IMMUNOLOGIC MECHANISMS IN RESISTANCE TO EXPERIMENTAL INFECTION WITH TAENIA TAENIAEFORMIS

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ABSTRACT

IMMUNOLOGIC MECHANISMS IN RESISTANCE TO EXPERIMENTAL INFECTION WITH *TAENIA TAENIAEFORMIS*

By

Antony J. Musoke

Mice were found to be protected against *Taenia taeniaeformis* infection by passive transfer of serum collected from donors 28 days after infection. The protective activity resided exclusively in the first fraction of 7S immunoglobulins eluting from DEAE cellulose at pH 5.8 with 0.05 M phosphate buffer. This fraction contained $7S\gamma_1$ and $7S\gamma_2$ immunoglobulins but no detectable γA, γM or skin sensitizing activity. Fractions containing $7S\gamma_2$ alone were ineffective in passive transfer.

Intraperitoneally implanted metacestodes of either *T. taeniaeformis* or *T. crassiceps* in rats provoked a high degree of resistance to oral challenge with eggs of *T. taeniaeformis*. This resistance was passively transferred to normal recipients with serum. Immunoglobulin fractions of immune serum containing $7S\gamma_1$ or γM were most effective in passive transfer and little activity was associated with $7S\gamma_2$ antibodies. No skin-sensitizing antibodies were detectable in immune sera. These findings are in sharp contrast to previous observations involving protective immunoglobulins and reaginic

antibodies in serum from rats with hepatic cysticerci of *T. taeniaeformis*.

Cysticerci implanted into normal rats survived for at least 21 days with no sign of host rejection, whereas those implanted into rats with hepatic infections with *T. taeniaeformis* were killed and encapsulated. Similar results were obtained by implanting cysticerci in normal rats given inoculations of complete Freund's adjuvant. Repeated inoculations of immune serum had no effect on the survival of implanted cysticerci, and it was concluded that exposure to infection by oncospheres provokes cellular defense mechanisms which can be effective against cysticerci in abnormal sites. Why these mechanisms are inoperative against hepatic cysticerci remains unclear.

Passive transfer of immunity to *Taenia taeniaeformis* was achieved with serum taken 14, 21, 49 and 63 days after infection. The protective capacity of serum collected at 14 and 21 days resided in the $7S\gamma_2$ immunoglobulins and appeared to be exclusively the result of $7S\gamma_{2a}$ antibody activity. However, as the infection progressed the range of chromatographic fractions showing protective capacity was extended to all those containing $7S\gamma_2$ and $7S\gamma_1$ immunoglobulins. Fractions enriched for γM did not confer protection.

Immune serum containing $7S\gamma_{2a}$ antibodies was able to kill developing parasites after they had left the intestine, and the hepatic postoncospheral forms retained their susceptibility to antibody over the first 5 days of growth. After that time they

rapidly became insusceptible to antibody both *in vivo* and *in vitro*. Prior to the 5th day their susceptibility to antibody mediated attack was shown to depend on the integrity of the complement system. This appears to be the first time that complement has been demonstrated to play a role in immunity to a helminth infection *in vivo*.

Weanling rats born of mothers infected with *Taenia taeniaeformis* were found to be passively protected against homologous challenge. Cross fostering of normal suckling rats onto immune mothers established that passive transfer occurred via the colostrum and milk. Immunoglobulin fractions from immune colostrum containing γA were fed to 12- to 14-day-old rats for 4 days via stomach tube. Significant passive protection against challenge with *T. taeniaeformis* was achieved with γA from 1 of 3 colostrum pools. The effect of colostral γA preparations on the infectivity of freshly hatched oncospheres of *T. taeniaeformis* was measured by the intraintestinal inoculation of immunoglobulin solutions into isolated gut loops containing hatched eggs of the parasite. γA from 1 of 3 pools of immune colostrum caused a significant reduction in the number of parasites which reached the liver. This appears to be the first time that protective activity against a helminth infection has been achieved with γA.

A fraction of immune colostrum containing both $7S\gamma_1$ and $7S\gamma_2$ immunoglobulins was found to confer passive protection when inoculated parenterally. In view of the prolonged period of absorption (ca. 18 days) of 7S immunoglobulins from the gut by

the suckling rat, it seems likely that these antibodies are primarily responsible for the natural passive transfer of protection from mother to young.

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By

Antony J. Musoke

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Dedicated to my parents

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INTRODUCTION

The cysticercoses, affecting domesticated animals and man, result from the tissue migration of larvae of parasites of the family *Taeniidae*. This group of diseases not only impinges significantly upon public health and economic production of animal protein in developing countries but, in recent years, has become a cause of concern to the meat producers of industrialized nations. For example, bovine cysticercosis probably reaches its greatest significance in East Africa where Froyd (1960) estimated that up to 35 percent of carcasses were infected with *Taenia saginata*. However, several diastrous outbreaks of *T. saginata* infection occurred in feedlots in the Southwest United States in 1968 and the percentage of carcasses showing signs of cysticercosis in federally inspected slaughter houses has risen markedly in the last decade (Schultz, Hatterman, Rich and Martin, 1969).

Over the years a variety of methods of control and prevention of cysticercosis have been proposed and applied, including programs of health education, improved sanitation and improvement of meat inspection to detect and eliminate infected meat. These control procedures, quite apart from being expensive to operate, particularly in the most severely affected countries, involve complex socio-economic factors and have therefore met with very

limited success. In the absence of any chemotherapeutic approaches to treatment of the tissue stages, an immunologic solution to the problem is increasingly called for. Large scale experimentation in domesticated animals is premature at the present stage of knowledge, but fortunately an experimental model for cysticercosis can be found in the natural host-parasite relationship between *T. taeniaeformis* and laboratory rodents. The work reported herein was designed to attempt to characterize some of the immunological mechanisms operating in resistance to challenge infection with *T. taeniaeformis*.

The literature review has therefore been organized firstly to provide background information on the two important taeniid organisms of man, *T. solium* and *T. saginata*. This has been done to point out the analogy between the biology of these important organisms and that of the experimental models which are used. The second section of the literature review deals with protective immune responses to taeniid parasites. Here emphasis is placed on the experimental evidence to date regarding participation of immune mechanisms in resistance to cysticercosis, although evidence from the field is also reviewed. In addition, current theories on the means whereby tissue dwelling metacestodes are able to evade or resist immunological attack have been identified and examined.

Lastly a section concerning the current classification and biological functions of rat and mouse immunoglobulins is provided. This information is considered crucial for an understanding of the work reported below much of which deals with exploration of the role of immunoglobulins in immunity.

LITERATURE REVIEW

Cestodal organisms of the family *Taeniidae*, order *Cyclophyllidea*, constitute a group of parasites of both medical and veterinary importance. Parasites of genera *Taenia* and *Echinococcus* invade the tissues of man or domesticated animals, which act as intermediate or definitive hosts. In this first section the biology of the important human pathogens is reviewed and an account is given of the principal experimental models used in research.

Biology of Taeniid Parasites of Man

Taenia solium

Taenia solium has a cosmopolitan distribution and is an important parasite wherever man consumes raw or insufficiently cooked pork. It is currently most commonly found in the Slavic people (Czechs, Serbs, etc.) but also occurs frequently in Mexico, many Latin American countries and North China (Soulsby, 1968).

Life Cycle. Adults of *T. solium* live attached to the wall of the small intestine of man. Eggs escape from the branched uterus through a ventral longitudinal slit either before or after the ripe proglottids become free. Like other taeniids, the egg is composed of an outer layer of keratinized blocks with several

membranes inside of the blocks and finally a 6-hooked or hexacanth embryo within the last membrane. The eggs remain viable on the soil for periods of up to 2 or 3 months.

On ingestion by hogs or man, the eggs hatch and activate in the intestine. The initial mechanism consists of the release of the oncosphere which is effected by digestion of the cement substance holding the keratin blocks. The conditions vary with the species of tapeworm: *T. taeniaeformis* and *E. granulosus* require pancreatin but not pepsin enzymes; for eggs of *T. saginata*, pancreatin is ineffective but pepsin is essential (Silverman, 1955; Smyth, 1963). Nothing is known about the hatching and activation conditions for *T. solium*. With the help of a combination of intestinal juices and other factors yet unknown, the larvae become activated and capable of penetrating the intestinal wall. Penetration into the gut wall by the oncosphere is rapid, occurring within 30 minutes. The parasites continue to migrate down the villus until a venule of sufficient size is reached. Penetration and eventual migration through the intestinal wall may be aided by enzymes as several workers (Silverman and Maneely, 1955; Heath, 1971) have noted clear zones around the oncosphere, possibly resulting from lysis of cells around them.

The oncospheres of *T. solium* are then carried to the skeletal musculature where they develop into mature cysticerci, called *Cysticercus cellulosae*, over a period of 2 months. Muscle infections in man, however, are abortive and represent a dead end.

Man is readily infected by ingesting the cysticercus in raw or inadequately cooked pork. The larva is digested free of the

host capsule and becomes activated in the small intestine where it attaches to the gut wall. The parasite develops into a mature worm producing large (10-12 mm) proglottids containing up to 40,000 eggs each.

Pathology. The adult *T. solium* in the small intestine may cause considerable irritation at the site of its attachment to the mucosa or may produce intestinal obstruction.

Human infection with the cysticerci may occur by the ingestion of eggs in contaminated food or by reverse peristalsis whereby eggs in the bowel are carried forward to the duodenum or stomach and here are stimulated to hatch. Cysticerci may then be found in every organ of the body of man but are most commonly encountered in the muscles, subcutaneous tissue, and the eye.

Like other taeniid metacestode infections, the location of the parasite is important. The larvae do not cause a clinical syndrome unless their location impinges on a vital organ.

The larvae are occasionally located in the brain. Here the parasite may cause little pathology while alive but upon death a great variety of neurological symptoms may develop, including epilepsy, incoordination, transient paresis, meningoencephalitis and failing vision (Faust, Russell and Jung, 1970).

The presence of the growing larvae in the tissue provokes a local cellular reaction which includes infiltration with neutrophils, eosinophils, lymphocytes, plasma cells, and macrophages. Fibrosis and necrosis follow this cellular picture with an eventual caseation and calcification of the cyst.

Taenia saginata

Taenia saginata has a wide world distribution but occurs most frequently in Africa, Asia, and the USSR. Sporadic outbreaks have been reported in the Southwest United States and cases occur sporadically in most developed countries. Immigration and tourism lead to these occurrences where infection is not endemic in the area.

Life Cycle. *Taenia saginata* resides in the small intestine of man and the life cycle is similar to that of *T. solium* except that cattle act as the intermediate host. Man is not susceptible to the tissue phase of this parasite. The fully grown "bladder-worm" (*Cysticercus bovis*) is usually situated in the intermuscular fascial layers surrounded by a connective tissue capsule. In heavy infections, however, organs such as liver, lungs, kidneys and abdominal fat may be infected.

After 4-6 months, the cysticerci begin to degenerate and by the ninth month most of them may be dead. This depends on the size of the original infection and also the age of the animal when it was infected. Man becomes infected by eating raw or inadequatly cooked meat containing the cysticerci of *T. saginata* and the larvae develop into the adult tapeworm in the small intestine. Gravid segments measuring about 16-20 mm in length and containing about 100,000 eggs each are passed out in approximately 2 months.

Pathology. In the intermediate host cysticerci degenerate and eventually die by the ninth month of infection. Because of its large size, the adult *T. saginata* is frequently responsible

for considerable disturbance in normal functions of the digestive tract. Diarrhea, hunger pains and loss of weight commonly accompany infection with this parasite. Leukocytosis is characteristic, with eosinophilia reaching 6-34 percent.

Treatment and Control of *T. solium* and *T. saginata*

Although treatment of infected persons can be carried out with certain taeniicidal agents (e.g., Yomesan), the effect of this effort is not long lasting. Control therefore has to be directed towards infection in domestic animals by way of appropriate meat inspection procedures. Also corrective hygienic methods have to be encouraged if successful control of the parasites is to be achieved (Silverman and Griffith, 1955).

Until recently chemotherapy of medically important larval tapeworms has been impossible. This may have been a result of the relative inaccessibility of the larvae to the drugs and the difficulty and danger of experimentation with some of these infections. Now, however, several chemical compounds have been tested for their activity in laboratory models, and some promising agents have emerged.

The cytostatic drug, cyclophosphamide, has been reported to kill the cysticercus of *T. taeniaeformis* in mice provided it is given at a critical time in the early development (Hinz, 1964). Salazar *et al.* (1972) showed some evidence that larvae of *T. solium* are affected by Trichlorfon and may undergo regression. Williams *et al.* (1973) demonstrated conclusively that eggs of various

taeniid parasites become noninfective if treated with Bunamidine hydrochloride *in vitro*.

Benzimidazoles are among the existing anthelmintics which seem to have broad spectrum activity against a variety of parasites. Their activity against larval cestodes has only recently been investigated. Prophylactic and therapeutic effects of thiabendazole or cambendazole have been demonstrated by Campbell and Blair (1974). They showed that when mice infected with *T. taeniaeformis* were fed either of these compounds, there was degeneration or complete destruction of the hepatic cysts. Another related compound, mebendazole, was also successfully used to treat mice infected with *T. taeniaeformis* (Thienpont, Vanpary and Heremans, 1974). These authors further reported that, apart from regression of the infection, the recovered mice were resistant to challenge infection with eggs of *T. taeniaeformis*.

In separate trials, Heath and Chevis (1974) fed mebendazole to mice and rabbits infected with *Echinococcus granulosus* and *T. pisiformis*, respectively. This treatment resulted in complete destruction of all hydatid cysts and cysticerci.

In some of these experiments, however, drugs were used at dosages much higher than would normally be tolerated in man or domestic animals. Also the effects of their repeated use needs to be investigated. Nevertheless, the results confirm that metacestodes are not necessarily invulnerable to anthelmintic treatment. Trials using cambendazole in cattle infected with *T. saginata* are already under way (Mann, personal communication) and, if these

drugs prove to be as effective as they are in laboratory models, eradication efforts for hydatidosis and cysticercosis may well become oriented toward their use.

Biology of Taeniid Parasites of Laboratory Animals

Taenia taeniaeformis

Taenia taeniaeformis occurs in the small intestine of the cat and other related carnivores and is of cosmopolitan distribution.

Life Cycle. The bladderworm stage, *Cysticercus fasciolaris*, develops in the livers of the intermediate hosts which are chiefly rats and mice and also the rabbit, the squirrel and muskrat. The intermediate host becomes infected by ingesting the egg. The oncosphere of *T. taeniaeformis* is freed by intestinal enzymes. The predilection site for the parasites is the liver, where the organism continues to migrate to the subcapsular area until encapsulated with a fibrous tissue layer (Singh and Rao, 1967; Smyth and Heath, 1970). The larvae continue to develop until they reach the infective stage by 60 days (Hutchison, 1958). The cat becomes infected by ingesting livers containing metacestodes and the parasites then complete their development by maturing in the small intestine. Tapeworm segments containing eggs begin to be passed out approximately 2 months after infection.

Pathology. The cysticercus appears to be fairly harmless in the intermediate hosts, even when it occurs in large numbers. A malignant tumor has been associated with this infection in livers

of chronically infected rats, but its presence does not seem to cause any clinical syndrome.

In the definitive host the head of the adult worm is buried deep in the mucosa causing irritation and, in rare cases, may cause a perforation.

Echinococcus granulosus

This species is found in the small intestine of dogs and wild carnivores and is widely distributed, reaching its greatest importance in Africa and South America.

Life Cycle. Adults of *Echinococcus granulosus* measuring 3 to 6 mm reside in the small intestine of the dog, jackal (*Canis aureus*) and wolf (*C. lupus*).

After the eggs have been ingested by the intermediate host, they hatch in the intestine and the embryos migrate in the blood stream to various organs, especially the liver and lungs.

Intermediate hosts include man, domestic animals, and numerous wild mammals. The oncosphere continues to grow in these tissues which become infiltrated with giant cells and eosinophils. On the outer layer are fibroblasts with many more eosinophils. By the fifth month the hydatid is approximately 1 cm in diameter. The hydatid consists of an outer friable non-nucleated layer and an inner nucleated germinal layer. From the inner layer scolices develop which may detach, being found free in the cyst fluid. Upon rupture of the mother cyst wall, daughter cysts develop from these scolices. The larval form of *E. granulosus* can also be

propagated in rats or gerbils by intraperitoneal implantation of the cysts or inoculation of the scolices, respectively. This secondary propagation of the parasite in laboratory animals makes it a highly suitable model for laboratory research.

The definitive host becomes infected by consuming the viscera of infected larval hosts.

Pathology. The damage produced by the hydatid cyst of *E. granulosus* in humans is both mechanical and allergic. The cysts, which may be lodged in vital centers, may interfere with the function of the organ with damaging, even fatal, results. In some cases the cyst when in relatively unconfined location grows to tremendous size causing a physical burden to the patient and at times may burst precipitating sometimes fatal anaphylactic reactions.

Treatment and Control. Surgical intervention when the cyst is located in operable sites is the only available treatment to date as nonsurgical procedures are unsuccessful. Control again involves proper hygienic methods as eggs of *E. granulosus* are highly resistant to disinfectants (Meymarian and Schwabe, 1962). Dogs should be prevented from eating carcasses of sheep, cattle and hogs in endemic areas.

Taenia crassiceps

Taenia crassiceps is a common cestode of the red fox (*Vulpus vulpes*) in Europe and of the Arctic fox (*Alopex lagopus invitus*). The metacestode, *Cysticercus longicollis*, has been reported in

various small rodents and lemmings (*Dicrostomyx groenlandicus*) (Freeman, 1962). It has also been found to develop to the adult stage in experimental dogs. The first case of human infection was reported recently in Canada (Shea, Marberley, Walters, Freeman and Fallis, 1973).

Life Cycle. The definitive hosts become infected by ingesting cysticerci of *T. crassiceps* from rodents. The metacestode develops in the small intestine and grows to an egg-producing adult within 5 to 6 weeks. The cycle is then maintained when the eggs are ingested by mice. The oncosphere migrates to the pleural cavity and becomes infective for the definitive host in approximately 2 months. The metacestode can also be maintained by intraperitoneal transfer from mouse to mouse for indefinite periods, but the strain loses its infectivity to dogs (Freeman, 1962). This characteristic does not detract from its value as a laboratory animal mode.

Pathology. Nothing is known about the pathology of *T. crassiceps* in intermediate hosts.

Occasional enteritis and digestive disturbances have been reported in the dog and phasic eosinophilia has also been noted (Freeman, 1962).

In the one case of human infection so far documented the larvae were located in the eye, causing impaired vision and marked eosinophilia (Shea *et al.*, 1973). Surgical removal of the cysticerci was effective in this case and may be the recommended treatment when possible.

Immunity to Taeniid Parasites

In this section, the literature review deals with protective immune responses to taeniid parasites. Emphasis is placed on the antibodies involved and their mechanism of action, and the antigens which provoke formation of these antibodies. A section on immunity and host-parasite relationships is also included.

Antibodies Involved in Immunity to Taeniid Parasites

The early work of Miller and Gardiner (1932) and Campbell (1938) established conclusively the participation of antibody in protection against *T. taeniaeformis*. Immunity to this larval cestode was first demonstrated by the successful passive immunization of rats with serum collected 28 days after experimental infection (Miller and Gardiner, 1932; Campbell, 1938a). Similar studies by Kerr (1935) and Hearin (1941) demonstrated passive immunization against *T. pisiformis* in rabbits and *Hymenolepis nana* in mice. Blundell-Hassell, Gemmell and Macnamara (1968) were able to transfer immunity to *T. hydatigena* via serum from artificially immunized lambs.

More recently, Leid and Williams (1974a) have demonstrated the ability of antibodies of a single well defined immunoglobulin class, $7S\gamma_{2a}$, to passively transfer resistance to *T. taeniaeformis* in the rat. A more complete conception of the role of antibody is essential if an understanding of the immune mechanism in this and other species is to be arrived at. The potential importance of locally produced γA antibodies on hatching and penetration of the

oncospheres in naturally or passively immunized animals also demands attention. Miller (1931) showed that immunity to *T. taeniaeformis* could be transferred from immune mothers to the offspring and later Gemmell, Blundell-Hasell and Macnamara (1969) reported some evidence that this may occur via colostrum in lambs born of ewes immunized with *T. hydatigena*. More recently Rickard and Arundel (1974) showed that lambs fed colostrum from ewes immunized with *T. ovis* were resistant to challenge infection. However, Urquhart (1961) has reported contrary results in identical experiments using animals infected with *T. saginata*.

In rodents the mechanism of passive transfer of immunity to cysticerci has not been studied, although in both rats and mice prenatal and postnatal transfer of some immunoglobulins occurs (Brambell, 1970). It is not known which immunoglobulin types participate in this passive transfer of immunity but, as Gemmell and Macnamara (1972) have pointed out, γA could be implicated in this phenomenon since it predominates in secretory and mucosal surfaces (Heremans and Vaerman, 1971). Almost nothing is known of γA function in newborn rats, although Hemmings, Jones and Williams (1973) could detect little or no human γA uptake across the gut of 12-day-old rats.

Mechanism of Action of the Antibody

Immunity to *T. taeniaeformis* is believed by some workers to operate during the initial stages of embryo penetration through the lamina propria to the liver (Campbell, 1938a; Miller and Gardiner, 1932). Campbell (1938b) reported that there is an

antibody-mediated destruction of parasites before encystment. Leonard and Leonard (1941) extended this observation and showed that, while passively immunized rabbits were resistant to oral infection with *T. pisiformis*, they were susceptible to the intravenous administration of hatched oncospheres. Thus they postulated that the intestine played a vital role in the mechanism of acquired immunity. Froyd and Round (1960) were able to substantiate this proposal by similar experiments in cattle with *T. saginata* and an "intestinal barrier" has been postulated by several authors in the field. Banerjee and Singh (1969) and Heath (1971) pursued this area of investigation by histopathologic studies of oncospheres of *T. taeniaeformis* in normal rats. They showed that epithelial cells may actually be lysed during penetration of the intestinal wall and that the oncosphere moves into the lamina propria seeking entry into the circulatory system en route to the liver. Heath (1971) further demonstrated that in immune animals oncospheres of *T. pisiformis* do not attempt to attach to the intestinal wall. This proposal is in agreement with Silverman (1955), who observed an immobilizing effect of immune serum on cestode oncospheres *in vitro*.

There is no experimental evidence to date regarding dependence of this phase of immunity upon such amplification systems as complement activation and vasoactive amine function which could be triggered by antigen-antibody reactions, although Murrell (1971) demonstrated changes in the permeability of larvae of *T. taeniaeformis* in the presence of heterologous antibody and showed that

this effect was complement dependent. The only attempt to implicate complement in immunity to a helminth infection was that of Jones and Ogilvie (1971). However, these authors could not involve complement in the sequence of events which cause expulsion of *Nippostrongylus brasiliensis* worms from the intestine of rats.

Smyth and Heath (1970) have reported a rapid and intense inflammatory reaction around degenerating larvae in cysticercosis and hydatidosis infections. It is thought that these reactions may be in part a result of a reaction involving antibody, antigen and mediator cells, particularly mast cells and neutrophils, causing release of substances like histamine, and slow reacting substance of anaphylaxis (SRS-A).

The capacity to mediate immunologic release of histamine and SRS-A has been associated with two rat immunoglobulins--γE and $7S\gamma_{2a}$--both of which show antibody activity in *T. taeniaeformis* infection (Leid and Williams, 1974a, 1974b). The release of vasoactive amines mediated by rat γE is dependent upon participation of the mast cell but does not require polymorphonuclear leukocytes or an intact complement system, as is the case in SRS-A release mediated by $7S\gamma_{2a}$ (Orange, Stechschulte, and Austen, 1972). The presence of accumulations of mast cells around cysticerci of *T. taeniaeformis* has been demonstrated by Varute (1971). It is not known to what extent such reactions may contribute to resistance to *T. taeniaeformis*, although some work has been done with other helminthic systems. Degranulation of eosinophils and basophils at the site of infection with a nematode, *Trichostrongylus*

colubriformis, has been observed by Rothwell, Dineen and Love (1971), and they have proposed participation of pharmacologically active amines produced by these cells in rejection of the parasite.

An experimental approach to this proposal has been made by Keller and Ogilvie (1972) using chemical inhibition of histamine, serotonin and SRS-A and a similar analysis of the situation in *T. taeniaeformis* should be fruitful.

Antigens Involved in Immunity

Immunity to *T. taeniaeformis* can be provoked artificially by the implantation of live parasites or extracts of metacestodes (Miller, 1932; Freeman, 1962). Miller (1932) immunized rats with cysticerci intraperitoneally and produced immunity in recipients and Campbell (1936) was able to provoke a strong resistance to infection by inoculating rats with extracts of *T. taeniaeformis*. Similarly, Kerr (1934) produced some detectable immunity in rabbits against *T. pisiformis* by vaccination with extracts. Immunization has generally been much more successful when animals are exposed to live parasite material. Thus rats given intraperitoneal implants of live cysticerci of *T. taeniaeformis* or *T. crassiceps* become solidly resistant to challenge (Miller, 1932; Freeman, 1962), although Heath (1973) was unable to do this with cysticerci of *T. pisiformis* implanted subcutaneously.

Campbell (1938a) observed that rats developed some immunity against *T. taeniaeformis* as early as 7 days after infection. Similar observations were recorded by Hearin (1941) on the early onset of immunity in mice against *H. nana*. Furthermore, Dow *et al.*

(1962) showed that embryos of *T. taeniaeformis* attenuated by irradiation were effective in immunizing rats. Similarly an infection of chemically or physically treated activated embryos of *T. hydatigena* induced strong resistance in sheep to a challenge infection (Gemmell, 1969). In these two experiments the oncospheres did not develop into metacestodes. Since killed eggs were ineffective in inducing immunity (Gemmell, 1964, 1969), all these experiments suggest that living organisms, or at least their metabolic products, may be essential for the induction of immunity. It would seem also from these observations that oncosphere survival but not necessarily complete reorganization is important in stimulating immunity.

Evidence that some of these antigens may be metabolic products is provided by the work of Rickard and Bell (1971a, 1971b). They showed that oncospheres of *T. taeniaeformis* or *T. ovis* contained within millipore chambers and implanted intraperitoneally in rats or lambs resulted in a high degree of immunity. Furthermore, antigens collected from *in vitro* cultures of *T. ovis* induced a strong resistance to challenge infection in lambs. More recently Rickard and Outteridge (1974) showed that *T. pisiformis* culture antigen induced a good protective immunity when injected into rabbits, although their results were by no means as clear cut as those of Rickard and Bell (1971a, 1971b). Nothing is known of the chemical and physical characteristics of the protective antigens at this time.

Immunity and Host-Parasite Relationships

Campbell (1938b) observed that *T. taeniaeformis* larvae outgrow their susceptibility to antibody by the sixth day of infection and proposed that this evasion of immunological attack by the parasites was derived from the host capsule surrounding the larvae. Recent work, however, indicates that this insusceptibility to antibody seems to result from inherent changes on the part of the parasite (Rickard, 1974). Furthermore, even in the case of the thick-walled metacestode of *E. granulosus* host proteins including immunoglobulins have been found to have reached the cyst fluid (Hustead and Williams, personal communication; Coltorti and Varela-Diaz, 1972).

Since the original proposal of Campbell (1938b) workers in the field of cestode immunology have advanced further theories. These include: (a) coating of the parasite surface with host material (including specific antibody) so that the cysticercus is not recognized as foreign (Varela-Diaz, Gemmell and Williams, 1972; Rickard, 1974); (b) hiding of the evolutionarily adapted parasite behind a self-made mask of molecular mimicry of host-tissue so that the host recognizes it as self (Sprent, 1959; Dineen, 1963; Damian, 1964); (c) induced synthesis by the parasite of components antigenically identical to host components (Capron *et al.*, 1968). These possible mechanisms of cestode survival in immune hosts are briefly discussed subsequently.

(a) Coating of the Parasite. Varela-Diaz *et al.* (1972) have proposed a mechanism involving two antibodies and two antigens in juxtaposition. When one of the antibodies having a neutral effect

on the parasite combines with the antigenic determinant, the resulting steric hindrance blocks the action of the lethal antibody. A similar hypothesis, involving one antibody and one antigen, has been advanced by Rickard (1974). He proposed that the parasites become coated with specific antibody which blocks their susceptibility to cell mediated immunity. This is analogous to the situation in tumor enhancement whereby cancerous cells are coated with antibody and shielded from cellular attack.

For both hypotheses (Varela-Diaz *et al.*, 1972; Rickard, 1974) to be operational one has to assume that metacestodes are capable of inducing formation of a variety of antibodies, some of which have no destructive effects while others have.

(b) Molecular Mimicry. This hypothesis suggests that host immune responses exert selective pressure on "fitness" for survival of the parasites. This selection for survival may only favor variants of the parasite which display reduced disparity with the host. A reduction of disparity is only necessary, of course, with those antigenic characteristics of the parasite which stimulate responses adverse to its survival in the host. As Coltorti and Varela-Diaz (1972) pointed out, this hypothesis is inadequate to account for the strict host specificity of the serum components in hydatid cyst fluid, for example.

(c) Synthesis of Host Substances by the Parasite. According to this hypothesis, when a parasite enters a given host, the formation of antigens resembling those of the host is induced and rejection of the parasite is avoided (Capron *et al.*, 1968). Chordi and Kagan (1965) and Varela-Diaz and Coltorti (1973) have

demonstrated presence of multiple components identical to the host serum in hydatid cyst fluid as well as in membranes in a variety of hosts. If a process of induction were responsible for their synthesis, a considerable portion of the parasite genome would be required in order to code for a variety of molecules specific to the different hosts. In cases, then, where a parasite is capable of becoming established in several hosts, mechanisms of repression and derepression would have to operate to turn on or off wanted or unwanted genes.

More recently Capron and his co-workers (Bout, Capron, Dupas and Capron, 1974) have provided some evidence in support of this hypothesis. They showed incorporation of radiolabeled isoleucine and lysine into host-like antigens produced by *Schistosoma mansoni* parasites *in vitro*, which suggested that these parasites possessed a system of codes of protein synthesis similar to that of the host.

Rat and Mouse Immunoglobulins

This portion of the literature review deals with classification and biological functions of rat and mouse immunoglobulins.

Immunoglobulins of the Rat

Escribano and Grabar (1962) published the first immunoelectrophoretic patterns of rat serum and later Arnason *et al.* (1963, 1964) distinguished three immunoglobulin classes, γA, γG and γM. Binaghi and Sarandon de Merlo (1966) further subdivided the γG class into γG_a and γG_b. More recently, Bazin *et al.* (1974) have recognized 5 major rat immunoglobulin classes, γA, $7S\gamma_1$, $7S\gamma_2$, γE

and γM, with $7S\gamma_2$ having 3 subclasses, a, b and c. Physical and biological properties of each class are discussed subsequently.

$\underline{7S\gamma_2}$. This 7S immunoglobulin class has been recently divided into 3 subclasses of a, b and c according to their electrophoretic mobility and antigenic differences. The subclasses are separable by DEAE cellulose chromatography, using low ionic strength buffers (Stechschulte, Austen and Bloch, 1967). Like human 7SγG subclasses (Gergely *et al.*, 1971) they differ in their susceptibility to trypsin digestion, $7\gamma_{2b}$ and $7\gamma_{2c}$ being susceptible while $7S\gamma_{2a}$ is not (Nezlin *et al.*, 1973). $7S\gamma_{2a}$ antibodies have the capacity to fix for short periods of time in the skin of recipient rats for participation in passive cutaneous anaphylaxis reactions and prepare rat peritoneal cells for antigen induced release of slow reacting substance of anaphylaxis (SRS-A) (Stechschulte *et al.*, 1967; Orange, Valentine, and Austen, 1968; Morse *et al.*, 1968).

Leid and Williams (1974a) have demonstrated that $7S\gamma_2$ immunoglobulins, especially $7S\gamma_{2a}$, were responsible for passively transferred resistance to *T. taeniaeformis*. Occasionally $7S\gamma_2$ immunoglobulins were found to be protective against *Nippostrongylus brasiliensis* (Jones, Edwards and Ogilvie, 1970).

$\underline{7S\gamma_1}$. The rat immunoglobulin class originally designated γA by Arnason *et al.* (1964) and Binaghi and Sarandon de Merlo (1966) has now been shown to be $7S\gamma_1$ (Jones, 1969), although it is not the biological equivalent of $7S\gamma_1$ from mice and guinea pigs (Binaghi, 1971). It binds complement and is active in hemolytic

assays (Jones, 1969; Morse *et al.*, 1969), although its lytic properties are not as pronounced as those of $7S\gamma_2$ antibodies. Immunoglobulins of this class have been shown to confer protection against *Nippostrongylus brasiliensis* (Jones, Edwards and Ogilvie, 1970).

γA. An antigenically distinct class of immunoglobulin has now been shown to exist and predominates in all mucosal surfaces and secretions (Nash, Vaerman, Bazin and Heremans, 1969; Stechschulte and Austen, 1970; Bistany and Tomasi, 1970). However, it is present in low concentration in serum (Nash and Heremans, 1972). Electrophoretically γA has a faster mobility than $7S\gamma_1$ or $7S\gamma_2$. Other characteristics include a relatively high carbohydrate content, presence in serum as a 7S immunoglobulin, and appearance in secretions as an 11S molecule. However, a polypeptide chain, termed secretory piece, has not been detected (Stechschulte and Austen, 1970; Bistany and Tomasi, 1970). The absence of the secretory piece is not peculiar to the rat alone, since it has not been demonstrated in sheep (Sullivan *et al.*, 1969) or horse (Genco *et al.*, 1969). Although γA immunoglobulins predominate on all mucosal surfaces and secretions, their importance in intestinal parasitic infections has not been investigated.

γM. This is a slow moving immunoglobulin in an electrophoretic field and has a half life of approximately 3 days (Van Breda Vriesman and Feldman, 1972). Biologically γM has more agglutinating activity and is approximately 300 times more effective in lysis of

sensitized red cells than 7S antibodies. These characteristics, together with its valence of 10 and M.W. of 900,000 (19S), make it similar to γM antibodies of other species.

Occasionally protective γM antibodies to *Nippostrongylus brasiliensis* have been found (Jones, Edwards and Ogilvie, 1970).

γE. Reagins constitute one of the most striking antibody responses to parasitic infections (Ogilvie, 1964; Leid and Williams, 1974b, 1975). These antibodies were first described for animal parasites by Ogilvie (1964), who used passive cutaneous anaphylaxis tests to demonstrate reagins in rats, monkeys and sheep infected with *N. brasiliensis*, *S. mansoni* and *T. colubriformis*, respectively. Later this antibody was defined by Stechschulte, Orange and Austen (1970) to be rat immunoglobulin γE, similar to the immunoglobulin class designated γE in humans (Ishizaka and Ishizaka, 1967). γE has the capacity to persist at a skin site in the rat for weeks as assessed by passive cutaneous anaphylaxis (PCA), and the ability to prepare mast cells passively *in vitro* for antigen-involved release of histamine and serotonin (Becker and Austen, 1966).

A possible competition for receptor sites on the peritoneal cells between γE and $7S\gamma_{2a}$ antibodies has been demonstrated both *in vitro* and *in vivo* (Bach *et al.*, 1971; Ohman and Bloch, 1972). The known physico-chemical properties of this homocytotropic antibody are its fast electrophoretic mobility, molecular size of approximately 8S and susceptibility to inactivation by heat or 2-mercaptoethanol.

Although there is no evidence to connect γE antibodies with protective immunity to parasitic infections thus far, it is possible that γE may act in concert with other specific antibodies, for example $7S\gamma_{2a}$ (Leid and Williams, 1974a).

Immunoglobulins of the Mouse

The classification of mouse immunoglobulins owes much to the work of Fahey and co-workers in the early 1960s and also to the availability of myeloma proteins which made further characterization possible (Askonas and Fahey, 1962; Fahey, Wunderlich and Mischell, 1964; Fahey and Sell, 1965). The nomenclature of these immunoglobulins is similar to that in the rat. As in the rat, five major classes have been recognized, γA, $7S\gamma_1$, $7S\gamma_2$, γE and γM with $7S\gamma_2$ divided into 3 subclasses, a, b and c (Kalpaktsoglou, Hong and Good, 1973; Prouvost-Danon, Binaghi, Rochas and Boussac-Aron, 1972). $7S\gamma_{2c}$ has sometimes been referred to as IgG_3 ($7S\gamma_3$) by some authors (Grey, Hirst and Cohn, 1971; Bazin, Beckers, Platteau, Mets and Kints, 1973). The major difference in classification seems to be in the short term skin fixing antibody, being $7S\gamma_{2a}$ in the rat and $7S\gamma_1$ in the mouse.

Physical properties of these mouse immunoglobulins such as molecular weight, electrophoretic mobilities are similar to those of the rat. So far association of purified, well defined classes of immunoglobulins with protective capacity against helminths has not been studied, although effects of immune serum or fractions on parasites have been reported. Haerin (1941) demonstrated passive immunization of mice with immune serum against *Hymenolepis nana*.

Using the same experimental model, DiConza (1969) found that the active serum factors were associated with the 7SγG immunoglobulin fraction of the infected mouse serum.

Cytophilic Antibodies

The sera of some animals immunized with different antigens, especially sheep red blood cells, contain antibodies with a pronounced affinity for macrophages and these antibodies were termed "cytophilic antibodies" by Boyden and Sorkin (1960). They can be demonstrated by autoradiography or by hemoadsorption (Boyden and Sorkin, 1960; Boyden, 1964). Cytophilic antibodies (CA) are known to be produced by rabbits (Boyden and Sorkin, 1960), guinea pigs (Boyden, 1964) and mice (Nelson and Mildenhall, 1967; Lokaj, 1968). However, they have not been described in other species. CA have been shown to belong to 7S globulins (occasionally 19S), with electrophoretic mobility of β or fast γ (Brown and Carpenter, 1971). Mercaptoethanol appears to have no effect on the cytophilic property of these antibodies, although Tizard (1969) had earlier reported contrary results.

On the basis of the molecular weight (180,000), mercaptoethanol resistance and pattern of chromatographic elution, it appears that CA belong to a 7SγG class of immunoglobulin, distinct from 7SγG hemoagglutinin.

The significance of CA *in vivo* is at present unknown, but several suggestions have been advanced by Nelson (1970). These include: (a) CA may function as an opsonin. In mice the factor was found to be capable of opsonizing erythrocytes for complete

phagocytosis. (b) They may serve as a recognition factor in the earliest phase of antibody production. (c) The factor could be involved in the expression of cell mediated immunity, especially delayed type hypersensitivity. CA have not been implicated in any way in parasitic diseases but they represent an important biologic activity of immunoglobulins which should be pursued in infectious diseases, including cysticercosis and hydatidosis.

REFERENCES

REFERENCES

Arnason, B. G., de Vaux St. Cyr, C., and Grabar, P. 1963. Immunoglobulin abnormalities of the thymectomized rat. *Nature,* 199, 1199-1200.

Arnason, B., de Vaux St. Cyr, C., and Relyveld, E. 1964. Role of the thymus in immune reactions in rats. IV. Immunoglobulins and antibody formation. *Int. Arch. Allergy,* 25, 206-224.

Askonas, B. A., and Fahey, J. L. 1962. Enzymatically produced subunits of proteins formed by plasma cells in mice. II. β_{2a} myeloma protein and Bence Jones protein. *J. Exp. Med.,* 115, 641-653.

Bach, K. M., Bloch, K. J., and Austen, K. F. 1971. IgE and IgGa antibody-mediated release of histamine from rat peritoneal cells. II. Interaction of IgGa and IgE at the target cell. *J. Exp. Med.,* 133, 771-772.

Banerjee, D., and Singh, K. S. 1969. Studies on *Cysticercus fasciolaris*. I. Studies on the early stages of infection in cysticerciasis in rats. *Indian J. Anim. Sci.,* 39, 149-154.

Bazin, H., Beckers, A., Platteau, B., Naze-DeMets, J., and Kints, J. P. 1973. Les immunoglobulines de la souris et du rat. *Experimentation Animale,* 6, 119-232.

Bazin, H., Beckers, A., and Querinjean, P. 1974. Three classes and four sub(classes) of rat immunoglobulins: IgM, IgA, IgE, and IgG_1, IgG_{2a}, IgG_{2b}, IgG_{2c}. *Eur. J. Immunol.,* 4, 44-48.

Becker, E. L., and Austen, K. F. 1966. Mechanisms of immunologic injury of rat peritoneal mast cells. I. The effect of phosphonate inhibition on the homocytotropic antibody-mediated histamine release and the first component of rat complement. *J. Exp. Med.,* 124, 379-395.

Binaghi, R. A. 1971. Biological activities of IgG in mammals, *Progress in Immunology*, ed. by B. Amos. Academic Press, New York, p. 849-858.

Binaghi, R., and Sarandon de Merlo, E. 1966. Characterization of rat IgA and its non-identity with the anaphylactic antibody. *Int. Arch. Allergy*, 30, 589-596.

Bistany, T. S., and Tomasi, T. B., Jr. Serum and secretory immunoglobulins of the rat. *Immunochemistry*, 7, 453-460.

Blundell-Hasell, S. K., Gemmell, M. A., and Macnamara, F. N. 1968. Immunological responses of the mammalian host against tapeworm infections. VI. Demonstration of humoral immunity in sheep by the activated embryos of *Taenia hydatigena* and *T. ovis*. *Exptl. Parasit.*, 23, 78-82.

Bout, D., Capron, A., Dupas, H., and Capron, M. 1974. Characterization of *Schistosoma mansoni* antigens. *Proc. Third International Congress of Parasitology*, D7, 1146-1148.

Boyden, S. V. 1964. Cytophilic antibody in guinea pigs with delayed type hypersensitivity. *Immunology* (Lond.), 7, 474-483.

Boyden, S. V., and Sorkin, E. 1960. The adsorption of antigens by spleen cells previously treated with antiserum *in vitro*. *Immunology*, 3, 272-283.

Brambell, F. W. R. 1970. *The Transmission of Passive Immunity from Mother to Young*. North Holland Publishing Company, Amsterdam, p. 102-141.

Brown, G. L., and Carpenter, P. L. 1971. Characteristics of cytophilic antibody in the mouse. *Infection and Immunity*, 3, 637-641.

Campbell, D. H. 1936. Active immunisation of albino rats with protein fractions from *Taenia taeniaeformis* and its larval form *Cysticercus fasciolaris*. *Am. J. Hyg.*, 23, 104-113.

Campbell, D. H. 1938a. The specific protective property of serum from rats infected with *Cysticercus crassicollis*. *J. Immunol.*, 35, 195-204.

Campbell, D. H. 1938b. Further studies on the "nonabsorbable" protective property in serum from rats infected with cysticercus crassicollis. *J. Immunol.*, 35, 465-476.

Campbell, W. C., and Blair, L. S. 1974. Treatment of the cystic stage of *Taenia crassiceps* and *Echinococcus multilocularis* in laboratory animals. *J. Parasit.*, 60, 1053-1054.

Capron, A., Biguet, J., Vernes, A., and Afchain, D. 1968. Structure antigenique des helminthes. Aspects immunologiques des relations hote-parasite. *Pathol. Biol.*, 16, 121-138.

Chordi, A., and Kagan, J. 1965. Identification and characterization of antigenic components of sheep hydatid fluid by immunoelectrophoresis. *J. Parasit.*, 51, 63-71.

Coltorti, E. A., and Varela-Diaz, V. M. 1972. IgG levels and host specificity in hydatid cyst fluid. *J. Parasit.*, 58, 753-756.

Damian, R. T. 1964. Molecular mimicry: Antigen sharing by parasite and host and its consequences. *Amer. Naturalist*, 98, 129-149.

DiConza, J. J. 1969. Protective action of passively transferred immune serum and immunoglobulin fractions against tissue invasive stages of the dwarf tapeworm, *Hymenolepis nana*. *Exptl. Parasit.*, 25, 368-375.

Dineen, J. K. 1963. Immunological aspects of parasitism. *Nature* (Lond.), 127, 268-269.

Dow, C., Jarrett, W. F. H., Jennings, F. W., McIntyre, W. I. M., and Mulligan, W. 1962. The production of immunity to *Cysticercus fasciolaris* using x-irradiated oncospheres. *Amer. J. Vet. Res.*, 23, 146-149.

Escribano, J. J., and Grabar, P. 1962. L'analyse immunoelectrophoretique du serum de rat normal. *C. R. Acad. Sci. Paris*, 255, 206-208.

Fahey, J. L., Wunderlich, J., and Mischell, R. 1964. The immunoglobulins of mice. II. Two subclasses of mouse $7S\gamma_2$-globulins: γ_{2a} and γ_{2b} globulins. *J. Exp. Med.*, 120, 243-251.

Fahey, J. L., and Sell, S. 1965. The immunoglobulins of mice. V. The metabolic (catabolic) properties of five immunoglobulin classes. *J. Exp. Med.*, 122, 41-58.

Faust, E. C., Russell, P. F., and Jung, R. C. 1970. *Craig and Faust's Clinical Parasitology*, 8th ed. Lea and Febiger, Philadelphia, p. 529-557.

Freeman, R. S. 1962. Studies on the biology of *Taenia crassiceps* (Zeder 1800) Rudolphi 1810 (Cestoda). *Canadian J. Zool.*, 40, 969-990.

Froyd, G. 1960. Cysticercosis and hydatid disease of cattle in Kenya. *J. Parasit.*, 46, 491-496.

Froyd, G., and Round, M. C. 1960. The artificial infection of adult cattle with *C. bovis*. *Res. Rev. Sci.*, 1, 275-282.

Gemmell, M. A. 1964. Immunological responses of the mammalian host against tapeworm infections. I. Species specificity of hexacanth embryos in protecting sheep against *Taenia hydatigena*. *Immunology*, 7, 489-499.

Gemmell, M. A. 1969. Immunological responses of the mammalian host against tapeworm infections. X. Immunization of sheep against *Taenia hydatigena* and *T. ovis* with chemically or physically treated embryos. *Exptl. Parasit.*, 26, 58-66.

Gemmell, M. A., Blundell-Hasell, S. K., and Macnamara, F. N. 1969. Immunological responses of the mammalian host against tapeworm infections. IX. The transfer via colostrum of immunity to *Taenia hydatigena*. *Exptl. Parasit.*, 26, 52-57.

Gemmell, M. A., and Macnamara, F. N. 1972. Immune response to tissue parasites. II. Cestodes. *Immunity to Animal Parasites*, ed. by E. J. L. Soulsby. Academic Press, New York, p. 235-272.

Genco, R. J., Yecies, L., and Karush, F. 1969. The immunoglobulins of equine colostrum and parotid fluid. *J. Immunol.*, 103, 437-444.

Gergely, J., Medgyesi, G. A., Wang, A. C., and Fudenberg, H. H. 1972. IgG myeloma subclass-typing by tryptic digestion of whole sera. *Immunochemistry*, 9, 589-592.

Grey, H. M., Hirst, J. W., and Cohn, M. 1971. A new mouse immunoglobulin: IgG_3. *J. Exp. Med.*, 133, 289-304.

Hearin, J. T. 1941. Studies on the acquired immunity to the dwarf tapeworm, *Hymenolepis nana* var. fraterna in the mouse host. *Amer. J. Hyg.*, 33, D71-D87.

Heath, D. D. 1971. The migration of oncospheres of *Taenia pisiformis*, *T. serialis*, and *Echinococcus granulosus* within the intermediate host. *Int. J. Parasit.*, 1, 145-152.

Heath, D. D. 1973. Resistance to *Taenia pisiformis* larvae in rabbits. I. Examination of the antigenically protective phase of larval development. II. Temporal relationships and the development phase affected. *Int. J. Parasit.*, 3, 485-489.

Heath, D. D., and Chevis, R. A. F. 1974. Mebendazole and hydatid cysts. *Lancet*, 7874, 218-219.

Hemmings, W. A., Jones, R. E., and Williams, E. W. 1973. Transmission of IgA to the rabbit foetus and suckling rat. *Immunology*, 25, 645-647.

Heremans, J. F., and Vaermann, J. P. 1971. In *Progress in Immunology*, ed. by B. Amos. First International Congress of Immunology. Academic Press, New York, p. 875.

Hinz, Von E. 1964. Chemotherapeutische Untersuchungen mit endoxan an der experimentellen Finneninfektion der weifsen Maus. *Ztschr. Tropenmed. Parasit.*, 15, 332-336.

Hutchison, W. M. 1958. Studies on *Hydatigera taeniaeformis*. I. Growth of the larval stage. *J. Parasit.*, 44, 574-482.

Ishizaka, K., and Ishizaka, T. 1967. Identification of γE antibodies as a carrier of reaginic activity. *J. Immunol.*, 99, 1187-1198.

Jones, V. E. 1969. Rat 7S immunoglobulins. Characterization of γ_2 and γ_1 antihapten antibodies. *Immunology*, 16, 589-600.

Jones, V. E., Edwards, A. J., and Ogilvie, B. M. 1970. The circulating immunoglobulins involved in protective immunity to the intestinal stage of *Nippostrongylus brasiliensis* in the rat. *Immunology*, 18, 621-633.

Jones, V. E., and Ogilvie, B. M. 1971. Protective immunity to *Nippostrongylus brasiliensis*: The sequence of events which expels worms from the rat intestine. *Immunology*, 20, 549-561.

Kalpaktsoglou, P. K., Hong, R., and Good, R. A. 1973. The five classes of immunoglobulins in normal 3H and Balb/c mice. *Immunology*, 24, 303-314.

Keller, R., and Ogilvie, B. M. 1972. The effects of drugs on worm expulsion in the *Nippostrongylus brasiliensis* infected rat: A discussion of the interpretation of drug action. *Parasit.*, 64, 217-227.

Kerr, K. B. 1934. Immunity in rabbits against one of its cestode parasites *Cysticercus pisiformis*. *J. Parasit.*, 20, 328, Abstr. 18.

Kerr, K. B. 1935. Immunity against a cestode parasite, *Cysticercus pisiformis*. *Am. J. Hyg.*, 22, 169-182.

Leid, W. R., and Williams, J. F. 1974a. Immunological response of the rat to infection with *Taenia taeniaeformis*. I. Immunoglobulin classes involved in passive transfer of resistance. *Immunology*, 27, 195-208.

Leid, W. R., and Williams, J. F. 1974b. Immunological responses of the rat to infection with *Taenia taeniaeformis* II. Characterisation of reaginic antibody and an allergen associated with the larval stage. *Immunology*, 27, 209-225.

Leid, W. R., and Williams, J. F. 1975. Reaginic antibody response in rabbits injected with *Taenia pisiformis*. *Int. J. Parasit.*, (in press).

Leonard, A. B., and Leonard, A. E. 1941. The intestinal phase of the resistance of rabbits to the larvae of *Taenia pisiformis*. *J. Parasit.*, 27, 375-378.

Lokaj, J. 1968. Cytophilic antibodies in mice immunized with sheep red cells. *Folia Microbiol. Praha*, 13, 97-105.

Meymarian, E., and Schwabe, C. 1962. Host-parasite relationships in echinococcosis. IV. Resistance to the ova of *Echinococcus granulosus* to germicides. *Am. J. Trop. Med. Hyg.*, 11, 360-364.

Miller, H. M. 1931. The production of artificial immunity in the albino rat to a metazoan parasite. *J. Prev. Med.*, 5, 429-452.

Miller, H. M. 1932. Further studies on immunity to a metazoan parasite, *Cysticercus fasciolaris*. *J. Prev. Med.*, 6, 37-46.

Miller, H. M. 1935. Transmission to offspring of immunity against infection with a metazoan (cestode) parasite. *Am. J. Hyg.*, 21, 456-461.

Miller, H. M., and Gardiner, M. L. 1932. Passive immunity to infection with a metazoan parasite *Cysticercus fasciolaris* in the albino rat. *J. Prev. Med.*, 6, 479-496.

Morse, H. C., Austen, K. F., and Block, K. J. 1969. Biologic properties of rat antibodies: III. Histamine release mediated by two classes of antibodies. *J. Immunol.*, 102, 327-337.

Morse, H. C. III, Bloch, K. J., and Austen, K. F. 1968. Biologic properties of rat antibodies. II. Time-course of appearance of antibodies involved in antigen-induced release of slow reacting substance of anaphylaxis ($SRS\text{-}A^{rat}$); Association of this activity with rat IgGa. *J. Immunol.*, 101, 658-663.

Murrell, K. D. 1971. The effect of antibody on the permeability control of larval *Taenia taeniaeformis*. *J. Parasit.*, 57, 875-880.

Nash, D. R., and Heremans, J. F. 1972. Intestinal mucosa as a source of serum IgA in the rat. *Immunochemistry*, 9, 461-464.

Nash, D. R., Vaerman, J. P., Bazin, H., and Heremans, J. F. 1969. Identification of IgA in rat serum and secretions. *J. Immunol.*, 103, 145-148.

Nelson, D. S. 1970. Studies on cytophilic antibodies. A mouse serum "antibody" having an affinity for macrophages and fast γ globulin mobility. *Aust. J. Exp. Biol. Med. Sci.*, 48, 329-341.

Nelson, D. S., and Mildenhall, P. 1967. Studies on cytophilic antibodies. I. The production by mice of macrophage cytophilic antibodies to sheep erythrocytes: Relationship to the production of other antibodies and the development of delayed-type hypersensitivity. *Aust. J. Exp. Biol. Med. Sci.*, 45, 113-130.

Nezlin, R. S., Krilov, M. Yu., and Rokhlin, D. V. 1973. Different susceptibility of subclasses of rat $Ig_{\gamma 2}$ to tryptic digestion. *Immunochemistry*, 10, 651-652.

Ogilvie, B. M. 1964. Reagin-like antibodies in animals immune to helminth parasites. *Nature*, 204, 91-92.

Ohman, J. L., Jr., and Block, K. J. 1972. Interaction *in vivo* of homocytotropic antibodies belonging to two different rat immunoglobulin classes: Effect of IGE on the passive cutaneous anaphylactic reaction mediated by IgGa antibodies. *J. Immunol.*, 108, 1637-1646.

Orange, R. P., Stechschulte, D. J., and Austen, K. F. 1972. Immunochemical and biologic properties of rat IgE. II. Capacity to mediate the immunologic release of histamine and slow-reacting substance of anaphylaxis (SRS-A). *J. Immunol.*, 105, 1087-1095.

Orange, R. P., Valentine, D., and Austen, K. F. 1968. Antigen induced release of slow reacting substance of anaphylaxis (SRS-A rat) in rats prepared with homologous antibody. *J. Exp. Med.*, 127, 767-782.

Prouvost-Danon, A., Binaghi, R., Rochas, S., and Boussac-Aron, Y. 1972. Immunochemical identification of mouse IgE. *Immunology*, 23, 481-491.

Rickard, M. D. 1974. Hypothesis for the long term survival of *Taenia pisiformis* cysticerci in rabbits. *Z. Parasitenk.*, 44, 203-209.

Rickard, M. D., and Arundel, J. H. 1974. Passive protection of lambs against infection with *Taenia ovis* via colostrum. *Aust. Vet. J.*, 50, 22-24.

Rickard, M., and Bell, K. 1971a. Immunity produced against *Taenia ovis* and *T. taeniaeformis* infection in lambs and rats following *in vivo* growth of larvae in filtration membrane diffusion chambers. *J. Parasit.*, 57, 571-575.

Rickard, M., and Bell, K. J. 1971b. Successful vaccination of lambs against infection with *Taenia ovis* using antigens produced during *in vitro* cultivation of the larval stages. *Res. Vet. Sci.*, 12, 401-402.

Rickard, M., and Outteridge, P. M. 1974. Antibody and cell-mediated immunity in rabbits infected with the larval stages of *Taenia pisiformis*. *Z. Parasitenk*, 44, 187-201.

Rothwell, T. L. W., Dineen, J. K., and Love, R. G. 1971. The role of pharmacologically-active amines in resistance to *Trichostrongylus colubriformis* in the guinea pig. *Immunology*, 21, 925-938.

Salazar, M., Gonzalez, D., and Vega, M. V. 1972. Ensayo de tratamiento de la cisticercosis con metrifonato. *Rev. Invest. Solud. Publica*, 32, 1-7.

Schultz, M. G., Hatterman, L. G., Rich, A. B., and Martin, G. A. 1969. An epizootic of bovine cysticercosis. *J.A.V.M.A.*, 155, 1708-1717.

Shea, M., Marberley, A. L., Walters, J., Freeman, R. S., and Fallis, A. M. 1973. Intraocular *Taenia crassiceps* (Cestoda). *Amer. Academy of Ophth. and Otol.*, 77, OP-778-OP-783.

Silverman, P. H. 1955. A technique for studying the *in vitro* effect of serum on activated taeniid hexacanth embryos. *Nature* (Lond.), 176, 598-599.

Silverman, P. H., and Griffith, R. B. 1955. A review of methods of sewage disposal in Great Britain with special reference to epizootiology of *Cysticercus bovis*. *Ann. Trop. Med. Parasit.*, 49, 436-450.

Silverman, P. H., and Maneely, R. B. 1955. Studies on the biology of some tapeworms of the genus, *Taenia*. III. The role of the secreting gland of the hexacanth embryo in the penetration of the intestinal mucosa of the intermediate host and some of its histochemical reactions. *Ann. Trop. Med. Parasit.*, 49, 326-330.

Singh, B. B., and Rao, B. V. 1967. On the development of *Cysticercus fasciolaris* in albino rat liver and its reaction on the host tissue. *Ceylon Vet. J.*, 15, 121-129.

Smyth, J. D. 1963. The biology of cestode life cycles. Tech. Comm. No. 34. Commonwealth Bureau of Helminthology. Farnham Royal, England, Commonwealth Agricultural Bureaux.

Smyth, J. D., and Heath, D. D. 1970. Pathogenesis of larval cestodes in mammals. *Helminthological Abstracts,* Series A, 1, 2-23.

Soulsby, E. J. L. 1968. *Helminths, Arthropods and Protozoa of Domesticated Animals.* (Monning.) p. 114-118. The Williams and Wilkins Company, Baltimore.

Sprent, J. F. A. 1959. Parasitism, immunity and evolution. *The Evolution of Living Organisms.* Symposium of the Royal Society of Melbourne, Dec. 1959, G. W. Leeper, ed. Melbourne University Press, p. 149-165.

Stechschulte, D. J., and Austen, K. F. 1970. Immunoglobulins of rat colostrum. *J. Immunol.*, 104, 1052-1062.

Stechschulte, D. J., Austen, K. F., and Block, K. J. 1967. Antibodies involved in antigen-induced release of slow reacting substance of anaphylaxis (SRS-A) in the guinea pig and rat. *J. Exp. Med.*, 125, 127-147.

Sullivan, A. L., Prendergast, R. A., Antunes, L. J., Silverstein, A. M., and Tomasi, T. B. 1969. Characterisation of the serum and secretory immune systems of the cow and sheep. *J. Immunol.*, 103, 334-344.

Thienpont, D., Vanpary, O., and Heremans, L. 1974. Anthelmintic activity of mebendazole against *Cysticercus fasciolaris.* *J. Parasit.*, 60, 1052-1053.

Tizard, I. R. 1969. Macrophage cytophilic antibody in mice: Differentiation between antigen adherence due to these antibodies and opsonin adherence. *Int. Arch. Allergy,* 36, 332-346.

Urquhart, G. M. 1961. Epizootiological and experimental studies on bovine cysticercosis in East Africa. *J. Parasit.*, 47, 857-869.

Van Breda Vriesman, P. J. C., and Feldman, J. D. 1972. Rat IgM immunoglobulin isolation and some biological characteristics. *Immunochemistry*, 9, 525-534.

Varela-Diaz, V., and Coltorti, E. A. 1973. The presence of host immunoglobulins in hydatid cyst membranes. *J. Parasit.*, 59, 484-488.

Varela-Diaz, V. M., Gemmell, M. A., and Williams, J. F. 1972. Immunological responses of the mammalian host against tapeworm infections. XII. Observations on antigen sharing between *Taenia hydatigena* and *T. ovis*. *Exptl. Parasit.*, 32, 96-101.

Varute, A. T. 1971. Mast cells in cyst-wall of hydatid cyst of *Taenia taeniaeformis* (Batsch). *Indian J. Exp. Biol.*, 9, 200-203.

Williams, J. F., Colli, C. W., Leid, R. W., and MacArthur, R. 1973. Effects of Bunamidine hydrochloride on infectivity of taeniid ova. *J. Parasit.*, 59, 1141-1144.

IMMUNOGLOBULINS ASSOCIATED WITH PASSIVE TRANSFER OF RESISTANCE TO *TAENIA TAENIAEFORMIS* IN THE MOUSE

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SUMMARY. Mice were found to be protected against *Taenia taeniaeformis* infection by passive transfer of serum collected from donors 28 days after infection. The protective activity resided exclusively in the first fraction of 7S immunoglobulins eluting from DEAE cellulose at pH 5.8 with 0.05 M phosphate buffer. This fraction contained $7S\gamma_1$ and $7S\gamma_2$ immunoglobulins but no detectable γA, γM or skin sensitizing activity. Fractions containing $7S\gamma_2$ alone were ineffective in passive transfer.

INTRODUCTION

Leid and Williams (1974a) have recently shown that passive transfer of resistance to *Taenia taeniaeformis* infection in the rat can be achieved with immunoglobulins of the $7S\gamma_{2a}$ type. The biological characterization of antihapten antibodies of this nature had previously been described by Morse, Bloch and Austen (1968). They demonstrated short-term sensitization of rat skin for passive cutaneous anaphylaxis (PCA) and antigen-induced release of SRS-A from neutrophils and histamine from mast cells. However, $7S\gamma_{2a}$ antibodies to *T. taeniaeformis* were inactive in PCA tests (Leid and Williams, 1974b), suggesting the possibility that subpopulations of this immunoglobulin with distinct biological functions might be stimulated by helminthic infections.

We have pursued the peculiar association of protective activity against *T. taeniaeformis* with physico-chemically distinct immunoglobulin types and report here on the localization of the protective capacity of infected mouse serum in an immunoglobulin fraction

normally associated with short-term PCA and mast cell sensitization in the mouse (Revoltella and Ovary, 1969; Binaghi, 1971).

MATERIALS AND METHODS

Parasite

The strain of *T. taeniaeformis* used in these experiments was derived from gravid segments obtained from Mr. C. E. Claggett in the Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, Maryland. The parasite was maintained as described by Leid and Williams (1974a).

Experimental Animals

Female 28-day-old mice and rats were obtained from Spartan Research Animals, Haslett, Michigan.

Antisera

Twenty-eight-day-old mice were infected orally with 400 eggs of *T. taeniaeformis* and 28 days later were exsanguinated by severing the major thoracic vessels under CO_2 anesthesia. Serum was stored at -20°C.

Fractionation of Antiserum

Antiserum was centrifuged at 10,000 g for 10 minutes and 4 ml portions were dialyzed overnight against 0.1 M Tris-HCl buffer pH 8.0, and applied to a Sephadex G-200 column (2.5 x 100 cm), equilibrated against the same buffer. The ascending portion of the first peak (Figure 1) was taken and this fraction contained γM and one β-globulin arc demonstrable in immunoelectrophoresis. The

Figure 1. Elution profile at 280 nm of mouse globulins obtained 28 days after infection with *T. taeniaeformis* and passed through Sephadex G-200. Fractions were pooled as indicated and F1 was used for passive transfer experiments. F2 was fractionated further on DEAE-cellulose columns.

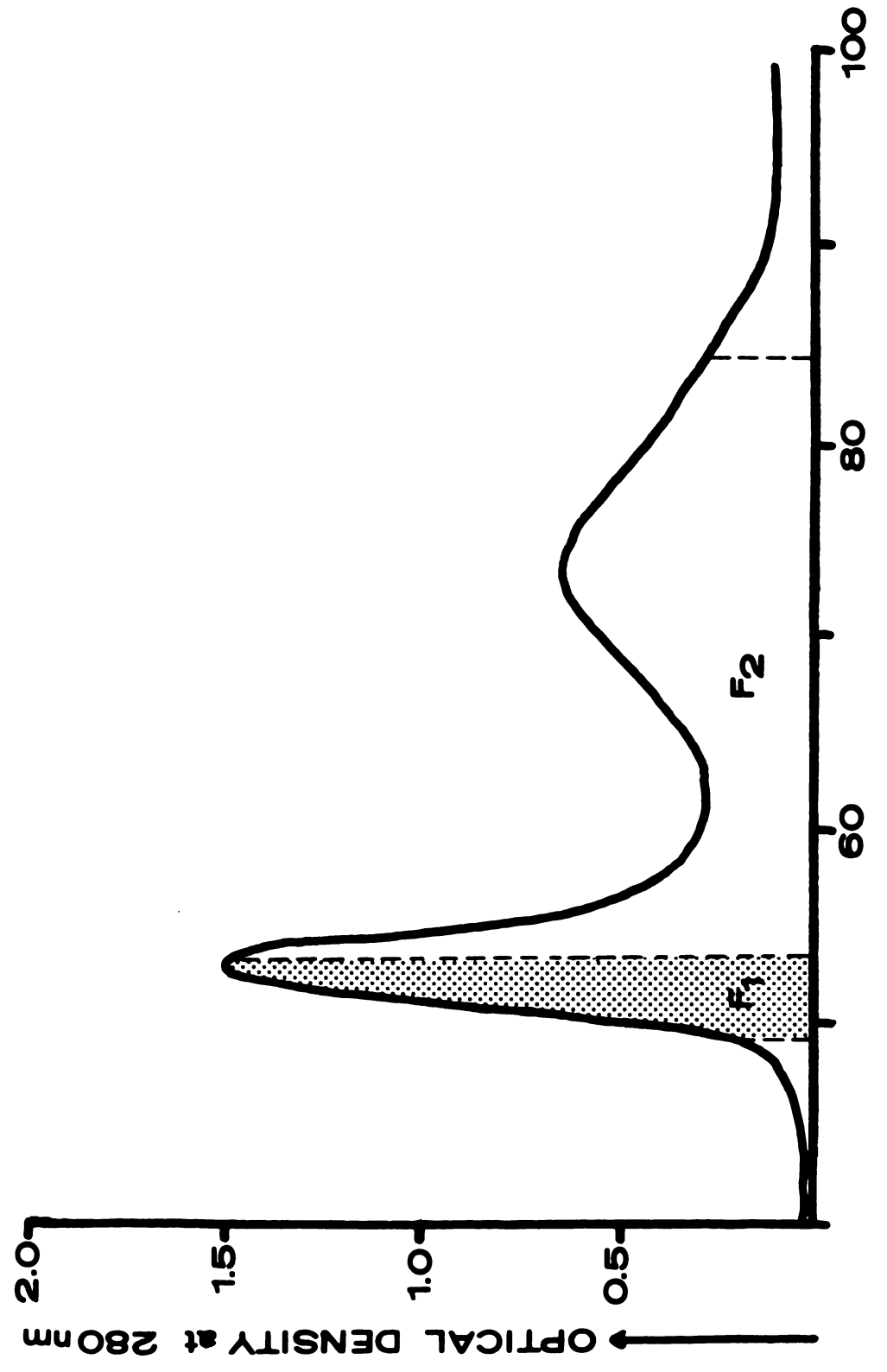
OPTICAL DENSITY at 280nm
2.0
1.5
1.0
0.5
F1
F2
60
80
100
TUBE NUMBER

Figure 1

descending portion of the first peak, together with the 7S peak, were further fractionated on DEAE-cellulose. Stepwise elution was performed using sodium phosphate buffers in the following sequence: 0.005 M, pH 7.8; 0.01 M, pH 7.8; 0.05 M, pH 5.8; 0.1 M, pH 5.8; and finally 2 M NaCl. All buffers were made 0.015 M in NaCl. The pooled fractions under each peak (Figure 2) were concentrated using polyethylene glycol and were tested against rabbit anti-whole mouse serum and anti-IgM and IgA (Meloy Laboratories, Springfield, Virginia) in both immunoelectrophoresis and double diffusion in gel tests, following the methods described by Leid and Williams (1974a).

Passive Transfer

Fractions 1-5 from DEAE chromatography and the ascending portion of the first peak from gel filtration were restored to the original serum volume with phosphate-buffered saline. Each fraction was tested for its capacity to confer protection against a challenge of 300 eggs of *T. taeniaeformis* in mice. Mice were killed 21 days later and the results were analyzed by a modified Student's t-test. This experimental procedure was repeated using two further batches of serum harvested in a similar manner from other groups of mice.

Passive Cutaneous Anaphylaxis (PCA)

All fractions from DEAE-cellulose chromatography were tested for their ability to provoke PCA in sensitized rats and mice following a modification of the procedure described by Revoltella and Ovary (1969). Positive samples were heated to 56°C for 1 hr,

Figure 2. DEAE-cellulose elution profile at 280 nm of 7S immunoglobulins from mice infected with *T. taeniaeformis*. Fractions F1-F5 were tested for protective capacity in passive transfer experiments and activity was localized in F3 (hatched area). PCA activity was restricted to F4.

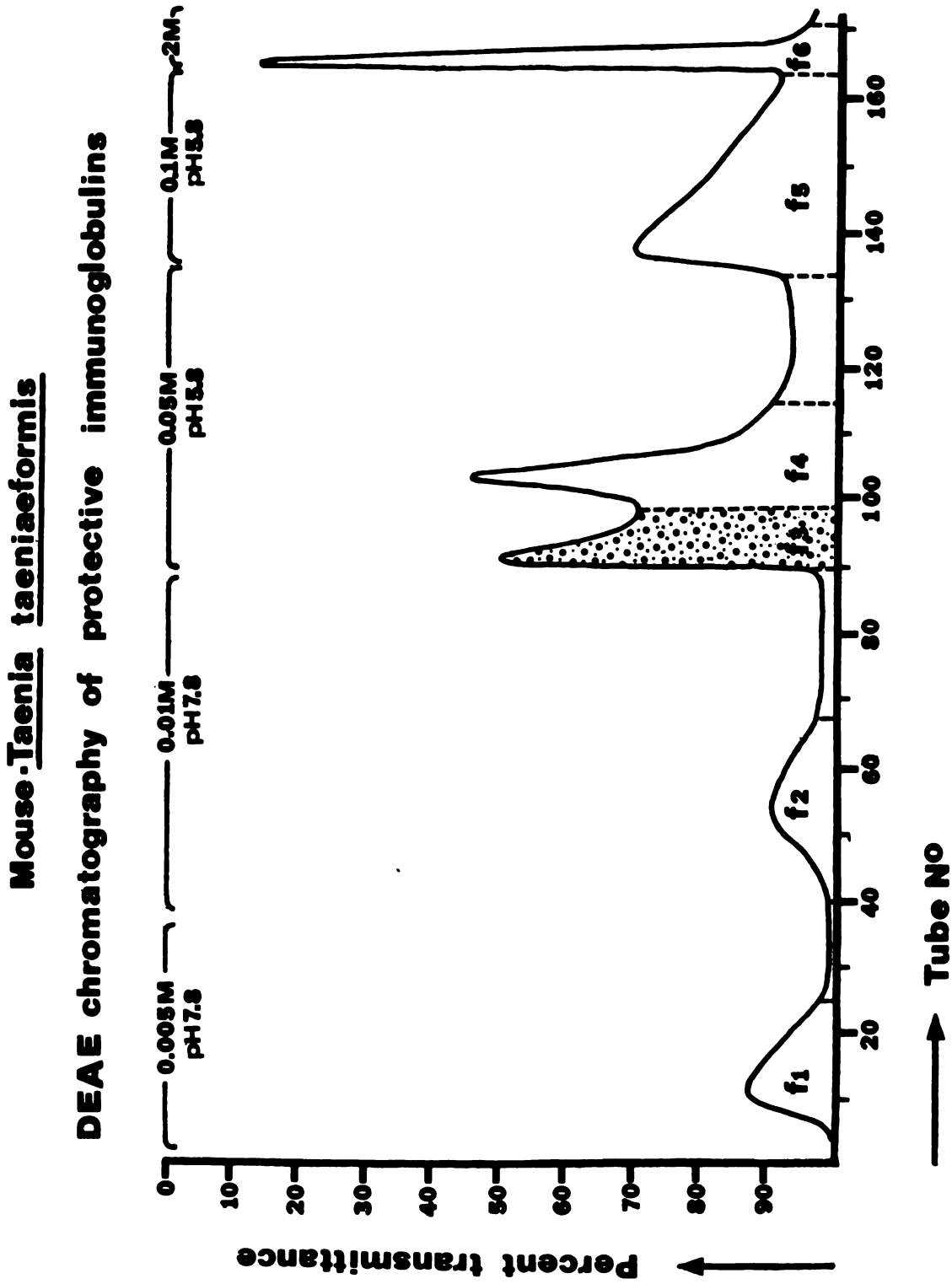
Mouse-Taenia taeniaeformis
DEAE chromatography of protective immunoglobulins
0.005M pH7.8
0.01M pH7.8
0.05M pH5.8
0.1M pH5.8
2M
f1
f2
f3
f4
f5
f6
Percent transmittance
Tube NO
0
10
20
30
40
50
60
70
80
90
20
40
60
80
100
120
140
160

Figure 2

or reduced and alkylated using the method of Nussenzweig, Merryman and Benacerraf (1964) and retested.

RESULTS

A typical DEAE-cellulose elution profile for 7S mouse immunoglobulins is shown in Figure 2. Two peaks were consistently eluted with the 0.05 M phosphate buffer, pH 5.8, and these were separated and identified as F3 and F4. These two fractions contained no detectable γA or γM, but immunoelectrophoretic analysis using rabbit anti-whole mouse serum showed that they contained distinct populations of $7S\gamma_1$ and $7S\gamma_2$ immunoglobulins (Figure 3).

The results obtained from a typical passive transfer experiment are shown in Table 1, where the average numbers of parasites developing in the livers in each group are recorded. Protective capacity was exclusively and consistently associated with F3, and there was no evidence of passive transfer with other fractions from Sephadex G-200 gel filtration (Figure 4).

Fractions 1-6 were tested for the ability to produce both homologous and heterologous PCA reactions in mice and rats, respectively. Latent periods of 2 hours and 72 hours were allowed prior to challenge. PCA activity was detected only in F4 (second peak 0.05 M eluate). This serum activity was shown in homologous and heterologous systems after both 2-hour and 72-hour sensitization periods, but was destroyed by heating to 56°C for 60 minutes and by reduction and alkylation with 2-mercaptoethanol and iodoacetamide.

Figure 3. Immunoelectrophoretic analysis of fractions of mouse 7S immunoglobulins eluted from DEAE-cellulose with 0.05 M phosphate buffer pH 5.8. F3 and F4 were the first and second peaks, respectively, and the troughs were filled with rabbit anti-whole mouse serum.

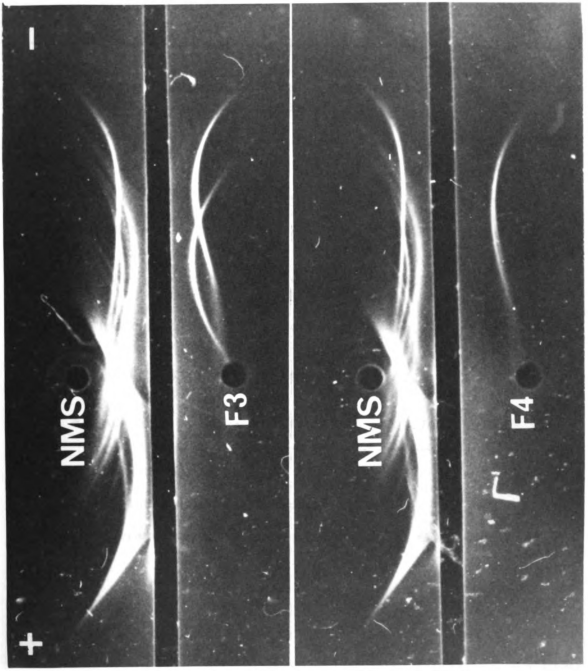

Figure 3

Table 1. Protective capacity of antiserum and immunoglobulin fractions isolated by column chromatography in recipient mice challenged by mouth with 300 eggs of *Taenia taeniaeformis*

Protein fraction transferred*	No. of mice	Mean no. of larvae ± s.d.†	S.E. of mean	P value
Normal mouse serum	6	75.5 ± 19.3	7.9	
Immune mouse serum	6	0.5 ± 0.83	0.34	<0.001
19S fraction of antiserum	6	76.0 ± 41.6	16.98	n.s.
F1-0.005 M DEAE-cellulose eluate	6	80.3 ± 46.78	19.1	n.s.
F2-0.01 M DEAE-cellulose eluate	6	68.8 ± 34.86	14.23	n.s.
F3-0.05 M (peak 1) DEAE-cellulose eluate	6	3.3 ± 4.5	1.83	<0.001
F4-0.05 M (peak 2) DEAE-cellulose eluate	6	64.67 ± 30.5	12.45	n.s.
F5-0.1 M DEAE-cellulose eluate	6	74.0 ± 21.74	8.88	n.s.

n.s. = not significant.

* - by intraperitoneal injection.

† - average number of larvae developing in the livers of each group.

Figure 4. Representative livers from groups of rats passively immunized with the DEAE fractions indicated. The animals were challenged *per os* with 300 eggs of *T. taeniaeformis* and killed 21 days later.

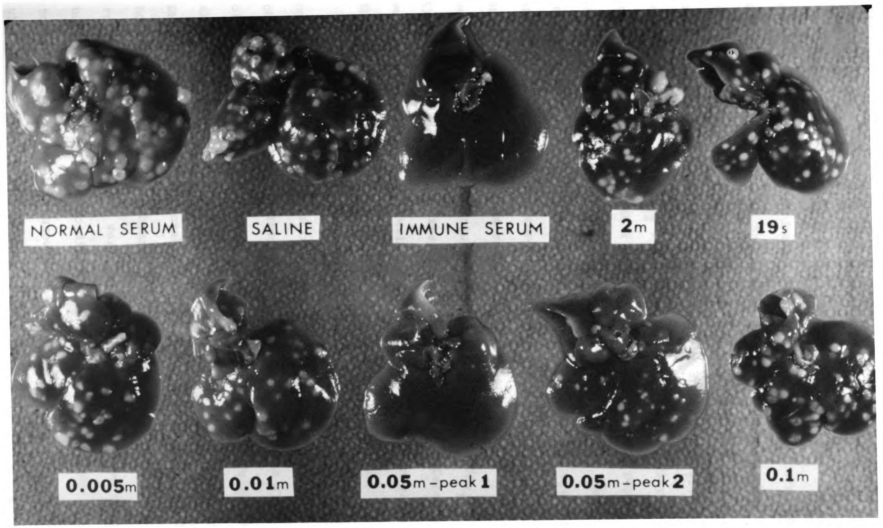
NORMAL SERUM
SALINE
IMMUNE SERUM
2m
19s
0.005m
0.01m
0.05m-peak 1
0.05m-peak 2
0.1m

Figure 4

DISCUSSION

In our experiments the distribution of protective activity against *T. taeniaeformis* in immune mouse serum appears to correspond most closely to that of the $7S\gamma_1$ immunoglobulins. Although the protective fraction, F3, contained both $7S\gamma_1$ and $7S\gamma_2$ immunoglobulins, fractions F1 and F2, which eluted earlier from DEAE-cellulose, contained only slow-moving $7S\gamma_2$ and showed no protective activity. It is possible, of course, that a physico-chemically distinct population of $7S\gamma_2$ antibodies, which does not elute prior to the step involving 0.05 M phosphate buffer, was responsible for the transfer of immunity. However, the abrupt appearance of both $7S\gamma_1$ and protective capacity in F3 is remarkably coincidental and is the basis for our tentative conclusion that antibodies of the $7S\gamma_1$ type are most likely to be responsible for the passive resistance we observed. Further work will be required in order to consolidate this position.

Clearly, protective antibodies were not of the γA or γM type nor had they demonstrable skin-sensitizing activity. The latter characteristic must be considered in relation to the recent findings of Revoltella and Ovary (1969). They were able to distinguish two skin-sensitizing antibodies in the sera of mice immunized against DNP, one of which was detectable after a short latent period of 2 hours and the other at 72 hours. These two antibodies, $7S\gamma_1$ and reagin, were shown to have very similar physico-chemical properties but were separated under conditions of anion exchange chromatography similar to those used in our experiment. The PCA activity which we detected in F4 was heat-labile, sensitive to reduction

and alkylation, and active in rat skin and therefore corresponds to the reagin of Revoltella and Ovary (1969) and other workers (Mota, Sadun and Gore, 1969; Bach and Brashler, 1973). The fact that PCA reactions could be provoked after only 2 hours of sensitization with reagin is in agreement with the observations of Stechschulte, Orange and Austen (1970).

Peculiarly, however, we were unable to demonstrate short-term homologous PCA reactions with F3 even though protective antibodies, probably $7S\gamma_1$ in type, were present in this fraction. This situation is reminiscent of the observations of Leid and Williams (1974a) on $7S\gamma_{2a}$ antibodies in rat serum, and lends support to the suggestion that some populations of antigenically identifiable immunoglobulins have biological functions in helminth infections which are quite distinct from those described for antibodies showing antihapten activity. Nevertheless, the tentative association of protective activity with $7S\gamma_1$ in the mouse and $7S\gamma_{2a}$ in the rat indicates that these two antibody types serve some analogous function, although it does not appear to involve the mast cell.

ACKNOWLEDGEMENTS

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REFERENCES

BACH, M. K. and BRASHLER, J. R. (1973). 'Activity of mouse reaginic antibodies in the rat: passive cutaneous anaphylaxis and *in vitro* passive histamine release.' *Immunol. Comm.*, 2, 85.

BINAGHI, R. A. (1971). 'Biological activity of IgG in mammals.' *Progress in Immunology* (ed. by B. Amos), p. 849.

LEID, R. W. and WILLIAMS, J. F. (1974a). 'Immunological responses of the rat to infection with *Taenia taeniaeformis* I. Immunoglobulin classes involved in passive transfer of resistance.' *Immunology*, 27, 195.

LEID, R. W. and WILLIAMS, J. F. (1974b). 'Immunological responses of the rat to infection with *Taenia taeniaeformis* II. Characterization of reaginic antibody and an allergen associated with the larval stage.' *Immunology*, 27, 209.

MORSE, C. H., BLOCH, K. J. and AUSTEN, K. F. (1968). 'Biological properties of rat antibodies II. Time course of appearance of antibodies involved in antigen-induced release of slow reacting substance of anaphylaxis (SRS-A^{rat}); association of this activity with rat IgGa.' *J. Immunol.*, 101, 658.

MOTA, I., SADUN, E. H. and GORE, R. W. (1969). 'Homocytotropic antibody response in mice infected with *Schistosoma mansoni:* A comparison with the response following *Trichinella spiralis* infection.' *Exptl. Parasit.*, 24, 251.

NUSSENZWEIG, R., MERRYMAN, C. and BENACERRAF, B. (1964). 'Electrophoretic separation and properties of mouse anti-hapten antibodies involved in passive cutaneous anaphylaxis and passive hemolysis.' *J. Exp. Med.*, 120, 315.

REVOLTELLA, R. and OVARY, Z. (1969). 'Reaginic antibody production in different mouse strains.' *Immunology*, 17, 45.

STECHSCHULTE, D. J., ORANGE, R. P. and AUSTEN, K. F. (1970). 'Two immunochemically distinct homologous antibodies capable of mediating immediate hypersensitivity reactions in the rat, mouse and guinea-pig.' *Excerpta Medica International Congress Series No. 232, Proceedings VII of the International Congress of Allergology*, p. 245.

IMMUNOLOGICAL RESPONSE OF THE RAT TO INFECTION WITH *TAENIA TAENIAEFORMIS*. III. PROTECTIVE ANTIBODY RESPONSE TO IMPLANTED PARASITES

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ABSTRACT

MUSOKE, A. J. and WILLIAMS, J. F., 1975. Immunological response of the rat to infection with *Taenia taeniaeformis*. III. Protective antibody response to implanted parasites. *International Journal of Parasitology*.

Intraperitoneally implanted metacestodes of either *T. taeniaeformis* or *T. crassiceps* in rats provoked a high degree of resistance to oral challenge with eggs of *T. taeniaeformis*. This resistance was passively transferred to normal recipients with serum. Immunoglobulin fractions of immune serum containing $7S\gamma_1$ or γM were most effective in passive transfer and little activity was associated with $7S\gamma_2$ antibodies. No skin-sensitizing antibodies were detectable in immune sera. These findings are in sharp contrast to previous observations involving protective immunoglobulins and reaginic antibodies in serum from rats with hepatic cysticerci of *T. taeniaeformis*. Possible reasons for this are discussed.

Cysticerci implanted into normal rats survived for at least 21 days with no sign of host rejection, whereas those implanted into rats with hepatic infections with *T. taeniaeformis* were killed and encapsulated. Similar results were obtained by implanting cysticerci in normal rats given inoculations of complete Freund's adjuvant. Repeated inoculations of immune serum had no effect on the survival of implanted cysticerci, and it was concluded that exposure to infection by oncospheres provokes cellular defense mechanisms which can be effective against cysticerci in abnormal sites. Why these mechanisms are inoperative against hepatic cysticerci remains unclear.

INDEX KEY WORDS: Implanted metacestodes; protective immunoglobulins; cellular defense mechanisms; *Taenia taeniaeformis*.

INTRODUCTION

There is considerable evidence that substances released by living metacestodes of taeniid parasites can stimulate protective immune responses. Miller (1931) reported that live metacestodes of *Taenia taeniaeformis* implanted into the peritoneal cavity of normal rats stimulated an absolute resistance to homologous oral challenge, and Gemmell (1965) found that viable embryos of *T. hydatigena*, *T. ovis*, or *T. pisiformis* inoculated intramuscularly produced a strong immunity against *T. pisiformis* in rabbits. More recently Rickard and Bell (1971a) successfully vaccinated lambs against *T. ovis* and *T. hydatigena* with antigens collected from embryos growing *in vitro*. They also showed that lambs and rats given intraperitoneally implanted membrane-diffusion chambers containing developing embryos of *T. ovis* or *T. taeniaeformis*, respectively, became highly resistant to homologous infection (Rickard and Bell, 1971b).

Although successful immunizations have been achieved with the metabolic products of taeniid larvae there has been little effort to characterize the immune mechanisms which are responsible for this resistance. However, Leid and Williams (1974a) recently showed that passive transfer of resistance could be achieved with immunoglobulins of the $7S\gamma_{2a}$ type isolated from the serum of rats with hepatic infections of *T. taeniaeformis*. The experiments reported here were therefore designed firstly to confirm the

findings of Miller (1931), and then to characterize the protective serum immunoglobulins produced in response to intraperitoneally implanted larvae of *T. taeniaeformis* in the rat. In addition, observations are presented on the effects of passively transferred immune serum on the survival of implanted parasites and on the influence of established hepatic cysticerci on the fate of implanted worms.

MATERIALS AND METHODS

Parasites

The strain of *T. taeniaeformis* used in these experiments was obtained from Mr. C. E. Claggett in the Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, Maryland, and has been maintained according to methods described by Leid and Williams (1974a).

Proliferative metacestode stages of *T. crassiceps* were passaged by intraperitoneal inoculations of cysticerci of CF_1 Carworth mice. The original inoculum was obtained from Dr. G. Esch, Wake Forest University, Winston-Salem, North Carolina.

Cysts of *Echinococcus granulosus* were propagated by a combination of intraperitoneal inoculation of protoscolices and surgical implantation of fertile cysts in rats as described by Varela-Diaz *et al.* (1974).

Experimental Animals

Sprague-Dawley rats 28-49 days old and CF_1 Carworth mice were purchased from Spartan Research Animals, Haslett, Michigan.

All animals were given proprietary brand food and water *ad libitum*.

Surgical Implantation of Parasites

Rats with infections of *T. taeniaeformis* of 5 months' duration were killed with carbon dioxide vapor and cysticerci were dissected from the livers and washed with sterile saline (3x) and Eagle's B.M.E. (3x). Washed cysticerci were implanted intraperitoneally into rats anesthetized with a combination of ketamine hydrochloride intramuscularly and methoxyfluorane by inhalation. Six larvae were inserted singly into each rat via a midline abdominal incision which was then sutured in two layers.

In experiments involving the effects of heterologous infection on immunity to *T. taeniaeformis*, rats were given intraperitoneal implants of either 50 washed cysticerci of *T. crassiceps* or 6 fertile cysts of at least 1 cm diameter of *E. granulosus*.

Collection and Fractionation of Immune Sera

Rats implanted with *T. taeniaeformis* larvae as described above were killed either 21 or 28 days later. All transferred cysticerci were recovered live and undamaged at autopsy of these animals. Blood was collected from the thoracic cavity under carbon dioxide anesthesia after severing the vessels anterior to the heart. Samples were allowed to clot for 2 to 3 hrs at 22 to 23 C and left overnight at 4 C. The serum was stored at -20 C without preservatives.

The globulins from 3 serum pools were precipitated with 50 percent saturated ammonium sulphate (x3) and dialyzed against phosphate buffered saline (PBS) until free of sulphate ions. The

procedure for anion-exchange chromatography of rat immunoglobulins was a modification of that described by Stechschulte, Austen and Bloch (1967). DEAE-cellulose (DE-52 Whatman Biochemicals Ltd., Springfield Mill, Maidstone, England) was prepared according to the directions of the manufacturer and poured in 1.5 x 30 cm siliconized glass columns. The cellulose was equilibrated against 0.005 M phosphate buffer pH 7.75. Proteins were eluted in a step-wise manner using 0.005 M buffer followed by 0.01 M, pH 7.75, 0.05 M, pH 5.8, 0.1 M, pH 5.8 and finally 2 M NaCl. All phosphate buffers were made 0.015 M in NaCl and the samples were dialyzed extensively against the starting buffer before application to the column. Column eluates were collected in 2.8 ml fractions and the elution pattern monitored by ultraviolet scanning at 280 nm (Gilson Medical Electronics, Middleton, Wisconsin). Protein peaks eluted with each buffer were pooled and concentrated back to the original serum volume.

Immunoelectrophoresis

Immunoelectrophoresis was performed following the method described by Williams and Chase (1971) in a Gelman apparatus (Gelman Instrument Company, Ann Arbor, Michigan) with a sodium barbital-HCl buffer μ = 0.038, pH 8.0. Monospecific antisera to rat immunoglobulins ($7S\gamma_1$, $7S\gamma_2$, γA and γM) were prepared following methods described by Leid and Williams (1974a).

Passive Cutaneous Anaphylaxis

The methods for performing PCA tests in rats and for the preparation of *T. taeniaeformis* extracts for antigen were described by Leid and Williams (1974b). All fractions from DEAE-cellulose chromatography were tested for their ability to provoke PCA reactions in sensitized rats. Latent periods of 4 hrs and 48 hrs were employed before injecting the antigen. Known positive controls were included in each rat.

Experimental Procedures

The ability of metacestodes of *T. taeniaeformis* of various ages to survive in the peritoneal cavity of normal rats was first studied. Larvae from rats which had been infected for periods of 2½, 3½, 4½, 5½, 6½, 9 and 14 months were implanted into groups of 6 normal recipients. These were killed 21 days later and the number of live larvae determined.

The influence of implanted larvae of *T. taeniaeformis* on susceptibility to homologous challenge was then determined. Three groups of rats were used: Group A1 received implants of 6 live 5-month-old larvae; Group A2 received implants of 6 dead larvae killed by freezing at -20°C; Group A3 served as normal controls. After 21 days all rats were challenged *per os* with 200 eggs of *T. taeniaeformis* and killed 3 weeks later. The number of cysticerci that established in the livers was determined for each group and the results were analyzed using a modified Student's t-test.

The influence of heterologous infection was measured by implanting groups of 6 rats with either *T. crassiceps* or *E. granulosus* as described above. Group B1 received 50 cysticerci of *T. crassiceps* and Group B2 served as normal controls. In another experiment Group C1 received 6

cysts of *E. granulosus* of at least 1 cm diameter, while Group C2 consisted of normal controls. Twenty-one days later these groups were challenged with 200 eggs of *T. taeniaeformis* and all rats were killed 3 weeks later and examined for liver cysts.

The protective capacity of serum pools and fractions of immunoglobulins from the serum of rats given implants was measured in passive transfer experiments. One milliliter samples of serum or immunoglobulin fractions were injected intraperitoneally into normal rats prior to an oral challenge with 200 eggs of *T. taeniaeformis*. Twenty-one days later the animals were killed with carbon dioxide vapor and the total number of cysticerci which established in each liver again determined.

Five-month-old live larvae of *T. taeniaeformis* were implanted in the peritoneal cavities of groups of 6 rats which had been given oral doses of homologous eggs 7, 14, 21 or 28 days previously. A control group of normal rats also received implants. All rats were killed 21 days later and the numbers of surviving implanted parasites compared in each group.

Finally 5-month-old larvae of *T. taeniaeformis* were implanted in the peritoneal cavities of normal recipients which were then given either:

(a) Three intraperitoneal inoculations at 6-day intervals of 1 ml of immune serum from rats which had been infected with *T. taeniaeformis* for 28 days.

(b) Three intraperitoneal inoculations at 6-day intervals of 1 ml of immune serum from rats which had been implanted with 5-month-old larvae of *T. taeniaeformis* 28 days previously.

(c) Three intraperitoneal inoculations at 6-day intervals of PBS.

(d) A total of 0.25 ml of complete Freund's adjuvant (CFA) distributed in the four footpads at the time of implantation.

All rats were killed 21 days later and the number of surviving implanted parasites compared.

RESULTS

The results of the experiment on the ability of larvae of different ages to survive in normal recipient rats indicated that all larvae older than 2½ months were able to survive free in the peritoneal cavity for a period of at least 21 days. Many of those aged 2½ months at the time of implantation were collapsed and encapsulated by the host-tissue response, but these stages were extremely delicate and it was difficult to collect and transfer them without causing traumatic damage. In subsequent experiments 5-month-old organisms were used to avoid this problem.

Implanted cysticerci either dead or alive were able to provoke a strong protective response to an oral egg challenge, as shown in Table 1. However, no hepatic parasites were established in rats receiving live metacestodes whereas 5 of 6 rats implanted with dead larvae had parasites developing in their livers.

The results of the experiment on the influence of heterologous infection on resistance to *T. taeniaeformis* are also shown in Table 1. Protection against oral challenge with *T. taeniaeformis* eggs was observed in rats implanted with *T. crassiceps* while rats with *E. granulosus* implants had the same number of parasites developing in their livers as the normal controls.

Immune serum obtained from rats implanted with live larvae of *T. taeniaeformis* was used in passive transfer experiments. One milliliter

Table 1. Effects of implanted metacestodes on resistance to *T. taeniaeformis*

Parasites implanted	No. of rats	No. of larvae implanted	Avg. no. of larvae in liver ± SD	SE of mean	P-value
A1 *T. taeniaeformis*	6	6 live	0.0 ± 0	0	<0.01
A2 *T. taeniaeformis*	6	6 dead	2.5 ± 2.8	1.1	<0.01
A3 None	5	none	35.2 ± 10.6	4.7	---
B1 *T. crassiceps*	4	50	11.5 ± 5.6	2.7	<0.01
B2 None	6	none	44.5 ± 12.3	5.0	---
C1 *E. granulosus*	6	6	21.3 ± 10.0	4.1	NS*
C2 None	6	none	17.3 ± 8.9	3.6	---

*NS = not significant.

of the serum was given intraperitoneally to one group of rats while the control group received normal rat serum. Rats receiving immune serum had an average of 9.8 $\pm$ 11 (SD) larvae in their livers after oral challenge as compared to control rats with an average of 40.9 $\pm$ 7 (SD) (Figure 1). This difference was highly significant ($P < 0.01$).

Globulins from 3 pools of serum from 30 rats given implants were precipitated with 50 percent saturated ammonium sulphate three times and subjected to anion-exchange chromatography. The DEAE-cellulose elution profiles for these rat immunoglobulin preparations are shown in Figure 2. Two peaks were consistently eluted with 0.05 M phosphate buffer pH 5.8 and these were collected separately. All protein peaks were concentrated back to the original volume of serum before being used in passive transfer experiments.

The results obtained from a typical passive transfer experiment using serum pool 1 are shown in Table 2. The average numbers of parasites developing in the livers in each group are recorded. Two more pools of immune sera were similarly tested and the results are depicted graphically in Figure 2. The protective capacity of these immunoglobulin preparations was not limited to any one fraction but was found to be distributed in several areas. However, fractions eluting with 0.05 M phosphate buffer pH 5.8 were consistently protective against an oral egg challenge. These eluates contained both $7S\gamma_1$ and $7S\gamma_2$ immunoglobulins but fractions enriched for $7S\gamma_2$, i.e., eluates of 0.005 M phosphate buffer pH 7.75, were only protective on one of three occasions and at a lower level of significance than fractions containing $7S\gamma_1$.

Figure 1. Anion exchange chromatograph of 3 pools of serum from rats given implanted cysticerci of *T. taeniaeformis*. Each fraction was used in passive transfer experiments and recipients were challenged with 200 eggs. Shaded areas represent protective fractions (dark shading $P < 0.01$, lighter shading $P < 0.05$).

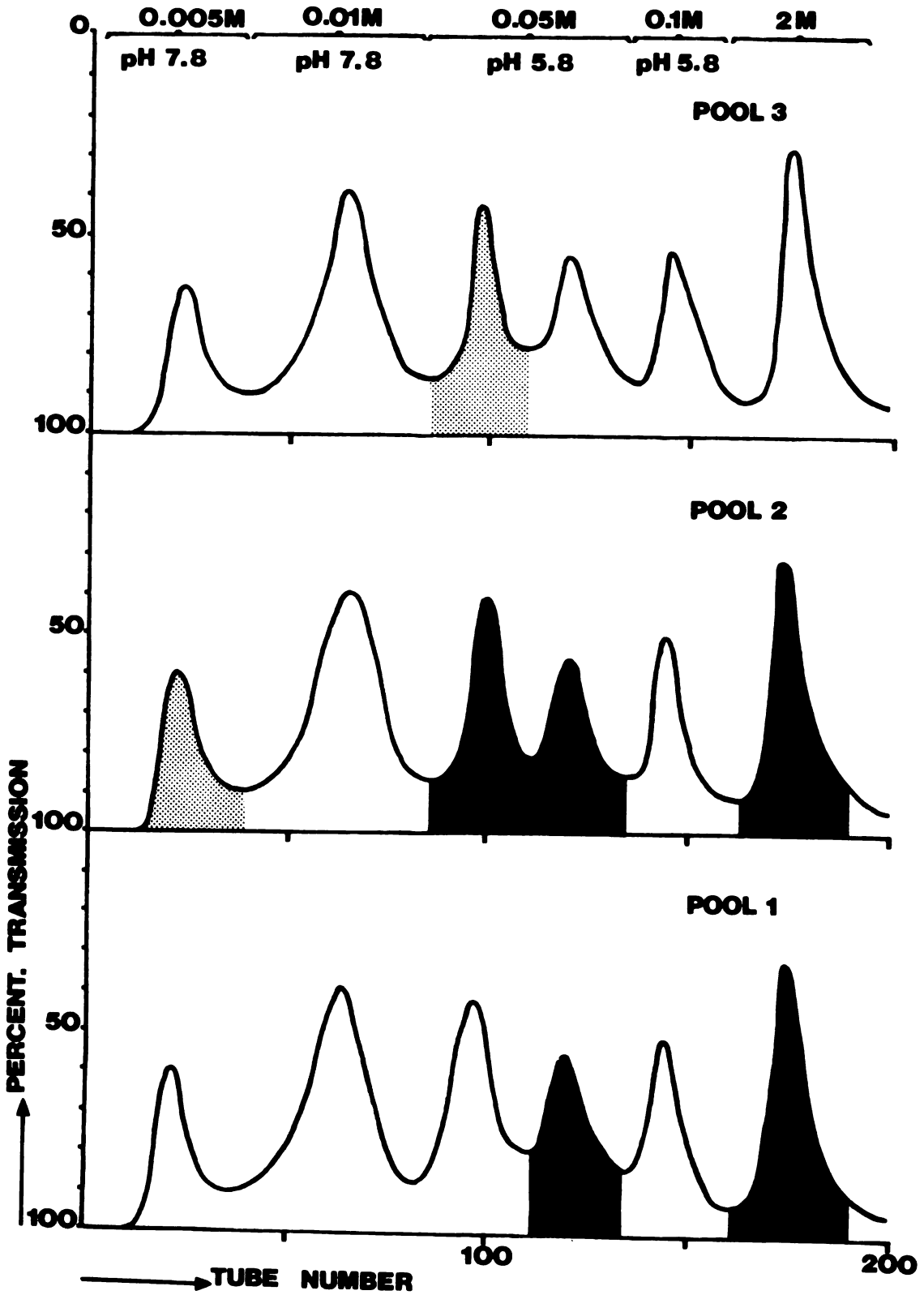
0
0.005M
pH 7.8
0.01M
pH 7.8
0.05M
pH 5.8
0.1M
pH 5.8
2M
POOL 3
50
100
POOL 2
50
100
POOL 1
50
100
PERCENT. TRANSMISSION
100
200
TUBE NUMBER

Figure 1

Figure 2. Representative livers from groups of rats passively immunized with serum from surgical implants. The animals were challenged *per os* with 300 eggs of *T. taeniaeformis* and killed 21 days later.

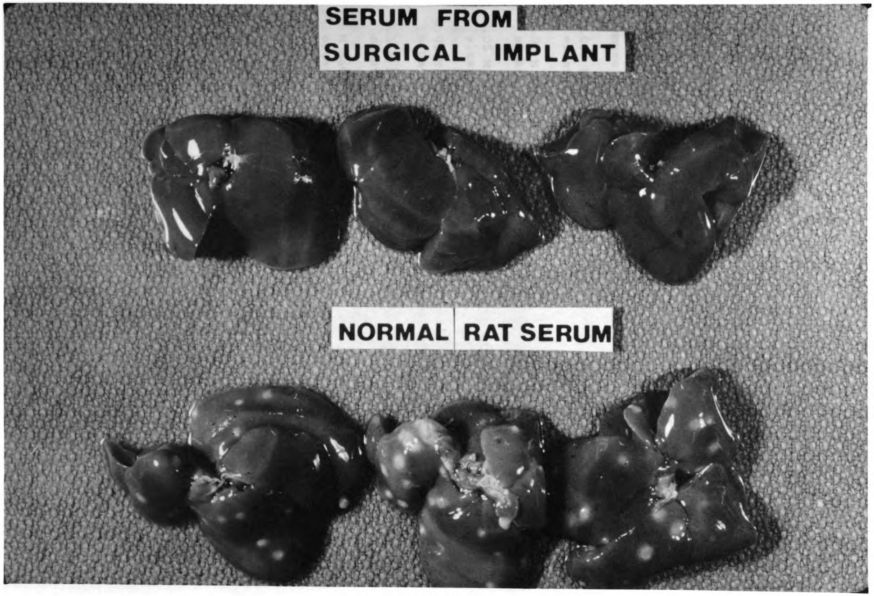

Figure 2

Table 2. Protective capacity of immune implant serum (Pool 1) and immunoglobulin fractions in recipient rats challenged with 200 eggs of *Taenia taeniaeformis*

Protein fraction transferred*	No. of rats in group	No. of larvae in liver ± SD	SE of mean	P-value
Normal whole rat serum	5	29.6 ± 3.7	1.6	---
Immune rat serum	4	11.8 ± 7.5	3.8	<0.01
0.005 M DEAE-cellulose eluate	5	17.0 ± 12.7	5.7	NS
0.01 M DEAE-cellulose eluate	5	21.2 ± 9.0	4.0	NS
0.05 M (1) DEAE-cellulose eluate	6	20.8 ± 10.6	4.3	NS
0.05 M (2) DEAE-cellulose eluate	5	10.0 ± 3.8	1.6	<0.01
0.1 M DEAE-cellulose eluate	5	25.6 ± 15.6	6.7	NS
2 M NaCl DEAE-cellulose eluate	6	17.8 ± 5.3	2.2	<0.01

NS = not significant.

* - by intraperitoneal injection.

In two of three pools fractions with detectable γM immunoglobulins were protective at the same level of significance as those fractions containing $7S\gamma_1$.

All eluates from DEAE-cellulose chromatography were tested for their ability to produce homologous PCA reactions in sensitized rats. Latent periods of 4 hrs and 48 hrs were allowed before challenge. Short-term and long-term PCA reactions were not elicited in any of these tests. Positive control reactions were obtained by sensitization of dermal sites with serum from rats infected with *T. taeniaeformis* orally.

The results of experiments carried out to determine the influence of established hepatic cysticerci on the fate of implanted larvae are shown in Table 3. All larvae which were implanted in rats given doses of homologous eggs, 7, 14, 21 and 28 days previously, were killed and encapsulated. All the cysticerci implanted in the group of normal rats survived and were lying free in the peritoneal cavity.

Passive transfer of immune serum to rats given implanted parasites resulted in no detectable effect on the larvae and the number of surviving organisms was not different from the controls. However, in rats inoculated with CFA, the larvae were all killed and enveloped in a fibrous response and in some instances only tiny fragments remained. It was not possible to identify and count the larvae which had originally been implanted. This result with CFA sensitization was duplicated in a second experiment using the same protocol.

Table 3. Survival of larvae of *T. taeniaeformis* implanted in the peritoneal cavities of rats dosed orally with homologous eggs

Day of infection	Worms implanted per rat	Live worms recovered per rat
7	6	0
14	6	0
21	6	0
28	6	0
control	6	36

DISCUSSION

Our results on the surgical transfer of cysticerci of *T. taeniaeformis* have shown that antigens of both live and dead metacestodes of this species can provoke a strong immunity to challenge infection. In addition, transfer of live metacestodes of *T. crassiceps* to normal rats induced a significant protective response against heterologous challenge with *T. taeniaeformis*. Comparable results showing that taeniid metabolic products can immunize against homologous challenge have been reported by Miller (1931) and Rickard and Bell (1971) and against heterologous challenge by Gemmell (1965).

The observation that surgically transferred mature cysticeri produced immunity in the recipient rats is in sharp contrast to Heath's finding that implanted cysticerci of *T. pisiformis* failed to stimulate resistance to challenge infection in rabbits (Heath, 1973). Clearly live cysticerci of *T. taeniaeformis* do represent a source of protective antigens and, in fact, products released by these stages when maintained in protein-free media *in vitro* can be used very successfully as immunogens (Musoke and Williams, unpublished observations). It is notable, however, that the degree of resistance manifested by rats given implants is by no means as impressive as that shown by rats with hepatic infections. Also in passive transfer experiments much larger volumes of serum from implanted rats are required to confer protection on recipients than is the case for serum from rats infected normally by oral dosing with eggs (Leid and Williams, 1974a). These results

suggest that early developmental stages may indeed be more effective producers of protective antigens than the mature cysticerci, as proposed by Heath (1973).

Alternatively these differences may be a reflection of the involvement of distinct antibody types in protective responses resulting from either implantation or infection. Our studies not only firmly establish an antibody mediated basis for the immune response induced by implanted worms but also point up a marked difference in the distribution of antibody activity among the immunoglobulin fractions from that observed by Leid and Williams (1974a) for infected rat serum. Protective activity in implant serum was not confined to one but appeared in several anion-exchange fractions. However, eluates enriched for $7S\gamma_1$ immunoglobulins were consistently associated with protection while $7S\gamma_2$ immunoglobulins were protective in only 1 of 3 pools and then to a lesser extent than $7S\gamma_1$.

Heterogeneity of protective antibody types does not appear to develop in the sera of infected rats until about the seventh week after exposure (Musoke and Williams, 1975) at which time $7S\gamma_1$ enriched fractions become highly protective. These results suggest that the effective immunogens of the mature cysticerci may be sufficiently distinct either qualitatively or quantitatively from those associated with the early post-oncospheral developmental phase to provoke the formation of antibodies of a different immunoglobulin class. Nevertheless, these immunogens must be shared or at least cross-react with those of the oncosphere against which the protective response is directed.

Protection was successfully transferred with DEAE fractions of implant serum which were devoid of reaginic activity and no skin-sensitizing antibodies were detectable in either unfractionated serum or chromatographic fractions. This is an interesting observation in that Leid and Williams (1974b, 1975) demonstrated that reaginic antibodies appeared in the sera of rats and rabbits infected with *T. taeniaeformis* and *T. pisiformis*, respectively, very early after oral exposure. Since the cysticerci have been shown to contain at least two allergens, one of which was released by cysticerci *in vitro*, we were surprised to find that implanted rats did not respond similarly. Possibly the manner in which these allergens are presented to and processed by the immunologic system of the host is an important determinant of the characteristics of the reaginic antibody response. Another factor which may be of significance is that reaginic antibody titers in infected rats begin to decline coincidentally with the appearance of antibodies of the $7S\gamma_1$ type in the serum. There is evidence that certain 7SγG immunoglobulin responses in the rat may suppress γE antibody formation (Tada and Okumura, 1970), and it is therefore possible that the rapid appearance of $7S\gamma_1$ antibodies in implanted rats could have had a similar effect on reaginic antibody production.

Antibodies were unable to influence the survival of implanted organisms in rats passively immunized with serum from either infected donors or from rats given implants. Once in the peritoneal cavity cysticerci are therefore able to provoke effective resistance to oncospheres but remain unaffected themselves even in the presence

of antibodies. However, we were never able to recover live implanted cysticerci from the peritoneal cavities of rats which had hepatic infections. In all cases these parasites were dead and fragmented and encapsulated in the omental tissues. The cysticerci were therefore susceptible to host defense mechanisms which were stimulated by exposure to the oncosphere and post-oncospheral developmental phases. Since immune serum from rats was not effective in transferring this response, the reaction was probably mediated by cells.

The absence of survival of implanted parasites which occurred in normal rats sensitized with Freund's adjuvant may therefore have come about because the adjuvant triggered a cellular type of specific defense mechanism to parasite antigens. The adjuvant effect may also possibly have been entirely non-specific and resulted from stimulation of the lymphoreticular system to mount an enhanced and effective defense reaction comparable to that which has been found to operate in animals which are able to reject tumors (Mathe and Pouillart, 1970) after sensitization with Bacillus Calmette-Guérin (BCG). Whatever the mechanism by which it acts, the observation that resistance is provoked by Freund's adjuvant is in accord with results reported by Silverman (1963) for *Haemonchus contortus* in sheep and by Varela-Diaz *et al.* (1974) for *Echinococcus granulosus* in gerbils.

The means whereby the cysticercus is able to evade cellular defense mechanisms in its normal location in hepatic tissue remain unclear, but we are pursuing this aspect of the host-parasite relationship in continuing studies in our laboratory.

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REFERENCES

GEMMELL, M. A. 1965. Immunological responses of the mammalian host against tapeworm infections. II. Species specificity of hexacanth embryos in protecting rabbits against *Taenia pisiformis*. *Immunology* 8: 270-280.

HEATH, D. D. 1973. Resistance to *Taenia pisiformis* larvae in rabbits. I. Examination of the antigenically protective phase of larval development. *International Journal for Parasitology* 3: 485-489.

LEID, R. W., and WILLIAMS, J. F. 1974a. Immunological response of the rat to infection with *Taenia taeniaeformis*. I. Immunoglobulin classes involved in passive transfer of resistance. *Immunology* 27: 195-208.

LEID, R. W., and WILLIAMS, J. F. 1974b. Immunological response of the rat to infection with *Taenia taeniaeformis*. II. Characterization of reaginic antibody and an allergen associated with the larval stage. *Immunology* 27: 209-225.

LEID, R. W., and WILLIAMS, J. F. 1975. Reaginic antibody response in rabbits injected with *Taenia pisiformis*. *International Journal for Parasitology* (in press).

MATHE, G., and POUILLART, P. 1970. Active immunotherapy of L1210 leukemia applied after the graft of tumor cells. In G. Mathe (Ed.), *Advances in the Treatment of Acute (Blastic) Leukemias. Results in Cancer Research*, Vol. 30. Springer-Verlag, New York.

MILLER, H. M. 1931. The production of artificial immunity in the albino rat to a metazoan parasite. *Journal of Preventative Medicine* 5: 429-452.

MUSOKE, A. J., and WILLIAMS, J. F. 1975. Immunological response of the rat to infection with *Taenia taeniaeformis*. IV. Sequence of appearance of protective immunoglobulins and the mechanism of action of $7S\gamma_{2a}$ antibodies. *Immunology* (submitted for publication).

RICKARD, M. D., and BELL, K. J. 1971b. Immunity produced against *Taenia ovis* and *T. taeniaeformis* infection in lambs and rats following *in vivo* growth of their larvae in filtration membrane diffusion chambers. *Journal of Parasitology* 57: 571-575.

RICKARD, M. D., and BELL, K. J. 1971a. Successful vaccination of lambs against infection with *Taenia ovis* using antigens produced during *in vitro* cultivation of the larval stages. *Research in Veterinary Science* 12: 401-402.

SILVERMAN, P. H. 1963. *In vitro* cultivation and serological techniques in parasitology, p. 45-67. In A. E. R. Taylor (Ed.), *Techniques in Parasitology*. Blackwell Scientific Publications, Oxford and Edinburgh.

STECHSCHULTE, D. J., AUSTEN, K. F., and BLOCH, K. J. 1967. Antibodies involved in antigen induced release of slow reacting substance of anaphylaxis (SRS-A) in the guinea pig and rat. *Journal of Experimental Medicine* 125: 127-147.

TADA, T., and OKUMURA, K. 1970. Regulation of homocytotropic antibody in rat. I. Feedback regulation by passive administration of antibody. *Journal of Immunology* 106: 1062-1071.

VARELA-DIAZ, V. M., WILLIAMS, J. F., COLTORTI, E. A., and WILLIAMS, C. S. F. 1974. Survival of cysts of *Echinococcus granulosus* after transplantation into homologous and heterologous host. *Journal of Parasitology* 60: 608-612.

WILLIAMS, C. A., and CHASE, M. W. 1971. *Methods in Immunology and Immunochemistry*, 1st edition, p. 103. Academic Press, New York.

IMMUNOLOGICAL RESPONSE OF THE RAT TO INFECTION WITH *TAENIA TAENIAEFORMIS*

V. SEQUENCE OF APPEARANCE OF PROTECTIVE IMMUNOGLOBULIN AND THE MECHANISM OF ACTION OF $7S\gamma_{2a}$ ANTIBODIES

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Immunological response of the rat to infection with *Taenia taeniaeformis*. V. Sequence of appearance of protective immunoglobulins and the mechanism of action of $7S\gamma_{2a}$ antibodies

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SUMMARY

Passive transfer of immunity to *Taenia taeniaeformis* was achieved with serum taken 14, 21, 49 and 63 days after infection. The protective capacity of serum collected at 14 and 21 days resided in the $7S\gamma_2$ immunoglobulins and appeared to be exclusively the result of $7S\gamma_{2a}$ antibody activity. However, as the infection progressed the range of chromatographic fractions showing protective capacity was extended to all those containing $7S\gamma_2$ and $7S\gamma_1$ immunoglobulins. Fractions enriched for γM did not confer protection.

Immune serum containing $7S\gamma_{2a}$ antibodies was able to kill developing parasites after they had left the intestine, and the hepatic postoncospheral forms retained their susceptibility to antibody over the first 5 days of growth. After that time they rapidly became insusceptible to antibody both *in vivo* and *in vitro*. Prior to the 5th day their susceptibility to antibody mediated attack was shown to depend on the integrity of the complement system. This appears to be the first time that complement has been demonstrated to play a role in immunity to a helminth infection *in vivo*. This finding is discussed in relation to the phenomenon of cestode parasite survival in immune animals.

INTRODUCTION

Experimental studies on acquired resistance to cysticercosis in laboratory and domesticated animals have provided ample evidence for the occurrence of protective serum antibodies (Miller and Gardiner, 1932; Campbell, 1938; Blundell-Hasell, Gemmell and Macnamara, 1968). However, the mechanism and site of action of these antibodies on the invading organisms remain unknown and the means whereby established tissue cysticerci survive in the absence of antibody attack have not been explored experimentally.

In our recent work we have therefore attempted to characterize some of the features of protective antibodies with a view towards clarifying these important aspects of the host-parasite relationship in taeniid metacestode infections. When serum samples were taken from rats and mice on the 28th day of infection with *Taenia taeniaeformis* we found a marked association between protective capacity and antibodies of certain well defined physico-chemical characteristics (Leid and Williams, 1974a; Musoke and Williams, 1975a). Antigenically these antibodies were recognizable as $7S\gamma_{2a}$ immunoglobulins in the rat and tentatively as $7S\gamma_1$ in the mouse, but in neither instance were we able to detect the homologous skin sensitizing activity which is manifested by antihapten antibodies in these classes (Morse, Block and Austen, 1968; Revoltella and Ovary, 1969; Binaghi, 1971). We have suggested that the migrating oncosphere may be attacked by protective antibodies acting in concert with reagins which may mediate local inflammatory reactions influencing the site and effectiveness of the response (Leid and Williams, 1974b, 1975).

In the present study we have extended our observations on the types of antibody responsible for protection in the rat by examining immunoglobulin fractions from serum samples taken at intervals over the first 9 weeks of infection with *T. taeniaeformis*. Several previous workers have proposed that protective antibodies in serum may only be effective on the very early stages of the parasite at the intestinal level (Leonard and Leonard, 1941; Froyd and Round, 1960), and some evidence is available to suggest that heat labile serum factors are involved in this resistance (Heath, 1971). We have therefore undertaken a series of experiments designed to characterize the mechanism of action of antibody in terms of the tissues in which the protective response occurs, the specific phases of the developing parasite which are vulnerable to immunological attack, and finally the role of complement in the process of destruction of the challenge organisms.

MATERIALS AND METHODS

Parasite

The strain of *T. taeniaeformis* used in these experiments was derived from gravid segments obtained from Mr. C. E. Claggett in the Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, Maryland. The parasite was maintained following the method of Leid and Williams (1974a).

Experimental Animals

Sprague-Dawley rats 28-42 days of age were purchased from Spartan Research Animals, Haslett, Michigan. They were given proprietary brand food and water *ad libitum*.

Preparation of Immune Sera

Rats were given 500 eggs of *T. taeniaeformis per os* and killed 14, 21, 49 or 63 days later using carbon dioxide vapour. Blood was collected from the thoracic cavity after severing the vessels anterior to the heart, allowed to clot at room temperature for 2-3 hrs and left overnight at 4 C. Serum samples were stored at -20 C without preservatives.

Passive Immunization

Recipient animals received intraperitoneal inoculations of serum or immunoglobulin fractions which had been filtered using a 0.45 μ filter (Millipore, Bedford, Massachusetts). One milliliter of serum or chromatographic fractions was injected at the time of oral challenge with 400 eggs. After 3 weeks animals were killed with carbon dioxide vapour and the total number of cysticerci in each liver was determined. The results were analyzed statistically using a modified Student's t-test.

Immunoelectrophoresis and Double Immunodiffusion

Immunoelectrophoresis (IEP) and double immunodiffusion (DID) were performed following the methods described by Leid and Williams (1974a).

Preparation of Antisera to Rat Immunoglobulins

Antisera to whole rat serum and rat immunoglobulins were prepared according to the procedures described by Leid and Williams (1974a).

Chromatography

The globulins from sera collected as above were precipitated with 50 percent saturated ammonium sulphate (3x) and dialyzed against phosphate buffered saline (PBS) until free of sulphate ions.

The procedure for ion-exchange chromatography of rat immunoglobulins was similar to that described by Musoke and Williams (1975a). Stepwise elution was performed using sodium phosphate buffers in the following sequence: 0.05 M, pH 7.8; 0.01 M, pH 7.8; 0.05 M, pH 5.8; 0.1 M, pH 5.8; and finally 2 M NaCl. All buffers were made 0.015 M in NaCl. The pooled fractions under each peak were concentrated back to the original volume of serum using polyethylene glycol.

Preparation of $7S\gamma_{2a}$ Immunoglobulin Fraction

In order to determine whether antibody activity is associated with $7S\gamma_2$ immunoglobulins other than $7S\gamma_{2a}$, advantage was taken of the recent observation of Nezlin, Krilov, and Rokhlin (1973) on the susceptibility of $7S\gamma_{2b}$ and $7S\gamma_{2c}$ to tryptic digestion. DEAE cellulose purified fractions of $7S\gamma_2$ from 21-day immune serum were subjected to trypsin digestion (D.C.C.-treated Trypsin, Sigma Chemical Co.) at 1:100 ratio to protein for 17 hrs at 37 C in 0.05 M Tris-HCl buffer pH 8.0. After addition of an equimolar

amount of trypsin inhibitor (Soy-bean-TS, Sigma Chemical Co.), the hydrolysates were fractionated on a 2.5 x 90 cm column of Sephadex G-200 using a 0.05 M Tris-HCl buffer in 0.28 M NaCl.

Column eluates were collected in 2.8 ml fractions and the optical density of each fraction was determined using a Perkin-Elmer Coleman 111 Spectrophotometer. Protein peaks were analyzed by IEP and DID with rabbit antiwhole rat serum and guinea pig anti-rat $7S\gamma_2$. The trypsin resistant fraction which consisted of $7S\gamma_{2a}$ alone was made up to the original serum volume with PBS and used in passive transfer experiments.

Isolation of *T. taeniaeformis* Larvae

In vitro hatching and activation of eggs of *T. taeniaeformis* was carried out following standard methods (Silverman, 1954; Rickard and Bell, 1971; Heath, 1973). However, activation rates were low and this procedure could not be used to obtain suspensions of organisms which had shed their oncospheral membranes. For this purpose we resorted to the recovery of organisms from the liver parenchyma of rats after oral dosing with eggs. Ten thousand eggs of *T. taeniaeformis* were given *per os* and parasites were harvested after intervals of 1, 2, 4, 6, 8 and 10 days. The livers from each group of rats were minced finely with scissors and 0.25 percent trypsin solution was added at the rate of 50 ml/gm of tissue. The supernatant fluid was collected after the larger fragments of liver tissue had settled and was centrifuged at 1000 g for 10 minutes. This sediment, consisting of free hepatic cells and parasites (Figure 1), was washed twice with Hanks' BSS (Grand

Figure 1. Six- (a) and 10-day-old (b) larvae of *T. taeniaeformis* liberated from liver tissue by a combination of trypsin and collagenase digestion.

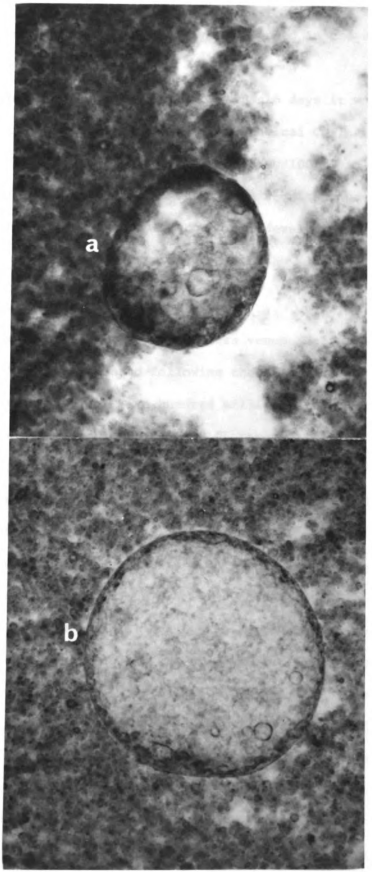


Figure 1

Island Biological Co., Buffalo, N.Y.) and suspended in the same medium.

For the liberation of parasites older than 6 days it was necessary to incorporate collagenase (Sigma Chemical Co.) at a concentration of 10 mg/100 ml and calcium at 5 mg/100 ml in the digestion mixture. This additional enzyme was required due to the formation of a host fibrous capsule around the developing cysticerci which was unaffected by trypsin digestion.

Isolation of Cobra Venom Factor (CoF)

The anticomplementary factor from cobra venom (*Naja haje*, Sigma Chemical Co.) was isolated following the method described by Ballow and Cochrane (1969). Five hundred milligrams of lyophilized cobra vanom were dissolved in 40 ml of 0.01 phosphate buffer pH 7.5. The solution was dialyzed overnight before application to a DEAE cellulose column (DE52, Whatman Co.) 2.5 x 40 cm equilibrated with the same phosphate buffer.

After the major toxic components had passed through the column in the initial eluate, the remaining bound proteins were eluted using a linear 0.5 M NaCl gradient. Column eluates were collected in 2.8 ml fractions and the protein elution pattern was monitored by ultraviolet scanning at 280 nm (Figure 2). Since most of the anticomplementary activity resides in the third protein peak (Maillard and Zarco, 1968), the eluates in this region were tested for their capacity to inhibit complement in normal rat serum using a haemolytic assay.

Figure 2. Elution profile at 280 nm of cobra venom on DEAE-cellulose with phosphate buffer and 0.5 M NaCl gradient. The third peak (stippled area) was used to deplete complement levels in rats.

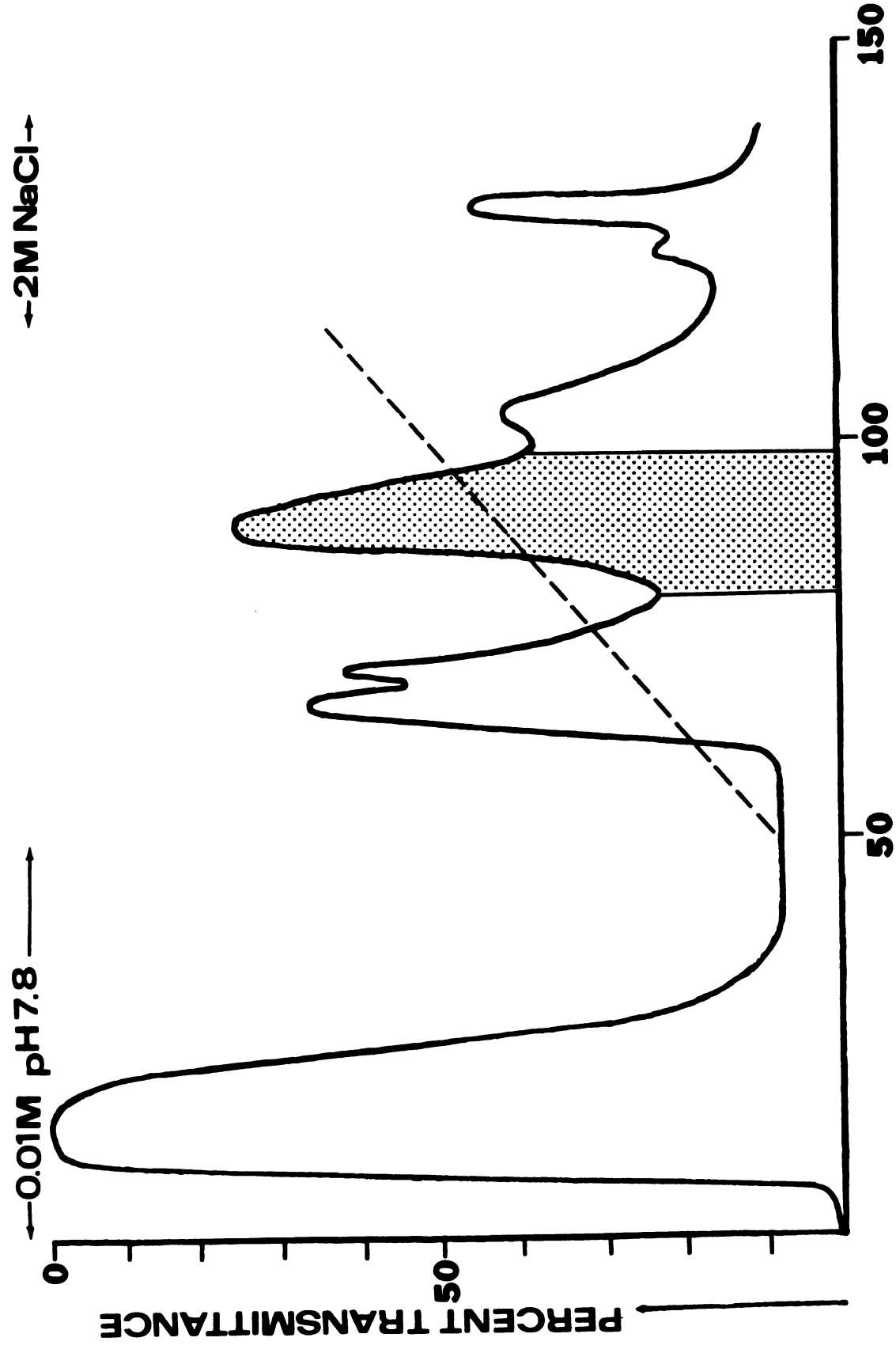

←0.01M pH7.8
←2M NaCl→
PERCENT TRANSMITTANCE
0
50
50
100
150
TUBE No.

Figure 2

One tenth milliliter of each eluate was incubated with 0.5 ml of normal rat serum diluted 1:20 for 30 minutes at 37 C. Sensitized sheep cells prepared according to the method described by Kabat and Mayer (1971) were added at a final concentration of 2×10^8. The mixture was further incubated for 30 minutes at 37 C. Two milliliters of cold PBS were added and the amount of haemolysis was determined by spectrophotometric analysis of the supernatant at 541 nm after centrifugation at 1000 g for 10 minutes. Tubes showing 50 percent inhibition of haemolysis were pooled and concentrated. CoF units were determined using this haemolytic assay on twofold dilutions of the concentrated eluates. One unit was defined as that amount in 0.1 ml of CoF required to cause 50 percent inhibition of lysis (Ballow and Cochrane, 1969). Further purification of the CoF was not attempted since active preparations produced no undesirable side effects in rats even after repeated injections.

Passive Cutaneous Anaphylaxis (PCA)

Homologous PCA tests were carried out on serum and immunoglobulin fractions following the procedure described by Leid and Williams (1974b).

RESULTS

Immunoglobulin preparations from 21-day immune serum containing all $7S\gamma_2$ subclasses were used in passive transfer experiments. After oral challenge with 300 eggs the average number of parasites which developed in the liver of passively immunized rats was 1.0

$\pm$ 1.7, while in the controls the average was 19 $\pm$ 6.8 ($P < 0.001$). After tryptic digestion purified $7S\gamma_{2a}$ preparations were also used to passively immunize rats, and these and control animals were challenged orally with 500 eggs. The experimental rats were found to harbour an average of 0.6 $\pm$ 1.6 while the controls had 118 $\pm$ 18.9 ($P < 0.001$).

Having established the exclusive association of protective antibody activity with immunoglobulins of the $7S\gamma_{2a}$ type, we proceeded to examine the distribution of protective antibodies in other immunoglobulin classes in sera collected at intervals after infection.

Globulins from immune sera collected at 14, 21, 49 and 63 days after infection were precipitated with 50 percent SAS (3x) and subjected to ion exchange chromatography. Sequential stepwise elution was followed. The protein peaks were analyzed for immunoglobulin types present in each protein pool by I.E.P. $7S\gamma_{2a}$ immunoglobulins were detected in 0.005 M phosphate buffer eluates while all the three subclasses of $7S\gamma_2$ were present in all the remaining fractions. γM immunoglobulins were detected in the 2 M NaCl eluate. Reaginic antibodies were distributed in the 0.05 M and 0.1 M fractions from sera taken at 21 and 49 days postinfection.

Groups of 28-day-old rats were dosed with 400 eggs orally followed by intraperitoneal inoculations of 1 ml quantities of each chromatographic fraction. Control animals received inoculations of normal rat serum. All groups of rats were killed 21 days later and the numbers of cysticerci developing in each group

compared to the controls. The results graphically represented in Figure 3 indicated that as the infection progressed the spectrum of chromatographic fractions showing protective activity was extended to involve all those containing 7S immunoglobulins. However, eluates with detectable γM immunoglobulins were consistently not protective.

Experiments were then designed to establish whether or not these antibodies to *T. taeniaeformis* exerted their action only at the intestinal level. Twenty-eight-day immune serum was used in this experiment since the major protective activity was limited to one class of antibody at this time and high levels were present (Leid and Williams, 1974a). Rats were inoculated intravenously with 1 ml of 28-day immune serum. Thirty minutes later the rats were anesthetized with a combination of ketamine hydrochloride intramuscularly and methoxyfluorane by inhalation and challenged via a mesenteric vein with 700 eggs. These had been exposed to artificial digestive juice *in vitro* and approximately 1/3 of the eggs had hatched, although no more than 10 percent had activated. Control rats were challenged with the same number of eggs *per os* after inoculations of either 1 ml of normal or immune serum intravenously. All rats were killed 21 days later and the number of cysticerci developing in the livers of each group are shown in Table 1.

These results suggested that passive immunity could not be bypassed by avoiding intestinal migration and that protective antibodies could function outside of the intestine. This finding was at odds with the proposals of Leonard and Leonard (1941)

Figure 3. Elution pattern at 280 nm of globulins (50 percent $[NH_4]_2SO_4$) of immune rat sera collected 14, 21, 49 and 63 days after infection, on DEAE-cellulose with phosphate buffers and 2 M NaCl. All buffers were made 0.015 M in NaCl. Each protein peak was used in passive transfer experiments. Lightly stippled areas indicate statistical significance of $P < 0.05$; vertical bars, $P < 0.01$; and heavily stippled $P < 0.001$.

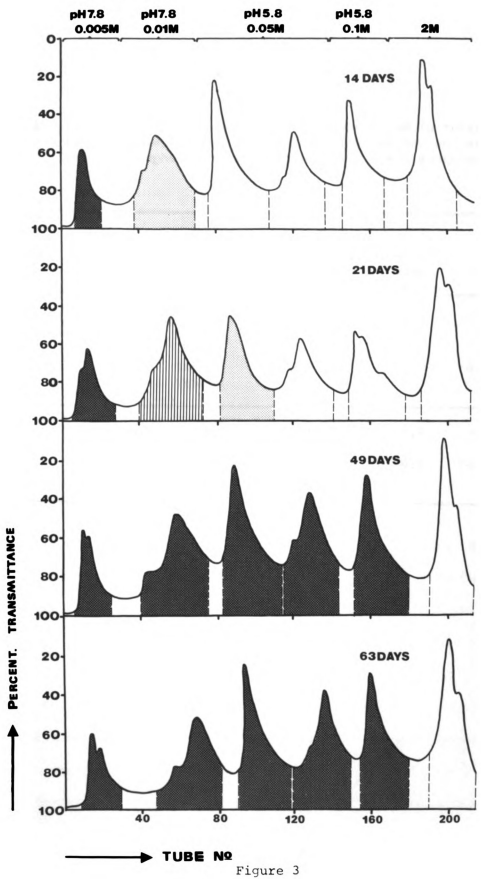

pH7.8
0.005M
pH7.8
0.01M
pH5.8
0.05M
pH5.8
0.1M
2M
14 DAYS
21 DAYS
49 DAYS
63 DAYS
PERCENT. TRANSMITTANCE
0
20
40
60
80
100
40
80
120
160
200
TUBE Nº

Figure 3

Table 1. Passive protective capacity of immune serum in recipient rats inoculated with 700 artificially hatched and activated oncospheres of *T. taeniaeformis* via a mesenteric vein

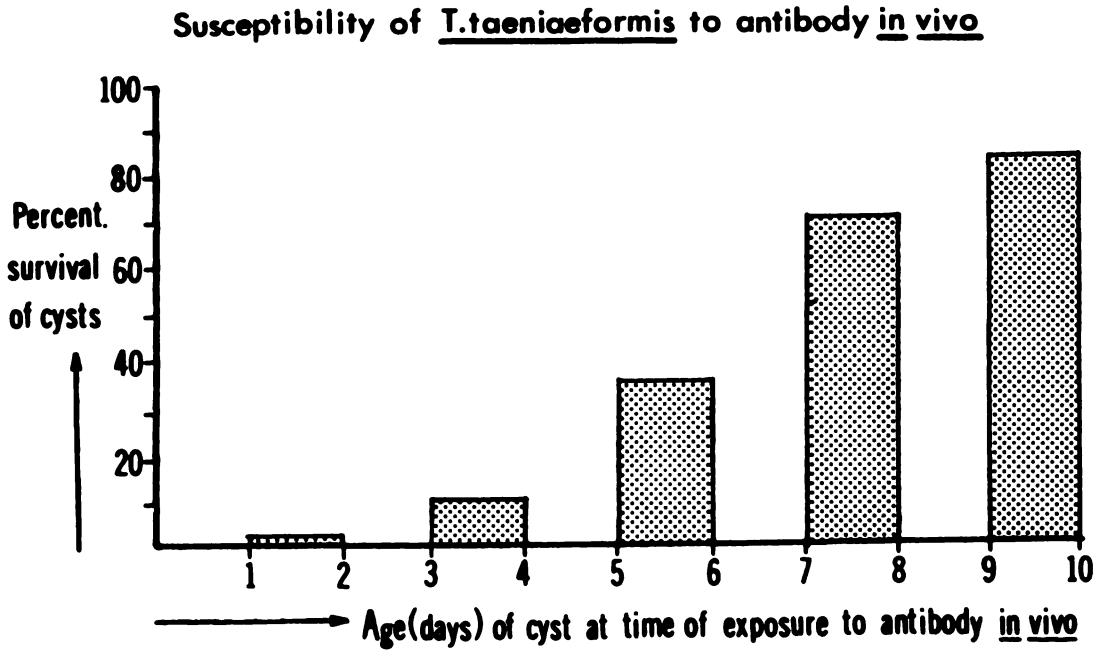
Treatment (i.v.)	No. of rats	Route of challenge	Mean no. of cysts in liver ± SD	SE of mean	P-value
Normal serum	5	intra-venous	100.2±32.5	14.5	---
Immune serum	6	intra-venous	0.0	0.0	<0.001
Normal	5	oral	171.4±17.5	7.8	---
Immune serum	6	oral	0.0	0.0	<0.001

and Froyd and Round (1960) regarding the site of action of protective antibodies, but was consistent with the results of an experiment reported by Campbell in 1938b. He had treated rats with immune serum at daily intervals after oral infection and shown that parasites within the liver remained fully susceptible to antibody for at least 4 days. He had speculated that the development of insusceptibility was due to formation of a fibrous host capsule which isolated the growing parasites from attack. We attempted to confirm his work on the development of insusceptibility in growing larvae and to establish if this change was derived from an isolating effect of the host capsule. Ten groups of rats were dosed with 500 eggs of *T. taeniaeformis* on day 0. Twenty-eight-day immune serum was administered to one group per day from day 0 to 10. All rats were killed 21 days later. The results depicted in Figure 4 showed that the effectiveness of antibody begins to wane by the sixth day.

Postoncospheral stages of *T. taeniaeformis* were liberated by enzymic digestion from livers of rats dosed orally with 10,000 eggs, at intervals of 1, 2, 4, 6, 8 and 10 days and exposed to normal or immune serum *in vitro*. The parasites were then injected via a mesenteric vein into recipient rats which were sacrificed 21 days later. The results, also depicted in Figure 4, demonstrated that the development of insusceptibility to antibody is derived from inherent changes on the part of the parasites.

Attempts were then made to determine the role of complement in the process of immunologic destruction of the parasites prior to 6

Figure 4. Percent survival of larvae of *Taenia taeniaeformis* of different ages after exposure *in vivo* and *in vitro* to immune serum containing $7S\gamma_{2a}$ antibodies.


Susceptibility of T.taeniaeformis to antibody in vivo
100
80
Percent.
survival 60
of cysts
40
20
1
2
3
4
5
6
7
8
9
10
Age(days) of cyst at time of exposure to antibody in vivo

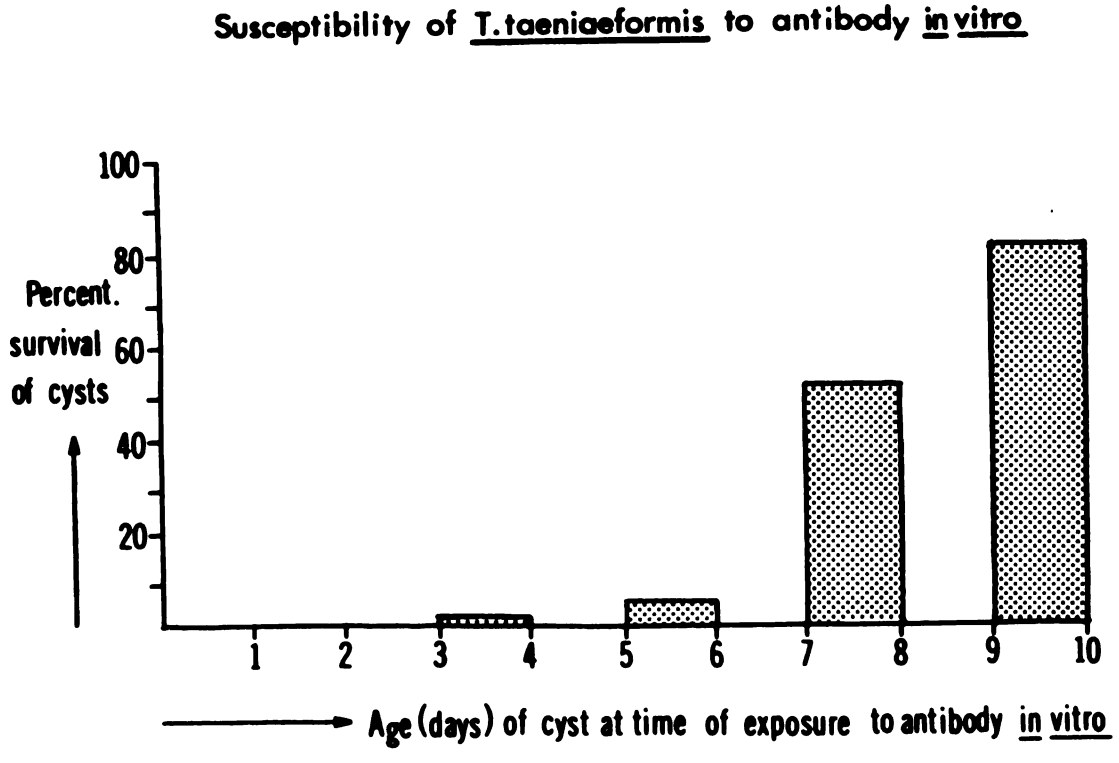

Susceptibility of T.taeniaeformis to antibody in vitro
100
80
Percent.
survival 60
of cysts
40
20
1
2
3
4
5
6
7
8
9
10
Age (days) of cyst at time of exposure to antibody in vitro

Figure 4

days. In view of the fact that serum complement levels can be effectively depleted using CoF for only 4-5 days (Maillard and Zarco, 1968), it was necessary to establish the optimum time at which to begin the CoF injections. Two groups of rats were inoculated with 1 ml of normal or immune serum intravenously and dosed with 2000 eggs of *T. taeniaeformis per os*. Twenty-four hours later the embryos were liberated from the liver tissue by tryptic digestion and injected via a mesenteric vein into normal recipients. The animals were killed 21 days later. The mean number of cysticerci in the rats receiving embryos from passively immunized rats was 21 $\pm$ 3.5 while the control group had 25 $\pm$ 10.2. These results indicated that there was a lag phase *in vivo* of at least 24 hrs before a 1 ml dose of antibody resulted in death of the parasites.

In the following experiment two groups of rats were dosed orally with 500 eggs of *T. taeniaeformis* followed by an intravenous inoculation of 1 ml of heat inactivated 28-day immune serum. Two other groups received an equivalent amount of inactivated normal serum. Twenty-four hours later, one of the groups injected with normal or immune inactivated serum began to receive intraperitoneal doses of 4 units per rat of CoF every 6 hours for 5 days. The daily levels of total complement were measured by the method described by Kabat and Mayer (1971) (Figure 5). All rats were sacrificed 21 days later and the mean number of larvae in each group is shown in Table 2. Representative livers from this experiment are depicted in Figure 6. These results clearly demonstrate that an intact complement system is required for successful passive transfer of resistance to *T. taeniaeformis*.

Figure 5. Percent inhibition of lytic complement in serum of rats inoculated with 4 units of CoF per rat every 6 hrs for 5 days.

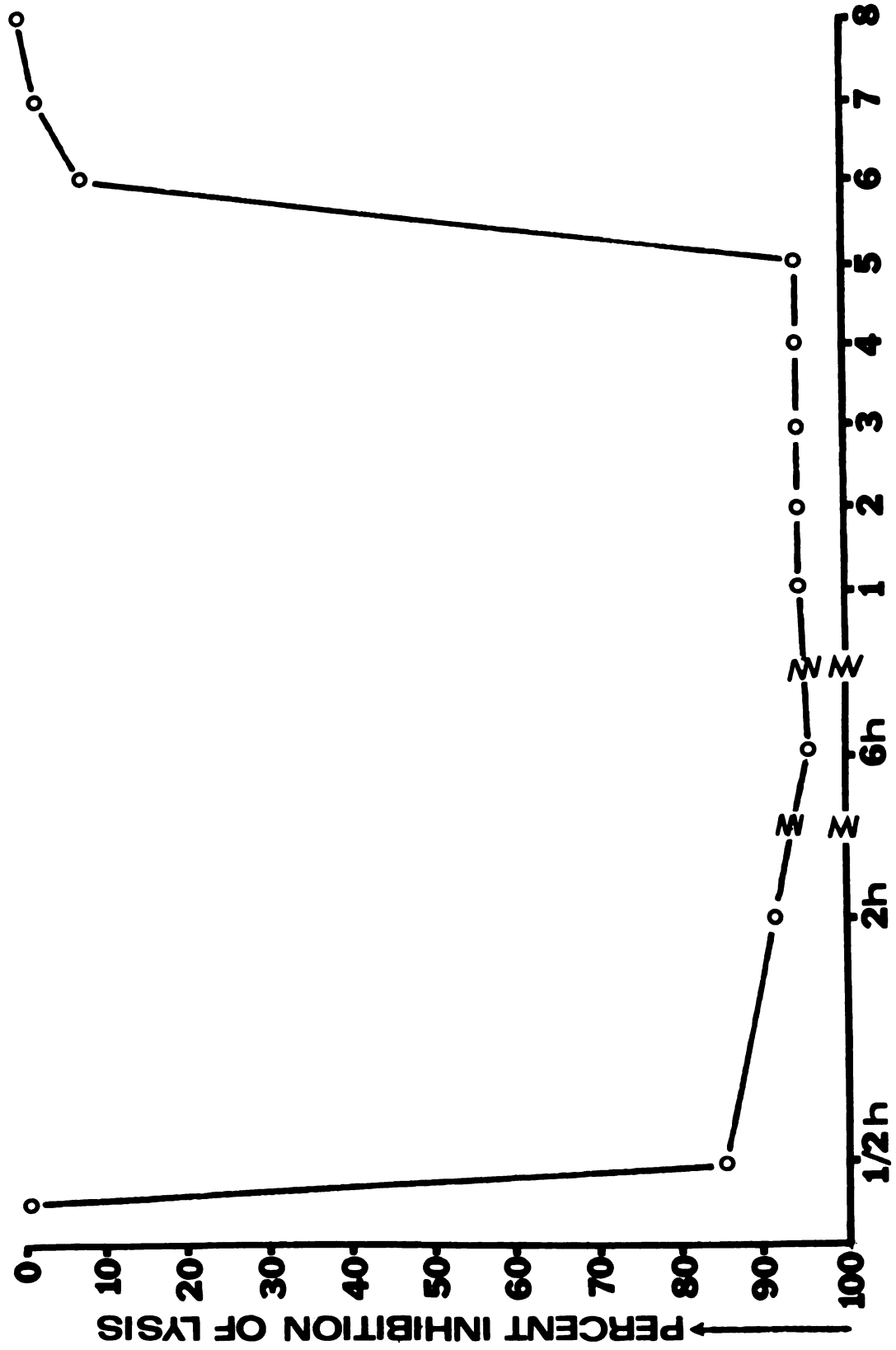

PERCENT INHIBITION OF LYSIS
0
10
20
30
40
50
60
70
80
90
100
1/2h
2h
6h
1
2
3
4
5
6
7
8
TIME (days)

Figure 5

Table 2. Effect of depletion of complement (C_3) on the establishment of *T. taeniaeformis* larvae in recipient rats injected with inactivated normal or immune serum. The animals were challenged with 500 eggs and killed 21 days later.

Treatment	No. of rats	Avg. no. of cysts in liver ± SD	SE of mean	P-value IRS x IRS-CoF
Inactivated normal serum + CoF	6	55.3 ± 14.2	5.8	---
Inactivated immune serum (IRS)	6	2.7 ± 2.2	0.9	---
Inactivated immune serum + cobra venom (IRS-CoF)	6	42.8 ± 13.0	5.3	<0.001
Inactivated normal serum	4	91.3 ± 13.7	6.9	---

Figure 6. Representative livers from four groups of rats, two of which were inoculated with normal or immune inactivated serum. The other two groups received inactivated normal or immune serum and CoF. The animals were sacrificed 21 days later.

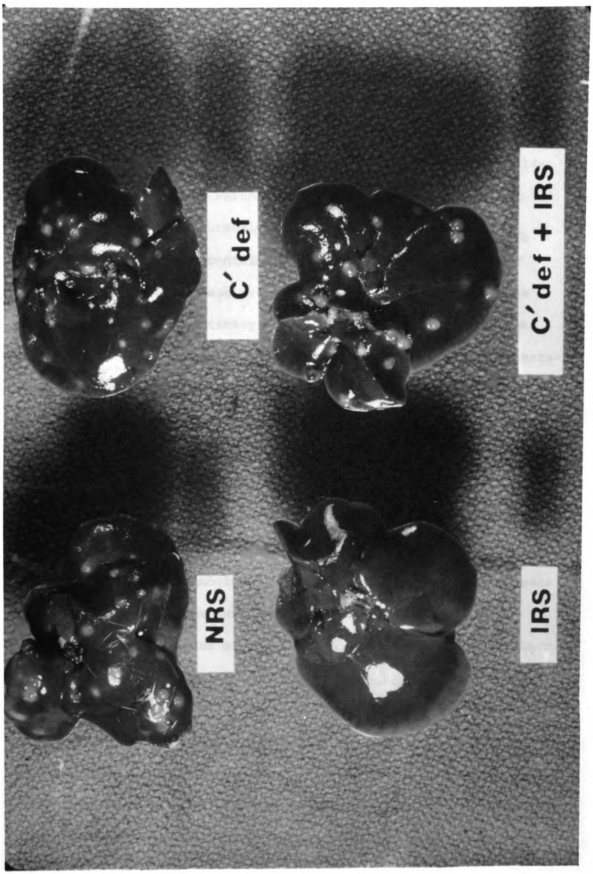


Figure 6

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DISCUSSION

Our results on the sequential appearance of protective antibodies in rats infected with *T. taeniaeformis* have confirmed the importance of $7S\gamma_{2a}$ immunoglobulins during the first 28 days of infection. Not only were those chromatographic fractions from 14- and 21-day serum samples which were enriched for $7S\gamma_{2a}$ the most effective for passive protection, but tryptic digestion of $7S\gamma_{2b}$ and $7S\gamma_{2c}$ from mixtures containing all three subclasses did not reduce the potency of these preparations. However, a remarkable extension of antibody activity to other chromatographic fractions was apparent with increasing time after infection until all preparations containing 7S immunoglobulins conferred highly significant protection upon recipients.

These results indicate either that a diversity of antibody responses develops to the protective antigen(s) produced by the developing parasite or that the antigens produced by older stages of the parasite differ sufficiently to cause the appearance of antibodies of distinct immunoglobulin classes. The protective antigens in more mature parasites must nevertheless either be shared or cross react with antigens of the early postoncospheral stages since the resistance mechanism is directed against these antibody labile forms.

None of the fractions enriched for γM immunoglobulin showed significant activity in any of our samples. This was particularly surprising since we have found that similar fractions prepared from serum of rats given surgical implants of mature metacestodes of *T.*

taeniaeformis are highly effective in protecting recipients (Musoke and Williams, 1975b), whereas fractions enriched for $7S\gamma_{2a}$ were much less effective. It seems likely that these contrasting findings derive from the fact that antigens are presented to the immunologic system of the host in a very different manner when live parasites are surgically implanted as opposed to their developing within the liver parenchyma.

With regard to the site at which protective antibodies exert their effect on the migrating organisms, our results clearly demonstrate that this effector mechanism can destroy parasites outside of the intestinal environment and that postoncospheral developmental stages in the liver retain a high degree of susceptibility to antibody for approximately 5 days. We were unable to bypass the effects of circulating protective antibody by administration of challenge doses via the mesenteric vein and a clear pattern of gradually acquired invulnerability of hepatic parasites was demonstrated both *in vivo* and *in vitro*. These findings are comparable to the recent observations of Heath (1973) and Rickard (1974) indicating that metacestodes of *Taenia pisiformis* remain susceptible to the effects of immune serum for at least one week, and give little support to the idea of circulating antibody participation in an "intestinal barrier" (Leonard and Leonard, 1941; Froyd and Round, 1960). This is not to say that there is no intestinal component to the resistance mechanism in cysticercosis, however, since we have been able to show that preparations of colostral γA from immune rats can be used to confer protection upon neonatal

recipients, and that the action of γA is confined to the intestinal lumen (Musoke, Williams, Leid, and Williams, 1975). It remains to be shown whether or not intestinal secretions containing γA contribute to the resistance shown by actively infected animals.

The observation that the effectiveness of protective antibody on the postoncospheral stages of *T. taeniaeformis* wanes as the parasites develop is in agreement with the results described by Campbell (1938b). However, he postulated that this decrease derived from formation of the fibrous host capsule around the parasite isolating the organism from antibody attack. The results of our experiments do not support this notion since larvae isolated at various stages *in vitro*, in the absence of the host capsule, also showed this shift toward the antibody-invulnerable phase, especially from day 6 onwards. It appears that the parasites themselves acquire some structural or metabolic characteristics which make them invulnerable to antibody mediated attack. The interesting observation that there is a 24-hour lag phase *in vivo* before protective antibody exerts its lethal effect is difficult to explain at this time but may indicate a requirement for other elements of the host defense system in destruction of the parasites.

The susceptibility of the early postoncospheral stages of *T. taeniaeformis* to antibody was shown to be dependent upon the integrity of the complement system in the host. Rats depleted of complement over this critical period were highly significantly deficient in their ability to destroy challenge organisms when given doses of immune serum which resulted in almost total destruction of all parasites in normal challenged animals. We believe

that this represents the first instance of the effectiveness of antibody against a helminth being dependent upon complement *in vivo*, although other workers have used similar experimental approaches in their investigations (Jones and Ogilvie, 1971). The latter authors used CoF to deplete C_3 levels in rats but were unable to implicate complement in the sequence of events which results in the expulsion of *Nippostrongylus brasiliensis* from the intestine. In our system the complement dependent antibody mediated attack on the early stages of *T. taeniaeformis* may be responsible for immobilization and destruction of the parasites by lytic effects and could result in the chemotactic attraction of either specific or non-specific cellular components of the defense mechanism.

In an attempt to clarify the means whereby older parasites evade antibody attack, we have made repeated efforts to demonstrate the presenceof amounts of hemolytic complement comparable to normal serum levels in the intracapsular fluid bathing the cysticerci of *T. taeniaeformis*. These have been uniformly unsuccessful (unpublished observations). However, a great many other serum proteins were detectable in this fluid in immunodiffusion tests. Although antigenic changes on the surface of the parasite (Varela-Diaz, Gemmell and Williams, 1972) or the masking effect resulting from adhesion of specific antibody (Rickard, 1974) have been postulated for the failure of rejection of cestode parasites in immune animals, it appears to us that anticomplementary factors produced by the metacestodes may be of prime importance in evading immunological damage. We have recently been able to demonstrate the release of

anticomplementary substances by cysticerci of *T. taeniaeformis* both *in vitro* and *in vivo* (Hammerberg, Musoke, Hustead and Williams, in preparation) and are pursuing the biological significance of this finding in continuing studies in our laboratory.

ACKNOWLEDGEMENTS

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REFERENCES

Ballow, M., and Cochrane, C. G. 1969. Two anticomplementary factors in cobra venom: Hemolysis of guinea pig erythrocytes by one of them. *J. Immunol.*, 103, 944.

Binaghi, R. A. 1971. Biological activities of IgG in mammals. *Progress in Immunology*, edited by B. Amos, p. 849-858. Academic Press, New York.

Blundell-Hasell, S. K., Gemmell, M. A., and Macnamara, F. N. 1968. Immunological responses of the mammalian host against tapeworm infections. VI. Demonstration of humoral immunity in sheep by the activated embryos of *Taenia hydatigena* and *T. ovis*. *Exptl. Parasit.*, 23, 78.

Campbell, D. H. 1938a. The specific protective property of serum from rats infected with *Cysticercus crassicollis*. *J. Immunol.*, 35, 195.

Campbell, D. H. 1938b. Further studies on the "nonabsorbable" protective property in serum from rats infected with *Cysticercus crassicollis*. *J. Immunol.*, 35, 465.

Froyd, G., and Round, M. C. 1960. The artificial infection of adult cattle with *C. bovis*. *Res. Vet. Sci.*, 1, 275.

Heath, D. D. 1971. The migration of oncospheres of *Taenia pisiformis*, *T. serialis* and *Echinococcus granulosus* within the intermediate host. *Int. J. Parasit.*, 1, 45.

Heath, D. D. 1973. Resistance to *Taenia pisiformis* larvae in rabbits. I. Examination of the antigenically protective phase of larval development. II. Temporal relationships and the development phase affected. *Int. J. Parasit.*, 3, 485.

Jones, V. E., and Ogilvie, B. M. 1971. Protective immunity to *Nippostrongylus brasiliensis*: The sequence of events which expels worms from the rat intestine. *Immunology*, 20, 549.

Kabat, E. A., and Mayer, M. M. 1971. *Experimental Immunochemistry*, 2nd Edn., p. 149. Charles C. Thomas, Springfield, Illinois, U.S.A.

Leid, W. R., and Williams, J. F. 1974a. Immunological response of the rat to infection with *Taenia taeniaeformis*. I. Immunoglobulin classes involved in passive transfer of resistance. *Immunology*, 27, 195.

Leid, W. R., and Williams, J. F. 1974b. Immunological responses of the rat to infection with *Taenia taeniaeformis*. II. Characterisation of reaginic antibody and an allergen associated with the larval stage. *Immunology*, 27, 209.

Leid, W. R., and Williams, J. F. 1975. Reaginic antibody response in rabbits injected with *Taenia pisiformis*. *Int. J. Parasit.*, (in press).

Leonard, A. B., and Leonard, A. E. 1941. The intestinal phase of the resistance of rabbits to the larvae of *Taenia pisiformis*. *J. Parasit.*, 27, 375.

Maillard, J. L., and Zarco, R. M. 1968. Decomplementation per un facteur extrait du venin de cobra. Effet sur plusieurs reactions immunes du cobaye et du rat. *Annales de L'Institute Pasteur*, 114, 756.

Morse, H. C., III, Bloch, K. J., and Austen, K. F. 1968. Biologic properties of rat antibodies. II. Time-course of appearance of antibodies involved in antigen-induced release of slow reacting substance of anaphylaxis (SRS-A^{rat}); Association of this activity with rat IgGa. *J. Immunol.*, 101, 658.

Musoke, A. J., and Williams, J. F. 1975a. Immunoglobulins associated with passive transfer of resistance against *Taenia taeniaeformis* in the mouse. *Immunology*, 28, 97.

Musoke, A. J., and Williams, J. F. 1975b. Immunological response of the rat to infection with *Taenia taeniaeformis*. III. Protective antibody response to implanted parasites. *Int. J. Parasit.*, (submitted for publication).

Musoke, A. J., Williams, J. F. Leid, R. W., and Williams, C. S. F. 1975. The immunological response of the rat to infection with *Taenia taeniaeformis* IV. Immunoglobulins involved in passive transfer of resistance from mother to off-spring. *Immunology*, (submitted for publication).

Miller, H. M., and Gardiner, M. L. 1932. Passive immunity to infection with a metazoan parasite *Cysticercus fasciolaris* in the albino rat. *J. Prev. Med.*, 6, 479.

Nezlin, R. S., Krilov, M. Yu., and Rokhlin, O. V. 1973. Different susceptibility of subclasses of rat Igγ2 to tryptic digestion. *Immunochemistry*, 10, 651.

Rickard, M. D. 1974. Hypothesis for the long term survival of *Taenia pisiformis* cysticerci in rabbits. *Z. Parasitenk.*, 44, 203.

Rickard, M. D., and Bell, K. 1971. Immunity produced against *Taenia ovis* and *T. taeniaeformis* infection in lambs and rats following *in vivo* growth of larvae in filtration membrane diffusion chambers. *J. Parasit.*, 57, 571.

Revoltella, R., and Ovary, Z. 1969. Reaginic antibody production in different mouse strains. *Immunology*, 17, 45.

Silverman, P. H. 1954. Studies on the biology of some tapeworms of the genus *Taenia*. II. Factors affecting hatching and activation of taeniid ova and some criteria for their viability. *Ann. Trop. Med. Parasit.*, 48, 207.

Varela-Diaz, V. M., Gemmell, M. A., and Williams, J. F. 1972. Immunological responses of the mammalian host against tapeworm infections. XII. Observations on antigen sharing between *Taenia hydatigena* and *T. ovis*. *Exptl. Parasit.*, 32, 96.

APPENDIX

THE IMMUNOLOGICAL RESPONSE OF THE RAT TO INFECTION WITH *TAENIA TAENIAEFORMIS*. IV. IMMUNOGLOBULINS INVOLVED IN PASSIVE TRANSFER OF RESISTANCE FROM MOTHER TO OFFSPRING

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SUMMARY

Weanling rats born of mothers infected with *Taenia taeniaeformis* were found to be passively protected against homologous challenge. Cross fostering of normal sucling rats onto immune mothers established that passive transfer occurred via the colostrum and milk. Immunoglobulin fractions from immune colostrum containing γA were fed to 12- to 14-day-old rats for 4 days via stomach tube. Significant passive protection against challenge with *T. taeniaeformis* was achieved with γA from 1 of 3 colostrum pools. The effect of colostral γA preparations on the infectivity of freshly hatched oncospheres of *T. taeniaeformis* was measured by the intraintestinal inoculation of immunoglobulin solutions into isolated gut loops containing hatched eggs of the parasite. γA from 1 of 3 pools of immune colostrum caused a significant reduction in the number of parasites which reached the liver. This appears to be the first time that protective activity against a helminth infection has been achieved with γA.

A fraction of immune colostrum containing both $7S\gamma_1$ and $7S\gamma_2$ immunoglobulins was found to confer passive protection when inoculated parenterally. In view of the prolonged period of absorption (ca. 18 days) of 7S immunoglobulins from the gut by the sucling rat, it seems likely that these antibodies are primarily responsible for the natural passive transfer of protection from mother to young.

INTRODUCTION

Natural passive transfer of resistance to cysticercosis from mothers to their offspring has been shown to occur in both

laboratory and domestic animals. Miller (1935) showed that female rats which were immune to infection with *Taenia taeniaeformis* transferred this resistance to their young. Gemmell, Blundell-Hasell and Macnamara (1969) reported that lambs born of ewes immunized against *Taenia hydatigena* were passively protected against challenge infection. More recently, Rickard and Arundel (1974) have shown that lambs were protected against *Taenia ovis* after suckling ewes which were either naturally or artificially immunized against this parasite.

In rats the mechanism of natural passive transfer of immunity to *T. taeniaeformis* has not been studied but serum antibodies in the $7S\gamma_{2a}$ immunoglobulin subclass have been implicated in artificial passive protection by Leid and Williams (1974). However, the possible involvement of secretory γA (SγA) antibodies in the natural protection of newborn animals against cysticercosis has been raised by Gemmell and Macnamara (1972), and antibodies of this type have been shown to play an important role in neonatal immunity to a variety of infectious organisms (Porter, Noakes and Allen, 1970; Stone, Stack and Phillips, 1974).

Although it has not been shown to be associated with a detectable secretory fragment, γA is certainly the major immunoglobulin in rat colostrum (Stechschulte and Austen, 1970). We have therefore investigated the characteristics of passively transferred resistance to *T. taeniaeformis* in newborn rats and report here on the contributions of colostrally derived antibodies of defined immunoglobulin classes in this process.

MATERIAL AND METHODS

Parasite

The strain of *T. taeniaeformis* used in these experiments was obtained from Mr. C. E. Claggett in the Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, Maryland. The parasite was maintained as described by Leid and Williams (1974).

Experimental Animals

Sprague-Dawley rats were purchased from Spartan Research Animals, Haslett, Michigan. They were given proprietary brand food and water *ad libitum*.

Immunoelectrophoresis and Double Immunodiffusion

Immunoelectrophoresis (I.E.P.) was performed following a slight modification of the method of Scheidegger (1955) in a Gelman apparatus (Gelman Instrument Company, Ann Arbor, Michigan), with a sodium barbital HCl buffer, μ = 0.038, pH 8.2 (Williams and Chase, 1971). Two percent Noble agar (Difco, Detroit, Michigan) was prepared with barbital buffer diluted 1:2 and contained 1:10,000 thiomersolate. Double immunodiffusion (D.I.D.) was performed according to a micromethod modified from that described by Williams and Chase, 1971). Two percent Noble agar (Difco, Detroit, Michigan) was prepared in a 0.1 M TRIS-HCl buffer, pH 8.1 with a final concentration of thiomersolate of 1:10,000.

Measurement of Protein Concentration

Protein concentrations were determined by the method of Lowry *et al.* (1951).

Harvesting of Rat Colostrum

Twenty-eight-day-old female rats were dosed orally with 1000 eggs of *T. taeniaeformis*, and four weeks later they were mated. Colostrum was harvested following the method described by Stechschulte and Austen (1970). Three separate pools of colostrum were prepared, each derived from at least 12 litters. The suckling rats were permitted to nurse for 2-3 hours after an overnight starvation period. They were then killed with carbon dioxide vapor and stomach contents were collected and taken up in phosphate buffered saline pH 7.2 (PBS) before homogenization. The homogenized colostrum was stirred overnight at 4°C and centrifuged at 4°C for 1 hour at 17,000 g. The clear fluid above the pellet and below the floating lipid layer was removed and concentrated with polyethylene glycol and dialyzed extensively against PBS. The globulins were precipitated with 50 percent saturated ammonium sulphate and dialyzed free of sulphate ions before chromatography.

Chromatography

Gel filtration chromatography was performed on a siliconized 2.5 x 100 cm column of Sephadex G-200 (Pharmacia, Uppsala), equilibrated with 0.1 M TRIS-HCl pH 8.0. The globulins from colostrum were dialyzed against the equilibrating buffer before application and eluted fractions were collected in 2.8 ml volumes. Elution profiles were prepared using the optical density of each fraction at 280 nm in a Beckman spectrophotometer (Beckman Instrument Company, Fullerton, California).

The procedure for ion exchange chromatography of rat colostrum was a modification of the method described by Stechschulte and Austen (1970). DEAE cellulose (DE-52, Whatman) was prepared according to the manufacturer's instructions and was poured in 1.5 x 20 cm siliconized glass columns. After initial equilibration against 0.01 M phosphate buffer pH 7.8, proteins were eluted in a stepwise manner using 0.01 M phosphate followed by 0.05 M phosphate pH 5.8 and 0.05 M phosphate + 0.5 M NaCl pH 5.8. The first peak from a Sephadex G-200 fractionation was dialyzed extensively against the starting buffer before application to the column. Column eluates were collected in 2.8 ml fractions and the elution pattern followed by ultraviolet monitoring at 280 nm. Protein peaks eluted with each buffer were pooled and concentrated with polyethylene glycol.

Preparation of Antisera

Antisera to rat immunoglobulins were prepared according to the methods described by Leid and Williams (1974).

Analysis of the Chromatographic Fractions

Protein peaks obtained by gel filtration on Sephadex G-200 columns were analyzed by IEP and DID, against antisera to rat immunoglobulins. F1 (Figure 1) contained predominantly γA with minor contamination by $7S\gamma_2$ immunoglobulins. F2 contained both $7S\gamma_1$ and $7S\gamma_2$ immunoglobulins and was not fractionated further. γM immunoglobulins were not detected in colostrum by the techniques employed. F1 was fractionated by anion-exchange chromatography to remove contaminating $7S\gamma_2$ immunoglobulins. The fraction eluted with 0.05

Figure 1. Sephadex G-200 gel filtration of globulin fraction of colostrum from the stomachs of 24-hour-old newborn rats. F1 contained predominantly γA with some $7S\gamma_2$ immunoglobulins. F2 contained $7S\gamma_1$ and $7S\gamma_2$ immunoglobulins throughout but no γA. γM was not detectable in any of the fractions tested.

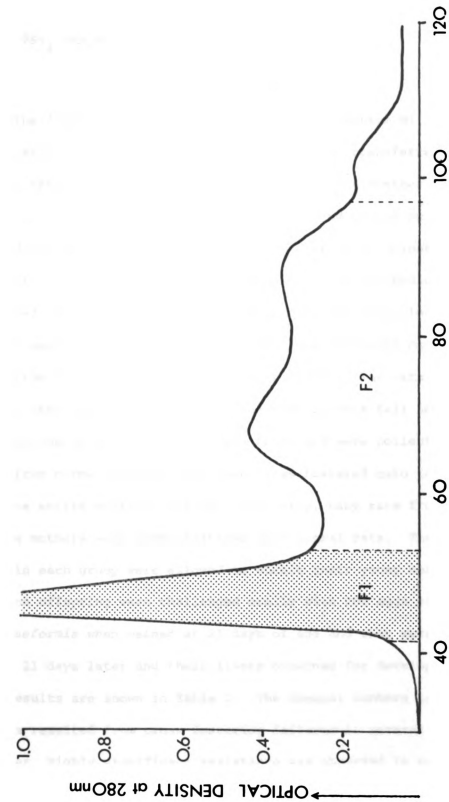
OPTICAL DENSITY at 280nm
1.0
0.8
0.6
0.4
0.2
F1
F2
40
60
80
100
120
TUBE NUMBER

Figure 1

M phosphate + 0.5 M NaCl buffer contained only γA, with no detectable $7S\gamma_2$ immunoglobulins (Figure 2).

RESULTS

The first experiment was carried out to confirm Miller's observation (1935) that immunity was passively transferred from mother rats to their offspring, and to determine whether the resistance manifested in young rats at the end of the suckling period was attributable wholly to the ingestion of colostrum and milk or in part to the prenatal transmission of antibodies. Six infected and 6 normal female rats were bred and when close to term 3 from each group were placed in wire floor maternity cages modified from those described by Hollander (1970). The rats littered on one inch (mink cage) mesh and the newborn rats fell immediately through the holes onto the bedding below and were collected. Baby rats from normal mothers were then cross fostered onto immune rats for the entire suckling period. Similarly, baby rats from the immune mothers were cross fostered onto normal rats. The other 3 rats in each group were allowed to suckle their young normally. All the offspring were challenged orally with 200 eggs of *T. taeniaeformis* when weaned at 21 days of age and they were sacrificed 21 days later and their livers examined for developing cysts. The results are shown in Table 1. The unequal numbers in these groups resulted from cross fostering failures in several litters. However, highly significant resistance was observed in normal rats which had suckled immune mothers ($P < .001$, Student's 't' test) and in rats which suckled their own immune mothers ($P < .001$) when

Figure 2. Anion exchange chromatography of F1 from gel filtration of rat colostrum. The stippled fraction contained only γA detectable by immunoelectrophoresis (below). Troughs contained rabbit anti-whole rat serum, and sheep anti-rat γA.

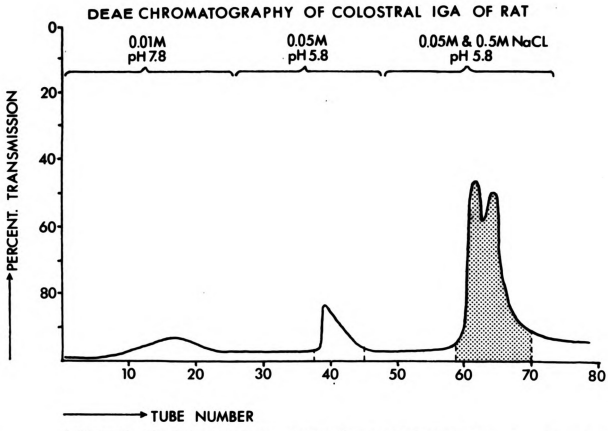
DEAE CHROMATOGRAPHY OF COLOSTRAL IGA OF RAT
0.01M pH 7.8
0.05M pH 5.8
0.05M & 0.5M NaCl pH 5.8
PERCENT. TRANSMISSION
0
20
40
60
80
10
20
30
40
50
60
70
80
TUBE NUMBER

Figure 2

Table 1. Effect of ingestion of colostrum and milk from immune mothers on resistance to infection with *Taenia taeniaeformis* in young rats

Experimental group	No. of rats	Avg. no. of cysts/liver ± SD	SE of mean	P-value
Normal rats suckling normal mothers	19	44.3 ± 21.3	4.9	---
Normal rats suckling immune mothers	9	2.2 ± 5	1.7	<0.001
Rats from immune mothers suckling normal mothers	20	54.5 ± 22.7	5.1	---
Rats from immune mothers suckling immune mothers	25	0.6 ± 2.3	0.5	<0.001

these groups were compared to controls. Rats born of immune mothers but suckling normal females showed no sign at all of resistance.

Prior to our experiments on oral dosage with purified immunoglobulins, it was necessary to determine the optimum age at which to challenge young rats. Litters were dosed orally at the ages of 10, 12, 14, 16, 18, 20 and 22 days, with 200 eggs of *T. taeniaeformis*, and all rats were sacrificed 21 days later. The results (Figure 3) indicated that young rats did not become appreciably susceptible to infection until at least eighteen days after birth and that dosing prior to this time resulted in very light and variable infections.

Experiments were then carried out to establish the role of immunoglobulins isolated from immune colostrum in passive transfer, by oral administration to suckling rats prior to challenge. Fourteen-day-old rats were fed fractions enriched for either γA or $7S\gamma_1$ and $7S\gamma_2$, twice a day for four days, using a stomach tube. A total of 1 mg of protein per rat was fed. The control group received unfractionated globulins precipitated from normal colostrum. All rats were challenged with 200 eggs of *T. taeniaeformis per os* two hours after the last feeding. Twenty-one days later the rats were killed and the average numbers of cysticerci per liver in each group are shown in Table 2. These results indicated that rats fed colostral γA had a significantly lower number of parasites (Ave 2.7) than the controls (Ave 12.3, $P < 0.05$). Although the group receiving fractions containing $7S\gamma_1$ and γ_2 had fewer larvae in their livers (Ave 4.8) than controls, this

Figure 3. Histogram demonstrating the association between susceptibility of suckling rats to infection with *Taenia taeniaeformis* and age at time of oral dosage with eggs. Vertical bars represent the S.E. of the mean for each experimental group.

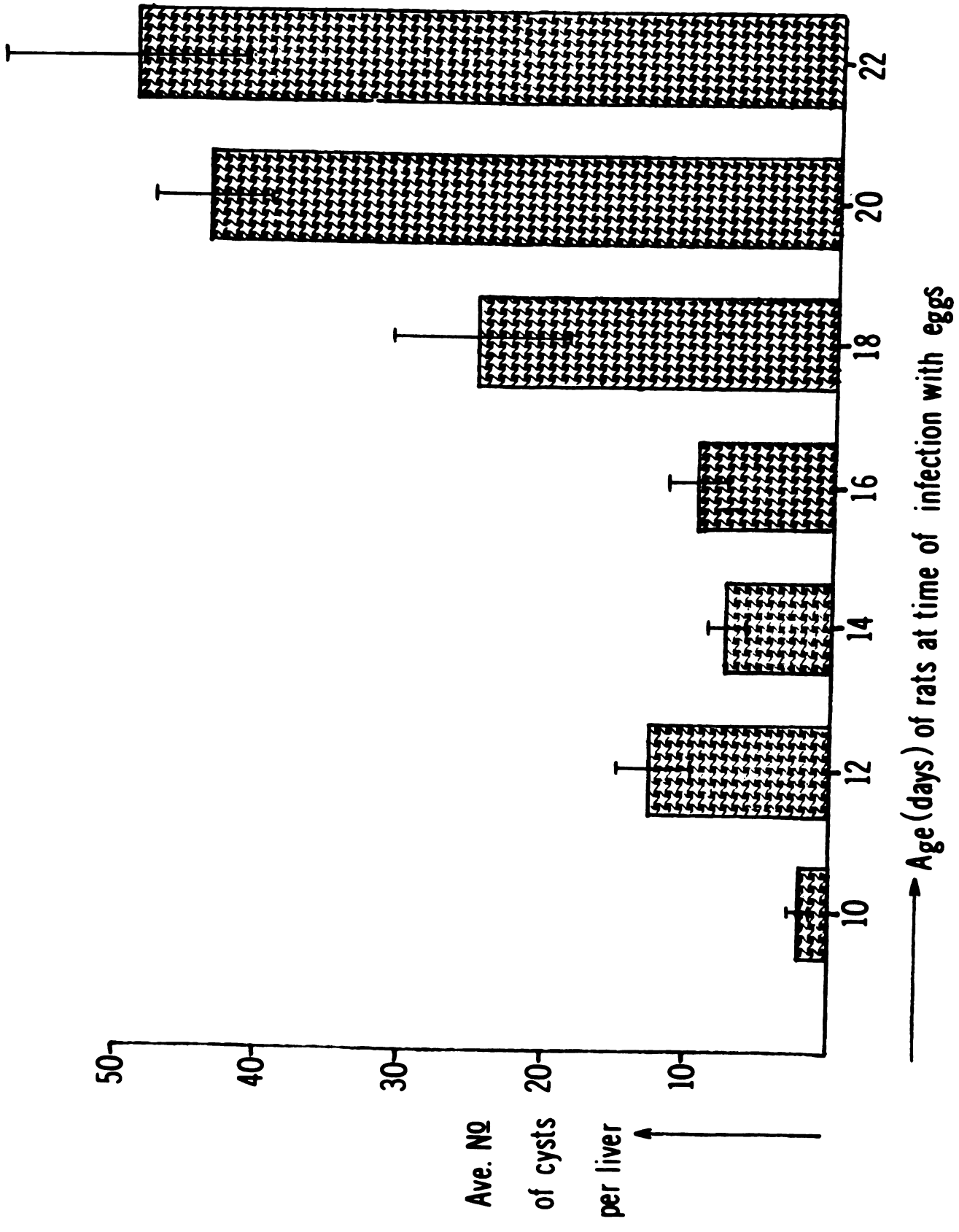
Ave. No of cysts per liver
50
40
30
20
10
10
12
14
16
18
20
22
Age (days) of rats at time of infection with eggs

Figure 3

Table 2. Effect of feeding chromatographic fractions of immune colostrum to normal 14-day-old rats on their resistance to infection with *Taenia taeniaeformis*

Experimental group (1 mg protein per rat)	No. of rats in group	Avg. no. cysts/ liver ± SD	SE of mean	P value
Normal colostral whey globulins	8	12.3 ± 9.2	3.2	---
Immune colostrum γA (Pool 1)	6	2.7 ± 3.1	1.3	<0.05
Immune colostrum $7S\gamma_1$ and $7S\gamma_2$ (Pool 1)	6	4.8 ± 3.8	1.5	N.S.

difference was not statistically significant. The experiment was repeated on 2 further occasions. In the third trial, feeding was begun at 12 days. We were unable to demonstrate significant protective capacity offered by γA from these pools of colostrum.

Since the results of oral feeding of colostral γA were inconsistent we attempted to measure an effect of this antibody on the infectivity of freshly hatched parasite oncospheres employing the "sausage" technique as described by Silverman and Maneely (1955). A portion of the small intestine of normal 21-day-old rats was exposed through a midline incision in the abdomen under inhalation anesthesia with methoxyfluorane. A section approximately 1 inch in length was tied off with silk ligatures and colostral immunoglobulin fractions containing γA were injected into the sausage 30 mins before the introduction of hatched oncospheres (Rickard and Bell, 1971). The oncospheres were permitted 2 hours for further activation and penetration of the intestine wall. The gut was then flushed thoroughly with PBS and the ligatures were removed before closing the midline incision in two layers. The rats were sacrificed 21 days later. The results of the first experiment (Table 3) clearly demonstrated that γA plays a significant protective role against *T. taeniaeformis*. The number of parasites establishing in the group receiving immune γA was significantly different from the control group which had been inoculated with γA preparations from normal colostrum ($P < 0.01$). This procedure was repeated in two further experiments, using two different pools of colostrum. Although the means of the groups were not statistically different, there was a remarkable reduction in parasite

Table 3. Effect of intraintestinal inoculation of chromatographic fractions of colostrum enriched for γA on the infectivity of oncospheres of *Taenia taeniaeformis* in isolated gut segments (sausages)

Experimental group (1 mg protein per rat)	No. of rats in group	Avg. no. cysts/ liver ± SD	SE of mean	P-value
Normal colostral γA	5	40.8 ± 17.3	7.7	---
Immune colostral γA (Pool 1)	5	10.2 ± 8.8	3.9	<0.01
Normal colostral γA	6	15.5 ± 13.6	5.6	---
Immune colostral γA (Pool 2)	4	6.25 ± 4.0	2.0	<N.S.
Normal colostral γA	5	11.67 ± 1.5	0.9	---
Immune colostral γA (Pool 3)	5	7.4 ± 4.6	2.1	N.S.

burden in those groups receiving immune γA fractions (Table 3).

In view of the fact that fractions containing $7S\gamma_1$ + $7S\gamma_2$ immunoglobulins from immune colostrum did not provide significant protection when fed, we inoculated these fractions parenterally in another group of rats in order to demonstrate any protective capacity. A total of 1 mg of protein was inoculated intravenously in 18-day-old rats, and the control group received the same quantity of protein from normal colostrum by the same route. The rats were challenged with 200 eggs of *T. taeniaeformis* orally and sacrificed 21 days later. Rats receiving $7S\gamma_1$ and $7S\gamma_2$ immunoglobulins from immune colostrum harbored a mean of 11.5 $\pm$ 3.4 (S.D.) cysts while controls had a mean of 24.7 $\pm$ 12.5 cysts and this difference was statistically significant ($P < 0.05$).

DISCUSSION

The results presented in this paper confirm the observations of Miller (1935) that young rats born of mothers infected with *T. taeniaeformis* are immune to challenge infection. It appears that the majority of this transfer occurs postnatally since normal rats cross fostered onto lactating immune mothers acquired a high degree of resistance. Rats born of immune mothers but fed only on normal colostrum and milk were not detectably less susceptible than control animals when challenged at 21 days of age.

It is possible that prenatally derived protective antibodies may have waned by this time, but it would be difficult to establish this by challenge infection at an earlier stage since our results show that rats do not become appreciably susceptible to infection

until late in the third week of life. Prior to this time neonatal rats have little or no proteolytic enzymic activity in their intestine (Brambell, 1970), and they may therefore be unable to hatch and activate taeniid eggs.

Our findings on the effectiveness of oral administration of colostral immunoglobulins clearly implicate γA antibodies in the mechanism of resistance shown by young rats. This appears to be the first time that protection against a helminth infection has been demonstrated using purified preparations of this type. Although it was possible to achieve passive protection with γA on only one of 3 occasions, substantiation of this finding was offered by our results using the "sausage" technique which permitted direct access of antibody to the freshly hatched oncospheres. Statistically significant evidence to indicate protective capacity of γA was found in one of three trials, but the results of the other two experiments showed a remarkable reduction in the number of cysticerci developing in livers of rats receiving similar purified preparations.

Little is known about the mechanisms of action of antibodies to taeniid parasites, but Silverman (1955) observed an immobilizing effect of immune serum on cestode oncospheres *in vitro* which could be responsible for immunity at the intestinal level. In support of this proposal, Heath (1971) has shown that in immune animals oncospheres of *T. pisiformis* do not attempt to attach to the intestinal wall. Experiments conducted in our laboratory have shown that γA is not detectable in serum of newborn rats until the

third week of life and that radiolabelled colostral γA fed to suckling rats is not absorbed from the intestine (Hammerberg, Musoke and Williams, in preparation). These findings therefore indicate that the effect of γA antibodies on the oncosphere must occur within the intestine. It is possible that they exert their effect directly by neutralizing secretions from the penetration glands of oncospheres, immobilizing them much in the manner proposed by Silverman (1955). An indirect effect resulting from inhibition of attraction or attachment of activated embryos to the gut mucosa, as appears to be the case for SγA antibodies to intestinal bacteria (Fubara and Freiter, 1973) cannot be ruled out at this time.

One milligram doses of colostral $7S\gamma_1$ and $7S\gamma_2$ immunoglobulins provided no statistically significant passive protection when administered orally but were effective when given intravenously. This difference can be attributed to the fact that only 1/3 of orally administered antibody is absorbed (Halliday, 1957) and the remaining 2/3 is apparently degraded within the endodermal cells lining the intestine (Brambell, Halliday and Hemmings, 1961). However, in view of the fact that our γA preparations were not consistently protective, we feel that the capacity of suckling rats to absorb protective 7Sγ globulins from colostrum and milk over an extended period is probably of prime importance in the successful natural passive transfer of immunity from mother to young.

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REFERENCES

Brambell, F. W. R. (1970) *The Transmission of Passive Immunity from Mother to Young*. North-Holland Publishing Company, 102.

Brambell, F. W. R., Halliday, R. R., and Hemmings, W. A. (1961) 'Interference by human and bovine serum and serum protein fractions with the absorption of antibodies by suckling rats and mice.' *Proc. Roy. Soc. Biol.*, 149, 1.

Fubara, E. S., and Freter, R. (1973) 'Protection against enteric bacterial infection by secretory IgA antibodies.' *J. Immunol.*, 111, 395.

Gemmell, M. A., Blundell-Hasell, S. K., and Macnamara, F. N. (1969) 'Immunological responses of the mammalian host against tapeworm infections. IX. The transfer via colostrum of immunity to *Taenia hydatigena*.' *Exptl. Parasit.*, 26, 52.

Gemmell, M. A., and Macnamara, F. N. (1972) 'Immune response to tissue parasites. II. Cestodes.' p. 235. In *Immunity to Animal Parasites*. E. J. L. Soulsby (Ed), Academic Press, New York.

Halliday, R. R. (1957) 'The absorption of antibody from immune sera and from mixtures of sera by the gut of the young rat.' *Proc. Roy. Soc. Biol.*, 148, 92.

Heath, D. D. (1971) 'The migration of oncospheres of *Taenia pisiformis*, *T. serialis* and *Echinococcus granulosus* within the intermediate host.' *Int. J. Parasit.*, 1, 145.

Hollander, W. F. (1970) 'Wire-floor maternity caging for studies of neonatal mice.' *Laboratory Animal Care*, 20, 512.

Leid, R. W., and Williams, J. F. (1974) 'Immunological response of the rat to infection with *Taenia taeniaeformis*. I. Immunoglobulin classes involved in passive transfer of resistance.' *Immunology*, 27, 195.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) 'Protein measurement with the Folin phenol reagent.' *J. Biol. Chem.*, 193, 265.

Miller, H. M. (1935) 'Transmission to offspring of immunity against infection with a metazoan (cestode) parasite.' *Am. J. Hyg.*, 21, 456.

Porter, P., Noakes, D. E., and Allen, W. D. (1970) 'Secretory IgA antibodies to *Escherichia coli* in porcine colostrum and milk and their significance in the alimentary tract of the young pig.' *Immunology*, 18, 245.

Rickard, M. D., and Arundel, J. H. (1974) 'Passive protection of lambs against infection with *Taenia ovis* via colostrum.' *Aust. Vet. J.*, 50, 22.

Rickard, M. D., and Bell, K. (1971) 'Immunity produced against *Taenia ovis* and *T. taeniaeformis* infection in lambs and rats following *in vivo* growth of their larvae in filtration membrane diffusion chambers.' *J. Parasit.*, 57, 571.

Scheidegger, J. J. (1955) 'Une micro-methode de immunoelectrophorese.' *Int. Arch. Allergy*, 7, 103.

Silverman, P. H. (1955) 'A technique for studying the *in vitro* effect of serum on activated taeniid hexacanth embryos.' *Nature* (Lond.), 176, 598.

Silverman, P. H., and Maneely, R. B. (1955) 'Studies on the biology of some tapeworms of the genus *Taenia*. III. The role of the secreting gland of the hexacanth embryo in the penetration of the intestinal mucosa of the intermediate host and some of its histochemical reactions.' *Ann. Trop. Med. Parasit.*, 49, 326.

Stechschulte, D. J., and Austen, K. F. (1970) 'Immunoglobulins of rat colostrum.' *J. Immunol.*, 104, 1052.

Stone, S. F., Stack, S. L., and Phillips, M. (1974) 'Transmissible gastroenteritis virus in neonatal pigs: intestinal transfer of colostral immunoglobulins containing specific antibodies.' *Am. J. Vet. Res.*, 35, 339.

Williams, C. A., and Chase, M. W. (1971) *Methods in Immunology and Immunochemistry*, 1st Edn. p. 103, Academic Press, New York.