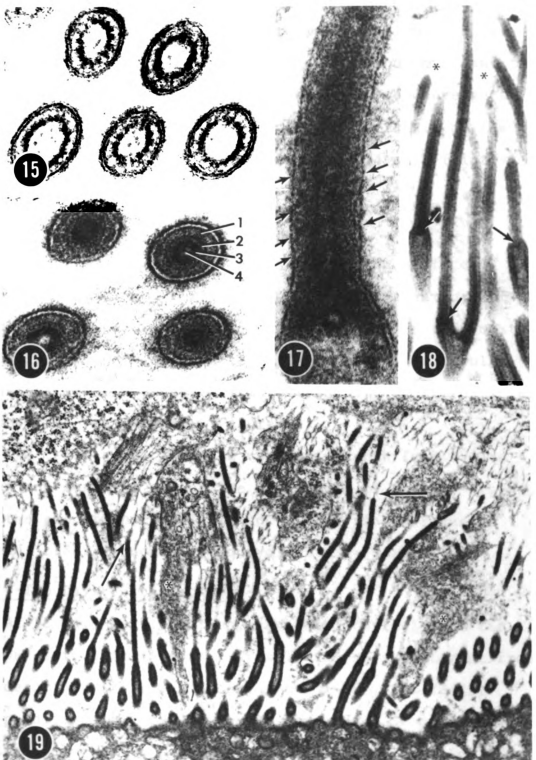
Figures 13-14

occur. However, several differences in microthrix structure are apparent: "streamers" are less evident and tips are markedly shorter (Figure 14). Subtegumental cells at this stage no longer show a perpendicular orientation to the tegumental free surface but instead are irregularly arranged between clear channels. Glycogen rosettes frequently appear within the channels. Muscle bundles are well formed and prominent.

High magnification electron micrographs of microtriches were obtained in order to examine their internal structure. However, even at 100,500 X (Figure 15), it was not possible to obtain a clear image of the composition of the inner ring which is seen in transverse sections of bases. It appears to consist of 15-22 spherical subunits, each about 9 nm in diameter, arranged in a circle. The dimensions of the rings varied from 0.12 x 0.09 μm to 0.15 x 0.11 μm. Amorphous opaque material, often in a clumped arrangement, was present within the ring. The microthrix base was bounded by a single, 9 nm thick plasma membrane.

Transverse sections of microthrix tips are shown in Figure 16. They are bounded by a single plasma membrane, 6-9 nm thick. The clear central area of the tip is surrounded by an extremely electron-dense ring, 6-9 nm in thickness and 16-26 nm in diameter. This ring is, in turn, surrounded by another less dense ring about 6 nm wide and 32-36 nm in diameter. The distance between the outer edge of the less dense ring and the inner edge of the plasma membrane is no more than 9 nm.

FIGURES 15-19. Characteristics of microtriches of *Taenia taeniaeformis* larvae, 14 and 18 days postinfection. 15. Transverse sections of bases at 14 days postinfection. Bases vary from 0.15 to 0.24 µm in width. They are bounded by a single plasma membrane. An electron-dense inner ring, 19-26 nm from the plasma membrane, appears to be composed of 15-22 spherical subunits. X 100,550. 16. Transverse sections of tips at 14 days postinfection. Tips vary from 46 to 77 nm in width, are bounded by a single plasma membrane, and contain four distinct inner zones (1-4). X 304,500. 17. Microthrix at 14 days postinfection. The internal organization of the microthrix can be seen. The single unit membrane of the base is continuous with that of the tip. The two dense bands parallel to the plasma membrane of the base converge centrally at the junction of the base and tip. At least two internal dense bands can be seen running the length of the tip. Just below the junction of base and tip is a dense circle, about 25 nm in diameter. Faint lines extend diagonally across the width of the tip (arrows). X 238,500. 18. Microtriches at 18 days postinfection. A branched microthrix has two tips (0.06 µm wide) arising from a base of normal width. Circular structures consistently appear at the junction of bases and tips (arrows). Dense internal bands run longitudinally in both bases and tips. "Streamers" (*) appear faint and wispy. X 47,700. 19. Host-parasite interface, 18 days postinfection. Host cell processes (*), not always demarcated by plasma membranes, probe down to the basal origins of the microtriches. Microthrix tips extend as far as 6.4 µm from the tegumental free surface and are in direct contact with host cell cytoplasm. Collapsed streamers continue beyond the electron-dense tips (arrows). Bases are 0.1 µm wide. X 20,500.



Figures 15-19

Longitudinal sections of microtriches show that the plasma membrane covering the tip is continuous with that of the base (Figure 17). An electron-dense oval to circular structure is usually evident at the junction of base and tip. The inner dense longitudinal lines of the base converge at this junction and continue along the entire length of the tip. The tip is traversed diagonally by narrow (4 nm), faintly staining strands.

Eighteen Days Postinfection

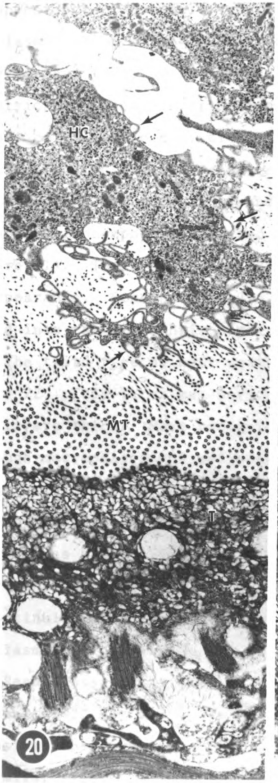
The most striking difference between the surface of 18 day old parasites and earlier stages is the appearance of the microthrix "streamers" (Figures 18-19). At this time most are collapsed and wispy, quite unlike the uniform tubular extensions seen in younger forms. Branching of microtriches was observed occasionally (Figure 18).

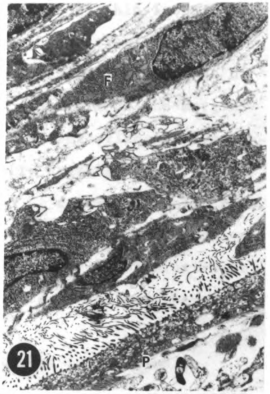
The tegumental cytoplasm varies in depth from 3.3 to 7.7 μm and contains all of the components of earlier stages, with the exception that lipid inclusions are infrequent. Instead, clear vacuoles of equivalent size were noted.

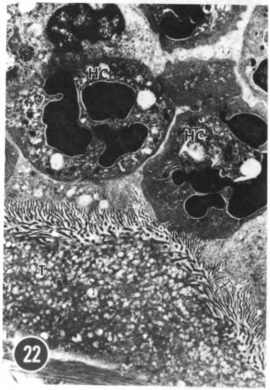
Twenty-Two Days Postinfection

At 22 days postinfection, the characteristics of the host-parasite interface are variable and very different from that described at 18 days postinfection (Figures 20-22). Some parasites (Figure 20) become surrounded by a clear space, containing very little of the amorphous material which had consistently appeared around microtriches in earlier developmental stages. Host cells, predominantly macrophages, contact the larva only at the level of microthrix tips. Numerous

FIGURES 20-22. Host-parasite interface at 22 days post-infection with *Taenia taeniaeformis*. Many thin loops of host cell (HC) cytoplasm have formed at the surface (arrows), and thin pseudopodia extend into the microthrix (MT) border. In addition to numerous small vacuoles, the parasite tegument (T) contains large (about 1.5 μm) clear areas. X 7250. 21. Some parasites (P) at this stage are surrounded by many flattened, fibroblast-like cells (F) in a laminar arrangement. X 4350. 22. Host cells (HC), including neutrophils, are in intimate contact with the microthrix border of the parasite. Inflammatory cells show degenerative changes with loss of cytoplasmic organelle integrity and nuclear fragmentation. The parasite tegument (T) appears undamaged, however. X 4850.







Figures 20-22

narrow pseudopodia extend into the microthrix border, giving the appearance of amoeboid veils. Although thin loops of cytoplasm develop at the surface of the host cells, these do not appear to be in the process of engulfing portions of microtriches. Amoeboid extensions also form at the margins of host cells not in contact with the microtriches. Lymphocytes and mast cells are occasionally present.

Other larvae become surrounded by a layer of host cells having a more compact, laminated or stratified organization (Figure 21). These cells are predominantly fibroblast-like in appearance. Some pseudopodia are evident, but they rarely extend into the microthrix border.

The third type of host-reaction is illustrated in Figure 22. This larva was surrounded by inflammatory cells, many layers thick. Most recognizable cells were neutrophils, although macrophages and eosinophils were also present. Some of the cells were in intimate contact with the parasite tegument and microthrix border, but no damage to the parasite membrane was seen. Virtually all of the host cells, on the other hand, appeared damaged. This damage took the form of discontinuity of plasma membranes, nuclear fragmentation, cytoplasmic vacuolization and loss of organelle integrity.

Regardless of the type of surrounding host reaction, the tegument of 22 day old parasites rarely contained the lipid inclusions observed in younger forms. However, clear spaces of similar size were scattered throughout the tegument (Figure 20). Certain features of the microtriches and subtegumental cells of 22 day old parasites were noteworthy.

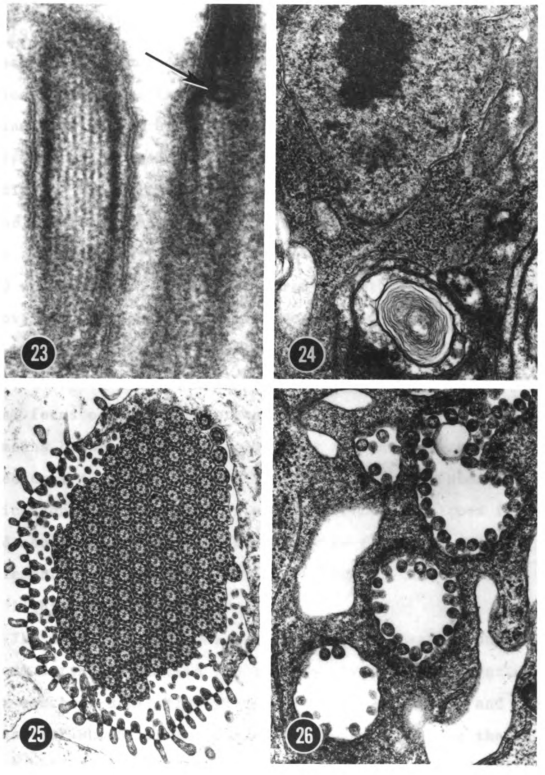
The microtriches often showed distinct longitudinal rod-like striations, 5-6 nm wide, running parallel with the dense outer bands seen in all previous stages (Figure 23). Intracellular membranous whorls, perhaps representing the origins of calcareous corpuscles, were present, as were flame cells and ducts (Figures 24-26). None of these structures had been observed in earlier developmental stages.

DISCUSSION

Our results demonstrate the dynamic nature of the host-parasite interface during the second and third weeks of infection, as the rapidly changing parasite surface makes contact with a succession of different host cell types. The consequences for inflammatory cells are generally adverse, whereas the parasite appears insusceptible to the influx of cellular and humoral defense factors. Circulating protective antibodies are detectable in the serum of rats by the second week postinfection (Leid and Williams, 1974; Kwa and Liew, 1978) and, since there is no obvious barrier to their arrival at the parasite surface, its characteristics are relevant to hypotheses on the immune evasive mechanisms which must come into operation over this period.

Clearly, the most important feature of the surface of 2-3 week old larvae was the dense covering of microtriches, contrasting sharply with the microvilli present on days 1-7 (Engelkirk and Williams, 1980). The abruptness of their appearance emphasizes the need for further work on one week old parasites in order to identify transitional stages. In this study all 8 day old larvae had microtriches, although

FIGURES 23-26. *Taenia taeniaeformis* larvae, 22 days postinfection. 23. Microtriches. On the left side of the micrograph, six longitudinal rod-like structures, 5-6 nm in width, can be seen between and running parallel with the two dense lines of the microthrix base. Within the microthrix at the right side is a dense circular to oval structure (arrow) frequently observed at the junction of base and tip; several of its subunits are visible at its left side. X 162,000. 24. Intracellular whorls of material which may represent a calcareous corpuscle in the process of formation. This structure was located about 12 μm from the tegument basal membrane. X 24,750. 25. Flame cell containing 125 cilia in transverse section. Each cilium has a hexagonal wall, 0.18 μm per side, and contains 11 microtubule doublets in the usual 9 + 2 conformation. X 16,400. 26. Ducts within a duct cell. Small (90-120 nm) oval nodules protrude into the lumina of the ducts. The nodules are membrane-bound, and a 60 nm-wide stalk provides continuity with the duct cell cytoplasm. X 30,000.



Figures 23-26

microvillar fragments were seen in the migratory trails they had made, and it seems likely that most microvilli are shed towards the end of the first week of infection. The acquisition of invulnerability to complement-dependent antibody-mediated attack at about this time (Musoke and Williams, 1975) may be entirely coincidental, but the possibility that the change in surface form is associated with the display of a different repertoire of antigenic determinants at the limiting membrane cannot be overlooked. If the vigorous and highly effective IgG_{2a} response which rats develop (Leid and Williams, 1974) were directed entirely against microvillar antigens, which are presented in such profusion over the first week of infection (Engelkirk and Williams, 1980), this might explain the susceptibility of early developmental forms of challenge organisms (Mitchell, 1979). Although the means by which older stages avoid recognition remain conjectural (Hammerberg and Williams, 1978), the future application of immunoelectron microscopical techniques for the demonstration of antigenic shifts or the presence of complement-fixing surface factors on microtriches may shed some light on this phenomenon.

The microtriches themselves certainly undergo changes in form during the second and third weeks of development. Their electron-dense tips elongate and then shorten, and the "streamers" which appear to contain cytoplasm during the 8-14 day period become withered by 18 days, perhaps indicating that they are no longer required by this stage of development. The functions of these structures are probably related to the

substantial surface area which they present for absorption, secretion and excretion. The distal portions seem to dip into a "soup" of amorphous material on Days 8-18. The cytoplasm of the tips, bases and distal tegument seems to be continuous, so that nutrients absorbed through the microthrix membrane at any site could ultimately be transported to the tegument and subtegumental cell bodies.

Alternatively, metabolic products might be eliminated from the parasite via microtriches, resulting in the discharge of materials at some distance from the tegument. If these were cytotoxic, as suggested for *Hymenolepis diminuta* by Ubelaker et al. (1970a), they could contribute to the "soup" within which the microtriches are immersed. It is interesting to note that Lewert and Lee (1955) demonstrated histochemically that 16 day old *T. taeniaeformis* larvae produce a collagenase-like enzyme. They hypothesized that it might soften intercellular materials and basement membranes, allowing the rapidly growing parasite to displace adjacent cells. This enzymatic activity could also make the surrounding area more fluid and permeable, thereby increasing the rate of diffusion of nutrients to the larva. Some of the amorphous debris which we found around the microtriches resembled tropocollagen, and although collagenous fibers were present within the developing host capsule, they were never in contact with the parasite.

Lipid inclusions, similar in size and appearance to the inclusions seen within 3-7 day old larvae (Engelkirk and Williams, 1980), were present in the tegument and subtegumental

cell bodies of all parasites 8-22 days of age, with maximum numbers on days 9-14. They were relatively rare on days 8 and 22, but at these stages clear vacuoles of similar size were seen. The electron-density of the inclusions may reflect differences in osmiophilic properties, which in turn may be affected by their lipid composition. Although they were occasionally close enough to the tegumental free surface to cause a bulging of the membrane, they were never seen being extruded. However, the similarities between the inclusions in the tegument and those in host cells suggest the possibility of their ejection and subsequent phagocytosis. Although clearly oil red O positive, they may contain substances other than lipids and may play some role in cytotoxicity or immunogenicity, similar to the role proposed for tegumental globules of *Raillietina cesticillus* by Baron (1971). In view of the proven requirement for preformed sterols and other lipids in larval cestodes (Smyth, 1969), the possibility that some inclusions accumulate as a result of absorption should not be discounted.

Structures resembling "coated pits" (Goldstein et al., 1979) were frequently seen at the base of the microtriches and may be involved in uptake, although they could also represent a means whereby excretory/secretory/metabolic products in tegumental vesicles are released onto the free surface. Braten (1978a) noted similar vesicles and pits in *Diphyllobothrium latum* procercoids and, based on their presence outside the parasite, suggested that they were secretory in nature. There is little doubt that some toxic agent must be released

from the parasites in order to account for the destructive effects we observed on host cells, many of which had degenerated into an unstructured mass of cytoplasm and organelles surrounding the microtriches. This phenomenon of lysis of host cells adjacent to taeniid larvae has been described previously by Silverman and Hulland (1961) and Siebert et al. (1979) for *T. saginata* and *T. crassiceps*, respectively, although the chemical characteristics of the substances responsible are not known. Our findings suggest that host cell processes may first probe between microtriches before ultimately losing their integrity, and that several different cell types including macrophages and granulocytes suffer this fate as the infection progresses.

The variability which we saw in the characteristics of the interface on Day 22 is consistent with previous light microscopic observations, which have shown not only differences in the rate at which collagenous encapsulation occurs around larvae but even some polarization of cell influxes around individual organisms (Ansari and Williams, 1976; Cook, 1979). The meshwork of host cell pseudopodia and microtriches evident in some cases (Figure 20) may account for the fact that when host capsules at this stage are incised some larvae are difficult to remove, whereas others, perhaps more comparable to those in Figures 21-22, slide out readily (unpublished observations).

Certain important features of the fine structure of microtriches, the underlying distal tegumental cytoplasm and the subtegumental cells themselves became evident in the

course of this study. Our interpretations of the images of microtriches presented in Figures 15-17 and 23 are synthesized in diagrammatic form in Figure 27. Our proposal is that the base contains 15-22 actin-like microfilaments, originating in the distal tegumental cytoplasm and running the entire length of the base and tip. The superimposition of the lateral filaments in longitudinal sections of bases accounts for the consistent appearance of two parallel outer dense bands. The density of microthrix tips is compatible with the presence of a single, central, 25 nm wide microtubule made up of 13 subunits, each 4-5 nm wide (Dùstin, 1978) and surrounded by continuations of the basal filaments. The microtubule extending down into the base is often sectioned obliquely, giving rise to a central dense ring at the base-tip junction.

The abrupt termination of the microtubule and its associated filaments distally is not coincidental with the end of the plasma membrane which invests them. The membrane continues as a "streamer", erect and elongated on Days 8-14 but collapsed by Day 18.

The microthrix structure which we have proposed is consistent with a transport function for these structures, in which directional guidance is provided by the stationary microtubule and contractile actin filaments are responsible for particle movement (Ochs, 1972; Rebhun, 1972). Obviously, further work will be necessary to examine this hypothesis both ultrastructurally and operationally, and techniques are available, for example, with which to improve the electron

FIGURE 27. Proposed structure of a microthrix of 14-22 day old larvae of *Taenia taeniaeformis*. A. Longitudinal view showing the circular arrangement of microfilaments in the base and their helical arrangement around a microtubule within the tip. B. Transverse section of "streamer." C. Transverse section of tip. D. Transverse section of junction of base and tip. E. Transverse section of base.

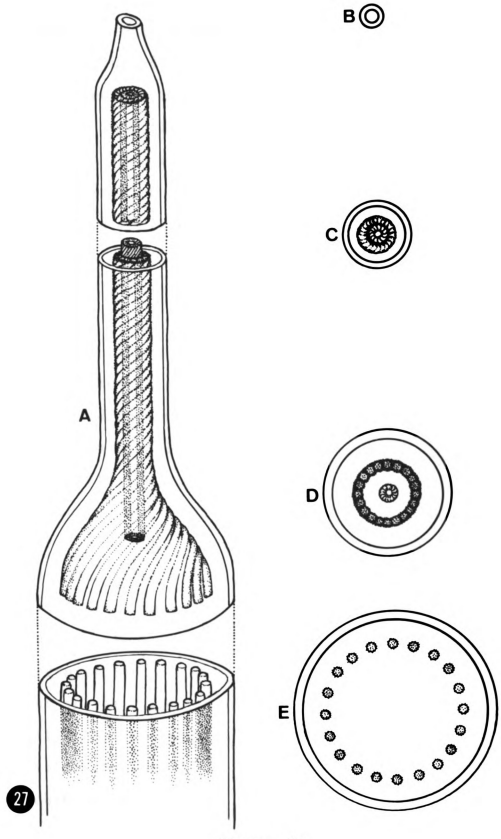


Figure 27

microscopic detection of microtubule subunits (Tilney et al., 1973), and to detect impaired fluxes of labeled molecules via pharmacologic disruption of microtubules (Dustin, 1978). The structure differs in several important respects from that proposed for microtriches of older cestode larvae and adults (Lee, 1966; Smyth, 1969; Lumsden, 1975), but it seems likely that specialized surface extensions occur whose functions are related to the changing host-parasite relationship during growth and development. Even on well formed strobilocerci of *T. taeniaeformis*, microthrix structure varies considerably from one site to another (Verheyen et al., 1978).

Organelles in the distal cytoplasm and subtegumental cell bodies were morphologically similar to those seen in much older taeniid larvae (Morseth, 1967; Baron, 1968; Nieland and Weinbach, 1968; Sakamoto and Sugimura, 1969; Slais et al., 1971; Lascano et al., 1975). These included flame cells, ducts, and intracellular whorls comparable to those described as calcareous corpuscles by Nieland and von Brand (1969). The presence of these organelles at this early phase in larval development has not previously been noted, but it emphasizes the extraordinary rate at which postoncospheral reorganization progresses over the initial period of establishment in rat liver. That these changes occur in an immunologically hostile environment is a tribute to the effectiveness of the evasive mechanisms at the parasite surface which we hope to explore in future work.

ACKNOWLEDGEMENTS

These studies were supported by NIH grant AI-10842. We are grateful to Mr. H. Stuart Pankratz for advice in matters pertaining to electron microscopy, to Dr. Betty Hamilton for preparing the microthrix diagram, and to Ms. Kate Baird for assistance in preparation of the manuscript. This is journal article number from the Michigan Agricultural Experiment Station.

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ARTICLE 3

INTERACTIONS BETWEEN *TAENIA TAENIAEFORMIS* LARVAE AND HOST CELLS *IN VITRO*. I. RESULTS AT 1 HOUR WITH 5-6 MONTH OLD STROBILOCERCI AND PERITONEAL CELLS FROM INFECTED AND NONINFECTED RATS

P. G. Engelkirk and J. F. Williams

ABSTRACT

Strobilocerci of *Taenia taeniaeformis*, incubated for 1 h *in vitro* with various combinations of antibody, complement and rat peritoneal cells, were examined at the ultrastructural level for evidence of host cell adherence and tegumental damage. Maximum adherence and parasite surface alterations occurred when larvae were incubated in the presence of cells and fresh serum. Comparable results were seen regardless of whether the cells or serum had been obtained from infected or noninfected donors. The predominant cells interacting with the larval surface were highly activated eosinophils which contained parasite material within phagosomes and were seen stripping microtriches from the tegumental free surface with finger-like pseudopodia. Mast cells, some of which were in the process of degranulating, also participated in the reactions. These results are discussed in light of current knowledge of eosinophil-mast cell-parasite interactions and the immunology of *T. taeniaeformis* infection in the rat.

INTRODUCTION

Recent studies on the interactions between helminth parasites and cellular components of host defense *in vitro* have led to the development of a number of hypotheses on resistance to trematodes and nematodes (for reviews see Butterworth, 1977; Capron, Dessaint and Capron, 1977; Mitchell, 1979). Furthermore, these investigations have provided new insights into the functions and capabilities

of various host cell types and their relationship to antibody and complement (Capron, Dessaint, Capron and Bazin, 1975; Capron, Torpier and Capron, 1979; Anwar, Smithers and Kay, 1979; Butterworth, Wassom, Gleich, Loegering and David, 1979). Interest in these systems derives in large part from the frequent occurrence of concomitant immunity in helminthiasis (Mitchell, 1979) and the need to characterize the protective resistance which operates against invasive stages.

Infections with the larval forms of cestodes offer especially good examples of the evasion of immune attack by established tissue parasites (Mitchell, 1979; Williams, 1979), but there has been little work done on the nature of the reactions *in vitro* between immune cells and the tegumental surface at which host and parasite interface. There are indications that antibody-dependent adherence of cells occurs to protoscolices of *Echinococcus granulosus in vitro* (Dumon, Vervloet and Quilici, 1976), and Kwa and Liew (1978) have reported that some cells adhere to the surface of larvae of *Taenia taeniaeformis* after 3 days *in vitro*. However, the conditions under which these reactions took place were not well defined.

In this study we report on the adherence of host cells to the tegument of *T. taeniaeformis in vitro* and the consequences for both host and parasite. In these short term experiments there was evidence of extensive damage to the tegument and profound changes in the appearance of adherent

cells, both of which were evaluated at the ultrastructural level.

MATERIALS AND METHODS

Animals. Experiment 1: Nine 59-66 day old female Spb[SD] rats (Spartan Research Animals, Haslett, Michigan, U.S.A.), which had been infected orally with 1000 *Taenia taeniaeformis* eggs 29-38 days previously, were used as sources of immune rat peritoneal cells (IR cells) and serum (IRS). Thirteen 52 day old female Spartan rats which had received intraperitoneal (IP) inoculations of 0.5 ml 3% $Al(OH)_3$ (Superfos a/s, Vedbaek, Denmark) 9 days earlier were killed to harvest eosinophil-enriched peritoneal cells (AL cells). Normal, age-matched, female Spartan rats were used as donors of normal rat serum (NRS). Experiment 2: Nine 59 day old female Spartan rats which had been infected orally with 1000 *Taenia taeniaeformis* eggs 30 days earlier were used as the source of IR cells and IRS. Thirteen 59 day old female Spartan rats which had received 0.5 ml IP injections of 3% $Al(OH)_3$ 5 days earlier were used as the source of AL cells. Normal, age-matched, female Spartan rats were used as the source of NRS.

Peritoneal cells. Infected and $Al(OH)_3$-injected animals were killed by exposure to CO_2 vapor in a dry ice chamber. Twenty milliliters of Tyrodes buffer (T) was injected IP into each rat. In Expt 1 the Tyrodes was modified to eliminate Ca^{++} and Mg^{++} and contained 0.01 M EDTA. Following the injection, the peritoneal contents were mixed by

massaging the abdomen for 1 min, and the cell suspension was collected into 50 ml polyethylene tubes through an incision in the ventral abdominal wall. Viscera were then rinsed with an additional 5 ml of buffer and the pooled collections from each group of rats were washed and centrifuged at 180 g for 10 min at 4 C. The cell sediment was washed twice in buffer. Wright's-stained smears of the final suspension were prepared for differential counting. Total white cell counts were made using a hemacytometer. Cells were pelleted once more and resuspended in 2.5 ml of buffer.

Serum. After cells had been harvested from infected animals, the thorax was opened and the major vessels severed so that blood could be aspirated from the pleural cavity. Collections from all infected animals were pooled. Normal rats were processed in the same way. Clotting was allowed to continue for 1 h at 22 C, followed by 1 h at 4 C. The clot was then removed and the tubes were centrifuged for 10 min at 150 g. Aliquots of NRS and IRS were heat inactivated at 56 C for 1 h (ΔNRS, ΔIRS). All sera were then brought to 37 C.

Parasites. Larvae for the first experiment were removed carefully from liver cysts of a female Spartan rat which had been infected orally with 1000 *T. taeniaeformis* eggs approximately six months prior to the experiment. In the second experiment the donor had been infected for about five months. Strobilocerci were washed in sterile saline before use.

Host cell:parasite ratios and differential counts. In Expt 1, 12.3×10^7 IR cells or 9.10×10^7 AL cells were used per larva. In the second experiment fewer cells were used: 1.87×10^7 IR or 1.66×10^7 AL cells per parasite. Differential counts of cell composition are shown in Table 1.

Incubation of larvae with peritoneal cells. Incubations were done in 95 x 16.8 mm polyethylene tubes. Cell/serum combinations were identical for each experiment and are detailed in Table 2. Serum, Tyrodes buffer and peritoneal cell volumes were all 0.5 ml, with the exception of those tubes containing buffer only, where the volume was 1.0 ml. One strobilocercus was added to each tube. Tubes were then incubated in a 37 C waterbath for 1 h with gentle rocking motion.

Processing of larvae for transmission electron microscopy (TEM). Parasites were processed for TEM by the procedure of McLaren, MacKenzie and Ramalho-Pinto (1977) with minor modification. Following incubation, each parasite was carefully transferred from the polyethylene tube to a small glass vial containing 2 ml of cold 2% glutaraldehyde in 0.2 M sodium cacodylate buffer, pH 7.3. After about 5 min, larvae were placed on a piece of dental wax, and several 2 mm wide slices from the anterior and posterior ends of each larva were obtained using an acetone-cleaned razor blade. After remaining in 2% glutaraldehyde overnight

Table 1. Results of differential counting (%)

	Experiment 1		Experiment 2	
	IR Cells	AL Cells	IR Cells	AL Cells
Mononuclear cells	45	65	58	66
Eosinophils	37	23	28	14
Lymphocytes	14	10	8	5
Neutrophils	2	0	3	13
Mast cells	2	2	3	2

at 4 C, the specimens were rinsed in cacodylate buffer, post-fixed in 1% osmium tetroxide in 0.2 M s-collidine buffer, pH 7.3, for 2 h at 22 C, and stained *en bloc* for 1.5 h (Expt 1) or 0.5 h (Expt 2) in 0.5% aqueous uranyl acetate at 22 C. Next they were dehydrated stepwise through increasing concentrations of ethanol terminating in 3 changes of 100% ethanol. Propylene oxide was used as a transition solvent, and samples were embedded in an epoxy-araldite mixture.

Microtomy and electron microscopy. Thick (3 μm) sections of the anterior and posterior portions were cut using glass knives and an LKB Ultratome III ultramicrotome. The sections were placed onto drops of filtered distilled water on a glass microscope slide. Following evaporation of the water, a drop of immersion oil and a coverslip were added. Four to 16 thick sections of each sample were examined using phase microscopy. A coding system was used to record the average number of host cells observed at the parasite surface and the extent of tegument damage. Host cell adherence was scored as - if an average of 0-9 host cells were detectable in thick sections, + for 10-25 cells, ++ for 26-50 cells, and +++ for greater than 50 cells. Tegumental damage was scored as - for normal appearance, + for generalized slight damage or moderately severe focal change, ++ for moderate damage over entire surface, and +++ if damage was extensive and generalized. Control larvae (buffer only) and parasites with large numbers of adherent

host cells and/or extensive tegument damage were then thin-sectioned for TEM. The block face was trimmed with an acetone-cleaned razor blade and thin-sectioned with a DuPont diamond knife. Three-hundred mesh copper grids were used to collect the 50-90 nm sections, which were subsequently stained for 10 min in 2% aqueous uranyl acetate and 5 min in 0.125% aqueous lead citrate. Grids were examined using a Philips 300 Transmission Electron Microscope.

RESULTS

Observations on Thick Sections

Tegument damage: Observations made by phase microscopy are summarized in Table 2. Different degrees of tegumental damage or host cell adherence sometimes occurred at the anterior and posterior ends of the same strobilocercus. In both experiments moderate to extensive tegumental damage was generally seen only when larvae were exposed to fresh NRS or IRS in the presence of cells. Cells from infected and noninfected rats were apparently equally effective. In contrast, when larvae were incubated in fresh NRS or IRS alone, no comparable tegumental damage occurred. With two exceptions (ΔIRS and T; ΔIRS and IR cells), there appeared to be no tegumental damage when larvae were incubated with ΔNRS or ΔIRS either in the presence or absence of cells, and no damage was seen when larvae were incubated with or without cells in the absence of serum.

Table 2. Host cell adherence and tegumental damage to larvae of *Taenia taeniaeformis* incubated in combinations of mixed peritoneal cells, antibody and complement

Tube Contents	Expt 1		Expt 2	
	Host Cells	Tegument Damage	Host Cells	Tegument Damage
T	-	-	-	-
T + IR Cells	-	-	-/+++	-
T + AL Cells	-	-	-	-
NRS + T	-	-	-	-
NRS + IR Cells	*+++/-	+++/++	+++/+++	-/++
NRS + AL Cells	-	+/+++	+++/+++	+/++
ΔNRS + T	-	-	-	-
ΔNRS + IR Cells	-	-	-	-
ΔNRS + AL Cells	-	-	-	-
IRS + T	-	-	-	-
IRS + IR Cells	++/++	++/++	+++/++	-/++
IRS + AL Cells	+/-	++/-	+++/+++	-/++
ΔIRS + T	-	-	-	-/++
ΔIRS + IR Cells	-	-	-/+++	-/+
ΔIRS + AL Cells	-	-	-/+	-

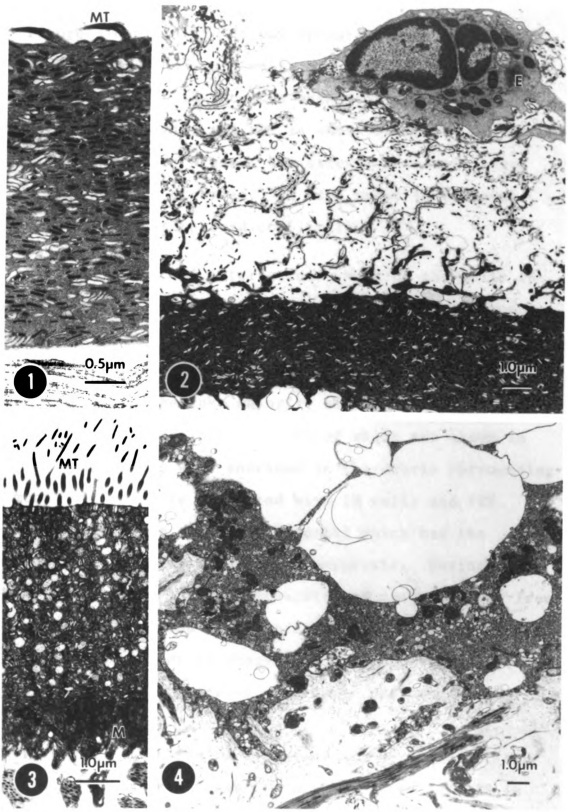
*Indicates scores on anterior/posterior portions.

Parasite/host cell interaction: The greatest degree of host cell adherence occurred under the same conditions as the greatest degree of tegumental damage, i.e., when larvae were incubated with cells in the presence of fresh NRS or IRS. There were some differences between the results of the two experiments. In Expt 1, where the buffer contained EDTA, large numbers of adherent host cells were seen only when IR cells were used. In Expt 2, on the other hand, large numbers of cells were present on larvae incubated with either IR or AL cells and NRS or IRS. Virtually no adherent cells occurred when larvae were incubated with buffer or ΔNRS, or ΔIRS. Two exceptions were noted: large numbers of cells were seen on the posterior sections of one parasite incubated with Tyrodes and IR cells, and one larva incubated with ΔIRS and IR cells. Cells were never seen adhering to control larvae which had been incubated in tubes to which no cells were added.

Ultrastructural Observations

Tegument damage: The normal appearance of the tegument at the anterior end of a 6 month old larva is shown in Figure 1. For comparison, Figure 2 shows the type of tegumental damage seen at the anterior end of a larva of the same age which had been incubated with IR cells and IRS. Areas of the surface appear to be stripping away and the tegument was very much thinner than in control larvae. Numerous host cells, almost all of which were eosinophils, were surrounded by debris; the latter consisted of microtriches, membranous

Figures 1-4. Tegument of normal 6 month old *Taenia taeniaeformis* larvae, and examples of the tegumental damage observed after 1 h incubation *in vitro* with serum and cells. 1. Appearance of normal tegument at the anterior portion of a strobilocercus incubated in Tyrodes buffer. The tegument here is 3.9 µm thick and microtriches (MT) can be seen at the outer surface. Only scanty debris occurred near the tegument surface, and no host cells were present. 2. Tegumental damage at the anterior portion of a larva incubated with IR cells and IRS. The outer surface has been disrupted and the tegument reduced to as little as 2.9 µm at one site. Debris, some of which appears membranous, and an eosinophil (E) can be seen near the parasite surface. 3. Appearance of normal tegument at the posterior portion of a strobilocercus incubated in Tyrodes buffer. The tegument is 5.8 µm thick. Microtriches (MT) extend from the outer surface and mitochondria (M) are clustered in the proximal cytoplasm. 4. Tegumental damage at the posterior portion of a larva incubated with AL cells and NRS. Extensive vacuolization has occurred, microtriches are no longer visible at the outer surface, and mitochondria are seen at all levels of the cytoplasm. Excluding the large vacuole, the tegument is only 2.9 µm deep in one area.



Figures 1-4

material and electron-dense rod-like structures from the damaged tegument.

Figure 3 depicts the normal appearance of the tegument at the posterior end of a 6 month old parasite. The extent of tegumental damage that occurs when these organisms are incubated with AL cells and NRS is shown in Figure 4. Microtriches are no longer present and extensive vacuolization of the tegument has occurred. In some areas (not shown) the tegument had disappeared completely.

Parasite/host cell interaction: Many of the cells in close proximity to the larvae examined with the electron microscope could not be identified because the portion of the cell within the thin section contained no identifying characteristics. The vast majority of the cells which could be identified were eosinophils, two of which are shown in Figure 5. These two were enmeshed in the debris surrounding a 6 month old parasite incubated with IR cells and IRS. The cells seem to be engulfing material which has the appearance of parasite tegumental components. Eosinophils were sometimes seen in close proximity to mast cells or free mast cell granules, and occasionally mast cell granules were present within eosinophil phagosomes (Insert, Figure 5). Fusion of eosinophil granules with these phagosomes was also seen (Figure 5).

The host cell-parasite interface at the posterior end of a 6 month old larva incubated with IR cells and IRS is illustrated in Figure 6. Surface projections from the

Figure 5. Eosinophils within debris near the tegumental surface of the anterior portion of a larva incubated for 1 h with IR cells and IRS (Expt 1). The cells are actively engulfing the debris and some of this material can be seen within phagosomes. Typical eosinophil granules (G) and mitochondria (M) can be seen. One phagosome (arrow) appears to be fusing with an eosinophil granule. Insert. Mast cell granules within an eosinophil phagosome.

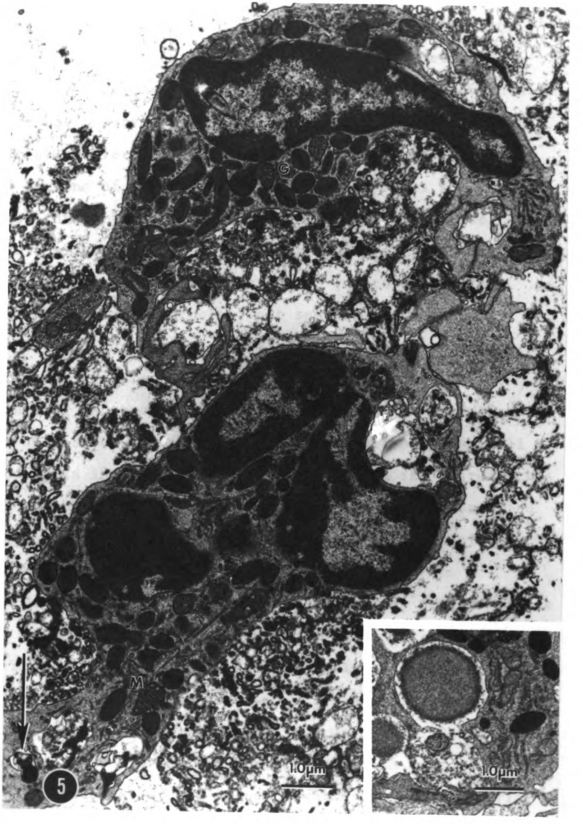


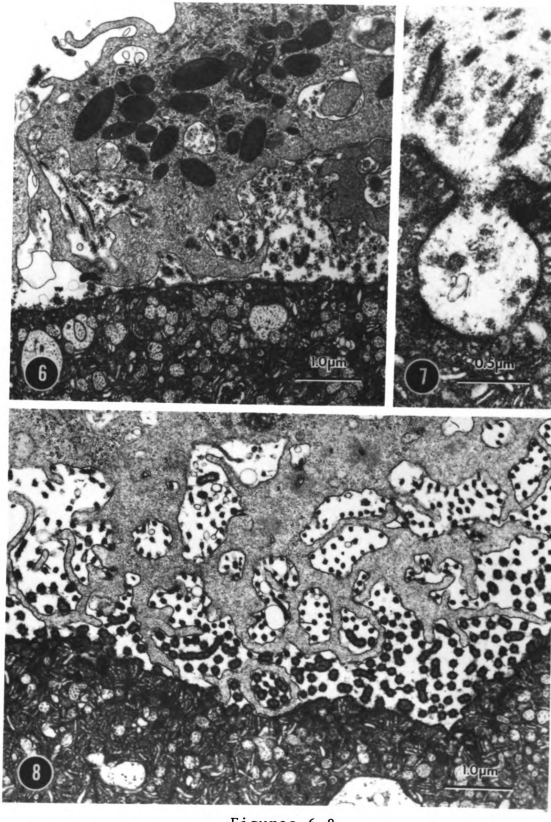
Figure 5

adherent eosinophil surround microtriches and amorphous material, which in many places appears to be adhering to the microtriches. In some areas (not shown) the host cell pseudopodia were seen intruding into shallow crevices in the distal cytoplasm of the tegument. Moderate tegumental damage was observed at many sites but did not appear to be especially severe at areas of contact with host cells. Some regions of the tegument contained vacuoles which were larger than normal (up to 1.7 μm in diameter). These occasionally opened to the parasite surface (Figure 7). Other areas contained large clusters (up to 3.8 μm in diameter) of small vacuoles, not seen in normal larvae.

Figure 8 demonstrates how extensively cells from uninfected rats interact with tegumental projections in the absence of exogenous antibody. This parasite (Expt 2) had been incubated with AL cells and NRS. Multiple host cell pseudopodia interdigitate with and surround the microtriches. Such amoeboid veil-like cell peripheries were common. The parasite tegument contained a mixture of vacuoles of abnormal sizes.

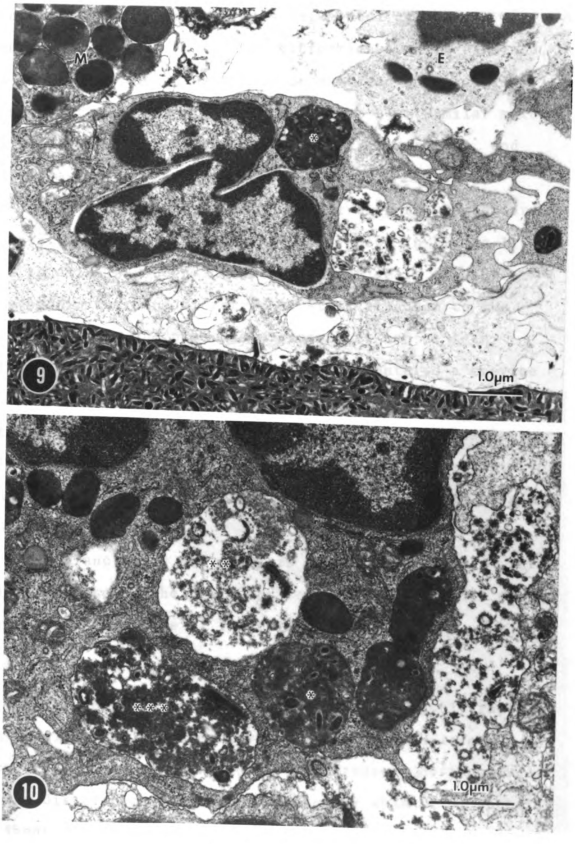
Figures 9 and 10 illustrate an additional feature of the host cell-parasite interaction, observed in this case when a 5 month old parasite was incubated with IR cells and IRS. The parasite had become surrounded by several layers of cells, amongst which eosinophils and mast cells were prominent. Many cells, including some which were recognizable as eosinophils, had vacuoles which contained material tentatively identified as microtriches and membranous debris

Figures 6-8. Events occurring at the outer tegumental surface of 5-6 month old *Taenia taeniaeformis* larvae incubated for 1 h with rat peritoneal cells. 6. Eosinophil close to the surface of the tegument of a posterior portion of a 6 month old larva incubated with IR cells and IRS. Pseudopodia surround microtriches, and there is debris within phagosomes. Some vacuoles within the parasite tegument are larger than normal. 7. Same parasite as Fig. 6. One of the tegumental vacuoles, approximately 1.0 μm in diameter, is opening to the surface. 8. Extensive interdigitation of host cell pseudopodia and microtriches of a 5 month old parasite incubated with AL cells and NRS. The large vacuole in the parasite tegument is 1.3 μm in diameter.



Figures 6-8

Figures 9-10. Events occurring at the outer tegumental surface of the anterior portion of a larva that had been incubated for 1 h with IR cells and IRS (Expt 2). 9. Host cells in close proximity to the parasite surface. A cell has surrounded one of the two remaining microtriches in this region. Some electron-dense amorphous material can be seen between the cell and the tegumental surface. Another cell (center) contains two vacuoles apparently filled with parasite material. One vacuole contains dense material (*) resembling tegumental cytoplasm. Another vacuole contains the debris frequently associated with tegumental damage. Portions of a mast cell (M) and an eosinophil (E) can be seen. 10. Higher magnification of an eosinophil near the surface of the larva shown in Fig. 9. Three types of phagosomes can be seen. Some appear to contain tegumental cytoplasm (*). Another (**) contains the same debris that can be seen outside of the eosinophil. A third type of vacuole (***) contains amorphous electron-dense material in addition to the debris.



Figures 9-10

resulting from tegumental damage. Other vacuoles contained material recognizable as tegumental cytoplasm. Pseudopodia surrounded the few microtriches which remained, and an electron-dense amorphous deposit was occasionally observed at the parasite surface (Figure 9). Similar material was occasionally seen within phagosomes (Figure 10), and may represent discharged eosinophil granule contents.

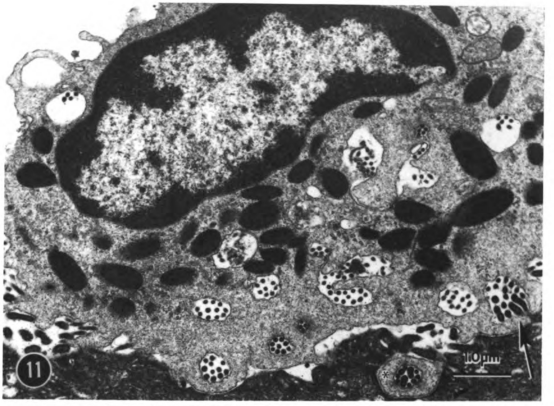
An eosinophil in intimate contact with the posterior tegument of a parasite from Expt 2 is shown in Figure 11. This parasite had been incubated with AL cells and IRS. Pseudopodia surround microtriches still attached to the larval surface, and numerous microtriches can be seen within phagosomes. One pseudopodium has entered a crevice at the tegumental surface. The nature of the interaction between the finger-like pseudopodia and microtriches is illustrated in Figure 12. This is a region of the posterior end of a 5 month old parasite, incubated with AL cells and IRS.

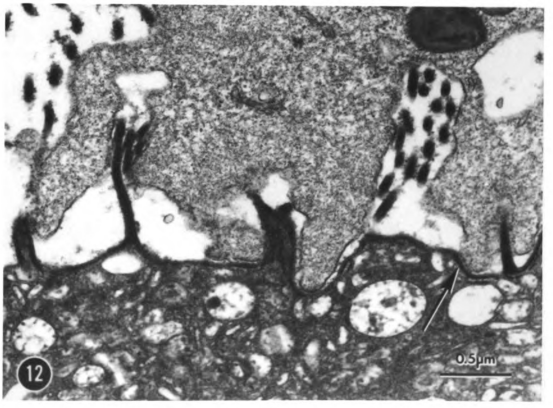
A degranulating mast cell is shown in Figure 13. This cell was close to the posterior tegument of a 5 month old larva in the presence of IR cells and ΔIRS.

DISCUSSION

These results provide evidence of a remarkably rapid non-specific cell adherence reaction to *T. taeniaeformis in vitro*, which is serum-dependent and intensely destructive to the distal tegument. The presence or absence of specific antibody (in IRS) did not alter demonstrably the outcome of

Figures 11-12. Events occurring at the tegumental surface of posterior regions of larvae incubated for 1 h with rat peritoneal cells (Expt 2). 11. An eosinophil in intimate contact with the surface of a larva which had been incubated with IR cells and NRS. Numerous microtriches are present within host cell phagosomes, and pseudopodia (arrow) are seen surrounding microtriches still attached to the parasite surface. A host cell pseudopodium (*) lies within an invagination of the tegumental surface. 12. Several finger-like host cell pseudopodia can be seen surrounding microtriches in this higher magnification electron micrograph. The parasite had been incubated with AL cells and IRS. Host cell-parasite membrane-to-membrane contact appears to have occurred at one point (arrow).





Figures 11-12

Figure 13. Degranulating mast cell in vicinity of tegumental surface of the posterior end of a parasite that had been incubated for 1 h with IR cells and ΔIRS (Expt 2). The granules swell and appear less dense when they make contact with the extracellular environment.

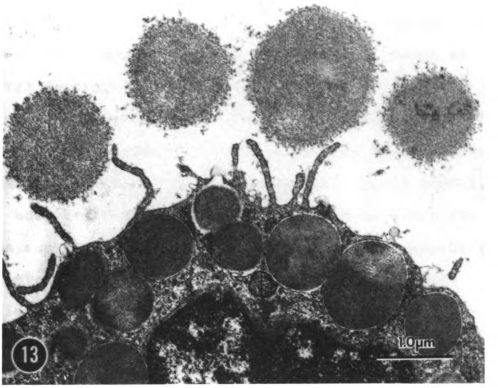


Figure 13

cell interaction with the parasite surface over the period of incubation studied. Furthermore, cell adherence and tegumental damage proceeded to the same degree whether the peritoneal cell populations were derived from immune or uninfected donors.

Since cells did not generally become fixed in the absence of fresh serum, the findings suggest that binding is mediated via interaction with some heat-labile humoral factor; however, the failure of .005 M EDTA to block the reaction suggests that active consumption of complement is not a prerequisite for cell adhesion and damage. This conclusion does not preclude the possibility of amplification in the presence of fresh complement, either non-specifically or specifically activated, and the enhanced reaction which we saw in the absence of EDTA may be indicative of this. Perhaps if the reaction were allowed to continue under conditions in which these effects could be more conveniently quantitated, differences might be manifested in terms of the rate at which damage proceeds or the relationship between cell numbers and the extent of membrane changes. Quantification of tegument damage through the release of absorbed Cr^{51} might provide a means of assaying these effects, although there is no precedent for this in cestodes, and the application of radiolabeled marker loss to other helminth systems *in vitro* has given variable and sometimes unsatisfactory results (Kassis, Aikawa, and Mahmoud, 1979).

It is also possible that cell adherence is effected via receptor interaction with complement and/or immunoglobulins which are already bound to the parasite limiting membrane prior to incubation *in vitro*. Kwa and Liew (1978) have detected the presence of rat immunoglobulin on the tegument of *T. taeniaeformis* larvae *in vivo*, and it is known that complement-fixing factors are associated with the surface of these parasites (Hammerberg and Williams, 1978). Receptors for bound C3b and Fc are present on several different inflammatory cell types in the rat (Capron, Rousseaux, Mazingue, Bazin and Capron, 1978; Capron et al., 1979), and their involvement in the reactions which we observed might be determined by blocking experiments with specific antisera in the future. Whatever the contribution of complement might be in fresh serum, it is highly likely that its effects would be short-term, because the incubation of larvae of *T. taeniaeformis* with serum *in vitro* under the conditions we employed leads to depletion of hemolytically active complement in the surrounding medium within 30 min (Hammerberg, Musoke, Hustead and Williams, 1976). There are other heat-labile factors in fresh serum which have been shown to interact with parasite surfaces (Rifkin, 1978), and further work will be required to determine if they, or comparable substances, play any role in the phenomena described here.

It seems clear that cells alone show little responsiveness to the parasite and, in the single instance where extensive adherence occurred in a serum-free environment,

it is possible that this was triggered by tegumental damage inflicted during dissection and removal of the larva from the liver. Verheyen, Vanparijs, Borgers and Thienpont (1978) have shown that a variety of phagocytic cells rapidly adhere to the tegument following chemically induced damage *in vivo*. In our study, eosinophils were particularly prominent amongst the peritoneal phagocytes which participated in the reactions *in vitro*, even though they constituted only 14-37% of the starting cell populations. Similarly, mast cells were quite frequently evident in the adherent cell mass, although they generally constituted no more than 3% of the original mixture. These observations are indicative of selective attraction to the parasite surface, especially in view of the fact that adherent neutrophils were rarely identified in any of the positive reactions.

There is increasing evidence that eosinophils can function as effector cells in a variety of human and mouse anti-parasitic systems *in vitro* and *in vivo* (for review see Butterworth, 1977), and eosinophils are known to participate in cytotoxic reactions to *Schistosoma mansoni* in the rat (Capron et al., 1978). Recently, it was shown that the effectiveness of eosinophils is influenced by cooperating mast cells (Capron et al., 1978), and it is interesting that mast cells were not only involved in the adherence response to *T. taeniaeformis* but were also seen to degranulate in combinations of cells and serum from immune animals. This observation, combined with our failure to detect cell

adherence under any circumstances when purified eosinophil populations were employed (unpublished observations), suggests that different cell types may collaborate in effecting damage on target organisms. It has been pointed out before that the use of mixed cell populations may more nearly approximate the conditions *in vivo* where cell-cell interaction in effector mechanisms is probably the rule rather than the exception (Beeson and Bass, 1977). Mast cells might affect eosinophils or even other inflammatory cells via the release of a variety of chemical mediators, and some hypotheses concerning their potential regulatory role in antiparasitic reactions have been discussed recently by Leid and Williams (1979).

The behavior of adherent cells in our experiment differed sharply from that reported in other systems. Surface fixation and tegument damage occurred very quickly (1 h), in contrast to the extended incubation periods (18-72 h) found necessary for demonstrable effects in schistosome systems, for example. The eosinophils in particular appeared to be especially aggressive, developing multiple pseudopodia intertwining with microtriches and incorporating tegumentary cytoplasm into large phagosomes. The eosinophil granules showed no propensity to become oriented toward the parasite surface, nor were they expelled or frequently seen fusing with the phagosomes. These differences could reflect the acute nature of the phenomenon, and longer term studies may reveal progressive alterations more consistent with previous reports (McLaren, MacKenzie and Ramalho-Pinto,

1977; McLaren, Ramalho-Pinto, and Smithers, 1978; Caulfield, Korman, Butterworth, Hogan and David, 1980). However, the rapidity of their responses and the severity of the tegumental changes could also be indicative of previously undetected potency of the non-specific effector capacity of these cells.

In our experiments we saw virtually no changes attributable to the effects of serum alone. Non-specific serum-mediated damage to *T. taeniaeformis* after *in vitro* exposure for 24 h had been reported previously by Chen (1950), although the mechanism involved was not determined. Some heat-labile serum factors other than complement appear to have been responsible. More recently, Siebert and Good (1979) showed that serum-induced changes could be produced in *T. crassiceps* after prolonged incubation (7 days) *in vitro*, but these were apparently antibody-dependent. Non-specific lytic effects on *Echinococcus* tegument have been described by Kassis and Tanner (1976), Herd (1976) and Rickard, MacKinlay, Kane, Matossian, and Smyth (1977), and these are believed to be complement-mediated. It may well be that the demonstration of these effects is related to the amount of complement available, the length of exposure, and the rate of release of complement-fixing factors by the parasites. In any event, the relationship between cells, complement and antibody on the parasite surface in *T. taeniaeformis* merits further attention.

Perhaps the most important outcome of our experiments is that they leave in no doubt the effectiveness of

host-defenses to recognize and attack the larval tegument when the opportunity is presented *in vitro*. Since the host and the established parasite coexist *in vivo* with no evidence of rejection, the balance must be tipped in favor of *T. taeniaeformis* so that effector mechanisms, whether specific or non-specific, are held in abeyance. This is the hallmark of concomitant immunity (Mitchell, 1979), and to the extent that it is especially characteristic of taeniiasis, our results suggest that the *T. taeniaeformis*/ rat model constitutes an ideal system for study of this phenomenon in the future.

ACKNOWLEDGEMENTS

This work was supported by NIH Grant AI-10842. We are grateful to Ms. Marla M. Signs for assistance in harvesting the peritoneal cells, and to Ms. Kate Baird for assistance in preparation of the manuscript. This is journal article number from the Michigan Agricultural Experiment Station.

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APPENDICES

APPENDIX A

PRODUCTION AND CHARACTERIZATION OF ANTISERA DIRECTED AGAINST EARLY POSTONCOSPHERAL STAGES OF *TAENIA TAENIAEFORMIS*

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PRODUCTION AND CHARACTERIZATION OF ANTISERA DIRECTED AGAINST EARLY POSTONCOSPHERAL STAGES OF *TAENIA TAENIAEFORMIS*

INTRODUCTION

Although rats can be immunized against challenge infection with *Taenia taeniaeformis* with a variety of materials derived from strobilocerci (Miller, 1932; Campbell, 1936; Kwa and Liew, 1977; Ayuya and Williams, 1979), little is known of the antigens responsible for this effect. Protective antigens have been partially characterized (Kwa and Liew, 1977), but whether these are shared with oncospheres or early postoncospheral stages is not yet clear. No comparative antigenic analysis of developmental stages has been attempted for *T. taeniaeformis* or any other taeniid cestode. This is in part because of the difficulty of *in vitro* culture of these parasites. However, large numbers of organisms can be grown in individual rat livers and the following results illustrate the potential for use of parasite-rich tissues in the preparation of antisera against developing forms.

MATERIALS AND METHODS

Animals Used for Preparation of Liver Homogenates

Eight 21 day old female Spb[SD] rats purchased from Spartan Research Animals, Haslett, Michigan, were infected orally with *Taenia taeniaeformis* eggs in accordance with the schedule shown in Table A1. A ninth rat served as an uninfected control. All animals used during the investigation received proprietary brand food and water *ad libitum*. Parasites were maintained as described previously (Engelkirk and Williams, 1980).

Preparation of Liver Homogenates

Animals were killed by exposure to CO_2 vapor in a dry ice chamber. The uninfected control rat was killed when it was 21 days old. Following removal, each liver was placed into a 50 ml graduated cylinder containing 10 ml of cold 0.1 M Tris-HCl buffer, pH 7.0, containing 0.15 M NaCl. The volume displaced by the liver was recorded. The liver was then placed on a piece of dental wax and cut into pieces of approximately 1 cm^3 using a razor blade. The pieces were placed into a 50 ml polyethylene centrifuge tube. An amount of cold Tris-HCl buffer equal to 1.5 times the liver volume was added to the tube, which was then placed into a beaker of ice, where it remained during homogenization. Homogenization was accomplished using a Polytron (Kinematica GmbH, Switzerland) at maximum speed for 30 sec. The homogenate was transferred to a 100 ml beaker and the mixture

Table A1. Dosage and necropsy schedule

Rat No.	Dosage (No. of eggs)	Postinfection Day on Which Necropsied
1	100,000	1
2	50,000	3
3	25,000	5
4	12,000	7
5	6,000	9
6	3,000	12
7	1,000	15
8	1,000	21

stirred overnight at 4°C. It was then transferred to polyallomer tubes and centrifuged for 30 min at 15,000 g and 4°C in a Sorvall OTD-2 Ultracentrifuge. The supernatant was stored at -70°C until used.

Preparation of Homogenate/ Adjuvant Inocula

Two milliliters of complete Freund's adjuvant (Calbiochem-Behring Corp.) was added to 2 ml of each of the 9 supernatants. A stable water-in-oil emulsion was prepared using a 1 ml glass syringe fitted with an 18 gauge needle.

Preparation of Antisera

Twenty-seven female Spartan "retired breeder" rats were divided into 9 groups of 3. The initial inoculation of each rat consisted of eight 0.1 ml IM injections (eight separate sites) of the homogenate/adjuvant emulsion, for a total volume of 0.8 ml. Rats received a total of three booster inoculations, each booster consisting of eight 0.1 ml IM injections. Boosters were administered not less than two weeks and not more than five months from the previous inoculation. Six animals died prior to completion of the immunization protocol: 2 in the control group, 2 in the Day 1 group, 1 in the Day 3 group, and 1 in the Day 12 group. Two weeks after receiving the third and final booster, each of the remaining 21 rats was killed, and blood was collected from the thoracic cavity following severance of the posterior vena cava. Serum from each individual animal was stored separately at -70°C until required for use. The experimental

procedure for the production and characterization of antisera is summarized diagrammatically in Figure A1.

Preparation of Saline Soluble Antigens and *in vitro* Products of *Taenia taeniaeformis*

Saline soluble antigens (SSA) and *in vitro* products (IVP) were prepared in the manner described by Ayuya and Williams (1979). Briefly, SSA is prepared by homogenizing washed strobilocerci (3 months old) in phosphate buffered saline (PBS) in a glass tissue grinder. The homogenate is stirred for 12 hrs at 4°C and then centrifuged at 50,000 g for 2 1/2 hrs. The supernatant is frozen at -20°C until required for use. IVP is prepared by incubating 50 strobilocerci for 24 hrs at 37°C in 150 ml of Hank's BME (GIBCO, Grand Island, New York) supplemented with antibiotics. The culture medium is dialyzed for 48 hrs against triple distilled water followed by 12 hrs against PBS. The medium is next concentrated by either negative vacuum dialysis or dialysis against polyethylene glycol (Carbowax, Union Carbide). When the latter method is used, the medium is further dialyzed for 48 hrs against PBS. IVP are stored at -20°C until used.

Gel Diffusion and Immunoelectrophoresis Procedures

Gel diffusion (Ouchterlony method) and immunoelectrophoresis (IEP) procedures were performed by standard techniques (Garvey et al., 1977). A 2% barbital buffer, pH 8.6, containing 0.04% EDTA and 0.01% thimerosal, was

Figure A1. Production and characterization of antisera: diagrammatic summary of experimental procedure.

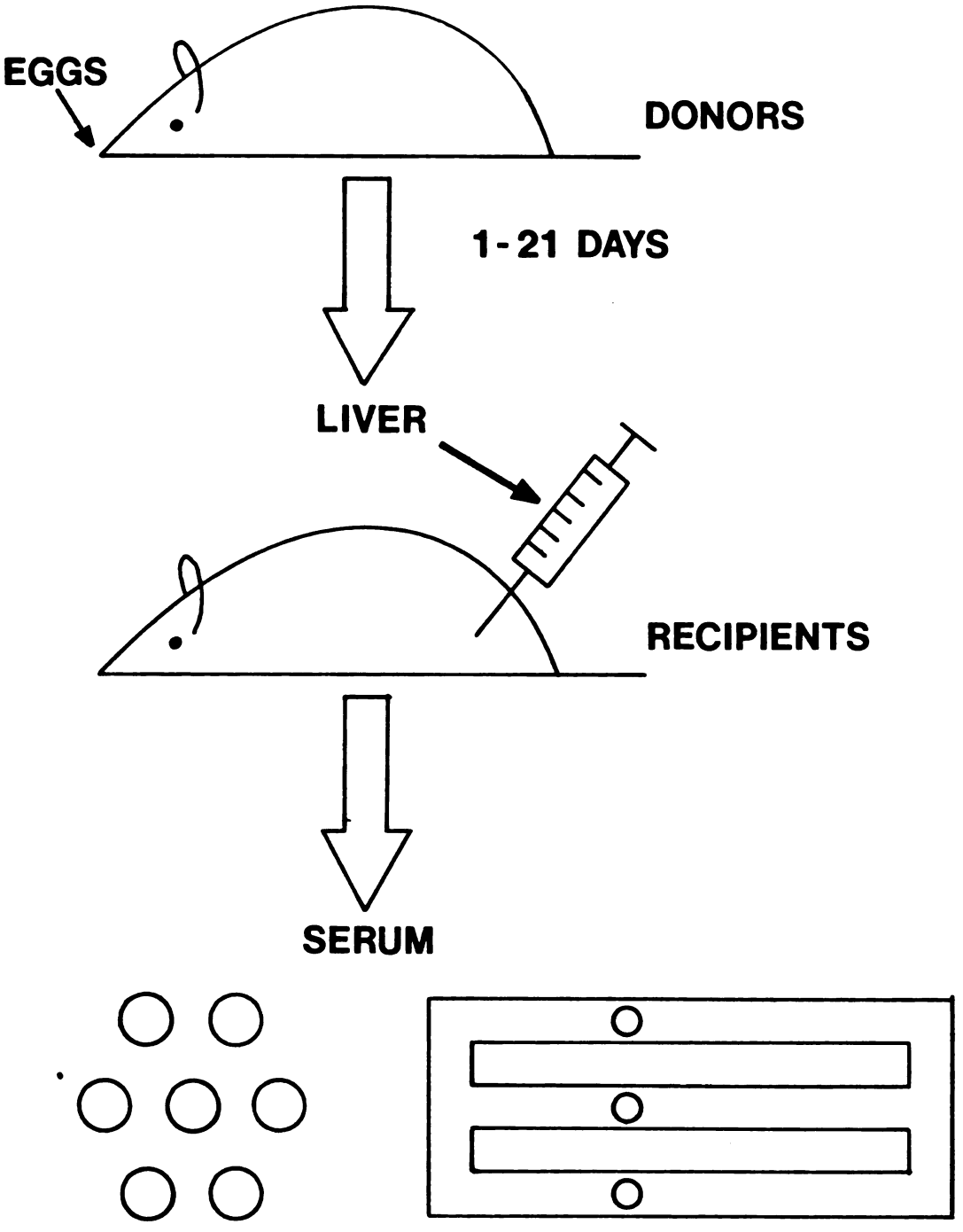

Figure A1

used for the IEP procedure and for preparation of the 2% Noble agar used in both procedures. IEP was performed in a Gelman apparatus (Gelman Instrument Company, Ann Arbor, Michigan).

RESULTS

Gel Diffusion Results

Of the 21 sera tested by the Ouchterlony method, 12 produced precipitin bands; these are presented diagrammatically in Figure A2. All 12 sera contained antibody which reacted with antigenic determinants present in SSA; 7 reacted with IVP.

IEP Results

The 12 sera which gave positive gel diffusion results were tested against IVP and SSA by IEP. The results are presented diagrammatically in Figure A3.

DISCUSSION

The results indicate that tissues derived from heavily infected rats can be used for the development of antisera against developing forms. Rats were selected for the production of antibodies because of the limited response expected from the inoculation of homogenized homologous liver. At least 5 precipitating antibody systems were demonstrable, and the results suggest that stages as early as 7 days share structural and possibly secretory/excretory components with strobilocerci. Homogenates containing stages earlier than 7 days postinfection may have contained

Figure A2. Diagrammatic representation of positive gel diffusion results. The circles represent the Ouchterlony wells. The lower well in each grouping of 3 contains the antiserum indicated by the label beneath it (e.g., 7A is the serum from one of the rats immunized with liver homogenate containing 7 day old parasites; 7B is from another rat which received the same homogenate). The upper left well in each grouping contains IVP and the upper right well contains SSA.

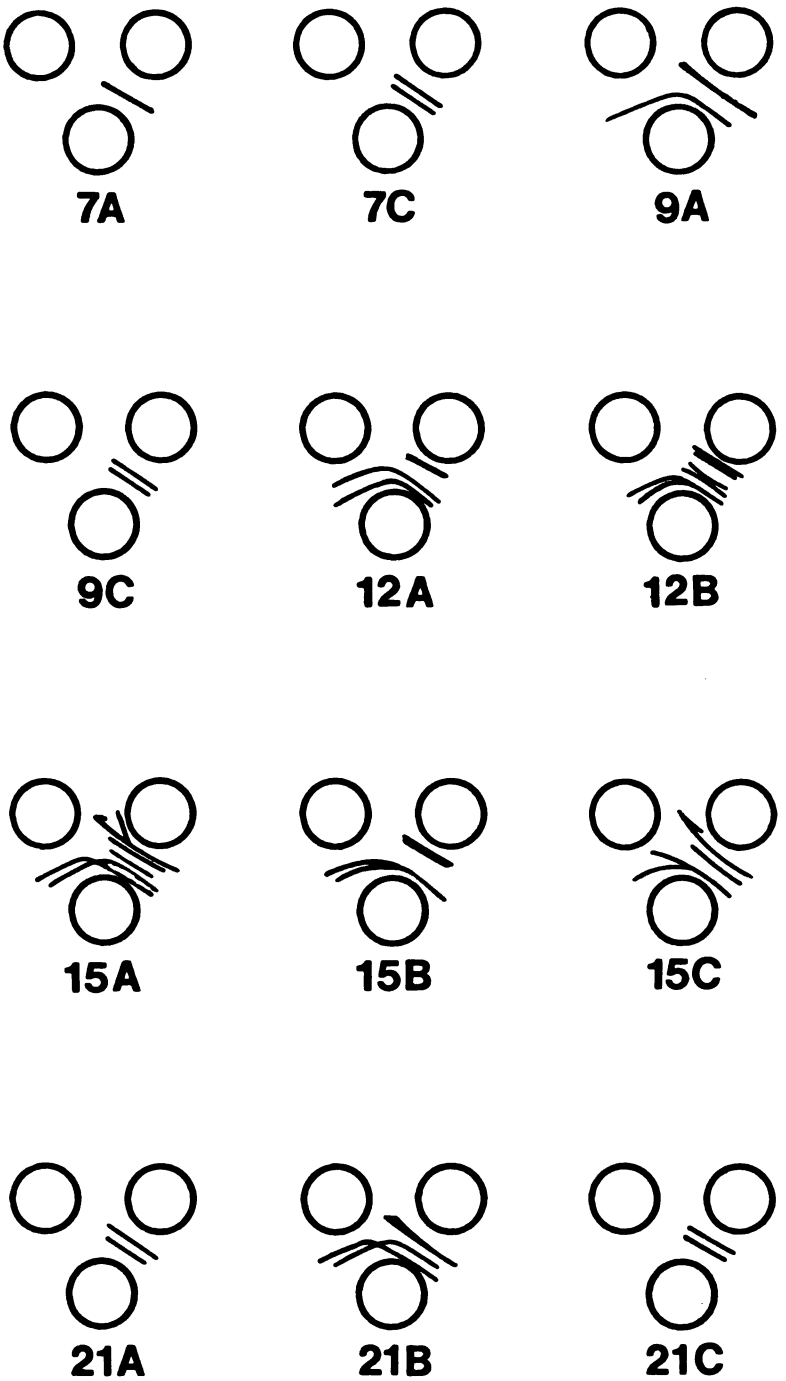

Figure A2

Figure A3. Diagrammatic representation of IEP results. Troughs are labeled to indicate the antisera they contained. The center well contained SSA; the top and bottom wells contained IVP. The anode (+) is to the left and the cathode (-) is to the right.

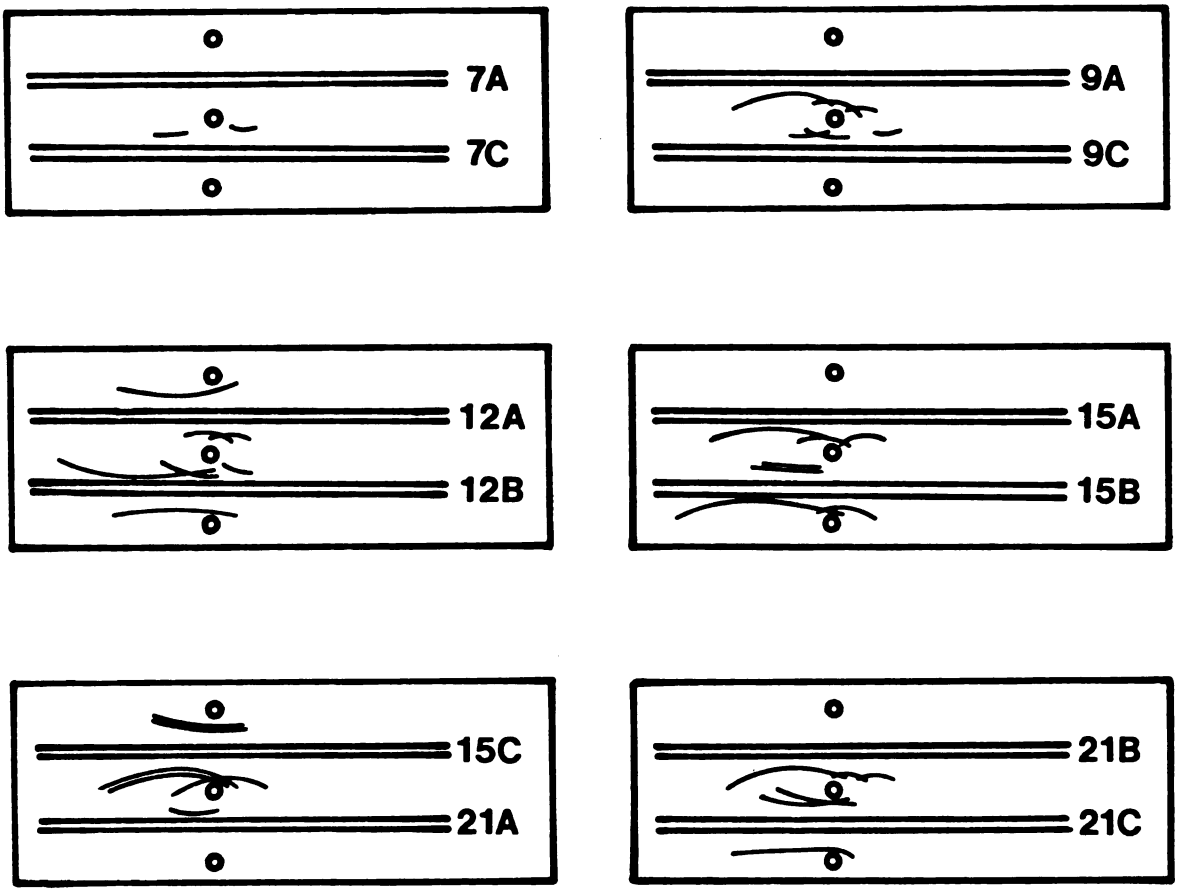

Figure A3

insufficient quantities of antigen to stimulate a host humoral response.

The method has advantages over the use of *in vitro* cultured parasites. Firstly, growth *in vitro* requires supplementation with a mixture of heterologous cells and serum which would be highly antigenic in recipient animals (Heath, 1973). Secondly, growth *in vitro* is slow and therefore parasites may not express antigens of tissue stages of equivalent age. The use of homologous recipients insures production of antiserum directed almost entirely against parasite antigens. Antibody directed against altered liver antigens can be readily absorbed. By selecting especially highly infected livers, it may be possible to enhance the antigenicity, and modification of the inoculation protocol or adjuvant may also be beneficial. Cross absorption with homogenates at different days post-infection may make it possible to demonstrate stage-specific antigens in the future.

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APPENDIX B

INDUCTION OF IMMUNITY TO HOMOLOGOUS CHALLENGE USING ANTIGENS OF EARLY POSTONCOSPHERAL STAGES OF *TAENIA TAENIAEFORMIS*

APPENDIX B

INDUCTION OF IMMUNITY TO HOMOLOGOUS CHALLENGE USING ANTIGENS OF EARLY POSTONCOSPHERAL STAGES OF *TAENIA TAENIAEFORMIS*

INTRODUCTION

It has been known for some time that rats can be immunized against challenge infection with *Taenia taeniaeformis* with antigenic materials from strobilocerci or their excretory/secretory products (Miller, 1932; Campbell, 1936; Kwa and Liew, 1977; Ayuya and Williams, 1979). Recent work has shown that antigens associated with oncospheres are also highly immunogenic in mice (Lloyd, 1979; Rajasekariah et al., 1980), but nothing is known of the immunogenicity of intermediate stages. In view of the marked and abrupt change in the appearance of developing larvae of *T. taeniaeformis* during the first 2 weeks of growth (Engelkirk and Williams, 1980a,b), the possibility that morphological changes might be associated with a loss of immunogens has arisen. Experiments were conducted to determine if parasites both before and after the development of microtriches have immunizing potential.

MATERIALS AND METHODS

Animals Used for Preparation of Liver Homogenates

Six 21 day old female Spb[SD] rats purchased from Spartan Research Animals, Haslett, Michigan, were infected orally with *Taenia taeniaeformis* eggs in accordance with the schedule shown in Table B1. A seventh rat served as an uninfected control. All animals used during the investigation received proprietary brand food and water *ad libitum*. Parasites were maintained as described previously (Engelkirk and Williams, 1980a).

Preparation of Liver Homogenates

Animals were killed by exposure to CO_2 vapor in a dry ice chamber. The uninfected control rat was killed when it was 22 days old. Liver homogenates were prepared and stored in the manner described in Appendix A, except that they were not centrifuged following overnight stirring at 4°C.

Preparation of Homogenate/ Adjuvant Inocula

Two milliliters of complete Freund's adjuvant (Calbiochem-Behring Corp.) was added to 2 ml of each of the homogenates. A stable water-in-oil emulsion was prepared using a 1 ml glass syringe fitted with an 18 gauge needle.

Inoculation of Animals

Experiment No. 1: Thirty-six 23 day old female Spartan rats were divided into 6 groups of 6. Five groups received

Table B1. Dosage and necropsy schedule

Rat No.	Dosage (No. of Eggs)	Postinfection Day on Which Necropsied
1	100,000	1
2	50,000	3
3	25,000	5
4	6,000	9
5	1,000	15
6	1,000	21

IM injections of homogenate/adjuvant emulsion: Day 1, Day 3, Day 5, Day 9, and uninfected control. One group received IM injections of adjuvant alone. Animals received 0.1 ml at each of five different sites, for a total of 0.5 ml.

Experiment No. 2: Forty-eight 21 day old female Spartan rats were divided into 8 groups of 6. Seven groups received IM injections of homogenate/adjuvant emulsion: Day 1, Day 3, Day 5, Day 9, Day 15, Day 21, and uninfected control. One group received IM injections of adjuvant alone. Animals received 0.1 ml at each of five different sites, for a total of 0.5 ml.

Challenge and Cyst Counting

Twenty-one days after immunization, the animals were bled from the orbital plexus (approximately 0.5 ml per rat) while anesthetized with ether. Blood from each group was pooled and sera were stored at -70°C. While still anesthetized, the animals were dosed orally with 150 *T. taeniaeformis* eggs. Sixteen days later the animals were killed in the manner previously described and their livers were removed and coded. Cysts were then counted by an individual unfamiliar with the coding system. The experimental procedure is summarized diagrammatically in Figure B1.

Preparation of *T. taeniaeformis* *in vitro* Products (IVP) and Saline Soluble Antigen (SSA)

IVP and SSA were prepared as described in Appendix A.

Figure B1. Induction of immunity to homologous challenge using antigens of early postoncospheral stages of *Taenia taeniaeformis*: diagrammatic summary of experimental procedure.

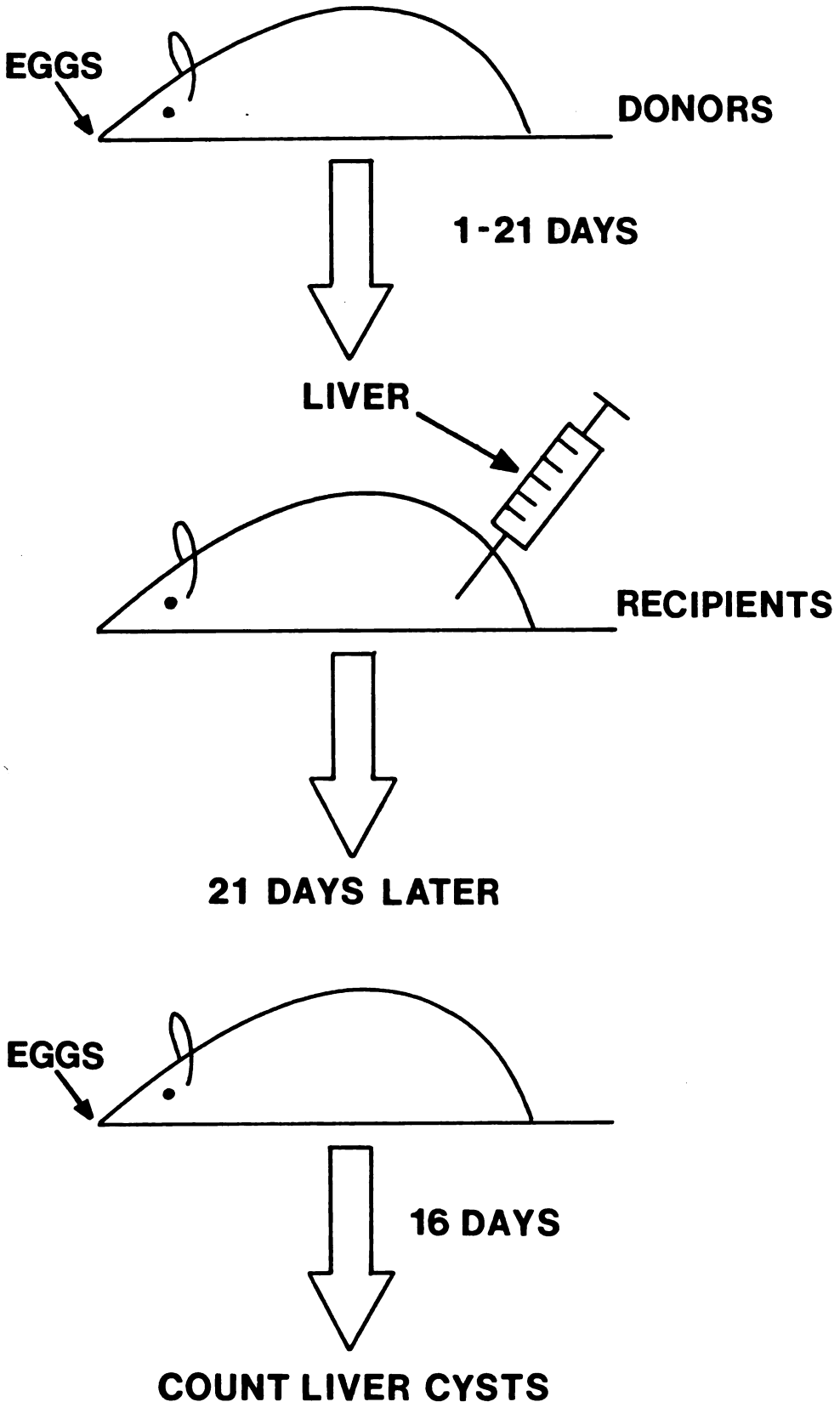
EGGS
DONORS
1-21 DAYS
LIVER
RECIPIENTS
21 DAYS LATER
EGGS
16 DAYS
COUNT LIVER CYSTS

Figure B1

Gel Diffusion

Gel diffusion was performed as described in Appendix A.

Challenge of Offspring

Two of the "retired breeder" rats that received Day 5 homogenate (Appendix A) were pregnant at the time of the original inoculations. A total of 24 offspring (13 females, 11 males) were born approximately 11 days later. The offspring were separated from the mother rats 7 days after the latter received their first booster inoculations. When the 13 female offspring were 25 days old, they were challenged orally with 200 *T. taeniaeformis* eggs. A total of seven 25 day old control rats were also challenged on the same day. Animals were killed 21 days later, their livers were coded, and the cysts were counted by an individual unfamiliar with the coding system. The experimental procedure is summarized diagrammatically in Figure B2.

RESULTS

Gel Diffusion

Pooled sera were tested against IVP and SSA in the manner described in Appendix A, with no evidence of formation of precipitin bands.

Liver Cyst Counts

The results of liver cyst counting are shown in Tables B2 (Expt. 1) and B3 (Expt. 2). One animal in the Day 5 group of Expt. 1 and three in the Day 5 group of Expt. 2 died during the experiment.

Figure B2. Challenge of offspring: diagrammatic summary of experimental procedure.

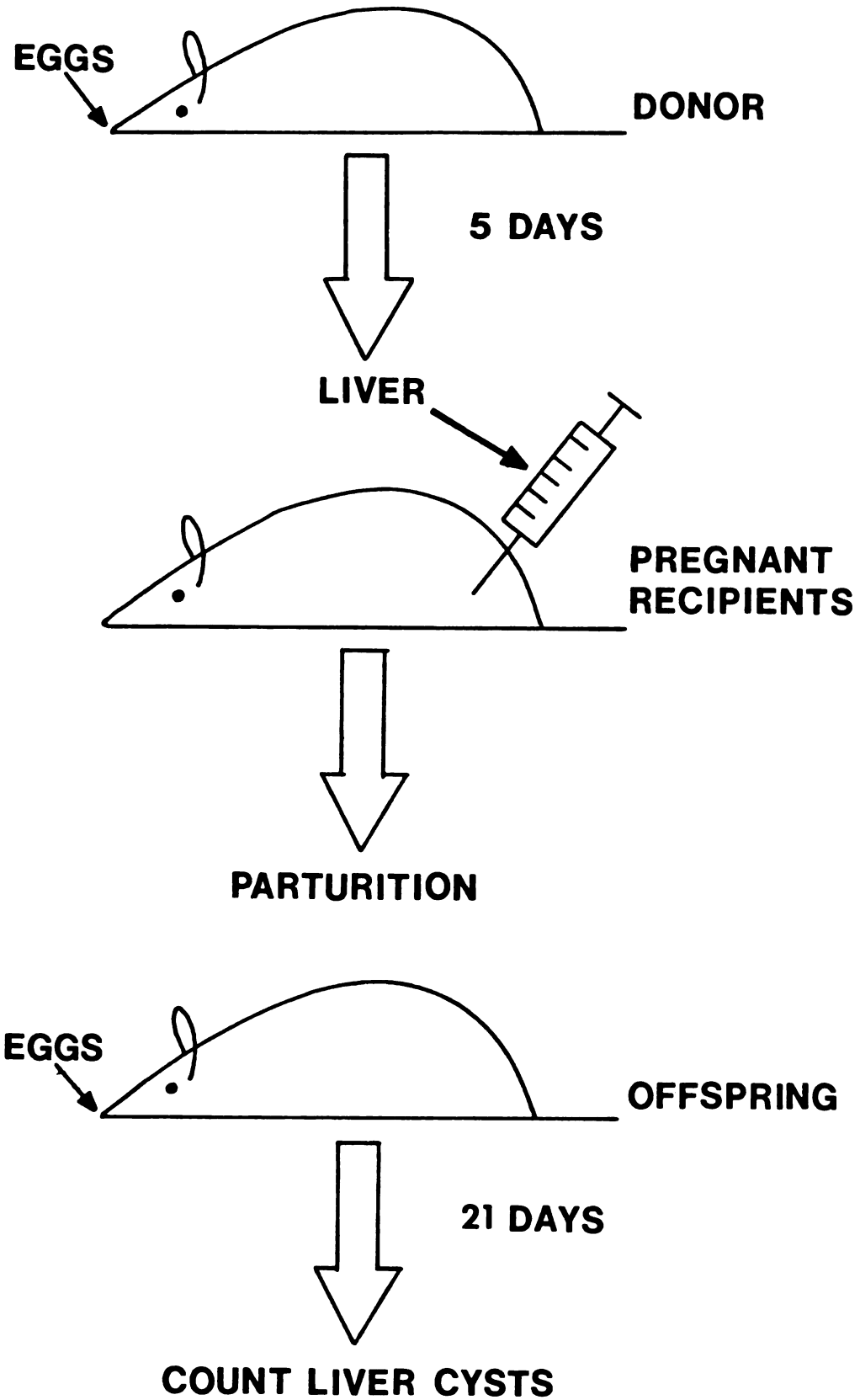
EGGS
DONOR
5 DAYS
LIVER
PREGNANT
RECIPIENTS
PARTURITION
EGGS
OFFSPRING
21 DAYS
COUNT LIVER CYSTS

Figure B2

Table B2. Liver cyst counts, Expt. 1

Group	Mean No. of Cysts (range)	P Value*
CFA only	15 (9-20)	-
Liver + CFA	13 (4-24)	NS
Day 1 + CFA	14 (7-21)	NS
Day 3 + CFA	15 (4-27)	NS
Day 5 + CFA	<1 (0-1)	<0.01
Day 9 + CFA	0 (0)	<0.01

*Analysis of variance.

Table B3. Liver cyst counts, Expt. 2

Group	Mean No. of Cysts (range)*
CFA only	23 (13-33)
Liver + CFA	24 (13-33)
Day 1 + liver + CFA	24 (17-30)
Day 3 + liver + CFA	7 (0-22)
Day 5 + liver + CFA	<1 (0-1)
Day 9 + liver + CFA	0 (0)
Day 15 + liver + CFA	<1 (0-5)
Day 21 + liver + CFA	18 (5-33)

*Statistical analysis not completed at time of publication.

Challenge of Offspring

Liver cyst counts of offspring and control rats are depicted in Table B4. All cysts in control rat livers were of appropriate size and appearance for 21 day old infections. Many of those in the livers of offspring rats, on the other hand, were dead (Figure B3).

DISCUSSION

These results demonstrate that intramuscular inoculations of early developmental forms of *Taenia taeniaeformis* can immunize rats against homologous challenge and, at least in the case of 5 day old parasites, that this resistance can be transferred from mother to young. Furthermore, protective immunogens are present both before and after the acquisition of microtriches (Engelkirk and Williams, 1980a,b) and before and after acquisition of invulnerability to passively transferred antibody (Musoke and Williams, 1975).

Since resistance can be passed to offspring, it may also be possible to passively transfer resistance to naive rats via serum or immunoglobulin fractions. Serum or fractions containing protective antibody could be labeled with fluorochrome, ferritin or peroxidase in an attempt to locate functional antigens using immunofluorescence or immunoelectron microscopy. Recent studies have demonstrated that oncospheres of *T. taeniaeformis*, products resulting from their disruption by freezing, thawing and sonication, or excretory/secretory antigens from their culture *in vitro* can

Table B4. Liver cyst counts, challenge of offspring and controls

Group	Mean No. of Cysts (range)	P Value*
Control	74 (39-100)	-
Offspring	27 (4-61)	<0.001

*Modified Student's t-test.

Figure B3. Liver cysts in *Taenia taeniaeformis*-infected rats. Top: Livers from offspring of rats immunized with a liver homogenate containing 5 day old parasites. Bottom: Livers from age-matched control rats.

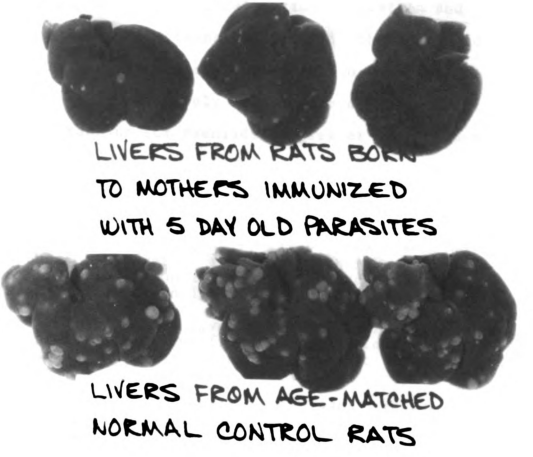
LIVERS FROM RATS BORN
TO MOTHERS IMMUNIZED
WITH 5 DAY OLD PARASITES
LIVERS FROM AGE-MATCHED
NORMAL CONTROL RATS

Figure B3

effectively immunize mice against heterologous challenge or calves against heterologous challenge with *T. saginata* (Lloyd, 1979; Rajasekariah, 1980). In addition, immunization of pregnant heifers with E/S antigens resulted in passive transfer of immunity against *T. saginata* to newborn calves (Lloyd, 1979). The localization, isolation and characterization of functional antigens in the *T. taeniaeformis*/rat system are therefore relevant to taeniiasis in general, and could ultimately lead to the development of an effective vaccine for taeniids of greater socioeconomic importance.

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