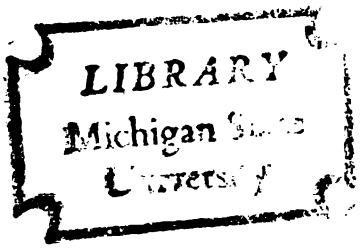
PERMEABILITY STUDIES ON TAENIID METACESTODES

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY
STEPHEN T. HUSTEAD
1976



ABSTRACT

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By

Stephen T. Hustead

Host immunoglobulins of several different classes were detected within the bladder fluids of *Taenia taeniaeformis, Taenia crassiceps,* and *Echinococcus granulosus*. Radioiodinated proteins were taken up *in vitro* by larvae of both *T. taeniaeformis* and *T. crassiceps* and were shown to retain their physicochemical and antigenic characteristics. Rates of uptake were similar in the two species and were not related to the molecular weight of the proteins. Immunoglobulins were taken up both *in vitro* and *in vivo* by larvae of *T. taeniaeformis*. Absorbed immunoglobulins were shown to retain both antigen binding capacity and biologic functions associated with the Fc portion of the molecules.

Not all cysts of *E. granulosus* contained detectable host proteins. This may be attributable to proteolysis within the bladder fluid, since uptake of I^{125} occurred when hydatid cysts were exposed to labeled proteins *in vitro*, but rapid degradation of the labeled carrier led to the appearance of dialyzable fragments.

Incubation in immune rat serum (IRS) was shown to increase the rate of absorption of I^{125} RNase-A but not I^{125} BSA by larvae of

T. taeniaeformis and *T. crassiceps*. This effect required a heat labile factor in serum, and partial activity could be restored in heat treated IRS (HI-IRS) by adding normal rat serum (NRS) as a source of complement (C'). In addition, the effectiveness of IRS in altering permeability was shown to be dependent on the available concentration of functional C'. Both live and dead larvae incubated in NRS rapidly depleted hemolytic C' levels in the surrounding medium.

Immunoglobulin fractions from IRS separated by anion exchange chromatography and gel filtration were tested in the presence of excess complement for their ability to increase uptake of I^{125} RNase-A. Enhanced permeability was observed in larvae incubated in each fraction.

It appears that taeniid metacestodes are capable of absorbing a variety of proteins without demonstrable loss in their structural or functional integrity following transport. However, this absorptive capacity, which most likely accounts for the presence of host serum components within the bladder fluids, can be altered *in vitro* by incubating larvae in antibody and complement. The observation that larvae restore normal control with time suggests that they avoid this immunologic effector mechanism *in vivo* by interfering with complement function.

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A THESIS

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

MASTER OF SCIENCE

Department of Microbiology and Public Health

1976

Dedicated to

Saundra

Her Spirit has been my inspiration

"Life is but
a tiny
piece
of our soul's
link
with eternity"

VKA

ACKNOWLEDGEMENTS

I wish to express my sincere appreciation to the Department of Microbiology and Public Health, Michigan State University, for the assistantship awarded to me during the course of my studies.

The advice, encouragement, and careful consideration in all aspects of this work by my guidance committee, Drs. Donald Twohy, Gordon Carter, Richard Patrick, and Ralph Costilow, are gratefully acknowledged.

I have particularly enjoyed the professional and social experiences with my fellow students, Drs. Antony Musoke, Roger Cook, Bruce Hammerberg, Wes Leid, Ashraf Ansari, Joseph Ayuya, Mr. John Picone, Ms. Anna Zajac, and Ms. Martha Calkins, which have led to lasting friendships, worldwide. I am extremely grateful for the expert technical assistance rendered by Ms. Marla Signs during each phase of this study.

Above all I wish to express my sincere appreciation to Dr. Jeffrey F. Williams, my major advisor. His enthusiasm, encouragement, and personal concern shown each of his students are an inspiration to us all.

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INTRODUCTION

Cysticercosis is characterized by infection with the larval stages of tapeworms belonging to the family *Taeniidae*. Although this group of diseases does pose a public health threat to man in endemic regions, they have become, above all, a major economic concern in most of the developing nations of the world, where well over 50 percent of the bovine carcasses show signs of infection. The legal sale of infected carcasses is prohibited, and thus cysticercosis has severely curtailed the growth of a profitable meat industry in these nations.

No chemotherapeutic agents specific for larval tapeworms have been marketed to date. Recently some promising agents have emerged, and the results from preliminary field studies suggest that satisfactory drug therapy may be possible shortly. However, because of the lack of specific treatment, a variety of methods of control have been attempted, including extensive programs in health education and increased control and tighter restrictions on meat inspection processes, each with limited effectiveness. Previously successful methods of control of other diseases were achieved following extensive investigation of the immunologic processes associated with infection. It is likely that such an approach is warranted with cysticercosis. However, at present even the basic biology of taeniid parasites is poorly understood, and must receive

more attention by trained investigators. The use of large domesticated animals in these studies is prohibited by cost, but infection with taeniid metacestodes does occur in small rodents and these parasite systems may serve as experimental models of cysticercosis. This study was designed to firstly identify the host immunoglobulin classes present in bladder fluid obtained from taeniid metacestodes, and then to conclusively demonstrate the origin of these macromolecules. Further studies were concerned with examining the effects of the immune response on permeability.

The literature review has been organized to provide the necessary background information considered crucial for the understanding of the work reported herein. The first section deals initially with the medically important taeniid parasites of man, and is followed by a comprehensive review of the experimental model systems used for investigative purposes. The second section deals with the immune responses associated with infection by taeniid organisms, including the current theories concerning concomitant immunity, a phenomenon frequently observed in helminth infections. The following section deals with membrane permeability and the theoretical models of small and large molecule transport. In the last section the mechanisms of transport associated with the Cestoda are reviewed in detail.

LITERATURE REVIEW

Biology of Taeniid Infections

In 1947 Stoll estimated that well over 40 million people in the world, particularly inhabitants of Asia, Africa, and the U.S.S.R., were infected with one of the two medically important species of the family *Taeniidae: Taenia solium* or *Taenia saginata*. Domestic animals, including the cow and the pig, are the primary intermediate hosts in these cyclo-zoonotic infections. Due to the increasing prevalence in food animals, taeniiasis has become an important economic problem affecting especially the less developed nations, which are prevented from exporting their contaminated meat products. In this section the biology of the two major human pathogens is reviewed, followed by a detailed account of the experimental models used in laboratory investigations.

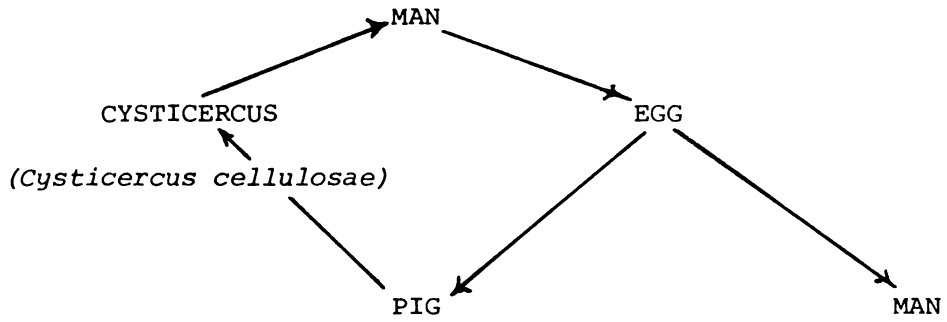
Parasites of Man

Taenia solium

Taenia solium, commonly referred to as the "pork tapeworm", resides as an adult cestode in the lumen of the small intestine of man, and the pig serves as the intermediate host. Although the parasite is cosmopolitan in distribution, the highest prevalence of infection is recorded wherever man consumes raw or partially cooked pork. The most afflicted endemic areas currently include the Slavic

countries, Latin American countries, Mexico, and North China (Soulsby, 1968).

Life Cycle



Man is the only definitive host and is readily infected by ingesting the cysticercus in inadequately prepared pork. However, humans are also susceptible to larval infection, commonly resulting from ingestion of eggs in contaminated food, or by the process of reverse peristalsis whereby eggs in the bowel are carried upwards to the duodenum and are stimulated to hatch.

The ingested eggs hatch following disruption of the embryophore, composed of keratinized blocks. Hexacanth embryos surrounded by only a single limiting membrane are liberated into the intestine and become activated before penetrating the mucosa. Little is known regarding the hatching and activating conditions for *T. solium*. Following penetration the oncospheres are carried via the blood to the skeletal musculature where they develop into mature cysticerci, called *Cysticercus cellulosae*. The mechanism of penetration is not known, but several investigators have proposed that enzymatic digestion occurs during this process, leading to the development of clear refractile zones surrounding oncospheres observed in

histologic sections of the intestinal wall (Silverman and Maneely, 1955; Heath, 1971).

Adult worms reside in the small intestine and may attain a length of several meters. The muscular scolex bearing four suckers and a double crown of prominent hooks functions to secure the worm to the mucosal surface. Large gravid segments are passed in the stool, and each may contain up to 40,000 eggs. Segments of *T. solium*, unlike those of *T. saginata*, are inactive in the feces, and eggs are not dispersed. This may perhaps explain the frequently acquired massive infections in pigs (Soulsby, 1968).

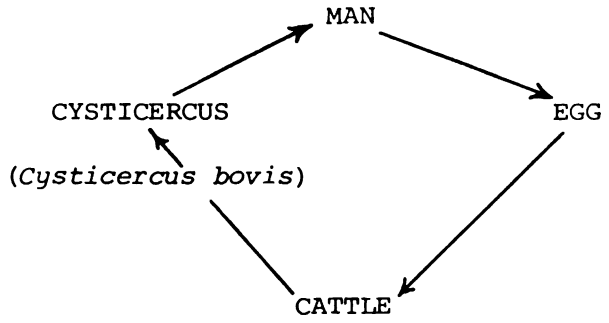
Pathology. For the most part, infection with the adult worm is asymptomatic. Occasionally, mucosal irritation or intestinal obstruction occurs where the worm burden is heavy. Clinical problems more commonly result from infection with the larval stage, resulting from either ingestion of eggs or reverse peristalsis. Since the infected patient is a constant source of viable eggs, he is both a danger to himself and to those with whom he comes in contact.

Cysticerci may be found in every organ of the body of man, but are primarily encountered in the subcutaneous tissues, the muscles, and the eye. Occasionally larvae develop in the brain. Surprisingly, little pathologic reaction occurs around the living parasite, but when the organism dies, intense tissue reaction follows and a variety of neurological symptoms may develop, including epilepsy, incoordination, transient paresis, meningoencephalitis, and failing vision (Faust *et al.*, 1970).

Taenia saginata

Taenia saginata, referred to as the "beef tapeworm", has a world-wide distribution, occurring most commonly in Africa, Asia, and the U.S.S.R. In contrast to *T. solium*, infection with *T. saginata* is frequently encountered in the United States, particularly in the Southwest.

Life Cycle



The life cycle of *T. saginata* is very similar to that of *T. solium*. The adult worm resides in the small intestine of man, who acquires the infection from the ingestion of raw or insufficiently cooked beef. The preference for very rare steak is one important feature of successful transmission in the United States. Embryonated eggs passed in the feces of man are ingested by cattle and result in the eventual development of infective cysticerci. Fortunately, man is not susceptible to the tissue phase of this parasite. The bladder form, termed *Cysticercus bovis*, is usually found in the intermuscular fascial layers surrounded by a connective tissue capsule. Larval viability is maintained from 4-6 months, at which time the cysticerci begin to degenerate and eventually die by the ninth month of infection.

Adults of *T. saginata* in man attain lengths exceeding twenty meters. The scolex bears four muscular suckers but, unlike *T. solium*, the armed rostellum is absent, and this alone may serve to differentiate the two species. Generally the gravid proglottids of *T. saginata* are longer than those of *T. solium*, and each may contain nearly 100,000 eggs.

Pathology. In view of the fact that *T. saginata* may reach lengths in excess of 20 meters, about three times as long as the entire human intestine, it is not difficult to understand that disruptive effects on normal digestive tract functions frequently occur following infection with this worm.

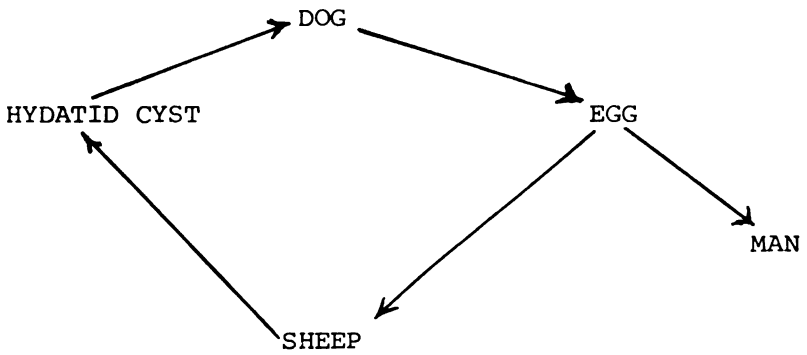
Since the majority of cysticerci die by the ninth month of infection, little clinical disease is manifested in infected cattle under normal conditions. The presence of cysticerci in beef carcasses results in a serious economic loss, particularly in developing nations. Successful treatment of infected persons is possible with various taeniicidal agents. Unfortunately, because of unsatisfactory control over meat inspection procedures, reinfection is inevitable. Until recently no chemotherapeutic agent specific for larval tapeworms was available. However, as a result of a number of intensive testing programs, primarily with laboratory models, some promising agents have emerged. Although the majority of these drugs are still in preliminary trial stages, successful drug therapy may become feasible in the future.

Parasites of Man and Laboratory Animals

Echinococcus granulosus

Adults of *Echinococcus granulosus* inhabit the small intestine of a number of wild carnivores, particularly the dog. Man is a frequent accidental intermediate host, chiefly in the endemic regions of Africa, South America, Australia, and New Zealand, but transmission normally occurs through a variety of herbivorous mammals.

Life Cycle



The minute adult tapeworm is seen only in dogs and other *Canidae*, and cannot develop in man. The worm measures between 3 and 6 mm and possesses a scolex, neck, and usually three proglottids: one immature, one mature, and one gravid segment. The ova released in the feces are indistinguishable from those of other taeniid species.

Intermediate hosts, including domestic and wild animals, and man are infected by ingestion of the eggs. Eggs hatch and activate in the small intestine, penetrate the mucosa, and enter the blood stream to be carried to internal organs. The hydatid cyst consists of an external acellular and internal germinative membrane similar

to that of adult cestodes (Bortoletti and Ferretti, 1973). Brood capsules attached to the germinative layer develop internally. They may detach from this layer and, upon rupture of the cyst wall, they are expelled and develop into daughter cysts.

Laboratory investigations with the larvae of *E. granulosus* have been limited to some extent by the high cost involved in maintaining adequate numbers of domestic herbivores. Inoculations of scolices or brood capsules have been used in attempts to propagate the organism in mice and rabbits (Deve, 1933, 1935), but more satisfactory growth rates have been attained by serial intraperitoneal implantation of the cysts into rats and gerbils (Varela-Diaz *et al.*, 1974).

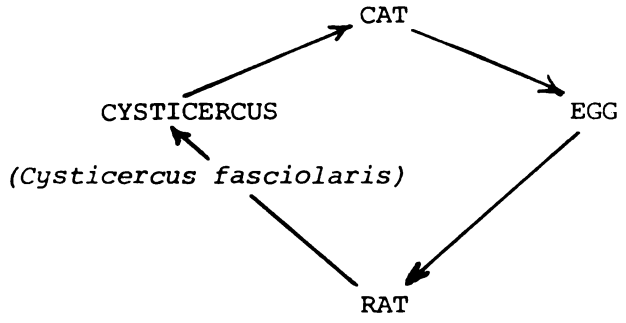
Pathology. Infection with the adult form is usually asymptomatic. The hydatid cyst grows slowly but steadily and may after years contain liters of fluid. The site of cyst development determines the rate of growth, but often serious functional impairment of vital structures results. This is of prime importance in the pathogenesis of hydatid disease. In addition, traumatic hydatid cyst rupture may provoke anaphylactic-like reactions which can be fatal. To date surgical intervention is the only available treatment, and this is possible only when cysts are located in operable sites. It is essential that dogs be prevented from feeding on animal carcasses in endemic regions if infection rates are to be limited in man and domestic animals.

Parasites of Laboratory Animals

Taenia taeniaeformis

The adult form resides in the small intestine of the cat and related carnivores, including the stoat, fox, and lynx, and is of cosmopolitan distribution. Rodents, chiefly rats and mice, serve as the intermediate hosts.

Life Cycle



The bladderworm stage, *Cysticercus fasciolaris*, occurs in the livers of the intermediate hosts. The original account of larval development from the egg was provided in 1855 by K. G. F. R. Leuckhart, and further details were given by Raum in 1883. As with all taeniid ova, the oncospheres hatch in the intestine, penetrate the intestinal tissue, and enter the circulatory system. Unfortunately, the invasive mechanism is poorly understood. There is some evidence that carbohydrate complexes in the intestinal mucosa are broken down in the area of oncospheral penetration (Banerjee and Singh, 1969), but at the moment all proposed modes of invasion are clearly speculative. Each larva which reaches the liver becomes encapsulated by dense proliferative fibrous tissue. Mast cells and eosinophils are

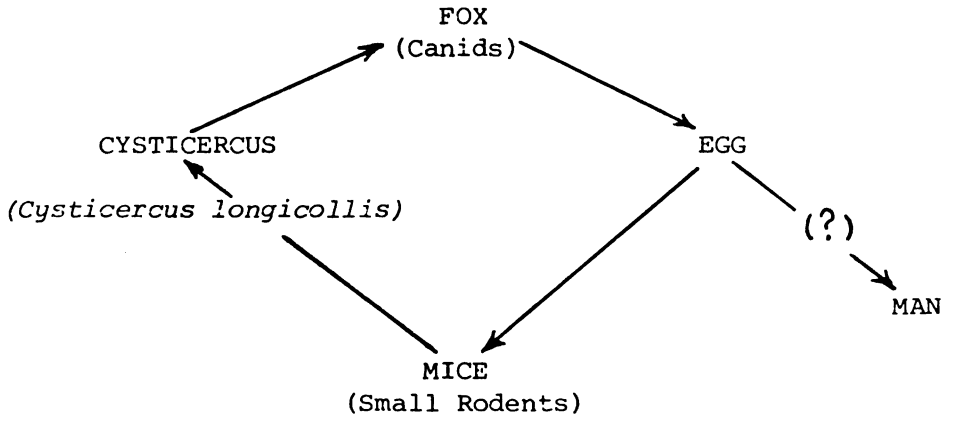
irregularly distributed throughout the fibrous capsule in the cyst-wall (Varute, 1971). Within this cyst wall the larva develops to a segmented strobilate stage in which the scolex has evaginated, giving it the appearance of a small tapeworm. Upon ingestion by the definitive host the strobila and attached scolex develop within 6 weeks to an adult tapeworm.

Pathology. Occasional digestive disturbance has been described. In addition, the scolex of the adult may become deeply embedded in the mucosa of the small intestine, leading to perforation. Although the cysticercus appear to be harmless even in large numbers, infection is believed to predispose rats to the development of a variety of hepatic tumors, described by Bullock and Curtis (1920).

Taenia crassiceps

Adult *Taenia crassiceps* frequently inhabit the intestine of the red fox (*Vulpus vulpes*) in Europe and the arctic fox (*Alopex lagopus invitus*) in North America. The metacestode, *Cysticercus longicollis*, commonly infects small rodents and lemmings (*Dicrostomyx groenlandicus*) (Rausch, 1952; Freeman, 1962). Recently the first human infection was described when a cysticercus, surgically removed from the eye of a Canadian woman, was positively identified as *T. crassiceps* (Shea *et al.*, 1973; Freeman *et al.*, 1973).

Life Cycle



In nature small rodents are infected by ingestion of the eggs passed in the feces of the fox or related Canids, who have previously eaten rodents infected with the developing metacestode. Typically the ingested eggs hatch to oncospheres in the intestine and then migrate to the subcutaneous tissues or the peritoneal cavity. Infectivity is achieved within 2 months. In the definitive host at least 5-6 weeks are necessary for development to a mature egg-producing adult to be completed.

In the laboratory experimental infection has been achieved in the dog. The metacestode stage has been maintained in white mice by serial intraperitoneal transfer for generations, but there is an associated loss of infectivity for dogs (Freeman, 1962). Numerous investigators have attempted to explain the morphologic, reproductive, and antigenic abnormalities which have been ascribed to the non-infective or ORF strain (Mount, 1968; Fox *et al.*, 1971). Data seem to substantiate the hypothesis that these features result from a genetic mutation in the ORF strain (Dorais and Esch, 1969; Smith *et al.*, 1972).

Pathology. Other than a few reports of enteritis and digestive disturbances in dogs, little is known about the pathology of *T. crassiceps* in either the definitive or intermediate hosts.

In the only reported human case partial visual function was restored upon surgical excision of the parasite from the retina. Interestingly, the family's pet dog was incriminated as the reservoir host pointing to yet another instance of taeniiasis as a public health problem. Perhaps the simplicity of maintaining this parasite in the laboratory combined with the recent report of human infection will arouse renewed interest in this rather neglected host-parasite system.

The Host-Parasite Relationship

In this portion of the literature review, a general discussion of the early and recent investigations in cestode immunology is presented with emphasis on the *Taenia taeniaeformis* system. This is followed by a detailed review of the mechanisms proposed to account for the phenomenon of concomitant immunity which characterizes many helminth infections, including cysticercosis.

Immunologic Responses to Infection with Taeniid Parasites

Early investigations in cestode immunology began with the work of Miller (1931a), who demonstrated conclusively that rats infected with *Taenia taeniaeformis* are resistant to super-infection. Further experimental work definitively established the role of antibody in protection against this infection. Resistance was shown to be passively transferable with serum, and was transferred naturally

from mother to young (Miller and Gardiner, 1932, 1934; Miller, 1931b, 1932, 1935; Campbell, 1936, 1938a,b,c). Successful artificial immunization procedures were also developed (Miller, 1931b; Campbell, 1936). Immunity to *T. taeniaeformis* and *T. crassiceps* was successfully stimulated by implantation of live parasites or extracts (Miller, 1932; Freeman, 1962). Vaccination with extracts of *T. pisiformis* has also been shown to produce detectable immunity in rabbits (Kerr, 1934). However, a greater degree of immunity is generally established when animals are vaccinated with live material. Similar findings have been reported for *Hymenolepis nana* in mice (Kerr, 1935; Hearin, 1941).

Leid and Williams (1974a) extended these findings by demonstrating that in the rat antibodies of a single immunoglobulin class (7Sγ2a) are primarily responsible for the passive transfer of resistance to *T. taeniaeformis*. Experimental infection was then studied in the mouse system and again antibodies from one immunoglobulin class (7Sγ1) were shown to mediate resistance (Musoke and Williams, 1975a). It should be noted that mouse 7Sγ1 immunoglobulins appear to have analogous biologic functions to rat 7Sγ2a immunoglobulins. In contrast, Musoke and Williams (in press) have recently demonstrated that immune serum fractions containing 7Sγ1 and γM immunoglobulins from rats infected with *T. taeniaeformis* by intraperitoneal implantation were most effective in passive transfer.

Miller's (1935) finding that immunity to *T. taeniaeformis* could be transferred from mothers to their young was recently substantiated by Musoke, Williams, Leid, and Williams (1975). In addition, they demonstrated that the protective activity resided in the γA rich

fraction of immune colostrum. Although colostrum mediated transfer of resistance has been reported in *T. hydatigena* and *T. ovis* infections, this appears to be the first report incriminating γA (Gemmell, Blundell-Hasell, and Macnamara, 1969; Rickard and Arundel, 1974).

The mechanism of action of protective antibodies is unknown, but there is some evidence that immune destruction occurs prior to encystment and that the intestine plays a vital role in the acquired resistance to super-infection (Leonard and Leonard, 1941; Froyd and Round, 1960). Histological studies have revealed that epithelial cells may be lysed by penetrating oncospheres as they move into the lamina propria en route to the liver via the blood circulatory system (Banerjee and Singh, 1969; Heath, 1971). Recently Musoke and Williams (1975b) have presented experimental evidence demonstrating the dependence of antibody on other humoral factors to achieve effective killing of invading oncospheres. They showed that prior to the 5th day of infection antibody mediated attack required an intact complement system in the host. The only other attempt to implicate complement in immunity to helminth infections was unsuccessful. Jones and Ogilvie (1971) failed to demonstrate the active involvement of complement in the phenomenon of worm expulsion by rats infected with *Nippostrongylus brasiliensis*.

There have been few studies on the effects of antibody on the physiology and metabolism of either protozoan or metazoan parasites. Incubation in immune serum apparently reduces oxygen consumption in *Trypanosoma vivax* and *T. lewisi* (Desowitz, 1956; Lincicome and Hill, 1965), disrupts protein and DNA synthesis in *T. lewisi* (Taliaferro and Pizzi, 1960), and inhibits amino acid incorporation

into protein by extracellular *Plasmodium knowlesi* (Cohen and Butcher, 1970). Murrell (1971) has presented some evidence that antibody-mediated complement dependent reactions were responsible for disrupting membrane permeability *in vitro* in larvae of *T. taeniaeformis*. More recently Dean *et al.* (1974) presented evidence that killing of schistosomula of *Schistosoma mansoni* by antibody and heat labile serum factors was greatly enhanced by the addition of polymorphonuclear leukocytes. Later they showed that although eosinophils and macrophages did not increase the rate of killing, they did react with schistosomula that had previously been damaged or killed by antiserum (Dean *et al.*, 1975).

It is becoming clear that in a number of parasitic infections acquired immunity may depend on the combined efforts of several immunological effector mechanisms, both humoral and cellular. Further studies characterizing these mechanisms are essential if the phenomenon of concomitant immunity is to be understood. Parasite induced inhibition of effector systems should not be overlooked as a contributing factor in this process.

Proposed Mechanisms of Survival of Tissue Parasites

As advances were made in the understanding of cestode immunology a number of hypotheses were proposed to account for the resistance of the developing tissue phases in immune animals to inherent host defense mechanisms.

Although concomitant immunity occurs in many helminthiases, this is a particularly perplexing aspect of taeniid metacestode infections since protective immunity is antibody mediated, yet

immunoglobulins appear to be present in and on the cystic larval forms surviving in the tissues. Chordi and Kagan (1965) first demonstrated that host-like proteins including immunoglobulins are present in hydatid cyst fluid. Further studies showed that immunoglobulins antigenically similar to host molecules could be extracted from cyst membranes (Varela-Diaz and Coltorti, 1973). In recent work attempts to determine the origin of these molecules have been inconclusive. Although their role in survival of the organsim is not at all clear, the fact that host or host-like plasma proteins are present is now well established and mechanisms proposed to explain the resistance of larval tissue phases in otherwise immune hosts must take this into account. Four major philosophies have evolved to explain concomitant immunity as follows:

A.) Molecular mimicry is the term applied to the occurrence of antigenic sharing between host and parasite as a result of evolutionary adaptation influenced by natural selection (Sprent, 1962; Dineen, 1963; Damian, 1964). Initially Sprent advanced the hypothesis that as both parasites and hosts adapt during evolutionary development they undergo reciprocal changes leading to the emergence of host specificity. It is postulated that as the host immune system evolved, those parasites were selected for an antigenic mosaic similar to that of the host. However, Dineen realized that following the principles of natural selection parasite antigens capable of stimulating resistance in the host would also be selected. In 1964 Damian was credited with unifying the general thoughts on this process of evolutionary adaptation and formally presenting it as a hypothesis of parasite survival.

B.) In the host induction hypothesis it is proposed that the parasite becomes able to synthesize macromolecules antigenically identical to host components, and incorporate them into external surfaces (Capron *et al.*, 1968). It has been pointed out that if a parasite were capable of responding to inductive stimuli by formation of host components an extraordinary proportion of the genome would be required to code for such a variety of antigenic determinants. For example, the presence of donor and recipient host-associated immunoglobulins has been revealed in hydatid cysts transplanted from mice to guinea pigs (Varela-Diaz and Coltorti, 1972). For the host induction hypothesis to be valid the parasite would either have to be capable of reverse transcription and reverse translation, although the latter has never been demonstrated in any model system, or contain an unusually diverse genome comprised of genes coding for various serum proteins antigenically identical to those found in the array of possible hosts. Nevertheless, recent findings reported by Capron and his co-workers have been interpreted in support of this hypothesis. They demonstrated uptake *in vitro* of radiolabeled isoleucine and lysine by *Schistosoma mansoni* and incorporation into host-like antigens recovered from the surrounding culture medium (Bout, Capron, Dupas, and Capron, 1974). This strongly suggests that indeed similar genetic codes are present in the parasite genome.

C.) Phenotypic adaptation differs from host induction in that host synthesized components are utilized and incorporated into external surfaces (Smithers and Terry, 1969). According to this hypothesis the invading parasite coats itself with antigenic determinants shed or actively cleaved from host cells. Host immune responses

are avoided since the parasite will antigenically resemble host tissue. Recent work with *Schistosoma mansoni* has provided most of the evidence in support of this view, but as yet conclusive and repeatable findings are lacking. In order to accept this hypothesis it is necessary to assume that parasites produce enzymes capable of cleaving membrane bound proteins and that their membranes contain receptors capable of binding these fragments in a similar configuration to self or host determinants.

D.) Bound host antibody, a hypothesis similar to phenotypic adaptation, was formally presented by Varela-Diaz *et al.* (1972) and supported in part by Rickard (1974). It is proposed that specific antibody produced against parasite antigens is responsible for neutralizing an effective immunologically mediated attack. Varela-Diaz *et al.* have proposed that two antibodies are involved, one neutral and one lethal. When both combine with the membrane bound antigenic determinants in juxtaposition, the resulting steric hindrance blocks the action of the lethal antibody.

Rickard (1974) could not accept the simplicity of Damian's molecular mimicry model. Indeed parasites and hosts have adapted during evolution to ensure mutual survival. This is well documented by the frequently encountered phenomena of concomitant immunity observed in numerous larval helminth infections. However, animals infected with developing larval forms have been shown to be insusceptible to super-infection. Should such a regulatory mechanism be lacking, larval forms would accumulate with lethal consequences. Conversely, the development of complete resistance with rejection of all forms would endanger the survival of the entire parasite

population. In an attempt to explain each of these phenomena Rickard proposed that invading embryos do elicit a significant immune response fully capable of mediating resistance, but that they develop rapidly to an antibody resistant stage before this specific immunoglobulin is produced in sufficient amounts. He further contends that the developing forms become coated with a second specific antibody which blocks the induction of cell mediated responses. This one antibody, one antigen proposal is somewhat analogous to the situation encountered in tumor resistance whereby neoplastic cells coated with specific antibody avoid cell mediated immunity.

To accept the feasibility of either of these hypotheses it will be necessary to demonstrate that developing tissue forms are capable of inducing formation of a variety of antibodies, some destructive, others neutral, or that other necessary humoral requirements such as complement are rendered ineffective.

Membrane Transport

The circumstances whereby host or host-like proteins appear within the fluid-filled cavities of taeniid parasites are susceptible to experimental analysis both *in vitro* and *in vivo*, and form the basis of much of the research which is presented in this thesis. A considerable body of evidence has accumulated regarding the characteristics of membrane transport systems in general and of cestode membranes in particular, and the conclusions are appropriately reviewed at this point.

Singer's fluid mosaic model of membrane structure is the currently accepted theory describing membrane organization (Singer and

Nicholson, 1972) and it proposes that membranes consist of a continuous phospholipid bilayer with randomly inserted protein molecules. Two major types of membrane proteins have been defined. Extrinsic proteins, such as spectrin of erythrocytes, can be removed from the membrane by increasing the salt concentration or upon addition of a metal ion chelating agent. Intrinsic proteins, on the other hand, can only be removed by organic solvents capable of destroying hydrophobic interactions. The model has numerous advantages in explaining observed phenomena. It permits for variations in lipid/protein ratios, the observed random movement of lipids and proteins, and the varying half-lives of protein and lipid molecules. In this portion of the literature review the theoretical models of membrane transport will be described. Emphasis is placed on the current models of protein uptake.

Theoretical Models of Small Molecule Transport

Generally three specific types of transport phenomena are recognized to explain small molecule uptake. Simple diffusion is merely the movement of a solute due only to the kinetic energy of the molecules. Movement occurs from a region of higher concentration to one of lower concentration. Usually in a diffusion system the rate of absorption versus the solute concentration will be a linear function. However, occasional anomalies do occur since the diffusion rate will depend to a certain extent on the physical and chemical properties of the membrane and particular solute in question. Diffusion requires no energy expenditure by the cell, and chemical stereospecificity is lacking.

A similar process termed facilitated diffusion also involves solute movement in relation to the prevailing concentration gradient. Again no energy is expended by the cell, but the process is stereospecific. Chemicals of similar structure will disrupt or inhibit the process. In facilitated diffusion the rate of diffusion characteristically follows saturation kinetics.

Active transport is identical to facilitated diffusion except that solute can be accumulated against a concentration gradient, and energy expenditure is required. The process is stereospecific and the rate of diffusion follows typical saturation kinetics. Included in this group of processes is the mechanism of co-transport, which involves carrier proteins in association with specific cations, and which has been used to account for transport of sugars through the villi of the small intestine. Specifically the binding of Na^+ ion is believed to enhance or increase the affinity of the receptor for the sugar. As the receptor-Na^+-sugar complex is moved into the cell the Na^+ ion dissociates due to a decrease in ion concentration, thus reducing the carrier affinity for the sugar.

With the transport of intact protein molecules numerous problems are encountered. Due to the size and molecular nature of membranes most transport processes are limited to small molecular weight substances and passage of large molecules or those of unusual conformational arrangement is prohibited. Consequently less conventional methods of transport have been postulated.

Theoretical Models of Protein Transport

Presently theorists recognize only one major method of protein absorption: pinocytosis, a process often termed cellular "drinking." Although similar to the process of phagocytosis carried out by many eukaryotic cell types, the actual mechanism of pinocytosis is poorly understood. The majority of studies have been carried out using amoebae, but recently significant contributions have been made in studies using erythroid cells, neural cells--especially at post-synaptic junctions, and fetal membrane and intestinal cells (Blanton *et al.*, 1968; Waxman and Pappas, 1969; Hayward, 1967). A charged molecule binding to a specific membrane receptor is necessary to initiate the cellular process. It has been proposed that once binding has occurred complex internal processes are activated within the cell leading to complete vesicle formation utilizing an original membrane fragment. A controversial and even less understood process of macromolecular uptake termed trans-membranosis was proposed by Tanaka (1962) to explain the absorption of vital stains in the lymph node cells of mice. He was unable to observe the typical vesicle formation until dyes had completely penetrated the cell membrane.

Particularly analogous to the question of uptake of plasma proteins by taeniid larvae is the transmission of immunity from mother to young. This involves immunoglobulin uptake by the yolk-sac membrane and through the neonatal intestine, and has been explored experimentally by Brambell and co-workers. Transmission of immunity occurs entirely before birth in the rabbit and early studies revealed that uptake occurred through the uterine lumen and

the yolk-sac, and not by way of the placenta (Brambell *et al.*, 1949). Conversely, in the rat the greater part of immunity is transmitted after birth and throughout most of lactation (Halliday, 1955b). Brambell's group has shown conclusively that the small intestine of the suckling rat is capable of absorbing intact antibodies present in the colostrum and milk of the mother (Brambell, 1958, 1966, 1970; Rodewald, 1973). The process of uptake shows a high degree of selection for homologous IgG immunoglobulins which are transported intact (Halliday and Kekwick, 1960), whereas heterologous gamma globulin may undergo changes with complete loss of functional activity in the process (Brambell *et al.*, 1961).

A hypothesis concerning the mechanism of transmission in the small intestine, which seems to be consistent with most of the observations, was proposed by Brambell (1958) and supported by Rodewald (1973). Uptake is believed to take place by pinocytosis. Vesiculation occurs between the bases of the microvilli at the apical pole of the cell. Each vesicle, which is derived or was at least in continuity with the cell membrane, has a limited number of specific immunoglobulin receptors. Attachment to these receptors protects the protein from lysosomal degradation. Experimental findings have shown that only a fraction of the protein absorbed is transmitted to the circulation intact. In fact, further studies indicate that as protein concentration increases a greater percentage of the absorbed protein is degraded while the amount released from the cell remains constant, suggesting that receptors do become saturated. Presumably these receptors have the greatest affinity for homologous gamma globulin, and since the Fc portion alone is

readily transmitted, it is believed to be the critical determinant. Release to the cellular interstitial fluid supposedly occurs through a process of reverse pinocytosis, similar to that followed by the endoplasmic reticulum in secretion of newly synthesized protein.

Membrane Permeability Associated with the Cestoda

Of the four major groups of helminth parasites the Cestoda have been studied most extensively. Unlike the trematodes and nematodes, cestodes lack a digestive system, and therefore all life-sustaining nutrients must be absorbed through the external tegument. Ultrastructurally the parenchymal covering is in the form of microvilli, distinctly resembling the mammalian intestinal brush border. This modification functions to increase the absorptive surface area.

Considerable work has been concerned with the uptake of sugars and amino acids by a number of larval and adult cestodes in hopes of gaining a basic understanding of the biochemistry and physiology of these parasites. Unfortunately, until the recent demonstration by Chordi and Kagan (1965) that intact "host-associated" proteins exist in hydatid fluid, very little work was done concerning the phenomena of macromolecule uptake. In this section the investigations in three major areas of study concerning membrane permeability and transport in the Cestoda will be reviewed.

Uptake of Sugars

Since the demonstration that specific cations are necessary for sugar transport through the villi of the small intestine in mammals, considerable evidence has accumulated indicating that mediated

processes are responsible for sugar transport in numerous cell types in higher animals. Recent work in lower metazoans, including tapeworms, suggests that similar mechanisms occur at this level.

In 1966 von Brand and Gibbs first demonstrated Na^+-dependent glucose transport in *Taenia taeniaeformis*. Subsequently identical findings were published for *Hymenolepis diminuta* (Dike and Read, 1971; Read *et al.*, 1974), *Calliobothrium verticillatum* (Fisher and Read, 1971; Pappas and Read, 1972a), *Hymenolepis microstoma* (Pappas and Freeman, 1975), and for larvae of *Taenia crassiceps* (Pappas *et al.*, 1973a). Their collective findings strongly suggest that glucose transport is inhibited in all cell types by the presence of phlorizin or ouabain in the medium. An apparent contradiction to this hypothesis was reported for the adult blood fluke, *Schistosoma mansoni* by Isseroff *et al.* (1972). They proposed that there may not be a common sugar transport system for flatworms as there appears to be in vertebrates. However, this has apparently been refuted by Uglem and Read (1975), who were able to demonstrate sensitivity to both of these inhibitors, further substantiating the similar nature of sugar transport in a wide variety of cell types.

Recently some interesting findings have been reported concerning the uptake of other hexoses. Fructose has been shown to enter larvae of *T. crassiceps* by diffusion, while galactose appears to be taken up by a combination of diffusion and a mediated process. In addition inhibitor studies indicate that glucose and galactose are mutually competitive inhibitors (Pappas *et al.*, 1973a).

Clearly studies involving a number of parasites have contributed significantly to the understanding of sugar transport. Because of

their ease of maintenance and value as a laboratory model, helminth parasite systems will continue to be used in future studies concerning the *in vivo* and *in vitro* processes of sugar absorption, particularly at the molecular level.

Uptake of Amino Acids

A great deal of work has been published concerning amino acid absorption in a variety of cestode species. The early experiments exploring the possibility of mediated transport mechanisms were carried out using *Hymenolepis diminuta* (Daugherty, 1957a,b; Daugherty and Foster, 1958). Since then the transport mechanisms for a large number of amino acids have been described for *H. citelli* (Senturia, 1964), *Calliobothrium verticillatum* (Read *et al.*, 1960a,b), and *Taenia crassiceps* (Haynes and Taylor, 1968; Hayes, 1970; Pappas and Read, 1973). Each of the three classical mechanisms of membrane transport has been incriminated at one time or another to explain the mode of uptake for specific amino acids. Often the solute concentration appears to determine whether uptake will occur through diffusion or by a combination of diffusion and a mediated process, e.g., methionine, phenylalanine, and lysine uptake by larvae of *T. crassiceps* (Pappas and Read, 1973). Uptake of other amino acids occurs solely through active transport processes, e.g., methionine uptake by *H. diminuta* (Pappas *et al.*, 1974).

It should be emphasized that the amount of data accumulated pertaining to amino acid transport in cestodes is far too vast and complex to be adequately covered in this review. Instead the basic considerations will be discussed. In general, research findings

reveal that the amino acid transport systems of the Cestoda are similar in many respects. Each species possesses more than one mechanism of uptake. Their transport systems appear to differ from the generalized mammalian systems in two respects. Firstly, they appear to lack stereospecificity. While the mammalian system has a much greater affinity for L-amino acids, cestode systems appear to absorb each equally, or occasionally even demonstrate a high affinity for D-isomers (Arms and Coatis, 1973). Secondly, cestode transport systems apparently lack the mechanism of ion-coupled active transport. As with sugar absorption, the mammalian transport system frequently utilizes the coupling of amino acids to the movements of cations (Pappas *et al.*, 1974). The co-transported ions are maintained at low intracellular levels through an active extrusion process often termed "pumping." The organic solute and ions move in relation to the prevailing concentration difference in the co-transported ion, and since this ion is actively removed transport will continue and solute will be accumulated.

Uptake of Macromolecules

Since the demonstration of host-associated proteins in hydatid fluid and the realization of the significance of this finding to the understanding of the phenomenon of concomitant immunity associated with cestode infections numerous investigators have sought to explain the mechanism of uptake. Most workers agree that there appear to be only two possible sources of proteins which accumulate in the bladder fluid of taeniid metacestodes. They are either synthesized in the parenchyma by the parasite and leaked into or stored

in the fluid, or they are of host origin. Unfortunately results from studies designed to determine the origin have been conflicting and inconclusive.

Rothman (1967), for example, sought to explain observed colloid transport in adult *Hymenolepis diminuta*. Unable to find evidence of pinocytosis he turned to a previously proposed process of transmembranosis described by Tanaka (1962). However, his results were effectively refuted by Lumsden and his co-workers (1970) and Rothman himself admitted that he had erroneously interpreted his electron microscopic photographs.

Later Esch and Kuhn (1971) described significant uptake of ^{14}C-chlorella protein by *T. crassiceps*. They associated this uptake with the presence of canals observed in sections of the larval membrane by light microscopy. However, they failed to demonstrate that the proteins were maintained intact following transport, and the tegumentary canals have not been detected by others.

In 1973 Pappas and Read questioned these results when they were unable to demonstrate uptake of inulin, an uncharged molecule similar in molecular weight to small proteins, by larvae of *T. crassiceps*. Because of the discrepancy in findings they indicated that further study was necessary but that their data suggested that intact protein uptake by larvae of *T. crassiceps* was highly improbable. Since inulin is an uncharged molecule, it has been argued that it is inappropriate to correlate the mechanism of inulin uptake with that of charged proteins. In general it has been shown that induction of pinocytosis is dependent on ionic charge. Specifically, proteins are most effective in activating the cellular mechanisms of

pinocytosis at environmental pH values which are on the cationic side of their isoelectric points (Brandt and Freeman, 1967).

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ARTICLE 1

PERMEABILITY STUDIES ON TAENIID METACESTODES: I. UPTAKE OF PROTEINS BY LARVAL STAGES OF *TAENIA TAENIAEFORMIS*, *TAENIA CRASSICEPS*, *ECHINOCOCCUS GRANULOSUS*

Permeability Studies on Taeniid Metacestodes: I. Uptake of proteins by larval stages of *Taenia taeniaeformis*, *Taenia crassiceps*, *Echinococcus granulosus*

SHORT TITLE: Protein Uptake by Taeniid Metacestodes.

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ABSTRACT

Host immunoglobulins of several different classes were detected within the bladder fluids of *Taenia taeniaeformis*, *Taenia crassiceps*, and *Echinococcus granulosus*. Radioiodinated proteins were taken up *in vitro* by larvae of both *T. taeniaeformis* and *T. crassiceps* and were shown to retain their physicochemical and antigenic characteristics. Rates of uptake were similar in the two species and were not related to the molecular weight of the proteins. Immunoglobulins were taken up both *in vitro* and *in vivo* by larvae of *T. taeniaeformis*. Absorbed immunoglobulins were shown to retain both antigen binding capacity and biologic functions associated with the Fc portion of the molecules.

Not all cysts of *E. granulosus* contained detectable host proteins. This may be attributable to proteolysis within the bladder fluid, since uptake of I^{125} occurred when hydatid cysts were exposed to labeled proteins *in vitro*, but rapid degradation of the labeled carrier led to the appearance of dialyzable fragments.

We conclude that taeniid metacestodes are capable of absorbing a variety of proteins, and that these macromolecules can retain their structural and functional integrity following transport. This absorptive capacity accounts for the presence of host serum components within bladder fluids.

INTRODUCTION

The fluid-filled bladders of taeniid metacestodes of several species have been shown to contain proteins physicochemically and antigenically identical to those present in host plasma (Chordi and

Kagan, 1965; Esch, 1964; Coltorti and Varela-Diaz, 1972). A number of hypotheses have been put forward to account for their presence (Damian, 1964; Capron *et al.*, 1968; Coltorti and Varela-Diaz, 1972) but experimental investigation of the phenomenon has produced conflicting results.

Coltorti and Varela-Diaz (loc. cit.) were able to demonstrate species specific determinants on immunoglobulin molecules in hydatid cysts of *Echinococcus granulosus* from a variety of hosts, and proposed that macromolecules entered the cysts by means of simple diffusion. Esch and Kuhn (1971) reported that larvae of *Taenia crassiceps* were able to take up whole C^{14} *Chlorella* protein and tentatively linked their findings to the occurrence of membranous pores which had been observed by light microscopy. However, Pappas and Read (1973) disputed the existence of pores and after failing to demonstrate uptake of intact C^{14} inulin, concluded that passage of entire macromolecules into larvae of *T. crassiceps* was very improbable.

An impressive degree of acquired immunity to infection by taeniid larvae has been shown to develop, and a conclusive demonstration of the origin of host or host-like proteins present in the bladder fluid of larvae growing in immune animals is essential if the mechanism of concomitant immunity in these infections is to be understood. In an attempt to characterize the relationship between permeability and the immune response, we have identified immunologically the host serum proteins present in cyst fluids of several taeniid metacestodes maintained in the rat and mouse, and report here

on the uptake of intact heterologous and homologous host proteins *in vitro* and *in vivo*.

MATERIALS AND METHODS

Maintenance of Parasites

The strain of *Taenia taeniaeformis* used in this investigation was maintained in Spartan (Spb:[SD]Br) rats and domestic cats as described by Leid and Williams (1974). The KBS strain of *T. crassiceps* was originally obtained through the generosity of Dr. G. W. Esch, Wake Forest University, Winston-Salem, North Carolina. The larvae were maintained in Carworth CF1 mice, and propagated by serial intraperitoneal injections of larvae as described by Freeman (1962). Hydatid cysts of *E. granulosus* were surgically implanted into the peritoneal cavity of rats and allowed to develop for periods ranging from 10 to 15 months (Varela-Diaz *et al.*, 1974).

Reagents

Proteins

Crystalline bovine serum albumin (BSA), bovine gamma globulins (BGG), and human gamma globulins (HGG) were purchased from Sigma Chemical Company (St. Louis, MO). A standard preparation of ribonuclease A (RNase-A) was obtained from Pharmacia Fine Chemicals (Piscataway, N.J.). Rat IgG_2 immunoglobulins were isolated from normal rat serum by DEAE-cellulose chromatography (Leid and Williams, 1974).

Four to five milligram quantities of rat IgG_2, BSA, and RNase-A were trace labeled with I^{125} by a slight modification of the

procedure of Helmkamp *et al.* (1960). Counting was performed in a Packard scintillation counter, and the values are expressed as counts per minute per microliter (cpm/µl) unless otherwise indicated.

Antisera

Anti-whole rat serum (anti WRS), and specific antisera for each immunoglobulin class in the rat were prepared as described by Leid and Williams (1974). Anti-mouse IgM and IgA were purchased from Meloy Laboratories, Springfield, Virginia. Rabbit anti-mouse IgG was prepared by immunization with a fraction purified by DEAE chromatography of normal mouse serum globulins precipitated with 40% ammonium sulphate. Sheep anti-rabbit IgG was similarly prepared.

Rabbit antisera were prepared against BSA and RNase-A by inoculating antigen solutions emulsified with an equal volume of Freund's complete adjuvant (FCA, Difco, Detroit). Injections of 0.5 ml portions were given intramuscularly in each hind leg, and 0.2-0.25 ml portions were inoculated subcutaneously in two sites along the back. Each rabbit received approximately 70 µg of antigen. Similar preparations were given as booster inoculations at 21 days and the rabbits were bled out 10 days later.

Anti-BGG and anti-HGG were prepared by sensitizing rats with a 1:1 suspension of antigen diluted in phosphate buffered saline (PBS), and homogenized in FCA. All animals received 250 µg of protein in the form of injections of 0.1 ml of the emulsion in each hind foot pad. Serum was harvested 14 days later.

Experimental Techniques

Immunoelectrophoresis (IEP) and Double Immunodiffusion (DID)

Immunoelectrophoresis and double immunodiffusion were performed following the method described by Leid and Williams (1974).

Polyacrylamide Gel Electrophoresis (PAGE)

Polyacrylamide gel electrophoresis was performed following the method of Weber and Osborn (1969). A 1% solution of aniline blue black was used to stain separated protein bands. All standard protein solutions were assayed for purity by PAGE prior to iodination.

Radioimmunoelectrophoresis (RIE) and Autoradiography

The presence of radioiodinated protein was detected in bladder fluid samples by radioimmunoelectrophoresis. Immunoelectrophoresis of the concentrated fluids was performed as described above. Two plexiglass plates were used to secure the dried, unstained IEP slides in position on the Kodak No-screen X-ray film. Exposure times were varied depending on the sample source, but never exceeded one half-life. The film was processed according to routine X-ray film development procedures. Control slides were included in order to detect any pressure induced artifacts.

Indirect Hemagglutination (IHA)

The procedure for indirect hemagglutination was a slight modification of that described by Stavitsky (1954). HA titers were determined using disposable microtiter plates and micro-diluters (Cooke Engineering Co., Alexandria, VA). Reactions were read after

incubation at room temperature for 4 hrs. Sheep red blood cells (SRBC) used in this procedure were collected directly into Alsever's solution and stored at 4 C.

Homologous Passive Cutaneous Anaphylaxis (PCA)

Homologous passive cutaneous anaphylaxis was performed following a slight modification of the procedure described by Ogilvie (1967). Rats were shaved and 0.1 ml quantities of serum or larval bladder fluid were injected intradermally (i.d.) along the back. Two hours later rats were challenged intravenously with a 1:1 solution containing BGG and a 1% solution of brilliant blue R (Bio Rad., California). Reactions were read 15-30 minutes after challenge. Whenever doubtful responses occurred the skin was removed and viewed from the underside.

Experimental Procedures

Larvae of *T. taeniaeformis*, 48-63 days old, were dissected free of liver cysts into chilled Eagle's medium. Groups of 50-75 larvae were washed 3X in distilled water, 3X in sterile water, and 3X in sterile normal saline. Fluid samples to be analyzed by IEP for host serum components were immediately procured by bladder puncture, and concentrated 2- to 5-fold by using polyethylene glycol (Carbowax, Union Carbide). Other larvae were transferred to sterile flasks containing 20 ml of Eagle's medium at 37 C for a pre-incubation period of one hour.

Uptake experiments were initiated by the addition of iodinated proteins to culture media. Incubations were terminated by removal

of individual larvae. Each larva was washed 3X in normal saline, blotted dry, and the bladder fluid collected in 5 mm x 40 mm test tubes. After the prescribed incubation period in each experiment the fluids obtained from a minimum of 5 larvae were pooled and the levels of radioactivity were determined. Fluid volumes were measured using either 10 µl or 50 µl Hamilton syringes. Pooled bladder fluid samples were also concentrated using polyethylene glycol and analyzed immunoelectrophoretically. Washed and dried IEP slides were examined for radioactive bands by autoradiography.

After 3-4 months of infection the larvae of *T. crassiceps* were removed from the peritoneal cavity and transferred to Eagle's medium. Only advanced larvae with well-formed bladders were selected for use in the experiments. The incubation procedures and methods of bladder fluid collection were identical to those outlined for *T. taeniaeformis*.

Hydatid cysts removed from the peritoneal cavity 10 to 15 months after transplantation were immediately washed as described for *T. taeniaeformis* larvae and then transferred to sterile incubation flasks containing 30-50 ml of Eagle's medium at 37 C. Fluid samples were obtained by puncture with a 30 gauge needle and 1.0 ml syringe. Otherwise the experiments were carried out as described for *T. taeniaeformis*.

At times it was necessary to maintain all three species overnight in Eagle's medium at 4 C before use in uptake experiments. Larvae of *T. crassiceps* and *E. granulosus* were stored under these conditions for periods exceeding two weeks without demonstrable loss of infectivity following transplant into mice and rats

respectively. In order to show that larvae had not degenerated during the course of our *in vitro* studies representative specimens of *T. crassiceps* and *E. granulosus* were reimplanted in animals at the end of a typical experiment. All these organisms successfully established in their respective hosts. We did not test the infectivity of larvae of *T. taeniaeformis* after maintenance *in vitro* because at this age (48-62 d) their ability to infect cats is questionable (Hutchinson, 1958). However, more advanced larvae (100-120 d) which had been maintained *in vitro* under similar circumstances for 24 hrs were able to infect cats and produce adult tapeworms equally as well as fresh worms obtained directly from rat livers.

RESULTS

The results of immunoelectrophoretic analyses of bladder fluids of each of the three species of taeniid metacestodes are shown in Figure 1. A number of host associated antigenic determinants, including albumin and a variety of globulins, were revealed with antisera to whole host serum. When class specific antisera against rat immunoglobulins were employed IgG_1, IgG_2, and IgM but not IgA were detected in bladder fluids of both *T. taeniaeformis* and *E. granulosus*. In bladder fluid from larvae of *T. crassiceps* mouse IgG, IgM, and IgA were present. Fluid samples from individual hydatid cysts of *E. granulosus* did not always contain detectable host associated components.

Preliminary results from studies in which larvae had been incubated in radioiodinated protein solutions indicated that

Figure 1. Host immunoglobulins detected by immunoelectrophoresis in the bladder fluids of *T. taeniaeformis*, *T. crassiceps*, and *E. granulosus*.

An array of host serum components were revealed in bladder fluid (3X) concentrated, obtained from larvae of *T. taeniaeformis* (a) and *E. granulosus* (b) by reaction against anti-whole rat serum (a-WRS). Rat IgG_1, IgG_2, and IgM were detectable by reaction against antisera specific for each of these immunoglobulin classes.

Bladder fluid from larvae of *T. crassiceps* (c) was analyzed similarly. Again an array of host serum components were revealed by reaction against anti-whole mouse serum (a-WMS). Mouse IgG, IgM, and IgA were detectable by use of antisera specific for each of these immunoglobulin classes.

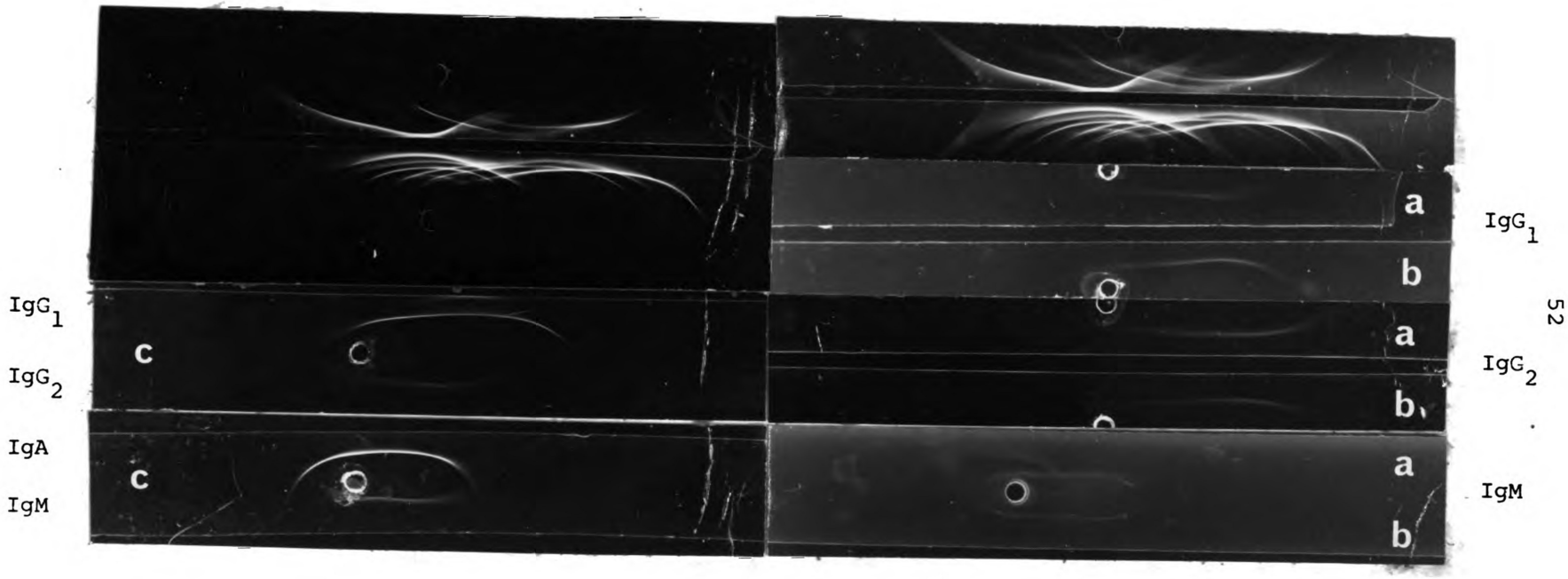

Figure 1

substantial uptake of I^{125} had occurred. In order to determine if uptake of intact protein molecules was involved we employed RIE analysis. Figure 2 shows the results of RIE tests with bladder fluids (5X concentrated) from larvae maintained for 4 hrs in Eagle's culture medium supplemented with I^{125} labeled rat IgG_2, BSA, or RNase-A. In samples from *T. taeniaeformis* and *T. crassiceps* radioactive bands were detected which corresponded in position to those of the parent molecules. In a number of cases considerable cpm/ml were observed in individual hydatid fluid samples but radioactive bands were detected in RIE on only one occasion.

In view of the fact that the labeled protein standards differed markedly in molecular weight we studied the rates at which they appeared in bladder fluid in order to determine if uptake of I^{125} was influenced by the molecular weight of the carrier. Larvae were incubated in isotopically labeled protein solutions in Eagle's medium as described above, and bladder fluid samples were taken at 30 min intervals for the first two hours, and then after 4, 8, and 24 hrs of incubation. Larvae of *T. taeniaeformis* and *T. crassiceps* showed remarkably similar rates of uptake for each protein (Figure 3), although there was considerable variation in uptake rates for individual parasites. In pools of hydatid cyst fluid levels of radioactivity were consistently low, and did not increase notably over the duration of the experiment.

Although I^{125} labeled proteins were detected within the bladder fluid of taeniid metacestodes further evidence was required in order to demonstrate the transport of functionally intact macromolecules. For this purpose we examined the appearance of

Figure 2. Autoradiography of bladder fluids from larvae of *T. taeniaeformis* (a) and *T. crassiceps* (b) incubated in I^{125} labeled RNase-A, BSA, or rat IgG_2. Supplemented Eagle's medium (c) was assayed similarly and served as the control.

Larvae were incubated in Eagle's culture medium supplemented with the labeled carriers for 4 hrs at 37 C. Bladder fluid was collected and concentrated (5X) prior to being assayed by RIE and autoradiography for the presence of the iodinated protein.

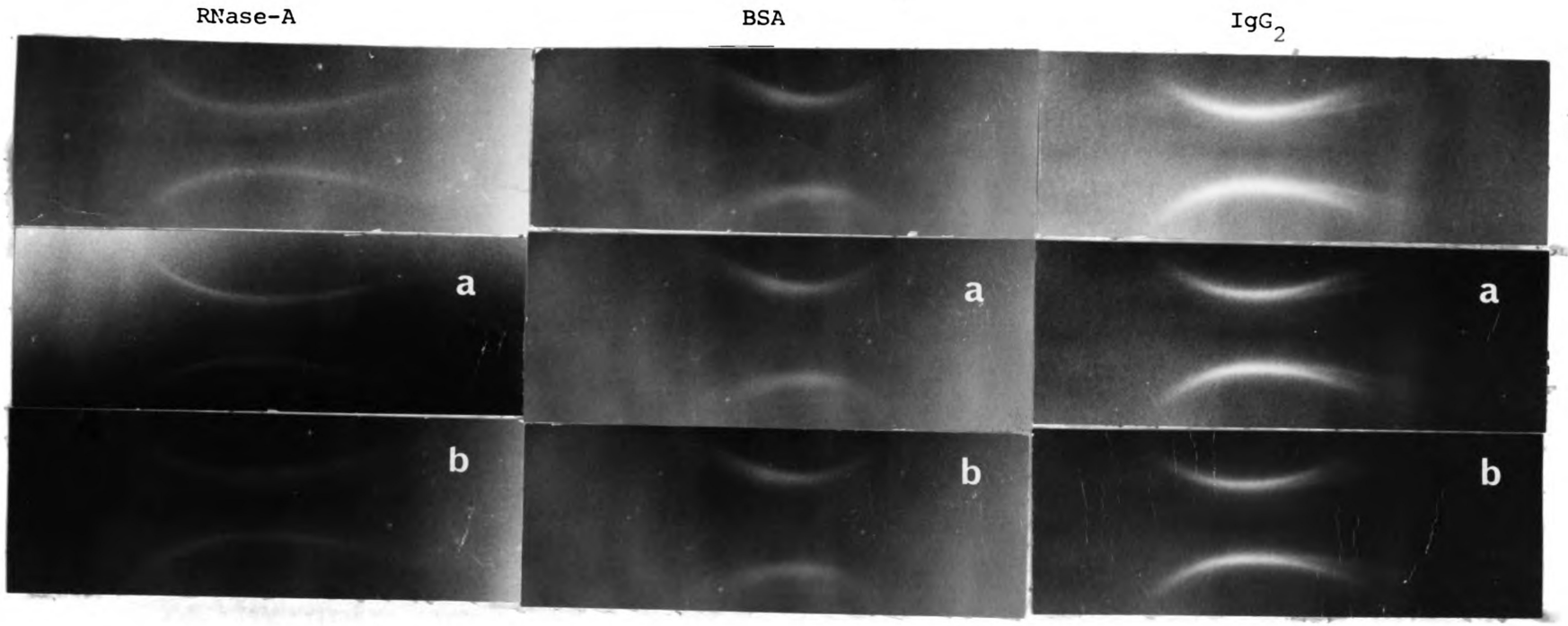

Figure 2

Figure 3. Rates of uptake of I^{125} labeled protein by larvae of *T. taeniaeformis* (★-★), *T. crassiceps* (☆-☆), and *E. granulosus* (O-O), incubated *in vitro* in Eagle's medium containing the labeled carriers.

Seventy-five larvae were incubated at 37 C in Eagle's culture medium supplemented with I^{125} labeled rat IgG (56,000 cpm/ml), BSA (64,000 cpm/ml) or RNase-A (47,000 cpm/ml). During the first 2 hrs, 5 larvae were removed every 30 mins, washed extensively in saline and their bladder fluids collected and pooled. The levels of radioactivity were assayed in a gamma scintillation counter. Additional samples were collected similarly after 4, 8, and 24 hrs of incubation.

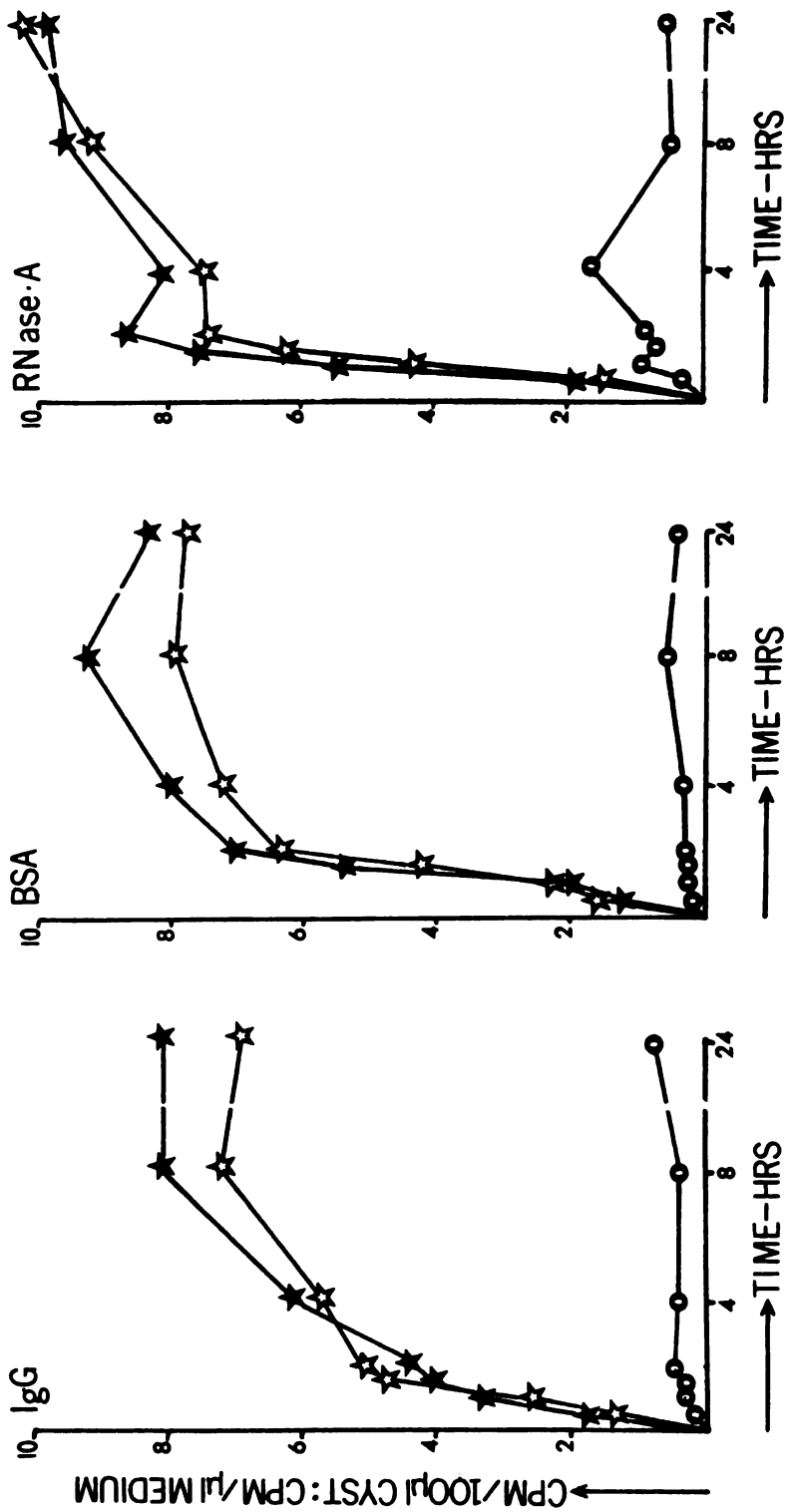
IgG
BSA
RNase·A
10
8
6
4
2
4
8
24
TIME-HRS
CPM/100μl CYST : CPM/μl MEDIUM

Figure 3

immunoglobulins of known antibody specificities within the bladder fluid of larvae of *T. taeniaeformis* both *in vitro* and *in vivo*. Larvae were incubated for 4 hrs at 37 C in Eagle's culture medium mixed with equal parts of either rat anti-BGG, rat anti-HGG, or rabbit anti-BSA. Bladder fluids were collected and assayed for antibody activity by indirect hemagglutination. The results are shown in Table 1. In each instance antibody activity was demonstrable within bladder fluid.

Table 1. Uptake of homologous and heterologous host antibody *in vitro* by larvae of *Taenia taeniaeformis* by indirect hemagglutination (IHA) and passive cutaneous anaphylaxis (PCA)

Antibody preparation		IHA titer	PCA titer
Rat anti-BGG:	serum	1280	640
	bladder fluid	16	4
Rat anti-HGG:	serum	640	--
	bladder fluid	8	--
Rabbit anti-BSA:	serum	1280	--
	bladder fluid	4	--

An opportunity to measure an additional parameter of immunoglobulin function was provided by our observation that anti-BGG antibodies produced in rats 14 days post-injection were of the IgG_{2a} class and therefore showed short-term homocytotropic activity. This activity could be assayed in serum and bladder fluid samples by homologous PCA tests following a 2 hour skin-sensitization period. Bladder fluid samples which showed anti-BGG activity in IHA were also capable of mediating homologous PCA reactions.

Uptake of intact antibodies *in vivo* was studied by passively immunizing infected rats. Sixty days after infection with *T. taeniaeformis* rats were given intraperitoneal inoculations of 75 mg of globulin from sheep anti-rabbit IgG serum each day for 4 days in order to establish high circulating antibody titers to rabbit IgG. Two days after the final inoculation rats were killed and serum and bladder fluids were tested for antibody activity by DID. Precipitating antibodies were detected within the fluid of the parasite.

Our failure to provide a convincing demonstration of the presence of labeled macromolecules in pools of hydatid fluid led us to examine firstly the extent of variation in uptake by individual cysts of *E. granulosus* and secondly the degree to which degradative processes within the cyst might be contributing to carrier breakdown. Ten cysts of *E. granulosus* were incubated in culture medium containing I^{125} BSA. After 2 hrs at 37 C fluid samples were obtained by needle puncture. Cysts were then incubated in culture medium alone at 37 C, to allow for membrane repair. After 8 hrs I^{125} BSA was added and fluid samples were collected 24 hrs later. The results are shown in Table 2, and indicate that only two cysts had taken up the label. Cyst fluids were examined for the presence of host serum components by IEP using anti-WRS. Although only 3 cysts reacted, two of these had taken up I^{125}. These observations were extended by examining 40 more cysts and a similar pattern of results was obtained.

Ten hydatid cysts were incubated in culture medium containing I^{125} rat IgG_2 for 24 hrs at 37 C. One milliliter aliquots of

Table 2. Uptake of I^{125} bovine serum albumin (BSA) by hydatid cysts of *Echinococcus granulosus in vitro*

Cyst	C.P.M./ml hydatid cyst fluid* 2 hrs	24 hrs	Reaction in gel with anti-WRS
1	80	212	
2	110	196	
3	93	159	
4	412	4822	+
5	93	230	
6	90	253	
7	552	2126	+
8	274	502	
9	107	210	+
10	87	174	

*Culture medium = 60,212 cpm/ml.

hydatid fluid from each cyst were pipetted into dialysis tubing and cpm/ml were determined before and after dialysis against PBS for 48 hrs at 4 C. Radioactivity counts were uniformly lower in dialyzed samples, whereas in those which had been stored without dialysis for the same period of time no more than a 6% reduction was obtained (Table 3). Comparable results were obtained with samples of culture medium which had been dialyzed for 48 hrs.

DISCUSSION

Our results strongly suggest that the host components which we were able to identify immunologically within the bladder fluid of

Table 3. Uptake of I^{125} rat IgG_2 by hydatid cysts of *Echinococcus granulosus in vitro*

Cyst	C.P.M./ml	C.P.M./ml Post-dialysis	Percent loss of activity
1	3096	1402	55
2	6157	2452	60
3	314	107	64
4	276	98	64
5	1947	538	72
6	2376	567	76
7	267	114	58
8	172	(164)*	5
9	212	(199)	6
10	200	(190)	5
Culture medium	62,411	59,812	5

*Values in parentheses correspond to hydatid fluid samples that were not dialyzed.

taeniid metacestodes were absorbed intact from the surrounding extravascular fluids *in vivo*. Larvae of *T. taeniaeformis* and *T. crassiceps* were shown to be able to take up both homologous and heterologous host proteins *in vitro* and the rates of uptake were not related to their molecular weights. Transport into the bladder fluid did not appear to result in any physicochemical or functional changes in these proteins since their electrophoretic and antigenic characteristics were preserved, and in the case of immunoglobulin uptake both

antibody combining sites and Fc portions of the molecules retained activity. Antibodies taken up both *in vitro* and *in vivo* by larvae of *T. taeniaeformis* were shown to be able to combine with their specific antigens, and the capacity of homocytotropic antibodies to adhere to rat skin and fix complement was unaffected following transport into larvae *in vitro*. Since the rates of uptake for each protein showed remarkably similar patterns in both *T. taeniaeformis* and *T. crassiceps* the absorption of macromolecules probably occurred by analogous mechanisms in each parasite.

These findings support the proposals of Esch and Kuhn (1971), and Coltorti and Varela-Diaz (1972) regarding the origin of host components within *T. crassiceps* and *E. granulosus* respectively. Esch and Kuhn (loc. cit.) were able to detect C^{14} in trichloroacetic acid precipitates from bladder fluid of larvae of *T. crassiceps* exposed to C^{14} *Chlorella* protein. However, they did not determine if structural or functional changes in the macromolecule had occurred during transport. Coltorti and Varela-Diaz (loc. cit.) and Varela-Diaz and Coltorti (1972) demonstrated host specific immunoglobulin determinants within hydatid cyst fluid and reasoned that these must have been host-derived since an inordinate proportion of the parasite genome would be required for synthesis of such a wide spectrum of proteins by the organism. They speculated that macromolecules entered the cysts by simple diffusion. Esch and Kuhn (loc. cit.) considered the possibility that tegumental pores in *T. crassiceps* might permit ready passage of large molecules to the interior of the bladder. Ultrastructural studies on a variety of taeniid metacestodes have not revealed evidence of pores which

might serve this function (Morseth, 1966; Bortoletti and Ferretti, 1971, 1973; Lascano *et al.*, 1975), nor have we been able to detect them in our own light and electron microscopic preparations (unpublished observations). In the absence of pores, transport of macromolecules across the complex tegumentary membrane seems more likely to involve an energy requiring mechanism than a process of simple diffusion.

Intact proteins are transported across the fetal membranes and intestinal epithelium of mammalian neonates (Brambell, 1966; Brambell *et al.*, 1961, 1964) and ultrastructural and functional analogies have been drawn between these epithelial surfaces and the tegument of larval cestodes (Beguin, 1966; Slais, 1966). A specialized process of pinocytosis has been postulated by Brambell (1966) and Rodewald (1973) to account for the specificity of protein uptake by trophoblast and intestinal epithelial cells. It seems possible that a comparable mechanism may be involved in cestodes. Proteins which do not become bound to membrane receptors are either not taken up by intestinal cells or are rapidly degraded in internalized pinocytotic vesicles by enzymatic digestion (Rodewald, 1973). Highly charged molecules are known to be the most effective in initiating pinocytotic vesicle formation (Brandt and Freeman, 1967) and for this reason proteins rather than uncharged substances should be employed in uptake studies. This may account for the fact that larvae of *T. crassiceps* failed to take up C^{14} inulin in the experiments described by Pappas and Read (1973). Although pinocytotic vesicles have not been observed in the tegument of taeniid metacestodes it may be that they are found only in response to certain

stimuli. Clearly a continued effort should be made to explore pinocytosis as a potential mechanism of transport in these organisms.

Quite apart from the necessity for further work on uptake, the functional significance of absorbed macromolecules also remains to be determined. While one may speculate that their presence is important in osmoregularity control (Dixon *et al.*, 1973) or that they provide a source of nutrients for the parasite, there is as yet no direct evidence to support these proposals. Perhaps even more challenging, in view of our recent work on the role of rat IgG_2 antibodies in protection against infection with *T. taeniaeformis* (Leid and Williams, 1974; Musoke and Williams, 1975), is the observation that antibodies of this subclass can not only be taken up by the parasite but retain their functional characteristics. Experimental exploration of the means whereby the growing organisms acquire the ability to evade immunologic damage mediated by these antibodies is crucial to an understanding of the host parasite relationship in metacestode infections.

Our results with respect to *E. granulosus* do not conform to the pattern obtained with *T. taeniaeformis* and *T. crassiceps*. We were only able to detect host derived substances in a small proportion of cysts and uptake of intact I^{125} BSA was revealed in one instance. Nevertheless, this observation, in conjunction with the fact that the I^{125} labeled substances which became internalized in other cysts were partially dialyzable, suggests that uptake of the entire proteins can occur but that they are rapidly fragmented. Proteolytic enzymes are known to be present within hydatid cyst fluid

(Lemaire and Ribère, 1935) and could be responsible for this effect. Evidently enzymatic degradation of proteins does not occur externally since we did not detect any fragmentation of labeled proteins in culture media used to maintain the organism *in vitro*.

The significance of the finding that few cysts contain host proteins and that only about 20% will take up the I^{125} label *in vitro* is not clear at this time. Coltorti and Varela-Diaz (1972) also found that only 20% of cysts contained detectable host immunoglobulins and reported that the concentrations of these proteins varied as much as 1000-fold from one cyst to another. The occurrence of intermittent or cyclic uptake activity combined with rapid degradation of internalized proteins could account for these anomalous results. Further work will be required in order to substantiate this notion, which is in part prompted by the consideration that cyclic activity by the parasite could also be responsible for the laminated appearance of the acellular membrane produced by these organisms.

ACKNOWLEDGEMENTS

This work was supported by USPHS Grant No. AI-10842. The authors are grateful for the excellent technical assistance of Miss Marla Signs and for the expert advice of Dr. A. J. Musoke. This is journal article No. _____ from the Michigan Agricultural Experiment Station.

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ARTICLE 2

PERMEABILITY STUDIES ON TAENIID METACESTODES: II. ANTIBODY MEDIATED EFFECTS ON MEMBRANE PERMEABILITY IN LARVAE OF *TAENIA TAENIAEFORMIS* AND *TAENIA CRASSICEPS*

Permeability Studies on Taeniid Metacestodes: II. Antibody Mediated Effects on Membrane Permeability in Larvae of *Taenia taeniaeformis* and *Taenia crassiceps*

SHORT TITLE: Antibody Mediated Effects on Permeability in Taeniid Metacestodes

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ABSTRACT

Incubation in immune rat serum (IRS) was shown to increase the rate of absorption of I^{125} RNase-A but not I^{125} BSA by larvae of *Taenia taeniaeformis* and *Taenia crassiceps*. This effect required a heat labile factor in serum, and partial activity could be restored in heat treated IRS by adding normal rat serum (NRS) as a source of complement (C'). In addition the effectiveness of IRS in altering permeability was shown to be dependent on the available concentration of functional C'. Both live and dead larvae incubated in NRS rapidly depleted hemolytic C' levels in the surrounding medium.

Immunoglobin fractions from IRS separated by anion exchange chromatography and gel filtration were tested in the presence of excess complement for their ability to increase uptake of I^{125} RNase-A. Enhanced permeability was observed in larvae incubated in each fraction.

We have shown that antibody in conjunction with complement is capable of disrupting larvae permeability control *in vitro*. However, the observation that larvae appear to restore normal control with time leads us to suggest that they avoid this immunologic effector mechanism by interfering with complement function.

INTRODUCTION

Murrell (1971) showed that the permeability of larvae of *Taenia taeniaeformis* to isotopically labeled sugars and amino acids *in vitro* was greatly enhanced in the presence of immune serum. Heat inactivation of the serum abolished this effect, suggesting that

complement may have been involved in the reaction. We have been able to demonstrate uptake of I^{125} labeled proteins by larvae of *T. taeniaeformis* and *Taenia crassiceps in vitro* (Hustead and Williams, 1976) and have pursued the possible interaction between immunologic factors and permeability in the following study. The results indicate that not only is the rate of absorption of certain macromolecules increased in the presence of antibody and complement, but that substances associated with the larvae *in vitro* can deplete functional complement levels in the surrounding medium, leading to restoration of normal permeability control.

MATERIALS AND METHODS

Maintenance of Experimental Infections

Taenia taeniaeformis and *T. crassiceps* were maintained in rats and mice respectively as described in our previous paper (Hustead and Williams, 1976).

Reagents

Proteins

Standard preparations of bovine serum albumin (BSA) and ribonuclease-A (RNase-A) were purchased from Sigma Chemical Company (St. Louis, MO). Purity was assayed by polyacrylamide gel electrophoresis (PAGE) by the method of Weber and Osborn (1969).

Immune Sera

Serum from rats infected *per os* with 500 eggs of *T. taeniaeformis* was obtained 28 days post-infection. The animals were killed

with CO_2 vapor and blood was collected from the thoracic cavity after severing the vessels anterior to the heart. The blood was allowed to clot 3-4 hours at room temperature and left overnight at 4 C. The serum was decanted, centrifuged, and used immediately. Normal rat sera were collected from retired female breeders purchased from Spartan Research Animals, Haslett, Michigan. When required, inactivation of functional hemolytic complement in serum was achieved by incubation at 56 C for 30 mins.

Antiserum to sheep red blood cells (SRBC) was prepared in rats by intraperitoneal inoculation of 5×10^8 cells suspended in phosphate buffered saline (PBS). The rats were killed 8 days later, and the serum harvested as described above.

Experimental Techniques

Labeling and Counting

The methods for I^{125} labeling and counting were described by Hustead and Williams (1976). All values are expressed as counts per minute per milliliter (cpm/ml) unless otherwise indicated.

Fractionation of Immune Serum

Immune rat serum was fractionated by anion exchange chromatography and gel filtration, and the fractions pooled as described by Leid and Williams (1974).

Immune Hemolysis

Complement fixation was demonstrated using a slight modification of the immune hemolysis assay described by Kabat and Mayer (1967).

Experimental Procedure

Larvae of *T. taeniaeformis*, 42-63 days old, were dissected from hepatic cysts of rats, and the bladder forms of *T. crassiceps*, 3-4 months old, were removed from the peritoneal cavity of mice as described previously (Hustead and Williams, 1976).

In general the effects of immune serum on permeability were assessed by incubation of larvae of either *T. taeniaeformis* or *T. crassiceps* for 30, 60, 90, or 120 mins in immune rat serum (IRS), heat-inactivated immune rat serum (HI-IRS), or normal rat serum (NRS) prior to a brief exposure to medium containing I^{125} labeled BSA or RNase-A. After 10 mins larvae were removed, washed 3X in saline, blotted dry, and bladder fluids were collected by puncture directly into scintillation vials. Levels of radioactivity were measured promptly and fluid volumes were determined using a 50 µl Hamilton syringe. Variations from this procedure are detailed in the results.

RESULTS

When larvae of *T. taeniaeformis* and *T. crassiceps* were incubated in IRS, HI-IRS, or NRS for 30, 60, 90, or 120 mins, and then transferred to a medium containing I^{125} BSA for 10 mins, minimal uptake of label occurred (Figure 1a). However, when larvae were treated similarly with the sera but then exposed to I^{125} RNase-A, the pattern of uptake of label seen in parasites incubated in IRS was different from that in HI-IRS and NRS groups (Figure 1b). After 60 mins in IRS uptake was approximately 10X greater than in the other two groups. By 120 mins uptake rates had returned to near control values.

Figure 1. Uptake of I^{125} BSA (1a) or I^{125} RNase-A (1b) by larvae of *T. taeniaeformis* and *T. crassiceps* following incubation at 37 C in IRS (★-★), HI-IRS (★-☆), or NRS (□-□). Larvae were removed from serum preparations at 30 min intervals and exposed to radiolabeled carriers for 10 mins in each case.

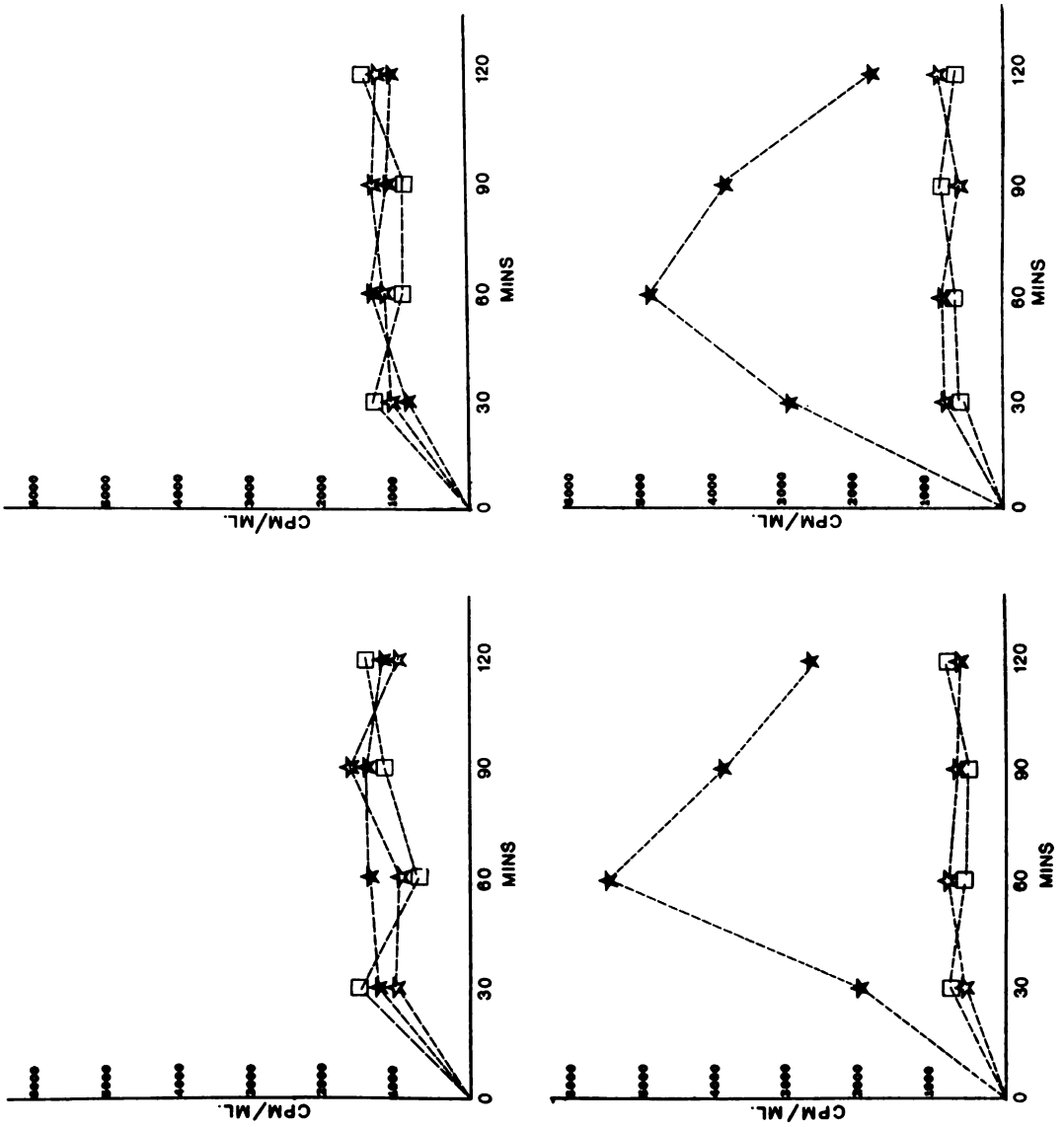

Figure 1

In view of the fact that heat-inactivation of immune serum abolished the effect on permeability to I^{125} RNase-A, we attempted to restore the activity of HI-IRS by adding a source of functional complement (C'). Preliminary results indicated that partial restoration could be achieved in this manner but again by 120 mins uptake rates in both IRS and HI-IRS + NRS groups had declined to near control values. It appeared that the contribution of the heat labile factor was diminishing during the course of incubation. We therefore undertook a serial study of functional hemolytic complement levels in the incubation medium during the course of an experiment.

Larvae were added to NRS diluted 1:4 with Eagle's medium and 1 ml samples were removed at 30 min intervals and assayed for complement by immune hemolysis. Control samples were taken from medium incubated at 37 C in the absence of parasites. The results are shown in Figure 2. A sharp decline in complement activity was apparent in medium incubated with live parasites. By 120 mins a depletion of approximately 80% of hemolytic activity had occurred, whereas no appreciable decline was observed in medium without parasites. Incubation of NRS with parasites killed by flash freezing at -160 C resulted in an even more abrupt decline of hemolytic activity. By 30 mins up to 90% depletion of complement was observed.

These results suggested that the effectiveness of IRS in disrupting permeability could be a function of the rate of decline in C' levels. This possibility was examined in the following experiment in which larvae of *T. taeniaeformis* were incubated initially

Figure 2. Effect of incubation with live ( ) or dead ( ) larvae of *T. taeniaeformis* or *T. crassiceps* on hemolytic complement levels in NRS. Fifty larvae were added to 50 ml of Eagle's medium containing 20% NRS. One milliliter samples were removed at 30 min intervals and assayed by immune hemolysis. Culture medium maintained without larvae but treated similarly served as the control ( ).

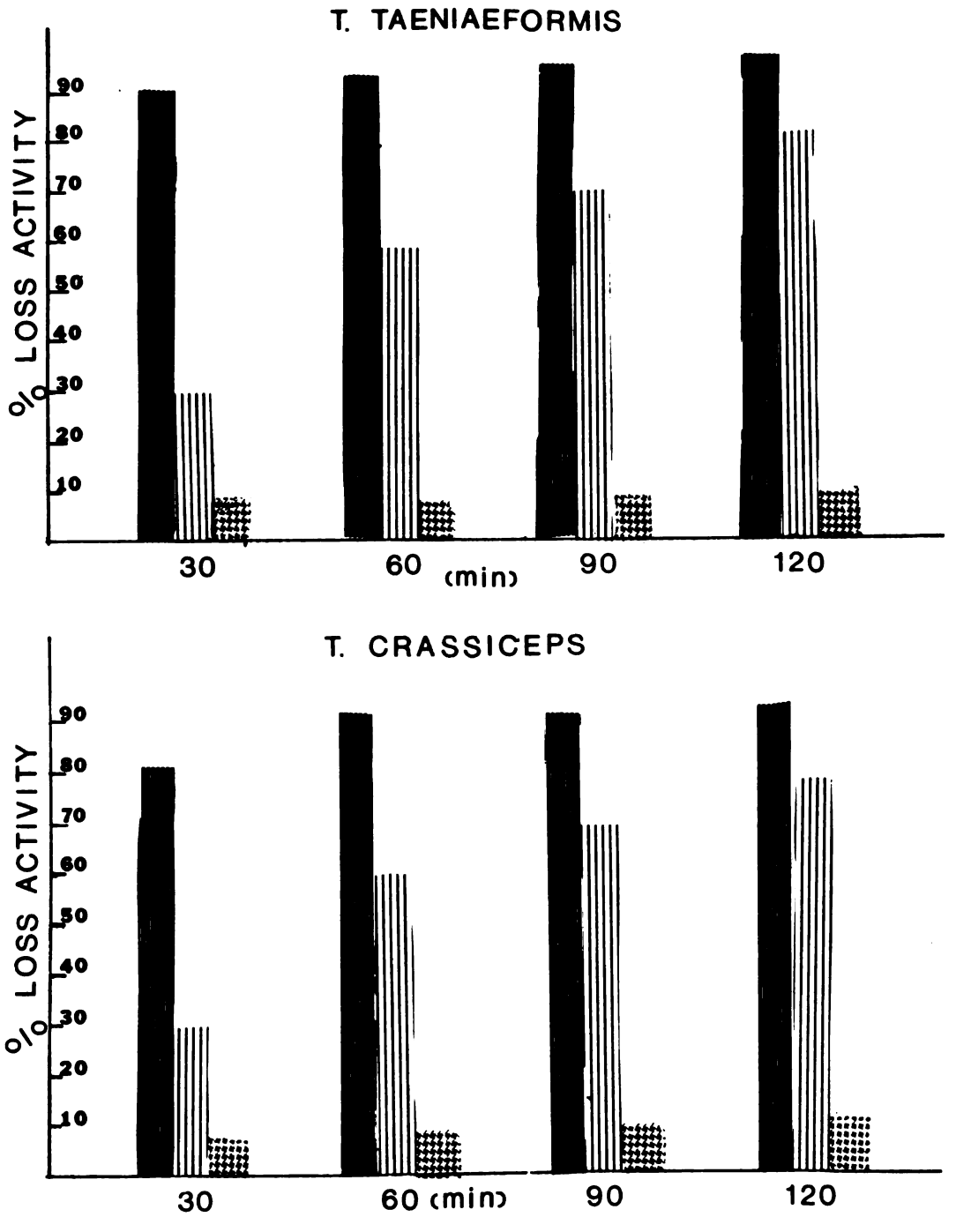
T. TAENIAEFORMIS
% LOSS ACTIVITY
90
80
70
60
50
40
30
20
10
30
60
(min)
90
120
T. CRASSICEPS
% LOSS ACTIVITY
90
80
70
60
50
40
30
20
10
30
60 (min)
90
120

Figure 2

in IRS diluted 1:2 with Eagle's medium. Under these circumstances C' levels rapidly declined in the medium and no observable effects on I^{125} RNase-A uptake were seen. However, when additional C' was added in the form of an equal volume of NRS, the effects of IRS were manifested and by 60 mins substantial uptake of label had occurred (Figure 3). Comparable results were obtained with *T. crassiceps*.

An attempt was then made to determine if the effects of IRS could be attributed to antibody activity in identifiable immunoglobulin fractions. Globulins from 28-day immune serum were separated by DEAE anion exchange chromatography and gel filtration on Sephadex G-200, and fractions tested for their ability to enhance uptake of I^{125} RNase-A. Fractions were restored to original serum volume prior to testing, and all were examined in the presence of excess complement. Increased uptake of label was observed in larvae incubated in each of the immunoglobulin fractions including the 19S void volume peak obtained from gel filtration.

DISCUSSION

These results provide clear evidence of immunologically mediated effects on permeability to macromolecules in taeniid larvae. Enhanced uptake of radiolabeled RNase-A was observed only in the presence of immune rat serum or globulins and required functional complement. Our findings are thus in agreement with those of Murrell (1971), who demonstrated alterations in the permeability of larvae of *T. taeniaeformis* to C^{14} labeled glucose, sucrose, and methionine in the presence of immune rat serum and a heat labile serum factor. His

Figure 3. Limiting effect of complement on IRS mediated alterations in permeability of larvae of *T. taeniaeformis* and *T. crassiceps* to I^{125} RNase-A.

a. Larvae were first incubated in IRS (★—★) or HI-IRS (☆—☆) diluted 1:2 with Eagle's medium before removal and exposure for 10 mins to I^{125} RNase-A.

b. After 6 hrs in the original medium larvae were removed, washed, and transferred to a second medium containing the same amount of IRS but an excess of complement (NRS). Again larvae were removed at 30 min intervals and exposed to I^{125} RNase-A for 10 mins before sampling.

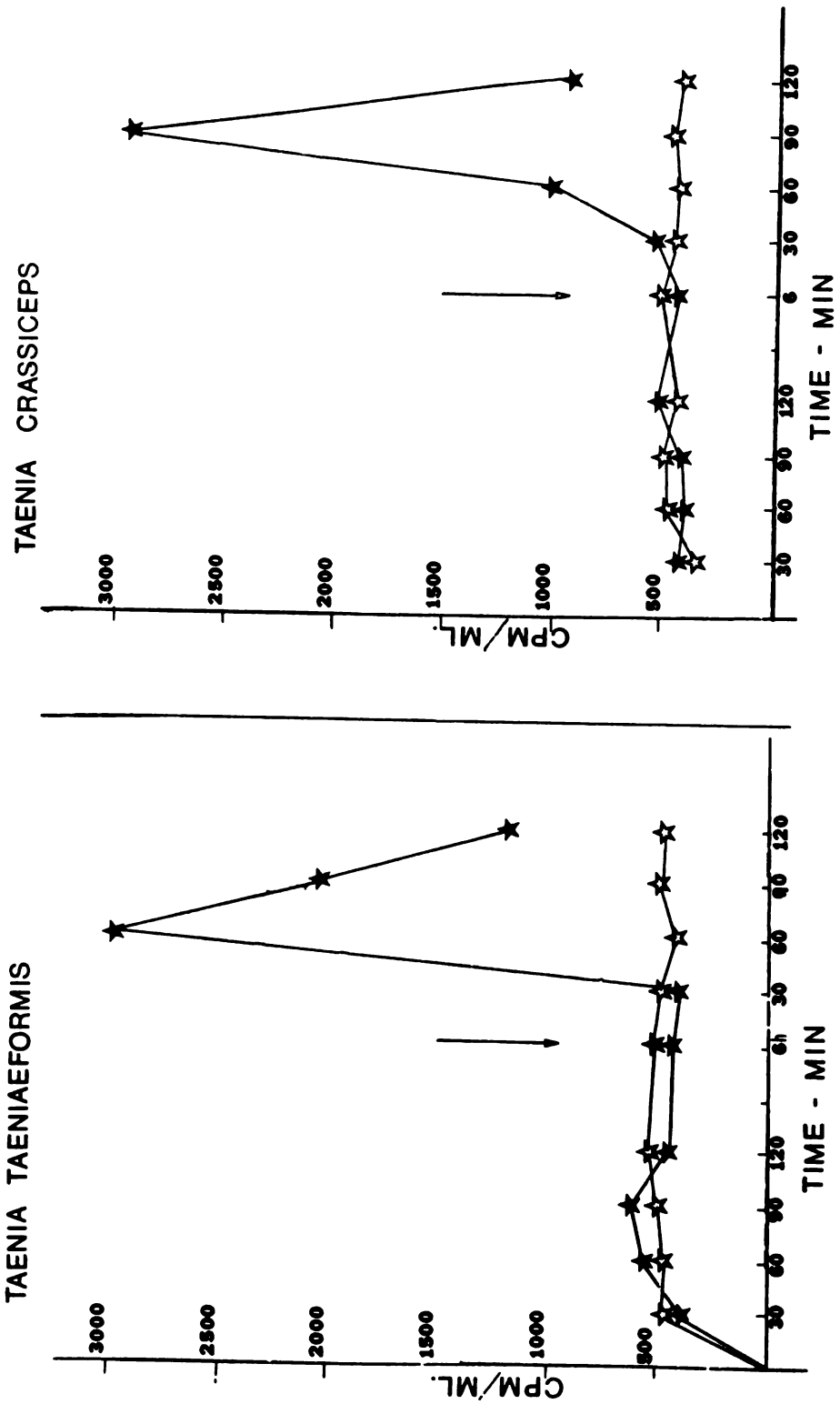
TAENIA CRASSICEPS
CPM/ML.
3000
2500
2000
1500
1000
500
TIME - MIN
30
60
90
120
TAENIA TAENIAEFORMIS
CPM/ML.
3000
2500
2000
1500
1000
500
TIME - MIN
30
60
90
120

Figure 3

suggestion that the effects were produced by antibody and complement is borne out by our observation that hemolytic complement levels were critical in determining if IRS could exert any influence on permeability. Furthermore, in our experiments uptake of I^{125} BSA was unaffected following exposure to antibody and complement, suggesting that lesions similar to those which occur in red blood cells during immune hemolysis were developing on the larval surface. Humphrey and Dourmashkin (1970) have characterized these lesions and shown that they are of the order of 100 Å in diameter and only permit the passage of macromolecules of less than 20,000 M.W.

The ability of larvae to restore permeability control during the course of incubation is attributable to the presence of factors which rapidly deplete functional complement in the surrounding medium. The abrupt decline in C' levels in NRS incubated with dead larvae suggests that these substances are preformed. Partial characterization has been achieved in our laboratory and we have shown that larval products are able to deplete circulating C' levels in rats *in vivo*, convert C3 to an electrophoretically faster species, and generate the production of anaphylatoxins from normal rat serum (Hammerberg *et al.*, 1976).

Immune serum from rats infected with *T. taeniaeformis* was equally effective in enhancing permeability to I^{125} RNase-A in both homologous and heterologous (*T. crassiceps*) metacestodes. This cross reactivity was not unexpected since rats infected with *T. crassiceps* are immune to oral challenge with *T. taeniaeformis*, and resistance is passively transferable with serum (Musoke and Williams, 1976). Whereas the protective capacity of IRS has been

shown to be associated with IgG_{2a} antibodies (Leid and Williams, 1974; Musoke and Williams, 1975), we were unable to demonstrate that antibody mediated effects on permeability were limited to this subclass of immunoglobulins. All immunoglobulin fractions of IRS separated by chromatographic means were able to enhance the uptake of I^{125} RNase-A in the presence of complement. It seems likely, therefore, that antibodies in several different immunoglobulin classes participate in the membrane associated reactions which we have examined. Certainly the sensitivity of this assay would be expected to exceed that of passive protective capacity employed in our previous work.

Although the protective effects of immune serum have been shown to be complement dependent, post-oncospheral stages of *T. taeniaeformis* rapidly became resistant to this immunologic effector mechanism (Musoke and Williams, 1975). It is tempting to link this phenomenon with the onset of the parasite's ability to interfere with complement function. Under normal circumstances larvae of *T. taeniaeformis* in the liver are surrounded by plasma proteins and can absorb them with impunity (Hustead and Williams, 1976). Apparently the necessary humoral factors are present *in vivo* to effect immunologic disruption of permeability control. Our results suggest that larvae may avoid these adverse immune reactions by producing and liberating factors which deplete complement levels.

ACKNOWLEDGEMENTS

The authors are grateful for the expert technical assistance of Miss Marla Signs in carrying out this work which was supported by USPHS Grant No. AI-10842.

This is journal article no. _____ from the Michigan Agricultural Experiment Station.

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