IMMUNOLOGICAL EVENTS ASSOCIATED WITH TAENIA TAENIAEFORMIS INFECTIONS IN THE LABORATORY RAT

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ABSTRACT

IMMUNOLOGICAL EVENTS ASSOCIATED WITH *TAENIA TAENIAEFORMIS* INFECTIONS IN THE LABORATORY RAT

By

R. Wes Leid

Serum from rats infected for 28 days with cysticerci of *Taenia taeniaeformis* was shown to protect recipient rats against homologous challenge. Furthermore, this activity resided in the globulin fraction of immune serum as determined by $(NH_4)_2SO_4$ precipitation. Gel filtration and anion exchange chromatography of immune globulin solutions resulted in a separation of rat globulins into γM, $\gamma_{2a} + \gamma_{2b}$, $\gamma_{2a} + \gamma_{2b} + \gamma E$ and $\gamma_{2a} + \gamma_{2b} + \gamma_1$ containing fractions. Highly significant protection ($P<0.001$, 0.001 and 0.01, respectively) was associated only with the latter 3 fractions, the DEAE eluates corresponding to 0.01M phosphate, 0.05M phosphate and 0.10M phosphate. Further chromatography gave rise to antibodies of a single well defined immunoglobulin class, $7S\gamma_{2a}$, which was able to confer highly significant protection ($P<0.01$) when given alone. The protective capacity exhibited by the 0.01M, 0.05M and 0.10M eluates may have been due to the presence of $7S\gamma_{2a}$ in all these fractions as it was a consistent feature detectable by double immunodiffusion and immunoelectrophoresis. The presence of the different immunoglobulin classes in each fraction was detected by monospecific antiserum to each class. A variety of absorption techniques were used in attempts to reduce the effectiveness of immunoglobulin

solutions. All were unsuccessful and may have resulted from a lack of effective antigens in extracts from cysticerci of *T. taeniaeformis*.

Infection with cysticerci of *T. taeniaeformis* resulted in the appearance of skin fixing antibody or reagin during the third week of infection. Peak titers were reached during the fifth week and declined thereafter. The physico-chemical and biological characteristics of this reagin were consistent with those of the rat immunoglobulin, designated γE. In no instance was it possible to demonstrate a short term skin-fixing response due to $7S\gamma_{2a}$ in protective serum from 28-day-old infections even though antibodies were present in this class. Nonetheless, short-term sensitization was possible at 2-6 hours and this reactivity was determined by physico-chemical means to be due solely to reagin or γE.

An allergen implicated in the reagin response was purified to a single defined protein, which contained carbohydrate but no lipid, was negatively charged and was able to provoke passive cutaneous anaphylaxis (PCA) in sensitized rats in approximately 5 μg quantities. A monospecific antiserum raised to this allergen removed all PCA reactivity from larval extracts. However, allergenic activity was only slightly reduced by absorption of adult worm extracts with this same antiserum. It was totally ineffective in reducing the ability of *in vitro* culture concentrates of the cysticerci to provoke PCA. Thus the presence of two different allergens was indicated. The partial removal of allergenic activity from adult worm extracts might indicate the presence of yet a third allergen. This is only the second instance in which a parasitic allergen has been so purified and the presence of more than one allergen shown to occur in helminthic extracts.

This work has demonstrated the ability of antibodies in a single well defined immunoglobulin class, $7S\gamma_{2a}$, to passively protect recipient rats. A pure allergen associated with the reaginic antibody response was isolated and partially characterized.

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By

R. Wes Leid

A DISSERTATION

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Dedication

This dissertation is dedicated to
my wife Katie and my sons Rory and Jeffrey

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I am extremely appreciative of my privilege in working with my mentor, Dr. J. F. Williams, during the course of this study. For his guidance and encouragement I am very grateful and will remember him as a teacher, colleague and friend throughout my career. I would also like to thank the following people for their valuable assistance during the course of this work: Ms. Anndy Whipple, Dr. David H. Bing, Dr. A. J. Musoke, A. Wayne Roberts, Dr. G. R. Carter, Dr. Kathy Morris and Ms. Sandy Spurlock.

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INTRODUCTION

Cysticercosis and hydatidosis are cyclo-zoonotic infections caused by members of the order *Cyclophyllidea*, Family *Taeniidae*. The cosmopolitan distribution of these parasites demonstrates that members of this parasite family are not limited by geographical boundaries and infection rates are high under a variety of socio-economic conditions. The larval or cysticercus stage of taeniid parasites is of greatest concern to man and domesticated food animals. Both the economic loss in protein from infected animals and the loss in human health and production have come to assume increased importance as other major world diseases are brought under control. These losses are of particular relevance in the developing countries of the world where reduction in total protein available for food consumption and lowered industrial output due to worker absence can be ill afforded. Since the cysticerci are not susceptible to any practical chemotherapy and indeed may only be treated by surgical excision if their location is amenable to surgery, immunological control of the cysticerci offers one avenue of therapy that should be pursued. The immunological events associated with specific acquired resistance to these helminths remain ill-defined and a further analysis of these phenomena in laboratory and food animals may permit specific prophylaxis and therapy.

The rat *Taenia taeniaeformis* system has served as an experimental model system for investigations on cysticercosis with particular emphasis on the immunology of such an infection. The literature review

has been divided into three main headings to provide a background on clinical and experimental cysticercosis and rat immunology. The first area of concern is the general biology, epidemiology, pathology and economics of infection with taeniid parasites. Here the emphasis is on *Taenia solium* and *T. saginata* and their significance both to public health and veterinary medicine.

The second section discusses the immunology of experimental infections in domestic animals and the biology and immunology of *T. taeniaeformis* infections in the rat. *Taenia taeniaeformis* is discussed in relation to the immunological reactions occurring during parasitism and how these may be comparable to those seen in the more costly large animal systems.

The last topic covers rat immunology with a particular emphasis on reagin mediated immediate hypersensitivity reactions. Earlier workers had indicated that serum antibody was important in passive protection to *T. taeniaeformis* and this section discusses the nomenclature, biological and physico-chemical properties of the different immunoglobulin classes in the rat. The allergic response is a prominent feature of helminthiasis in general and of cysticercosis in particular and with the pathogenesis of taeniid infections resulting from an intense hypersensitivity reaction. Therefore, reagin mediated hypersensitivity has been reviewed at some length with respect to the type of antibody involved, the molecular characteristics of such a protein and the means by which clinical allergic disease is manifested.

LITERATURE REVIEW

General Biology, Epidemiology, Pathology and Economics of Taeniid Infections

Cestodes of the genera *Taenia* and *Echinococcus* are members of the Family *Taeniidae*, Order *Cyclophyllidea*, one of the two medically important orders of cestodes in both man and domesticated animals. Members of this family have a life cycle which includes a larval stage in an intermediate host with the adult worm in a definitive host.

Taenia saginata

Biology. The life cycle of *Taenia saginata* is shown in Figure 1 and is typical of taeniid parasites. *Taenia saginata*, the beef tapeworm, resides as an adult in the small intestine of man with the larval stage present in the muscles of cattle. It is a cosmopolitan parasite with a more widespread distribution than that exhibited by *T. solium*. Man becomes infected by the consumption of raw or undercooked beef containing the cysticerci. Cattle become parasitized by the ingestion of eggs present on pastures contaminated by human feces.

Epidemiology. Urquhart (1961) has suggested that dairy calves in East Africa may also become infected during the first few days of life by the native stockman when mucus is cleaned from the calves' mouths or when the fingers are used to induce the calf to drink from a pail. A small number of eggs underneath the fingernails would be capable of

producing the infections seen and it has been noted that approximately 30% of the dairy cattle in East Africa have cysticerci (Soulsby, 1965). Silverman and Griffiths (1955) have suggested that an indirect means of transmission may occur in Great Britain whereby sea birds pick up eggs as a result of feeding on raw sewage, with the subsequent passage of these eggs through the birds' digestive tracts. This provides for contamination of pastures where cattle are grazing. This mechanism would help explain infection of cattle in the absence of adult *T. saginata* in people on the farm or in the surrounding locality. Although *T. saginata* is present in most countries of the world, the incidence of both the larval stage and the adult are particularly high in Africa with East African states having the highest rates. Froyd (1960) has found 31.7% of the people in Kenya harboring the parasites. Urquhart (1961) has reviewed reports on infection levels in Africa and these figures reach levels of 75-86% of the cattle parasitized with cysticerci of *T. saginata* depending upon the locality.

Economics. Presence of cysticerci in cattle represents a serious economic loss not only in terms of outright condemnation of the carcass but also in a reduction of the value of the meat containing small numbers of the larvae. In the case of carcasses with low numbers of the parasites, the meat must be kept frozen for several weeks to kill the cysticerci and as a result a lower price is obtained at marketing.

Pathology and Control. Under normal conditions infection with larvae does not cause clinical disease in cattle. The adult worms bring about little pathology in the human host and can be successfully removed by various chemical agents. Prevention and control of the

Figure 1. Life cycle of *Taenia saginata*.

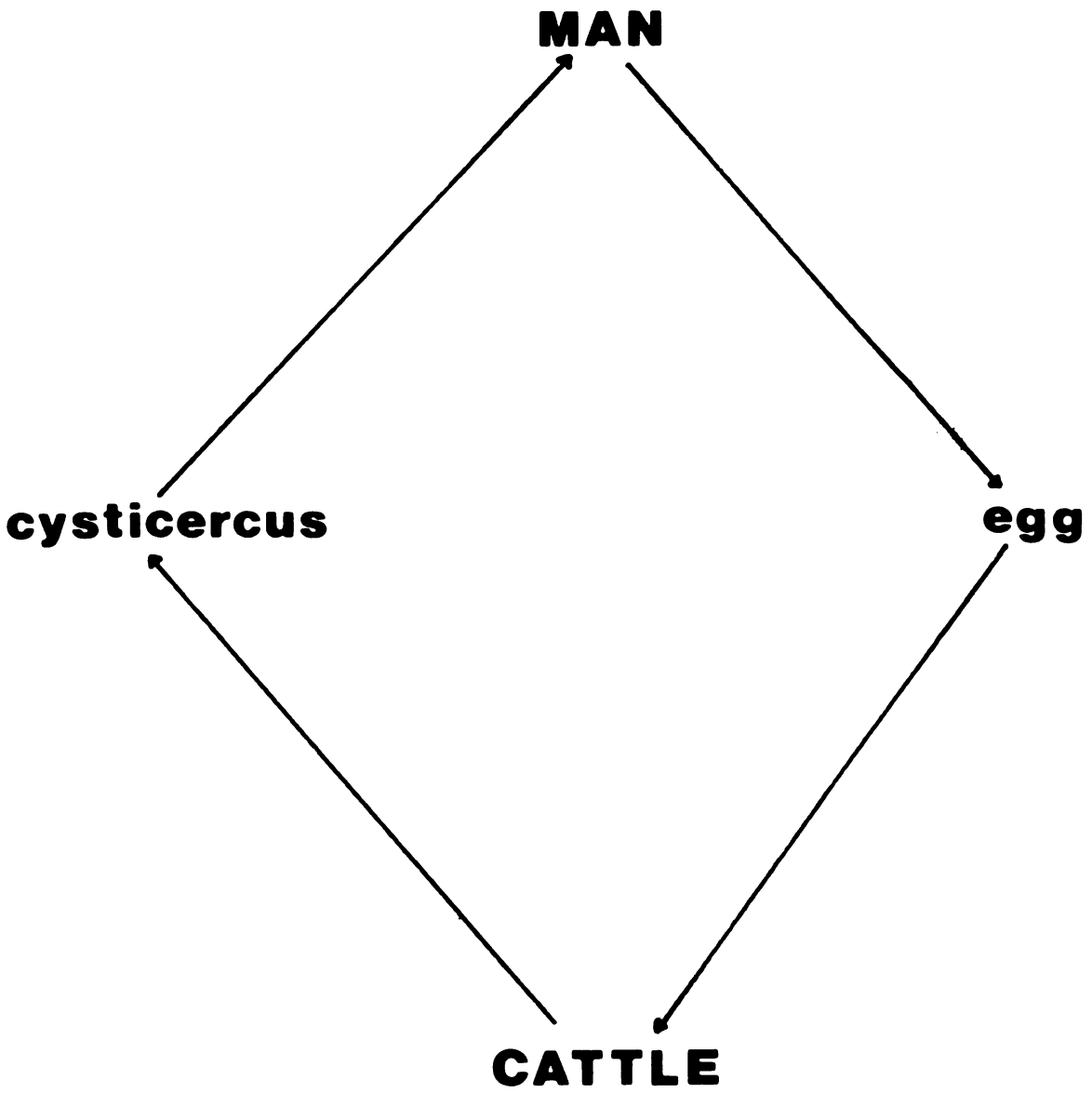

Figure 1

disease could be effected should generally accepted personal hygienic and community health standards be followed.

Taenia solium

Biology and Epidemiology. *Taenia solium*, the pork tapeworm, resides as an adult cestode in the small intestine of man with the pig serving as the usual intermediate host. Man becomes infected with the adult parasites by the consumption of raw or undercooked meat and may also become infected with the larval stage by the ingestion of infective eggs (Soulsby, 1965). The global intensity of this infection is not as high as that of *T. saginata*.

Pathology. If the larval stage is present in man, serious complications may be brought on as there is a marked tendency for the cysticerci to localize in the brain (Faust, Russell and Jung, 1970). In a study of cysticercosis in Mexico, 25 out of 100 cases diagnosed as human cerebral tumors were actually a result of infection with cysticerci of *T. solium* (Mazzoti, 1944). Also, epileptiform attacks are frequently seen in human beings after death and degeneration of the cysticerci (Soulsby, 1965). Clinically these larvae when found in the brain have been referred to as *Cysticercus racemosus* because of their tendency to expand within the cerebral cavities. The larval stage has also been found in the eye, musculature, heart, liver, lungs and abdominal cavity. The presence of the growing parasite provokes a local cellular reaction which includes infiltration with neutrophils, eosinophils, lymphocytes, plasma cells and sometimes giant cells. Fibrosis and necrosis follow this cellular picture with an eventual caseation and calcification of the cyst. These pathological findings are of tremendous importance should the larvae localize in the brain. Here the parasite may cause

little pathology while alive but upon death a great variety of neurological symptoms may develop, which can lead to a rapid demise of the patient. Such parasitized individuals may show varied clinical signs, including epilepsy, epileptiform seizures, disordered behavior, transient pareses, intermittent obstructive hydrocephalus, dysequilibrium, meningoencephalitis and failing vision (Faust, Russell and Jung, 1970).

Economics. In addition to the pathology of the disease in humans, this infection in the intermediate host, swine, constitutes both an important financial loss and also a loss in available protein. In a 3-year study of cysticercosis in slaughter houses of 6 Latin American countries, Garrick (1967) showed that 2.13% of all hogs slaughtered were infected with cysticerci of *T. solium*, with a resultant loss of over $500,000 in condemned carcasses.

Control and Treatment. The only known treatment for infection with the larval stage in man is surgical removal and, if the larvae have spread throughout the cerebral cavities, the prognosis is very grave. As with *T. saginata*, adult worms cause little pathology and can be removed by chemical means. Prevention of this disease revolves around the two basic concepts, personal hygiene and general sanitary measures (Faust, Russell and Jung, 1970). As long as people in endemic areas continue to eat raw or undercooked pork and do not dispose of human excreta according to accepted modern sanitary practices, the parasite and the disease which it causes will flourish.

Immunology of Experimental Infections. Immunologically-mediated host defense reactions have been shown to develop in domestic animals against cysticerci of taeniid parasites. Naturally acquired resistance

has been demonstrated in sheep infected with *T. hydatigena* (Gemmell, 1961, 1969a; Sweatman, Williams, Moriarty and Henshall, 1963), *T. ovis* (Gemmell, 1969a) and *Echinococcus granulosus* (Sweatman, 1957). Also cattle under field conditions are resistant to superinfection with *T. saginata* (Urquhart, 1961). Experimentally, sheep infected or artificially immunized with *T. hydatigena*, *T. ovis* or *E. granulosus* are resistant to homologous challenge infections (Gemmell, 1962, 1964a,b, 1965a, 1966; Rickard and Bell, 1971a). Soulsby (1963) showed that cattle infected with *T. saginata* were resistant to superinfection by the same parasite. Furthermore, resistance to challenge infections with *T. hydatigena* in sheep could be passively transferred to recipient animals by serum (Blundell, Gemmell and Macnamara, 1968) and also by colostrum (Gemmell, Blundell-Hassell and Macnamara, 1969).

Taenia hydatigena embryos treated by physical or chemical means were capable of eliciting a strong resistance in sheep to challenge infection (Gemmell, 1969b). These embryos did not develop into mature metacestodes and, since killed eggs or activated embryos did not induce immunity (Gemmell, 1964b, 1969b), it seems that elaborated antigens of the parasite are responsible for induction of acquired resistance. In this regard the experiments of Rickard and Bell (1971b) are important. Their studies indicated that antigens excreted or secreted by growing larvae of *T. ovis* contained in membrane diffusion chambers and implanted into peritoneal cavities of sheep were responsible for protection to challenge infections.

Taenia taeniaeformis

Biology. The laboratory animal model for cysticercosis of *T. taeniaeformis* in the rat has served in the study of the immunological

reactions to these tissue invading parasites. The life cycle of this tapeworm is shown in Figure 2. This particular parasite in the field is cycled through rodents (rats and mice) as intermediate hosts while the cat serves as the definitive host in a predator-prey relationship. The intermediate host becomes infected by the ingestion of eggs.

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 (Morseth, 1965, 1966). The embryophoric blocks are passively digested away as the egg is carried through the stomach and into the small intestine. At the latter site the intestinal enzymes set in motion, through an as yet undetermined means, the activation of the larvae. The embryo initiates intense movement and tears the oncospheral membrane, moving to the villi of the small intestine (Silverman and Maneely, 1955; Banerjee and Singh, 1969a,b; Heath, 1971). Penetration of the villus takes place within 15 minutes (Banerjee and Singh, 1969a) and occurs through lysis of host tissues, destruction of the same tissue by the hooks of the embryos or a combination of both methods (Silverman and Maneely, 1955; Banerjee and Singh, 1969a,b; Heath, 1971). The embryo migrates until it reaches a venule which carries the larvae passively to the site of predilection, a place that varies according to the species described (Heath, 1971).

In the case of *T. taeniaeformis* the final site is the liver, through which the embryo migrates until such time as the host encapsulates it with fibrous tissue (Singh and Rao, 1967; Smyth and Heath, 1970). The larvae continue development until the parasite reaches a fully infective metacestode stage by 60 days after infection (Hutchison, 1958). It is termed at this time a bladder worm and

Figure 2. Life cycle of *Taenia taeniaeformis*.

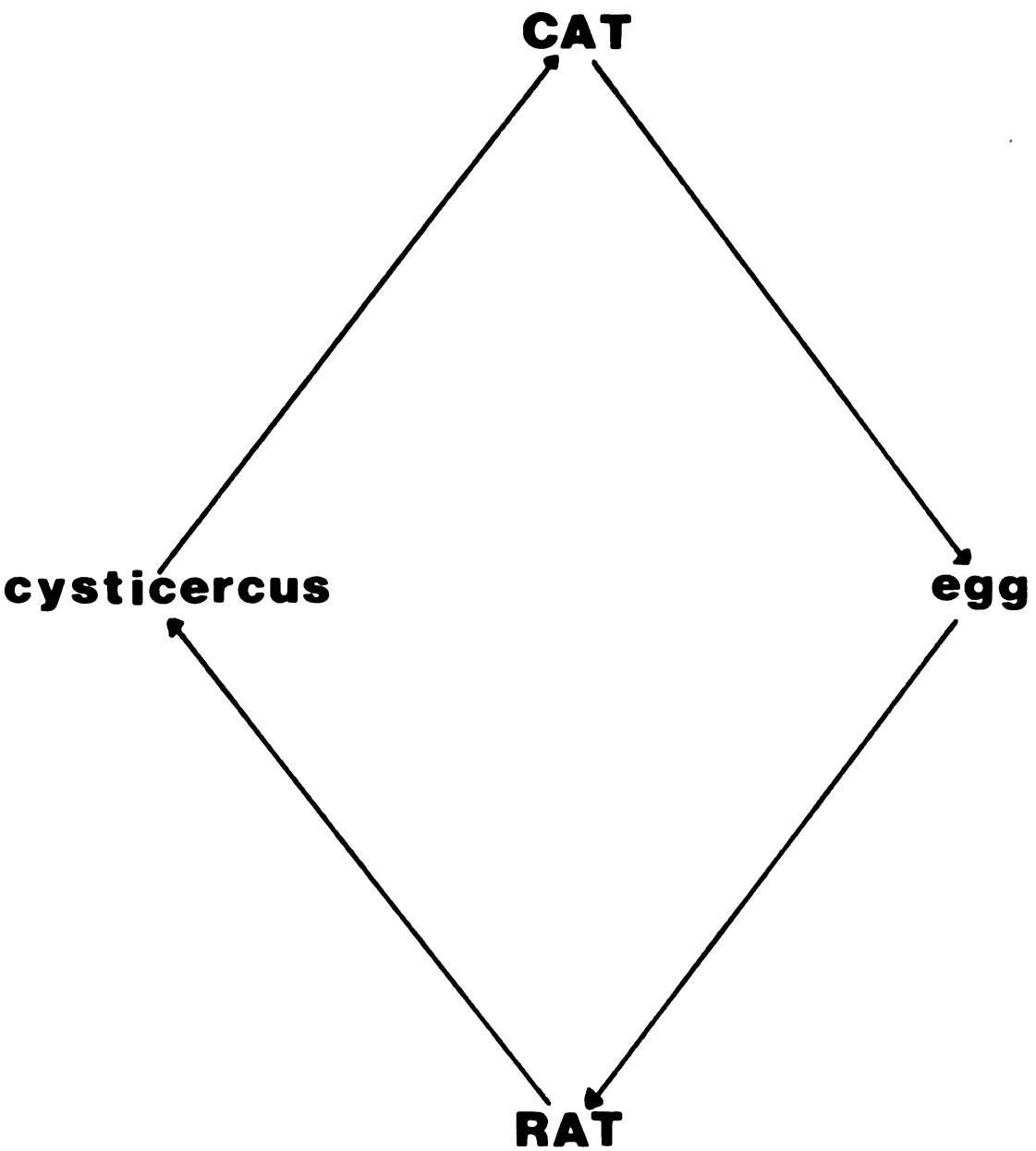

Figure 2

morphologically shows several analogies to the mammalian embryo (Smyth, 1969).

Upon oral consumption of this infective cysticercus by the definitive host the scolex evaginates from the bladder, attaches to the small intestine, usually in the crypts and initiates new growth, finally developing into an adult cestode. Infective eggs and gravid proglottids are passed in the cat feces 45 days later and thus the cycle is maintained. Similar cycles are perpetuated using other intermediate and definitive hosts for the remaining members of this family, *Taeniidae*.

Immunology. Miller (1931a) demonstrated conclusively that the rat becomes resistant to superinfection with *T. taeniaeformis*. Further experimental work by Miller and his colleagues showed that resistance could be artificially induced, passively transferred with serum from infected rats and maternally transferred to young rats from females with experimental infections (Miller and Gardiner, 1932, 1934; Miller, 1931b, 1932, 1935). A more detailed examination of this work was accomplished by Campbell (1936, 1938a,b,c). He confirmed Miller's early observations on the success of artificial immunization in the *T. taeniaeformis* system and extended those findings by testing a variety of antigenic and immunogenic preparations. He demonstrated that serum obtained at increasing times post infection was capable of conferring greater resistance to challenge in recipients than serum taken early in the infection.

His further studies on the passive transfer of resistance led him to postulate the possible existence of two distinct populations of antibodies capable of mediating resistance to challenge. He visualized two processes which he termed "early" and "late" immunity. The former

was characterized by its appearance early in the infection, ability to cause parasite death before liver establishment and susceptibility to removal by absorption with larval extracts. The "late" immunity involved serum antibodies that not only brought about destruction of larvae before establishment but also under appropriate conditions were able to result in death of parasites after their successful invasion of the liver. This late protective capacity was not for the most part reduced by any absorption procedures.

Rickard and Bell (1971) showed that implantation into recipient rats of growing larvae of *T. taeniaeformis*, contained within membrane diffusion chambers, was capable of inducing a significant protection to challenge. Reduction in parasite numbers was observed with 1 week implants and complete resistance was demonstrated at 3 weeks. This would indicate that the living parasite is important in the induction of a protective response by the host.

Murrell (1971) showed that larvae incubated in serum obtained 35 days after infection with *T. taeniaeformis* were unable to control efflux and influx of certain radiolabelled amino acids and sugars. Furthermore, this reaction was shown to be in part complement dependent.

The direct extrapolation from the rat *T. taeniaeformis* model to taeniid infections in domestic animals and man is not justified. Nonetheless, the knowledge gained from the rat model on the immunological events associated with *T. taeniaeformis* infections may serve to illuminate areas for fruitful exploration in the larger animals.

Rat Immunology

The physico-chemical and biological properties of immunoglobulins in general, and those of the rat in particular, have been studied

extensively over the past decade. In the rat at the present time, there are 6 classes of distinct immunoglobulins demonstrable: $7S\gamma_{2a}$, $7S\gamma_{2b}$, $7S\gamma_1$, γA, γE, and γM.

Arnason, de Vaux St-Cyr and Relyveld (1964) demonstrated that the rat had at least 3 immunoglobulin classes as revealed by immunoelectrophoresis: γG, γM and γA. No attempt was made at this time to isolate the γA protein. The γA molecules were so named because of their electrophoretic migration. Banovitz and Ishizaka (1967) were able to show antigen binding in 5 precipitin arcs using radioimmunoelectrophoresis.

The γG class has been separated electrophoretically into 2 components (Nussenzweig and Binaghi, 1965) designated by $7S\gamma_{2a}$ and $7S\gamma_{2b}$ (Binaghi and Sarandon de Merlo, 1966). These 2 classes were able to be separated chromatographically on DEAE-cellulose using a low ionic strength initial buffer (Stechschulte, Austen and Bloch, 1967; Bloch, Morse and Austen, 1968). Both of the $7S\gamma_2$ antibody classes have agglutinating and hemolytic properties (Morse, Bloch and Austen, 1968). The $7S\gamma_{2a}$ antibodies also have the capacity to fix for short periods of time in recipient rats for participation in passive cutaneous anaphylaxis (PCA) reactions. They also prepare rat peritoneal cells for the antigen induced release of slow reacting substance of anaphylaxis, SRS-A (Stechschulte *et al.*, 1967; Orange, Valentine and Austen, 1968; Morse *et al.*, 1968).

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 a $7S\gamma_1$ (Jones, 1969), although it is not the biological equivalent of $7S\gamma_1$ from mice and guinea pigs (Binaghi, 1971). This antibody class has been shown to have agglutinating activity. It also binds complement and is active in hemolytic assays (Jones, 1969; Morse

et al., 1968), although its lytic properties were not as pronounced as with the $7S\gamma_2$ antibodies.

There has now been shown to be an immunoglobulin class designated γA which predominates in secretions and is prominent at mucosal surfaces (Nash, Vaerman, Bazin and Heremans, 1969; Stechschulte and Austen, 1970; Bistany and Tomasi, 1970; Nash and Heremans, 1972). It is, however, present at very low levels in serum and all of its physicochemical characteristics have been shown to be similar to γA molecules in other species.

The γM immunoglobulin class has been studied extensively by Binaghi and his colleagues (Binaghi and Oriol, 1968; Oriol, Binaghi and Coltorti, 1971). They have defined an antibody class which has 30 times more agglutinating activity than the 7S antibodies and is approximately 300 times as effective in lysis of sensitized red cells. They have shown that the rat γM antibody molecule has a valence of 10, which is similar to that now demonstrated experimentally for γM antibodies of other species. Other workers (Van Breda Vriesman and Feldman, 1972) have shown that the half-life of electrophoretically slow γM is between 60-65 hours.

Reagin

Rat reaginic antibody, which appears in response to both immunization and infection (Binaghi and Benacerraf, 1964; Ogilvie, 1967) has now been defined by Austen and his co-workers (Stechschulte, Orange and Austen, 1970) to be the rat immunoglobulin γE. This antibody was similar to the immunoglobulin class designated γE in humans by Ishizaka and Ishizaka (1967). Orange, Stechschulte and Austen (1970) demonstrated that this γE antibody sensitized rat peritoneal cells for antigen

induced release of histamine and SRS-A. γE antibody was also shown to be responsible for long-term skin fixation (Stechschulte *et al.*, 1970). The optimal conditions for the *in vitro* release of histamine has been further studied by Bach, Bloch and Austen (1971a,b). A possible competition for receptor sites on the peritoneal cells between the γE and $7S\gamma_{2a}$ antibodies has been demonstrated both *in vitro* and *in vivo* (Bach *et al.*, 1971b; Ohman and Bloch, 1972).

Immediate hypersensitivity reactions have been shown to be mediated by skin sensitizing antibodies. In many animal species studied, there are two types of these skin fixing antibodies, one which fixes to target cells for short periods of time (γG) and another (γE) which sensitizes cells for an extended period (Binaghi, 1971; Bloch, 1968; Bloch and Ohman, 1971; Ishizaka, 1971). In humans, however, no short-term activity due to γG molecules has been conclusively demonstrated. Reagin or γE mediated hypersensitivity reactions appear to be one of the main causes of clinical allergic disease.

It has been known for over 50 years that this immediate type hypersensitivity reaction could be passively transferred with serum. This fact indicated that a serum antibody was responsible for the immediate reactions rather than a cellular involvement of the delayed type as seen with a tuberculin skin reaction. As the separation of plasma proteins became more refined and the immunoglobulins of man and other animal species were determined, more intensive investigations on the nature of the skin sensitizing antibody were initiated.

Early investigators (Ishizaka, Ishizaka and Hornbrook, 1963; Vaerman, Epstein, Fudenburg and Ishizaka, 1964) suggested that the immunoglobulin class γA might be the carrier of reaginic hypersensitivity. However, later it was shown through the use of an elaborate

physico-chemical separation procedure and a sensitive radioimmunoassay that reaginic activity was confined to a new immunoglobulin class, γE (Ishizaka, Ishizaka and Hornbrook, 1966a,b). Most of the previous work was confirmed when a myeloma protein was obtained which did not cross react with the known heavy chain specific antisera, but did show the presence of immunoglobulin light chains (Johansson, Bennich and Wide, 1968). The biological, physical and chemical properties of this immunoglobulin class were determined in a series of investigations by Johansson and his colleagues (Johansson and Bennich, 1967; Bennich, Ishizaka, Ishizaka, and Johansson, 1969; Johansson, Bennich and Berg, 1970; Bennich, 1971a,b). The Ishizakas and their colleagues (Ishizaka and Ishizaka, 1968a,b, 1969a,b; Ishizaka, Ishizaka and Lee, 1970a; Ishizaka, Tomioka and Ishizaka, 1970b; Ishizaka, Johansson and Bennich, 1969; Ishizaka and Ishizaka, 1971; and Ishizaka, 1971) also played a major role in investigations on γE.

These studies showed a molecule with an approximately 8S sedimentation coefficient, a molecular weight of 190,000-200,000, a fast gamma electrophoretic ability, a susceptibility to heat and reduction and alkylation. This antibody also failed to cross the placenta and had a half-life at passively sensitized sites of 8.5-14 days as opposed to 2-3 days in the serum. The levels in normal individuals were shown to be around 100-300 ng/ml. This antibody lacked any complement fixing activity even when aggregated (Ishizaka, Soto and Ishizaka, 1972).

Through inhibition studies, it has become evident that the γE antibody binds to target cells by the Fc portion of the immunoglobulin molecule (Stanworth, Humphrey, Bennich and Johansson, 1968; Ishizaka *et al.*, 1970a). This fixation by the Fc portion of the immunoglobulin would allow binding of specific antigen by the active sites located on

the Fab region. The result would be a process whereby release of vaso-active components from the target cells would occur by secretory means. This is in contrast to the cellular destructive pathways as would be the case should γG molecules be involved (Lichtenstein, 1972). The vasoactive mediators, primarily SRS-A and histamine, would be responsible for the clinical signs observed as these agents have profound effects on smooth muscles and vascular permeability (Lichtenstein, 1972).

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THE IMMUNOLOGICAL RESPONSE OF THE RAT TO INFECTION WITH *TAENIA TAENIAEFORMIS* I. IMMUNOGLOBULIN CLASSES INVOLVED IN PASSIVE TRANSFER OF RESISTANCE

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

I. IMMUNOGLOBULIN CLASSES INVOLVED IN PASSIVE TRANSFER OF RESISTANCE

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Summary. Passive transfer of immunity to *Taenia taeniaeformis* infections in the rat was achieved with serum taken 14, 21 and 28 days after infection, with maximal activity at 28 days. The protective capacity resided in the globulin fraction, which was further fractionated by gel filtration and anion exchange chromatography. The immunoglobulins present in each passively transferred fraction were detected with specific antisera to $7S\gamma_2$, $7S\gamma_1$, γM and γA. Protective activity was confined to those fractions containing 7S immunoglobulin. Fractions enriched for γM were unable to confer protection and it was possible to protect recipient rats against challenge with fractions devoid of γA and reaginic antibody activity. $7S\gamma_{2a}$ antibodies were able to confer passive protection when given alone, and probably contributed to the protective capacity of mixtures containing $7S\gamma_2$ and $7S\gamma_1$ immunoglobulins. A mechanism for specific acquired resistance to *T. taeniaeformis* is proposed based upon the recently established biological properties of $7S\gamma_{2a}$.

Absorption of protective activity from immune rat serum was unsuccessful using a variety of techniques, and an explanation is offered for this finding.

The results are discussed in relation to the current understanding of acquired resistance in cysticercosis and hydatid disease in domesticated food animals.

INTRODUCTION

Cysticercosis and hydatidosis are cyclozoonotic helminth infections with a widespread distribution and significance in both humans and domesticated food animals. Immunological reactions leading to the occurrence of specific acquired resistance in these diseases remain ill defined, but the laboratory animal model of *Taenia taeniaeformis* infection in the rat has been used by several investigators to approach the phenomenon experimentally. Miller and Gardiner (1932, 1934) and Campbell (1938a,b,c) showed that resistance to challenge infection could be transferred passively with serum of infected rats. Resistance in recipient animals was manifested by a highly significant reduction in the number of cysticerci successfully developing in the tissues. These workers followed contemporary procedures for establishing the role of serum antibody in immunity to infectious disease and their results were the first to demonstrate conclusively the importance of antibody in resistance to helminth infection.

Since that time successful passive transfer with serum from infected donors has been achieved in a variety of helminthiases, but only recently has it become possible to identify the immunoglobulins participating in these reactions on the basis of their biological and physico-chemical characteristics (Ogilvie, 1970; Jones, Edwards and Ogilvie, 1970; Wilson, 1966). In the present study we have confirmed the original observations of Miller and Gardiner (1932, 1934) and Campbell (1938a,b) and determined the contribution of identifiable serum immunoglobulins in the passive transfer of resistance to *T. taeniaeformis*.

MATERIAL AND METHODS

Maintenance of the parasite

T. taeniaeformis occurs naturally as a parasite in the intestine of the domestic cat and is maintained by the predator-prey relationship existing between cats and sylvatic rodents. Eggs released from the terminal segments of the worms appear in the faeces of the cat and are ingested by rats or mice. Embryos hatch from the ingested eggs and migrate to the liver where they establish as a metacestode or "cysticercus" stage. This larval form is infective for the cat.

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. Eggs were routinely teased from proglottids into saline containing 50 µg/ml of amphotericin; 2,500 µg/ml streptomycin; 333 U/ml of polymyxin B; and 1,000 U/ml of penicillin G and stored at 4 C. Egg doses were quantitated by a simple dilution method and administered by stomach tube to rats anaesthetized with ether. At least 6 weeks later cysticerci were removed from infected rat livers and 10-15 larvae placed in a gelatin capsule and given orally to each cat. Ten or more weeks thereafter gravid proglottids were obtained either directly from the faeces of the cat or from purged material after dosing with drocarbil (Nemural, Winthrop Labs, New York).

Experimental animals

Sprague Dawley rats 28-42 days old were purchased from Spartan Research Animal, Haslett, Michigan. Random source cats were vaccinated against feline enteritis and acclimatised to laboratory facilities and food for 2-3 weeks before experimental infection. Albino guinea pigs were obtained from the Michigan State Health Laboratories

(Lansing, Michigan). New Zealand white rabbits were purchased from local suppliers and the sheep were members of a flock maintained by one of the investigators (J.F.W.). All laboratory animals were given proprietary brand food and water *ad libitum*.

Passive transfer

Recipient animals received intraperitoneal (I.P.) inoculations of serum or immunoglobulin fractions using a tuberculin syringe at the time of oral challenge with 300-600 eggs of *T. taeniaeformis*. Samples of sera, globulin and chromatographic fractions were filtered through a 0.45 μ filter (Millipore, Bedford, Massachusetts) prior to inoculation. A period of 3 weeks was allowed for migration and establishment of the metacestodes on the surface of the liver. The animals were then sacrificed using carbon dioxide vapor and the total number of cysticerci in each liver determined. The results were statistically analysed using a computerized programme for the student's "t" test on a Monroe 1766 statistical calculator.

Preparation of immune sera

Rats were infected *per os* with 300 eggs of *T. taeniaeformis* and sacrificed 28 days later using carbon dioxide vapor. Blood was collected from the thoracic cavity after severing the vessels anterior to the heart, allowed to clot for 2-3 hours at 22-23 C and left overnight at 4 C. The serum was decanted, centrifuged and stored at -20 C without preservatives until used.

Immunoelectrophoresis (I.E.P.) and
double immunodiffusion (D.I.D.)

Immunoelectrophoresis was performed following a slight modification of the method of Scheidigger (1955) in a Gelman apparatus (Gelman Instrument Co., 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 merthiolate.

Double immunodiffusion was performed according to a micromethod modified from that described by Williams and Chase (1971). Two percent Noble agar was prepared in a 0.1M Tris-HCl buffer, pH 8.1, with a final concentration of merthiolate of 1 in 10,000.

Measurement of protein concentration

Protein concentrations were generally determined by the method of Lowry, Rosebrough, Farr and Randall (1951). In the case of immunoglobulin solutions, concentrations were calculated from the optical density at 280 nm, multiplied by a factor derived from the extinction coefficient (Oriol, Binaghi and Boussac-Aron, 1968; Binaghi and Oriol, 1968).

Rat $7S\gamma_2$ immunoglobulin levels in passively transferred fractions were quantitated using the radial immunodiffusion technique described by Mancini, Carbonara and Heremans (1965).

Chromatography

Descending flow gel filtration chromatography was performed on a siliconised 2.5 X 100 cm column of Sephadex G-200 (Pharmacia, Uppsala), equilibrated with 0.1M Tris-HCl, pH 8.0. A modification of the method of Sachs and Painter (1972) was introduced in order to maintain satisfactory flow rates (25-30 ml/hour) through repeated use of the column. Six millimeter glass beads were siliconised and filled the bottom 2 cm of the column and swollen Sephadex G-200 was poured over the bead layer. Samples were dialyzed against the equilibrating buffer before application and eluted fractions 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 Instr. Co., Fullerton, California).

The procedure for ion-exchange chromatography of rat immunoglobulins was a modification of that described by Stechschulte, Austen and Block (1967). DEAE Cellulose (Cellex D., BioRad, Richmond, California) was prepared according to the directions of the manufacturer and poured in 1.5 X 30 cm siliconised glass columns. The cellulose was equilibrated against either 0.01M phosphate buffer, pH 7.75, or 0.005M phosphate buffer also at pH 7.75. The 0.005M buffer was used initially where separation of the $7S\gamma_{2a}$ and $7S\gamma_{2b}$ immunoglobulins was required. Proteins were eluted in a stepwise manner using 0.01M phosphate buffer followed by a 0.05M pH 5.8, 0.1M pH 5.8 and finally 2M NaCl. All phosphate buffers were made 0.015M in NaCl and the samples were dialyzed extensively against the starting buffers 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, Wisconsin). Protein peaks eluted with each buffer were pooled and concentrated back to the original serum volume.

Preparation of antisera

Anti-whole rat serum (aWRS) was prepared in rabbits. Whole normal rat serum was diluted 1:5 with phosphate buffered saline (PBS) and emulsified with an equal volume of Complete Freund's Adjuvant (C.F.A., Difco, Detroit). Rabbits received 0.5 ml by I.M. inoculation in each hind leg and 0.1-0.2 ml portions were injected subcutaneously (S.Q.) at several sites along the back. Booster inoculations of a similar preparation were given 34 and 71 days later and the rabbits were bled out ten days after the last injection.

Anti-rat γM (aγM) was prepared in both rabbits and sheep. Normal rat serum immunoglobulins were precipitated 3 times with 50% saturated ammonium sulfate and passed through a Sephadex G-200 column. The ascending portion of the first peak was allowed to react in I.E.P. with aWRS and the precipitin arcs corresponding to IgM were excised from 18 slides. These agar slices were homogenised in a tissue grinder with a minimal volume of PBS and emulsified with an equal volume of C.F.A. Rabbits were inoculated I.M. with 0.5 ml of the emulsion and 0.2 ml portions injected at several sites over the back. Twenty days later the animals were boosted with a similar preparation and bled out ten days afterward.

Sheep anti-rat γM was prepared according to an extensive modification of the procedure described by Van Breda Vriesman and Feldman (1972). Sheep red cells (2×10^9) were inoculated intravenously (I.V.) or I.P. into rats and the animals bled out 10 days later. The resultant serum was subjected to Sephadex G-200 gel filtration and the ascending side of the first peak heated at 56 C for 1 hour. This protein solution was mixed with 1 ml of packed sheep red cells in the presence of 10 mg/ml of EDTA. The red cells were washed 3 times and taken up in 1 ml of PBS. One half of a milliliter of the suspension was mixed with an equal volume of C.F.A. and injected I.M. in the neck. The other 0.5 ml was diluted to a 10 ml volume with PBS and given I.V. to the same sheep. The sheep was bled 20 days later. Both rabbit and sheep anti-γM, prepared as described, reacted in I.E.P. with γM and several other serum components. After absorption with foetal rat serum polymerized according to the method of Avrameas and Terynck (1969) each antiserum recognized only one arc in I.E.P. with normal rat serum, corresponding to γM (FIG. 1).

FIG. 1. Immunoelectrophoretic analysis of normal rat serum (NRS) versus Fc specific guinea pig anti-7Sγ_2 (1), guinea pig anti-7Sγ_1 (2), sheep anti-γM (3) and sheep anti-γA (4).

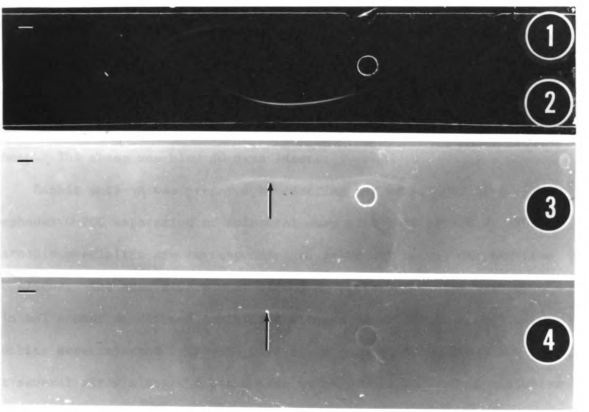

Figure 1

Anti-rat secretory γA (aS γA) was also prepared in both rabbits and sheep in a manner similar to that described by Stechschulte and Austen (1970). Stomach contents from freshly suckled 1- to 3-day-old rats were homogenised in a tissue grinder, stirred overnight at 4 C and centrifuged at 20,000 X g for 30 minutes. The supernatant was passed firstly through glass wool and then through a 0.45 μ filter. The whey was dialysed extensively against 0.1M Tris-HCl and applied to a Sephadex G-200 gel filtration column. The protein in the first peak was concentrated to the original volume and emulsified with an equal volume of C.F.A. and 1 ml injected I.M. into each hind leg of a sheep. The sheep was bled 30 days later.

Rabbit anti-γA was prepared by reacting the first peak of a Sephadex G-200 separation of colostral whey with aWRS in I.E.P. The cathodic precipitin are corresponding to secretory γA was excised from 18 slides. The agar slices were ground in a tissue grinder with a minimal amount of PBS and emulsified with an equal volume of C.F.A. Rabbits were injected I.M. with 0.75 ml in each hind leg and 0.1 ml at several sites along the back. The animals were boosted in a similar manner 20-30 days later and the sera collected 10 days afterward. Both rabbit and sheep anti-secretory γA reacted to I.E.P. against normal rat serum to produce an arc recognizable as γA and several other minor arcs. After absorption with the foetal serum immunoadsorbent these anti-sera recognized only γA in normal rat serum (FIG. 1).

Anti-γ_2 was prepared in guinea pigs rather than rabbits or sheep since it has been shown that rats and guinea pigs recognize only the Fc portion of the immunoglobulin molecule (Oriol *et al.*, 1968). The 7S immunoglobulin peak from a G-200 Sephadex gel filtration of normal rat globulin was dialysed against a 0.01M phosphate buffer made

0.015M in NaCl and applied to a DEAE cellulose column equilibrated against the same buffer. The initial peak, containing only $7S\gamma_{2a+b}$, was emulsified with an equal volume of C.F.A. Each guinea pig received 30 µg of protein in 1 ml with 0.5 ml I.M. in each hind leg and the animals were bled out 20-25 days later. The antisera recognised only $7S\gamma_2$ in I.E.P. against normal rat sera (FIG. 1).

Anti-$7S\gamma_1$ was also prepared in guinea pigs by taking advantage of the method of Henney and Ishizaka (1968) to render the animals tolerant to $7S\gamma_2$ immunoglobulins. Guinea pigs were each inoculated with 15 µg of total protein eluted from the DEAE cellulose column with the 0.1M phosphate buffer and emulsified with an equal volume of C.F.A. This preparation contained both $7S\gamma_2$ and $7S\gamma_1$ immunoglobulins as revealed by I.E.P. against aWRS. At the same time the guinea pigs were given 5 mg of rat $7S\gamma_2$ I.V. to suppress antibody formation to this immunoglobulin class. This antiserum produced only one arc in I.E.P. when tested against normal rat serum (FIG. 1).

Anti-rat Fab was prepared in sheep according to a method based upon that described by Oriol *et al.* (1968). The $7S\gamma_2$ immunoglobulin was purified by DEAE chromatography and 10 mg dialysed overnight against a 0.1M phsophate buffer made 0.003M in EDTA and 0.01M in cysteine. Insoluble papain (20 mg) was added and digestion allowed to proceed for 3 hours while stirring at 37 C. The reaction was stopped by centrifugation and the supernatant dialysed overnight against a 0.005M phosphate buffer pH 6.8. The supernatant was applied to DEAE cellulose equilibrated against the same buffer and the Fab fraction obtained in the first eluted peak while the Fc portion was retained on the column. One milliliter of the eluate containing 0.8 mg Fab was emulsified with an equal volume of C.F.A. and injected

I.M. into the hind limbs of a sheep. Thirty days later the sheep was bled. This antiserum reacted with γA, γM, γ_{2a+b} and γ1 in I.E.P. with fractions containing these proteins and it therefore served to identify the arcs corresponding to each immunoglobulin class.

Metacestode saline extracts

Larvae of *T. taeniaeformis* from 2- to 9-month-old infections were washed in distilled water several times and homogenised in a glass tissue grinder with a minimal volume of PBS. The suspension was stirred overnight at 4 C, centrifuged at 17,000 X g and stored at -20 C. In some instances the undissolved residue was taken up a second time in PBS and the above procedure repeated. In preparation for absorption experiments the larvae were homogenised without PBS and the thick suspension added directly to the immunoglobulin solutions.

Absorption of immune globulins

Absorption of the protective capacity of passively transferred globulin fractions of immune serum was attempted using a variety of techniques. Globulins were reacted in one instance with an immuno-adsorbent prepared by crosslinking the larval saline extract with glutaraldehyde (Avrameas and Ternynck, 1969). The mixture was stirred at 22-23 C for 24 hours followed by centrifugation and filtration throughout a 0.45 μ filter before passive transfer. In another experiment larval extract was coupled to polyacrylamide beads (P-300, -400 mesh, BioRad) using the methods outlined by Ternynck and Avrameas (1972). Sensitised beads were allowed to react with immune globulins for 24 hours at 22-23 C and removed by centrifugation before passive transfer of the filtered supernatant. In the third case the thick larval suspension was added to a solution of immune globulins such that the final concentration was 10% (v/v) and this was stirred for

24 hours at 22-23 C. After incubation overnight at 4 C, the suspension was centrifuged and the supernatant filtered.

RESULTS

Immune serum was obtained 7, 14, 21 and 28 days after infection with 300 eggs of *T. taeniaeformis*. Precipitating antibody activity was detected by day 21 in D.I.D. tests against concentrated larval extract. One milliliter of each sample was used in passive transfer experiments in order to determine at which time the serum contained the greatest protective capacity. Rats receiving 7 day serum harbored a mean of 48.8±15.79 (SD) larvae while those animals given 14 day serum had 12.75±11.53. A mean of 4.60±7.02 cysticerci was present in rats which received 21 day serum and no metacestodes were observed in any of the livers of rats receiving 28 day serum. Therefore serum obtained 28 days after infection with *T. taeniaeformis* eggs was used in all further experiments. These observations on the increased protective capacity of serum taken during the development of the parasite in the liver of infected rats confirm the observations of Miller and Gardiner (1934) and Campbell (1938a).

In the following experiment 0.2, 0.4, and 1 ml quantities of immune serum were passively transferred to recipient rats in order to determine the quantity of serum required for significant protection. The mean number of cysticerci developing in control animals was 8.67±4.08 (SD) but both the 0.4 and 1 ml quantities of immune serum completely prevented the establishment of larvae. As little as 0.2 ml of immune serum resulted in a highly significant reduction in the number of larvae establishing in the recipients (0.60±1.34). In this same experiment the globulin fraction was removed from immune serum by 50% $(NH_4)_2SO_4$ precipitation (3X) and passively transferred after

dialysis against PBS. The mean number of cysticerci in these rats receiving the globulin fraction was 1.86±1.95 ($P<0.01$). The globulin fraction of immune serum was therefore used for all subsequent chromatographic separations. Since the infection levels in this experiment were relatively low, higher levels of challenge were used thereafter as a routine.

Globulins were precipitated with 50% SAS 3 times and subjected to gel filtration on Sephadex G-200 to separate 19S from 7S immunoglobulins (FIG. 2). Both peaks were concentrated back to the original serum volume. The first peak contained γM detectable by both sheep and rabbit antisera specific for Fc determinants of this immunoglobulin plus a β globulin, detected by aWRS. The second peak from the G-200 column, containing 7S immunoglobulin, was dialysed against a 0.01M phosphate buffer made 0.015M in NaCl and applied to DEAE cellulose equilibrated against the same buffer. Sequential step-wise elution was followed and the results are shown in FIG. 3. The presence of $7S\gamma_2$ globulins was monitored in each fraction using guinea pig antiserum specific for the Fc portion of each class. Both $7S\gamma_{2a}$ and $7S\gamma_{2b}$ were detected in the 0.01, 0.05 and 0.10M phosphate buffer eluates. Rat γA was detected with Fc specific antiserum produced in sheep and rabbits and was present only in the 2M NaCl eluate. Reaginic activity was limited to the 0.05M phosphate buffer eluate and its presence was detected by homologous passive cutaneous anaphylaxis (Leid and Williams, 1973). No monomeric γM was detected in any of the DEAE cellulose fractions which were passively transferred. An aliquot of the original globulin solution was absorbed with an immunoadsorbent prepared from a saline extract polymerised with glutaraldehyde.

FIG. 2. Elution profile at 280 nm of the globulin fraction (50% $(NH_4)_2SO_4$) of immune rat serum after gel filtration on Sephadex G-200. Fraction 1 was concentrated and tested for protective activity. Fraction 2 was further fractionated on DEAE-cellulose.

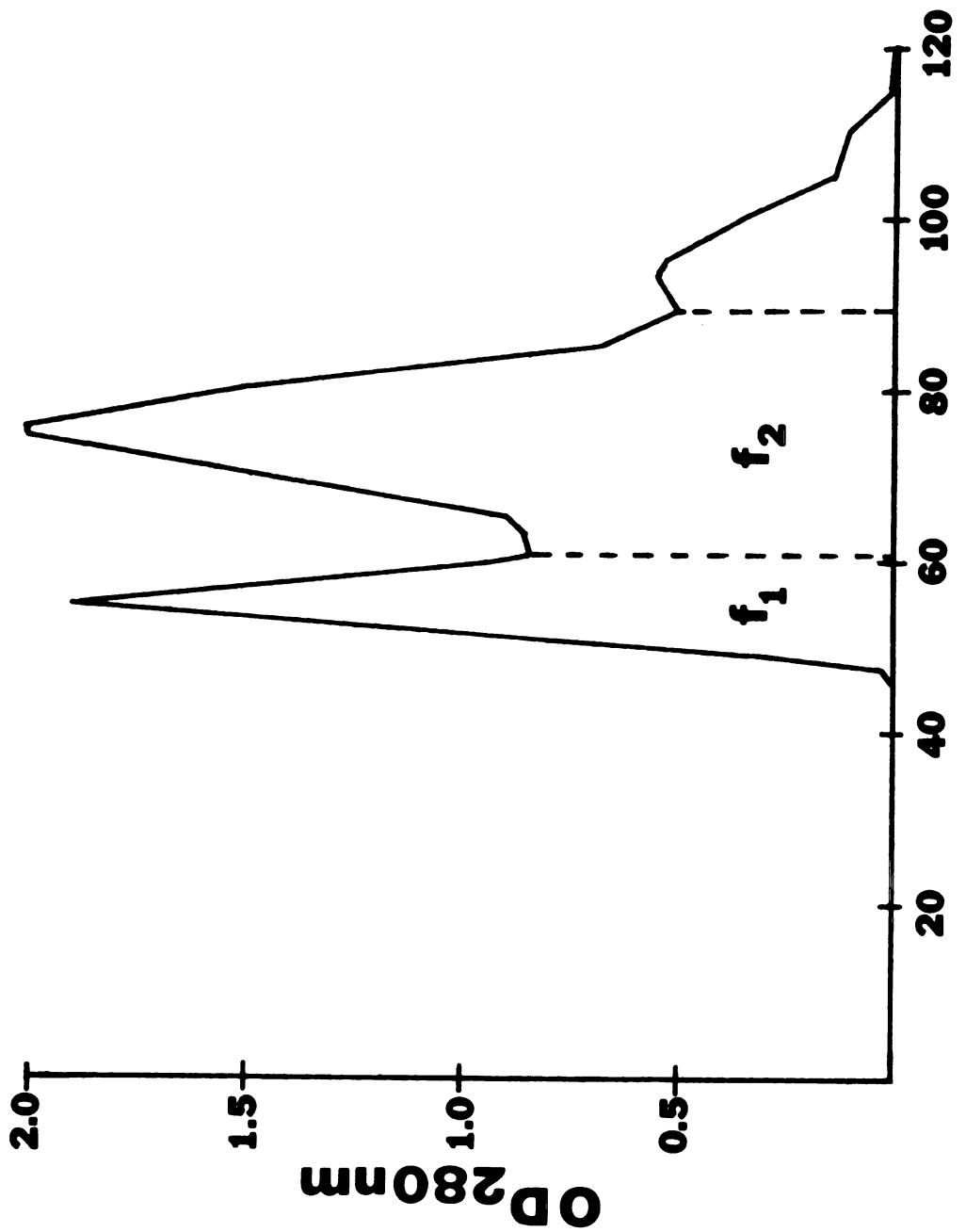

Figure 2

FIG. 3. Elution profile at 280 nm of the 7Sγ globulin fraction (50% $(NH_4)_2SO_4$) of immune rat serum fractionated on DEAE cellulose with phosphate buffers and 2M NaCl. All phosphate buffers were made 0.015M in NaCl. Fractions 1, 2 and 3 were tested for activity in passive transfer experiments.

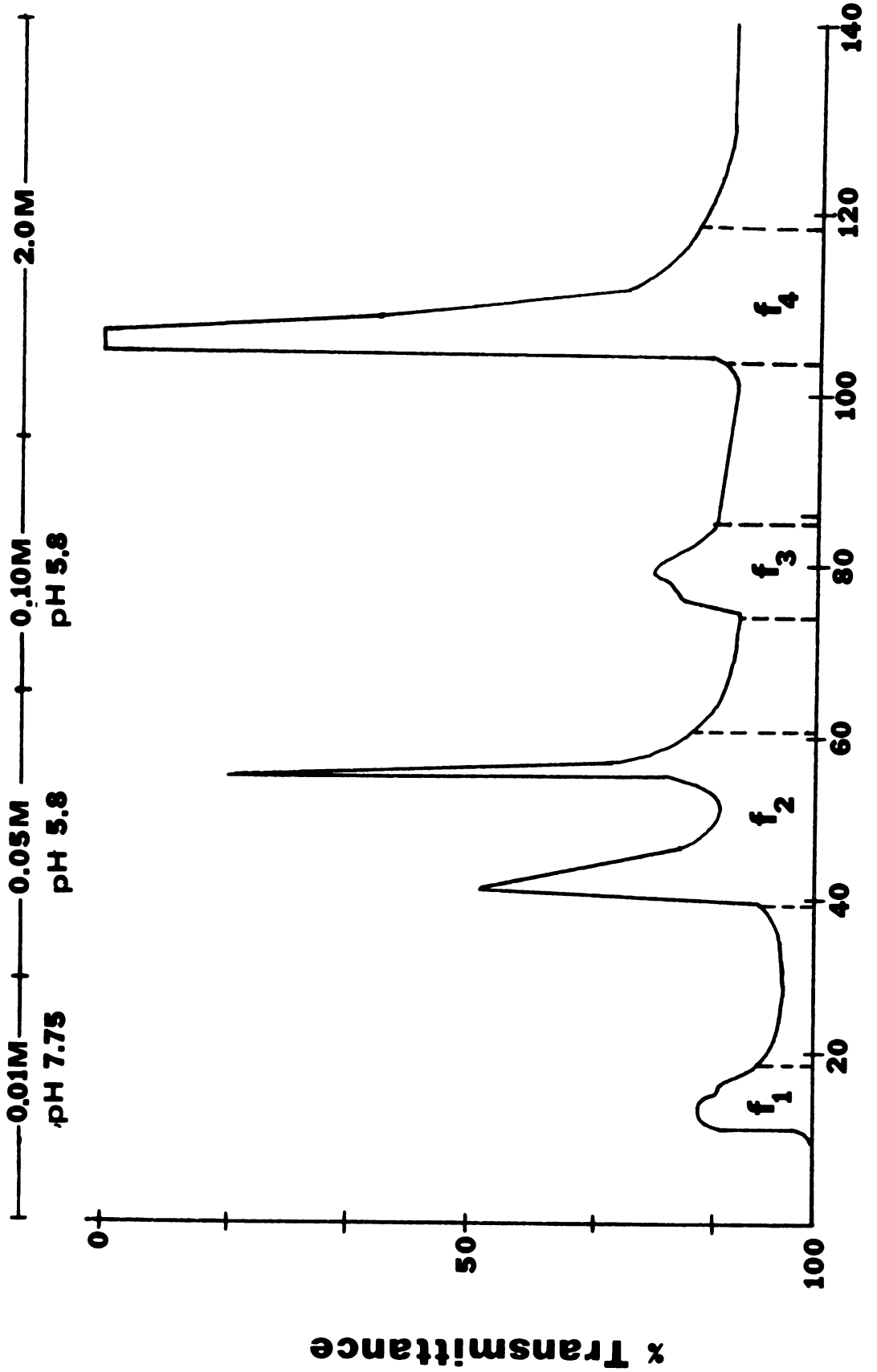
0.01M
pH 7.75
0.05M
pH 5.8
0.10M
pH 5.8
2.0M
x Transmittance
0
50
100
Tube Number
20
40
60
80
100
120
140
f_1
f_2
f_3
f_4

Figure 3

Groups of 28-day-old female rats were given 300 eggs *per os* followed by an I.P. inoculation of 0.8 ml quantities of each of the chromatographic fractions, PBS or normal 28 day serum. One milliliter portions of the absorbed solution and 0.4 ml of unfractionated immune serum and globulin were given. All groups were sacrificed 21 days later and the numbers of cysticerci developing in each group compared to that of the controls. The results of the passive transfer of fractions enriched for the various rat immunoglobulin classes and the effects of absorption on protective capacity of globulin solutions are shown in Table 1. The 19S or γM fraction did not confer protection and the absorption with the polymerised larval extract did not reduce the protective quality of the globulin solutions. All three phosphate buffer eluates (0.01M, 0.05M and 0.10M) produced a highly significant reduction in parasite burdens ($P<0.001$, 0.001 and 0.01, respectively) when compared to control animals. The levels of $7S\gamma_2$ were determined in each of these phosphate buffer eluates and were as follows: 0.4 mg/ml for 0.01M, 6.6 mg/ml for 0.05M and <0.4 mg/ml for the 0.10M.

In view of the fact that $7S\gamma_{2a}$ appeared in all three phosphate buffer eluates a further experiment was performed taking advantage of the technique devised by Stechschulte *et al.* (1967) for separation of $7S\gamma_{2a}$ from the $7S\gamma_{2b}$ by lowering the molarity of the initial buffer in DEAE chromatography. In this case a 0.005M phosphate buffer made 0.015M in NaCl was used as the starting buffer. Both the 19S and 7S peaks were obtained from Sephadex G-200 gel filtration of an immune globulin solution and the 19S peak was concentrated and dialysed against PBS extensively before passive transfer. The 7S peak was concentrated, dialysed against the 0.005M phosphate buffer and applied to a DEAE cellulose column. Again step-wise elution was followed

TABLE 1

PASSIVE PROTECTIVE CAPACITY OF IMMUNE SERUM AND IMMUNOGLOBULIN FRACTIONS IN RECIPIENT RATS CHALLENGED WITH 300 EGGS OF *TAENIA TAENIAEFORMIS*

Protein fraction passively transferred	Mean number of larvae ± S.D.	Number of rats per group	P value
Phosphate buffered saline	48.89 ± 17.95	9	ns
Normal rat serum	66.83 ± 26.35	6	ns
19S fraction of immune globulin	48.66 ± 24.59	9	ns
0.01M DEAE eluate of immune globulin	9.89 ± 6.45	9	<0.001
0.05M DEAE eluate of immune globulin	12.33 ± 13.65	9	<0.001
0.10M DEAE eluate of immune globulin	26.22 ± 3.56	9	<0.01
Immune rat serum	15.71 ± 14.00	7	<0.01
Immune rat globulin	4.86 ± 9.35	7	<0.001
Immune rat globulin absorbed with polymerised antigens of cysticerci	3.71 ± 8.98	7	<0.001

(FIG. 4), and the 0.005M and 0.01M eluates were concentrated to the original serum volume and passed through a 0.45 μ filter before inoculation. In this passive transfer experiment further adsorption procedures were attempted. Preliminary studies using immunoelectrophoresis and radioimmunoelectrophoresis (unpublished observations) had indicated antigen binding activity in the γ_2 immunoglobulins, and absorption procedures were therefore monitored by D.I.D. tests for removal of precipitins. The globulin fraction was first absorbed with larval extract coupled to polyacrylamide beads. A total of 1.6 mg of protein was bound to each ml of packed beads. This amount was approximately equivalent to the maximum achieved for the series of protein antigens studied by Ternynck and Avrameas (1972). The absorption was carried out at 22-23 C for 24 hours after which the globulin solution was tested in D.I.D. against concentrated larval extract. All the precipitating antibody activity was not removed by this process and the solution was then absorbed with a freshly homogenised thick larval suspension as described above. This treatment removed all precipitating antibody activity. A second volume of globulin solution was treated only with the thick larval suspension following the procedures outlined previously.

Groups of 28-day-old female rats were given 600 eggs of *T. taeniaeformis* orally followed by I.P. injections of the absorbed or unabsorbed immunoglobulin preparations. The animals were sacrificed 21 days later and the results are shown in Table 2 and FIG. 5. Again the IgM fraction conferred no passive protection while absorbed immunoglobulin preparations remained effective in passive transfer. The fraction eluted with the 0.005M phosphate buffer resulted in highly significant protection in recipients ($P<0.01$) while the 0.01M

FIG. 4. Elution profile at 280 nm of the 7Sγ globulin fraction (50% $(NH_4)_2SO_4$) of immune rat serum fractionated on DEAE cellulose with phosphate buffers and 2M NaCl. All phosphate buffers were made 0.015M in NaCl. Fractions 1 and 2 were tested for activity in passive transfer experiments.

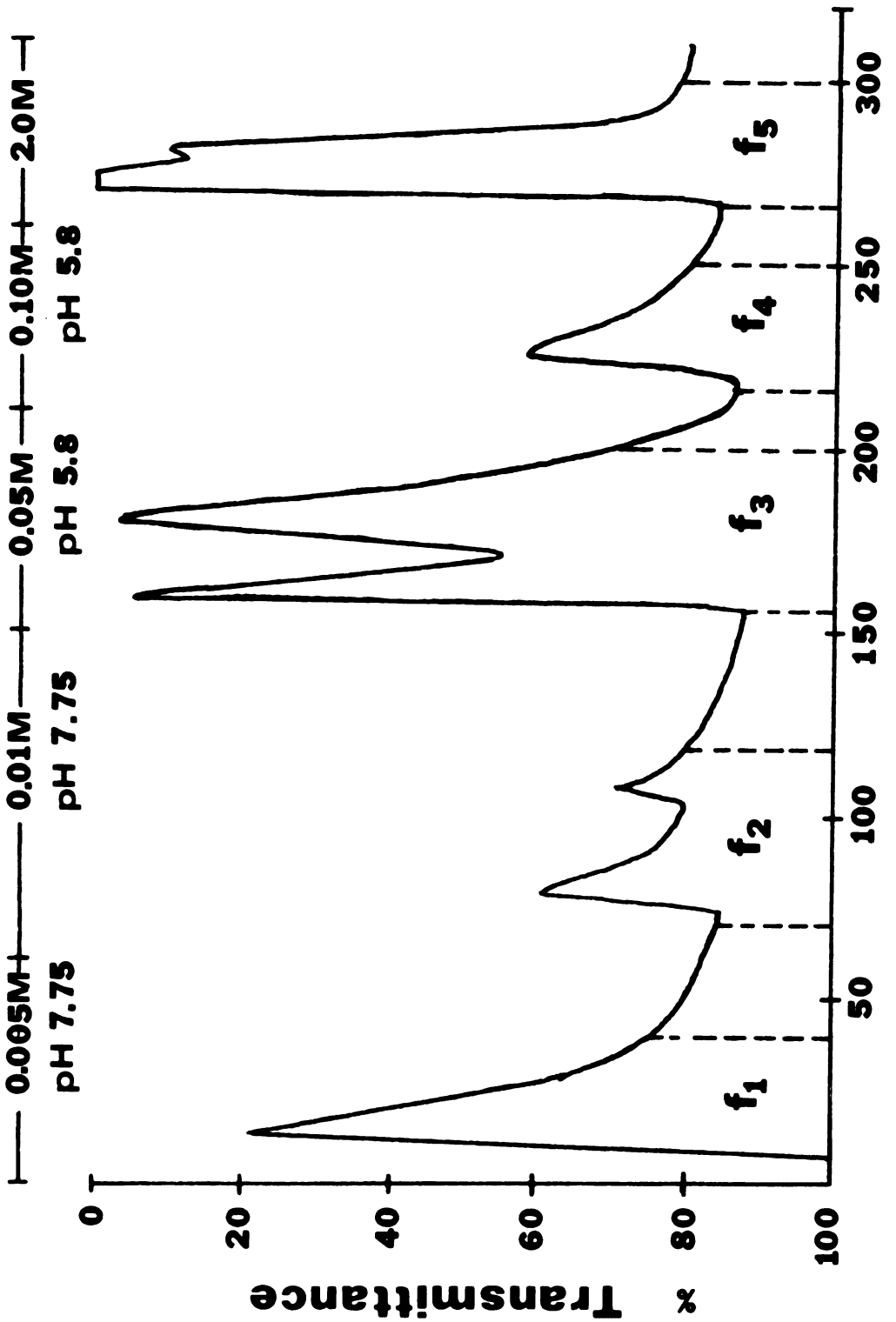
0.005M
pH 7.75
0.01M
pH 7.75
0.05M
pH 5.8
0.10M
pH 5.8
2.0M
% Transmittance
0
20
40
60
80
100
50
100
150
200
250
300
Tube Number
f1
f2
f3
f4
f5

Figure 4

TABLE 2

PASSIVE PROTECTIVE CAPACITY OF IMMUNE SERUM AND IMMUNOGLOBULIN FRACTIONS IN RECIPIENT RATS CHALLENGED WITH 600 EGGS OF *T. TAENIAEFORMIS*

Protein fractions passively transferred	Mean number of larvae ± S.D.	Number of rats per group	P value
Normal rat serum	111.16 ± 37.85	6	ns
Immune rat globulin	32.11 ± 36.24	9	<0.01
19S fraction of immune globulin	85.50 ± 21.41	8	ns
0.005M DEAE fraction of immune globulin	44.33 ± 35.32	9	<0.01
0.01M DEAE fraction of immune globulin	93.89 ± 13.00	9	ns
Immune rat globulin absorbed (1)	52.67 ± 77.97	9	<0.2
Immune rat globulin absorbed (2)	19.86 ± 12.24	7	<0.001

(1) Absorbed with antigen coated polyacrylamide beads, followed by larval suspension (10% v/v).

(2) Absorbed with larval suspension (10% v/v).

FIG. 5. Representative livers from groups of rats passively immunised with the DEAE fractions indicated. The animals were challenged *per os* with 600 eggs of *T. taeniaeformis* and sacrificed 21 days later.

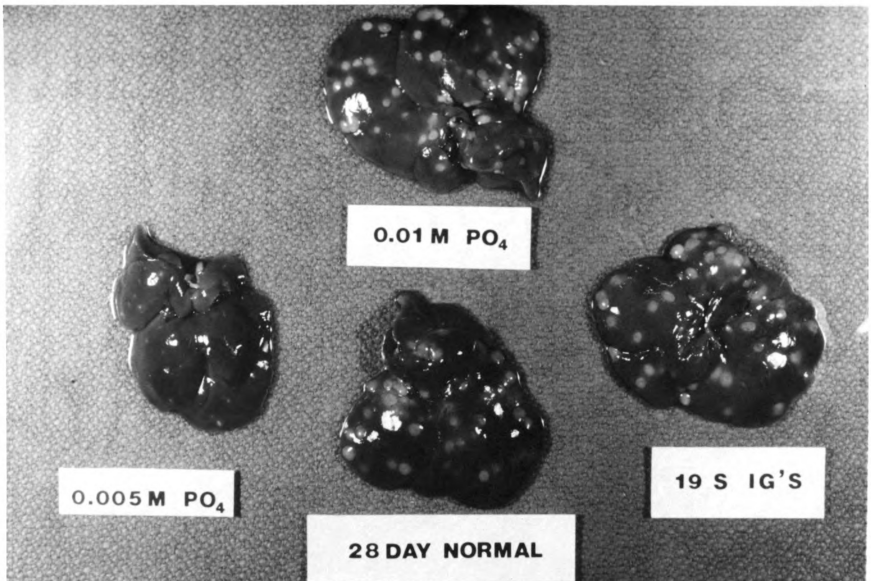

Figure 5

did not. The 0.005M eluate was tested in I.E.P. with anti-whole rat serum and a single arc appeared, corresponding to $7S\gamma_{2a}$ (FIG. 6). It was also tested in D.I.D. against guinea pig anti-γ_2 and again only a single band formed.

DISCUSSION

The results of our experiments confirm the role of serum antibody in passively transferred resistance to *T. taeniaeformis* as established by Miller and Gardiner (1932, 1934) and Campbell (1938a,b,c). Protective activity was confined to fractions of immune serum containing 7S immunoglobulins and fractions enriched for 19S antibodies were ineffective. Protection was successfully conferred with fractions devoid of γA and reaginic antibody activity. The results obtained with further fractionation of the 7S component of 28 day immune serum suggest that $7S\gamma_{2a}$ immunoglobulins contain the majority of protective antibodies to infection with *T. taeniaeformis*. We do not exclude the possible participation of $7S\gamma_{2b}$, $7S\gamma_1$ or γE in the successful passive transfer of resistance observed with fractions containing these immunoglobulin classes. However, these DEAE eluates were contaminated with $7S\gamma_{2a}$ which was able to confer protection when given alone. Furthermore, the eluate obtained with 0.1M phosphate buffer contained less $7S\gamma_{2a}$ antibodies than the 0.01M and 0.05M fractions and conferred a lesser degree of protection. Enrichment with $7S\gamma_1$ type antibodies was therefore not associated with enhanced protective capacity. A more conclusive demonstration of the quantitative contribution of antibodies of each immunoglobulin class might be achieved by the use of immune serum selectively depleted by absorption with antisera specific for $7S\gamma_{2a}$, $7S\gamma_{2b}$, $7S\gamma_1$, γA, γM and possibly γE. This approach has recently been applied successfully by Saif, Bohl and Gupta (1972) in their

FIG. 6. Immunoelectrophoretic analysis of normal rat serum (bottom well) and fraction 1, 0.005M eluate (upper well) from DEAE cellulose fractionation (FIG. 4) versus rabbit anti-whole rat serum (aWRS).

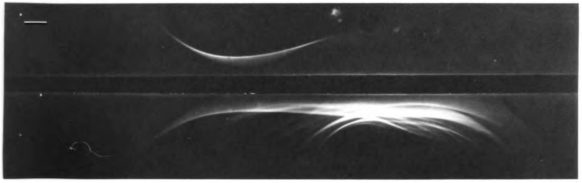

Figure 6

studies on the immunoglobulin classes containing neutralizing antibody *in vitro* to transmissible gastroenteritis virus in the pig. We are presently pursuing a similar objective in passive transfer experiments with the *T. taeniaeformis* system.

Nevertheless, our present findings on the involvement of $7S\gamma_2$ antibodies, especially $7S\gamma_{2a}$, in resistance to infection are of importance both in the context of the biological properties of rat immunoglobulins and their association with protective responses, and also in terms of the relevance of the rat-*T. taeniaeformis* model to immunity to cysticercosis in general.

$7S\gamma_2$ antibodies in the rat appear in response to a variety of artificial immunisation procedures (Block, Morse and Austen, 1968; Jones, 1969) and were shown by Jones (1969) to be responsible for short term homologous skin sensitisation. Morse, Block and Austen (1968) demonstrated that $7S\gamma_{2a}$ antibodies were capable of preparing rat tissue for antigen induced release of SRS-A, and $7S\gamma_{2a}$ antibodies inhibited histamine release from mast cells mediated by reaginic antibodies in rats infected with *Nippostrongylus brasiliensis* (Bach, Block and Austen, 1971). $7S\gamma_2$ antibodies were capable of passively transferring resistance to infection with *N. brasiliensis* but only after the donor rats had received multiple infections with this parasite (Jones, Edwards and Ogilvie, 1970). Passive protection was conferred predominantly by fractions enriched for $7S\gamma_1$ immunoglobulins if serum was obtained from rats after primary infections. Preparations of $7S\gamma_2$ which were active in passive transfer did not provoke 5 hour PCA reactions and the authors state that they had never observed anaphylactic antibodies of the $7S\gamma_{2a}$ type in rats with *N. brasiliensis*, although they did not separate this class chromatographically (Jones

et al., 1970). We have been unable to demonstrate short-term skin sensitisation at 2, 4 and 5 hours with $7S\gamma_{2a}$ antibodies against *T. taeniaeformis* (Leid and Williams, 1973), but this was readily achieved following artificial immunisation (unpublished observations). Possibly those $7S\gamma_{2a}$ antibodies which appear in response to helminth infections represent a biologically distinct population within this immunoglobulin class which is not capable of mediating PCA reactions. Alternatively the antigens required for provocation of this short term sensitisation may have been present in challenge solutions at levels insufficient to elicit the PCA reaction.

In the latter case a mechanism may be postulated for specific acquired resistance to *T. taeniaeformis* which implicates γ_{2a} antibodies in the release of vasoactive amines, perhaps at the level of the intestinal mucosa. The destruction of parasites before their establishment in the liver, designated "early immunity" by Campbell (1936), has been suggested to occur within the intestinal mucosa (Leonard and Leonard, 1941). Antigen production by the parasite embryo at or within the intestinal surface might trigger the release of cell bound SRS-A resulting in changes of vascular permeability which permit the increased accumulation of antibody and cells at the site. Complement fixing antibodies are known to occur in the sera of rats infected with *T. taeniaeformis* (Campbell, 1938b; Murrell, 1971) and a complement dependent attack on the embryo could be responsible for immobilisation or destruction of the parasite. γ_{2a} antibodies have also been shown to fix complement (Morse *et al.*, 1968) and chemotactic attraction of either specific or non-specific cellular components might also be a factor.

The role which secretory γA might play in immunity at the intestinal level is not known, although there is no direct evidence of its involvement in resistance to cysticercosis. However γA secreting cells are very prominent in the lamina propria of the small intestine of the rat (Nash, Vaerman, Bazin and Heremans, 1969) and it seems likely that secretory γA antibodies may contribute to specific acquired resistance in this infection. Indirect evidence in favor of this possibility derives from the observations of colostral transfer of protection in rats infected with *T. taeniaeformis* (Miller, 1935) and sheep with *T. hydatigena* (Gemmell, Blundell-Hasell and Macnamara, 1969).

We were unable to absorb the protective capacity of immune serum using a variety of procedures and this finding is in accord with the results reported by both Miller and Gardiner (1932) and Campbell (1938b). Again the concentration of certain critical antigens in the extracts or preparations used for absorption may have been insufficient to effect complete removal of the protective antibodies. The experiments of Rickard and Bell (1971) have some bearing on this suggestion. In their studies the degree of resistance to challenge infection with *T. taeniaeformis* produced by implanted membrane diffusion chambers containing growing larvae was dependent upon the duration of implantation. This might indicate a requirement for the elaboration and release of antigens over an extended period and therefore the concentration of such antigens in the developing larvae may not be high at any one time. *In vitro* maintenance of the cysticerci may offer a means to secure enriched preparations of these important antigens.

Little is known of the specific antibodies or antigens involved in immunological events in naturally occurring cysticercosis and

hydatidosis in domesticated animals. However, certain features of the biology of these infections support the belief that an acquired resistance analogous to that seen in our experimental model, and possibly mediated by comparable mechanisms, is manifested under field conditions. Resistance to superinfection has been observed with *T. saginata* in cattle (Urquhart, 1961) and with *T. hydatigena* and *T. ovis* in sheep (Gemmell, 1969). Experimentally Sweatman (1957) and Sweatman, Williams, Moriarty and Henshall (1963) have demonstrated acquired resistance in sheep to *T. hydatigena* and *E. granulosus*, respectively, and Soulsby (1963) was unable to superinfect calves exposed to *T. saginata* shortly after birth. Furthermore, resistance to challenge infection with eggs of *T. hydatigena* in sheep has been shown to be due in part to serum antibodies (Blundell, Gemell and Macnamara, 1968).

While direct extrapolation of results obtained with the rat-*T. taeniaeformis* model is not justified, the characterisation of immunological phenomena in this readily manipulated laboratory animal infection may serve to delineate areas for experimental exploration in the more costly domestic animal systems. In this regard we feel that the demonstrated association of protective resistance with an immunoglobulin of rather well defined biological reactivity may be considered an important advance.

ACKNOWLEDGEMENTS

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THE IMMUNOLOGICAL RESPONSE OF THE RAT TO INFECTION WITH *TAENIA TAENIAEFORMIS* II. CHARACTERISATION OF REAGINIC ANTIBODY AND AN ALLERGEN ASSOCIATED WITH THE LARVAL STAGE

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II. CHARACTERISATION OF REAGINIC ANTIBODY AND AN ALLERGEN ASSOCIATED WITH THE LARVAL STAGE

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Summary. Skin sensitising antibody or reagin was detected in rats 19 days after infection with *Taenia taeniaeformis* eggs. Peak titres were reached on day 32 and thereafter declined. A second dose of eggs was capable of increasing the levels of circulating reagin even though it was highly unlikely that the embryos had survived more than a transient period of time in the intestinal mucosa. The physico-chemical and biological characteristics of this reagin are consistent with those of the rat immunoglobulin designated γE. In no instance was it possible to demonstrate short-term skin sensitisation by the rat $7S\gamma_{2a}$ immunoglobulin class even though previous work had shown that antibodies of this type are produced during infection. All short-term reactivity at 2-6 hours appeared to be a result of reaginic antibody fixation. Reagins were not observed to cross the placenta or be transferred by the colostrum from highly immune females to their offspring. The role of reagin in passive and specific acquired resistance to *T. taeniaeformis* is discussed and the mechanisms whereby it might be contributing to immunological events in this infection are outlined.

An allergen was isolated from cysticerci of *T. taeniaeformis* which was capable of provoking passive cutaneous anaphylaxis (PCA) reactions

in sensitised rats in approximately 5 µgm quantities. A single band was obtained in polyacrylamide gel electrophoresis which stained for protein and carbohydrate but not for lipid. Chromatographic and electrophoretic studies indicated that the allergen was very negatively charged at slightly alkaline pH. Activity was completely removed from larval extracts by absorption with a monospecific antiserum prepared against the allergen. The possibility of more than 1 allergen being associated with cysticerci and adult worms of *T. taeniaeformis* is discussed.

Cross reactions with other taeniid parasitic extracts were observed but no positive PCA reactions were elicited with extracts prepared from two dissimilar helminths, *Schistosoma mansoni* and *Fasciola hepatica* although these parasites are known to cause cross reactions in field tests. The potential value of the purification procedure is pointed out in the context of the continuing effort to improve the specificity of clinical diagnostic tests based on intradermal reactions.

INTRODUCTION

Immediate hypersensitivity reactions are consistently associated with helminthic infections (Sadun, 1972; Ogilvie, 1970) and constitute a particularly prominent and important aspect of the immunological response to cysticercosis in man and domestic animals. Allergic clinical signs occur at the time of tissue invasion by *Taenia solium* in man (Dixon and Hargreaves, 1944) and the pathogenesis of the disease in chronic infections often involves the sudden onset of intense inflammatory reactions around degenerating cysticerci (Faust, Russell and Jung, 1970). Humans and pigs infected with *T. solium* and cattle parasitised with *T. saginata* develop immediate reactions at the site of intradermal inoculations of crude extracts of these parasites. Attempts to

capitalise upon this hypersensitivity in clinical or antemortem diagnosis have met with very limited success (Froyd, 1963; Machnicka-Roguska and Zweirz, 1970) and the necessity for specific allergen purification has recently been stressed (Euzeby, 1966; Arundel, 1972).

In spite of the practical significance of this phenomenon in taeniid infections, there is almost no information on experimental analysis of the reaction in terms of characterisation of the antibodies and antigens involved. Our investigations on the rat-*T. taeniaeformis* model afforded us an opportunity to establish some of the parameters of immediate hypersensitivity in experimental cysticercosis and its contribution to protective resistance. Observations on the pattern of appearance of reactivity, the physico-chemical and biological features of reaginic antibodies and the partial purification of one of the allergens implicated are presented in this report.

MATERIALS AND METHODS

Maintenance of the parasite

T. taeniaeformis was maintained in rats and cats as described previously (Leid and Williams, 1973).

Experimental animals

Sprague Dawley rats were obtained from Spartan Research Animal (Haslett, Michigan). Adult female rats were used as recipients for passive cutaneous anaphylaxis (PCA) tests. New Zealand white rabbits for antiserum production were purchased from local suppliers and the sheep were members of a flock maintained by one of the investigators (J.F.W.). All laboratory animals were given proprietary brand food and water *ad libitum*.

Reaginic serum

Whole blood was obtained from the thoracic cavity by severance of the blood vessels anterior to the heart 28-35 days after infection with approximately 300 eggs of *T. taeniaeformis*. The blood was allowed to clot at 22-23 C for 2-3 hours and remained overnight at 4 C before the serum was decanted, centrifuged and stored at -20 C.

Immunoelectrophoresis (I.E.P.) and double immunodiffusion (D.I.D.)

These procedures were performed as described previously (Leid and Williams, 1973).

Preparative electrophoresis

Pevikon C-870 (Mercer, New York) block electrophoresis was carried out using a slight modification of the method described by Osterland (1968). Sodium barbital-HCl buffer, $\mu = 0.1$, pH 8.4, was diluted 1:2 and used for the inner electrode vessels and a phosphate buffer $\mu = 0.2$, pH 7.5 in the outer buffer chambers (Williams and Chase, 1968). The dialysed sample was applied to a slit 8 cm from the cathodic end of the gel. Several drops of a 1% solution of bromophenol blue were added 1-2 cm from the anodic side of the slit to serve as an indicator of the progress of migration. A plexiglass container (32 X 18 cm) was cooled by circulating tap water at approximately 4 C underneath the pevikon layer and electrophoresis performed for 48 hours at 13.75 volts/cm. On completion of the separation, 1 cm segments of the block were removed and the proteins eluted by displacement filtration in phosphate buffered saline (PBS). Each fraction was concentrated to the original sample volume and the protein concentrations determined. Fractions were dialysed against PBS and stored at -20 C.

Measurement of protein concentrations

The concentrations of immunoglobulin and antigen solutions were measured as described in a previous paper (Leid and Williams, 1973).

Chromatography

Gel filtration and ion exchange chromatography of serum samples and parasite extracts were performed as described by Leid and Williams (1973). Anion exchange fractionation of the parasite extract was carried out on DEAE cellulose (Cellex D, BioRad). Step-wise elution was achieved with buffers of increasing molarity and decreasing pH, in the following sequence: 0.01M phosphate buffer, pH 7.9; 0.05M phosphate buffer, pH 5.8; 0.10M phosphate buffer, pH 5.8; and finally 2M NaCl. All phosphate buffers were made 0.015M in NaCl and the elution patterns were monitored by ultraviolet scanning at 280 nm. Fractions were collected in 2.8 ml volumes and the peaks eluted with each buffer were pooled and concentrated to the original sample volume. These pools were dialysed against PBS overnight at 4 C and tested for their ability to provoke PCA reactions in sensitised rats.

Cation exchange fractionation of a saline extract of *T. taeniaeformis* larvae was accomplished using CM-Sephadex A-25 (Pharmacia). The gel was prepared according to directions of the manufacturer, packed in a siliconised 1.5 X 30 cm glass column and equilibrated with 0.0175M phosphate buffer, pH 6.2. Samples were extensively dialysed against this same buffer before application. Step-wise elution was carried out using phosphate buffers of increasing molarity and increasing pH in the following sequence: 0.0175M phosphate buffer, pH 6.2; .03M phosphate buffer, pH 6.6; 0.05M phosphate buffer, pH 6.85; and 0.10M phosphate buffer, pH 7.77. Elution profiles were monitored and the fractions treated as described above.

Homologous passive cutaneous anaphylaxis (PCA)

The procedure for homologous PCA tests was a slight modification of that described by Ogilvie (1967). Rats were shaved and 0.1 ml quantities of serum or chromatographic fractions were injected intradermally (I.D.) over the back. Twenty-four to 72 hours later rats were challenged intravenously (I.V.) with 0.5 ml of the parasite antigen to be tested together with 0.5 ml of a 1% solution of brilliant blue R (BioRad). Reactions were read 15-60 minutes after challenge and graded on a scale from 0 to +++. Positive reactions varied from small areas of intense blueing several millimeters in diameter up to circular zones 15 mm or greater in diameter, which were classified as +++. Positive and negative control sera were included in each recipient and at least 3 rats were used for each sample tested. Whenever doubtful responses had occurred the skin was reflected and viewed from the underside. In preliminary experiments brilliant blue R was found to be superior to Evans blue since it was rapidly cleared and background blueing was eliminated. Positive reactions were extremely well delineated by this means.

Active cutaneous anaphylaxis (ACA) was used to detect allergenic activity in fractions of parasite extracts. Rats infected for at least 2 months were inoculated I.D. with 0.1 ml of the antigen and I.V. with 0.5 ml of a 1% solution of brilliant blue R dye 30 minutes later. Reactions were read as described for PCA. In some instances Prausnitz-Küstner (PK) tests were used to monitor the presence of allergens in parasite extracts. The skin site was first sensitised with 0.1 ml of reaginic serum and then challenged 24 hours later with 0.1 ml of the antigen I.D. followed by 0.5 ml of the brilliant blue R dye I.V. 30-60 minutes afterward. Reactions were read as for PCA.

Polyacrylamide gel electrophoresis (PAGE)

Polyacrylamide gel electrophoresis was performed following the methods of Clarke (1964) for 5% gels and Weber and Osborn (1969) for 10% gels. Separated protein bands were stained with a 1% solution of aniline blue black. Lipid and glycoprotein components of the bands in the gel were detected using the methods of Turner and MacGregor (1969).

Parasite extracts

Larval extracts of *T. taeniaeformis* were prepared as described by Leid and Williams (1973). Adults of *T. taeniaeformis* were obtained by purging infected cats. These worms were washed extensively in tap water followed by PBS. The washed parasites were homogenised with PBS in a glass tissue grinder immersed in crushed ice. The solution was stirred overnight at 4 C and centrifuged at 17,000 X g. The clarified supernatant was removed and frozen in aliquots at -20 C. Extraction of the insoluble residue was generally repeated to increase the yield of antigen.

Antigenic extracts of heterologous parasites were similarly prepared from adults of *T. pisiformis*, cysticerci of *T. crassiceps* and protoscolices of *Echinococcus multilocularis*. Parasite cyst fluid was obtained from cysticerci of *T. hydatigena*. These parasites were either maintained in our laboratory according to established procedures or were acquired from natural infections after treatment or at autopsy. In addition extracts were prepared from adults of *Schistosoma mansoni* and *Fasciola hepatica*, kindly supplied by Drs. S. W. Berry and Terrence J. Hayes, respectively.

In vitro maintenance of *T. taeniaeformis*

Cysticerci were teased from livers of rats infected for 5-6 months and washed in distilled water (3X), sterile distilled water (3X) and finally sterile PBS (4X). Thirty to forty larvae were placed in 200 ml capped culture vials containing Hanks BME (Grand Island Biological Co., Grand Island, New York) with 1,000 U/ml of penicillin, 1,000 µg/ml of streptomycin and 500 U/ml of polymyxin B and incubated at 37 C. The levels of antibiotics were reduced by half at the end of the first week. The pH of the medium was maintained at neutrality by the daily addition of sterile sodium bicarbonate solution. The medium was replaced at weekly intervals under these circumstances and larvae remained active and grossly normal in appearance for periods up to 6 weeks. The collected culture medium samples were pooled and concentrated 50 fold with Carbowax (Fisher Scientific Co., Pittsburgh, Penn.) before overnight dialysis against PBS (1:10) at 4 C and subsequent lyophilisation. Lyophilised preparations were reconstituted with distilled water before use.

Preparation of antisera

Anti-*T. taeniaeformis* larval extract (aTLE) was prepared in rabbits. The extract of cysticerci was emulsified with an equal volume of complete Freund's adjuvant (CFA, Difco, Detroit). Each rabbit received intramuscularly (I.M.) and subcutaneously (S.Q.) a total of approximately 2.0 mg protein. Booster inoculations of a similar preparation were administered I.M. 17 and 55 days later. The rabbits were bled 11 days after the final injection and the antisera collected and stored at -20 C.

Antisera to adult *T. taeniaeformis* extract (aTAE) and to products excreted or secreted by the cysticerci (anti-*in vitro* concentrate,

aIVC) was prepared in rabbits in a manner similar to the aTLE.

Antiserum directed against an allergen prepared by preparative block electrophoresis of *T. taeniaeformis* cysticerci was prepared in a sheep. One hundred micrograms of the allergen contained in 5 ml of PBS was emulsified with an equal volume of CFA and given by I.M. inoculation. The animal was bled 15 days later and this antiserum recognised only one protein when tested in I.E.P. against *T. taeniaeformis* larval extract.

RESULTS

The reaginic antibody response during infection with *T. taeniaeformis* was initially detected by testing in PCA pooled serum samples taken on days 7, 14, 21, 28 and 35 post infection. Slight positive reactions at 72 hours post sensitisation were observed at 21 days with strongly positive reactions evident by day 28 and 35.

A more detailed account of the pattern of reagin production following oral doses of eggs of *T. taeniaeformis* was obtained from PCA tests on serial bleedings taken from the retro-orbital plexus. Rats were bled at frequent intervals particularly during the time period at which reagin was first expected to appear and following the second booster dose of eggs. At least 5 animals were bled for each sample tested and the results are shown in FIG. 1. Reagin first appeared on day 19 post infection, reached a peak titre of 1:8 on day 32 and then declined with activity persisting only with undiluted serum through day 81. A dose of 1,000 eggs was given at this time and within 9 days the titre had reached a peak equivalent to that shown earlier. These high levels were maintained with some fluctuation until a second peak of 1:32 was reached on day 117 post infection. Thereafter a gradual decline occurred.

FIG. 1. An analysis of the pattern of appearance of skin sensitising antibody (or reagin) to infection with *Taenia taeniaeformis* in the rat. Serial bleedings were obtained at the times indicated and PCA tests done with the sample. A booster dose of 1,000 eggs was given 81 days after primary infection (arrowed).

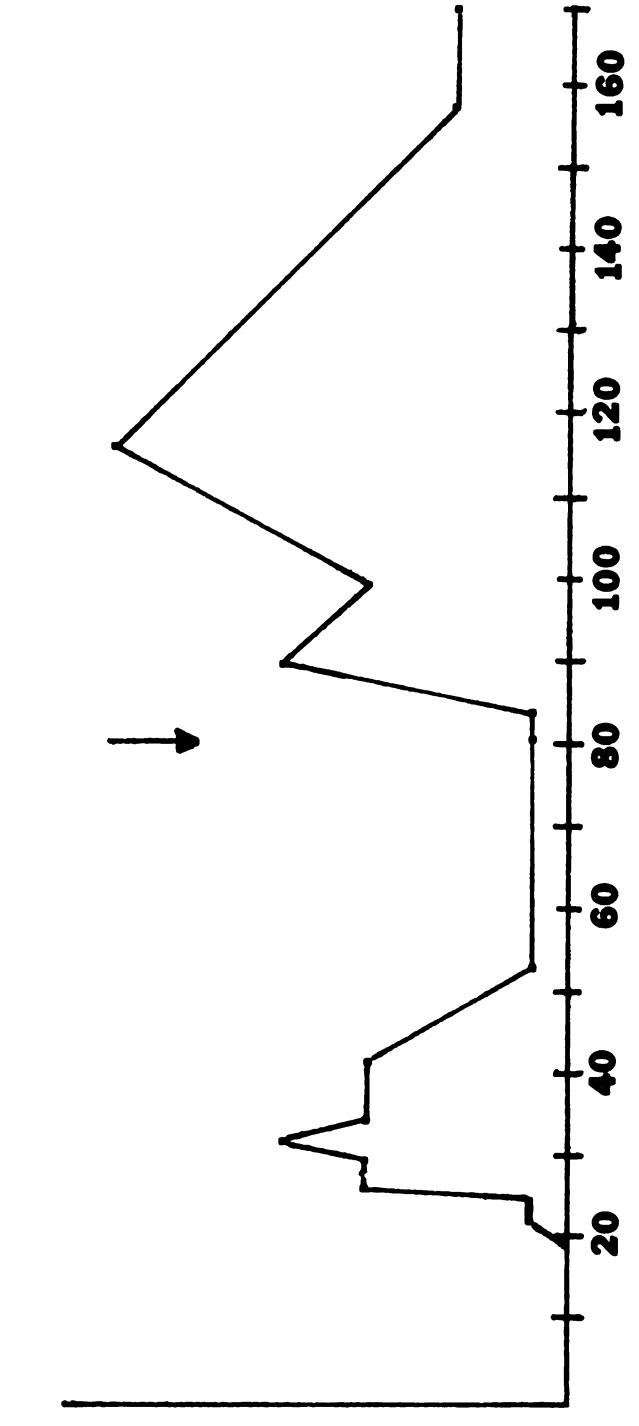
PCA TITRE
1:32
1:16
1:8
1:4
1:2
NEAT
20
40
60
80
100
120
140
160
DAYS POST INFECTION

Figure 1

The time period for optimal sensitisation of the skin of recipients was determined by challenging rats at 4, 24, 48, 72 hours and 9 days after intradermal inoculation of serial two-fold dilutions of 35 day serum. The results are shown in Table 1. Reactivity was observed by 4 hours with maximal levels at 24-72 hours and strong positive activity was also evident at 9 days post sensitisation. Subsequent PCA tests were performed with latent periods of 24-72 hours.

Heat susceptibility of reaginic antibody was established by titration of reactivity after 1-2 hours incubation of 35 day serum at 56 C. The results are presented in Table 2. Reaginic activity was abolished after only 1 hour at 56 C.

Serum obtained 35 days after infection with *T. taeniaeformis* was dialysed against 0.15M mercaptoethanol at 22-23 C for 2 hours followed by dialysis against either 0.02M iodoacetamide or PBS at 4 C overnight. Reactivity of the treated samples was determined by titration and the results are also shown in Table 2. Reduction with 2-mercaptoethanol alone reduced the titre from 1:8 to 1:2 while reduction followed by alkylation completely abolished PCA activity.

After ammonium sulfate fractionation of 35 day serum, reaginic reactivity was absent from the globulin fraction precipitated at 33% saturation but was precipitable at the 50% level.

Gel filtration of reaginic serum was carried out on Sephadex G-200 and the elution pattern is presented in FIG. 2. The fractions indicated were pooled and concentrated to the original sample volume and tested in PCA. Reactivity was confined to the ascending portion of the second peak indicating a molecular size slightly larger than that of the rat 7S immunoglobulins.

TABLE 1. Optimal period of sensitisation for reagin to *T. taeniaeformis* in serum obtained 35 days after infection with 300 eggs. Serial twofold dilutions of this serum were used to sensitise rats 4, 24, 48, 72 hours and 9 days before allergen challenge. PCA lesions were graded from negative to +++ according to the criteria described in the Materials and Methods.

TABLE 1

OPTIMAL SENSITISATION OF RAT SKIN WITH REAGINIC ANTIBODIES TO *TAENIA TAENIAEFORMIS*

4 hr.	24 hr.	48 hr.	72 hr.	9 day	PCA titre
+++	+++	+++	+++	+++	neat
++	+++	+++	++	++	1:2
++	++	++	++	+	1:4
+	++	++	++	+	1:8
-	+	+	+	-	1:16
-	-	-	-	-	1:32

TABLE 2. Sensitivity to heat treatment (56 C) and reduction and alkylation of rat reagins to *T. taeniaeformis* in serum obtained 35 days after infection with 300 eggs. PCA lesions were graded from negative to +++ according to the criteria described in the Materials and Methods.

TABLE 2

SENSITIVITY OF RAT REAGIN TO *T. TAENIAEFORMIS* TO HEAT TREATMENT AND REDUCTION AND ALKYLATION

Lability at 56 C			Susceptibility to reduction and alkylation			
2 hours	1 hour	Control	Reduced & Alkylated	Reduced	Control	PCA Titre
-	-	+++	-	+++	+++	neat
-	-	+++	-	++	++	1:2
-	-	++	-	+	++	1:4
-	-	++	-	-	+	1:8
-	-	+	-	-	-	1:16
-	-	-	-	-	-	1:32

FIG. 2. Elution profile at 280 nm of rat serum obtained 35 days after infection with *T. taeniaeformis* and passed through Sephadex G-200. Fractions were pooled as indicated and those with positive PCA reactivity are shown in black.

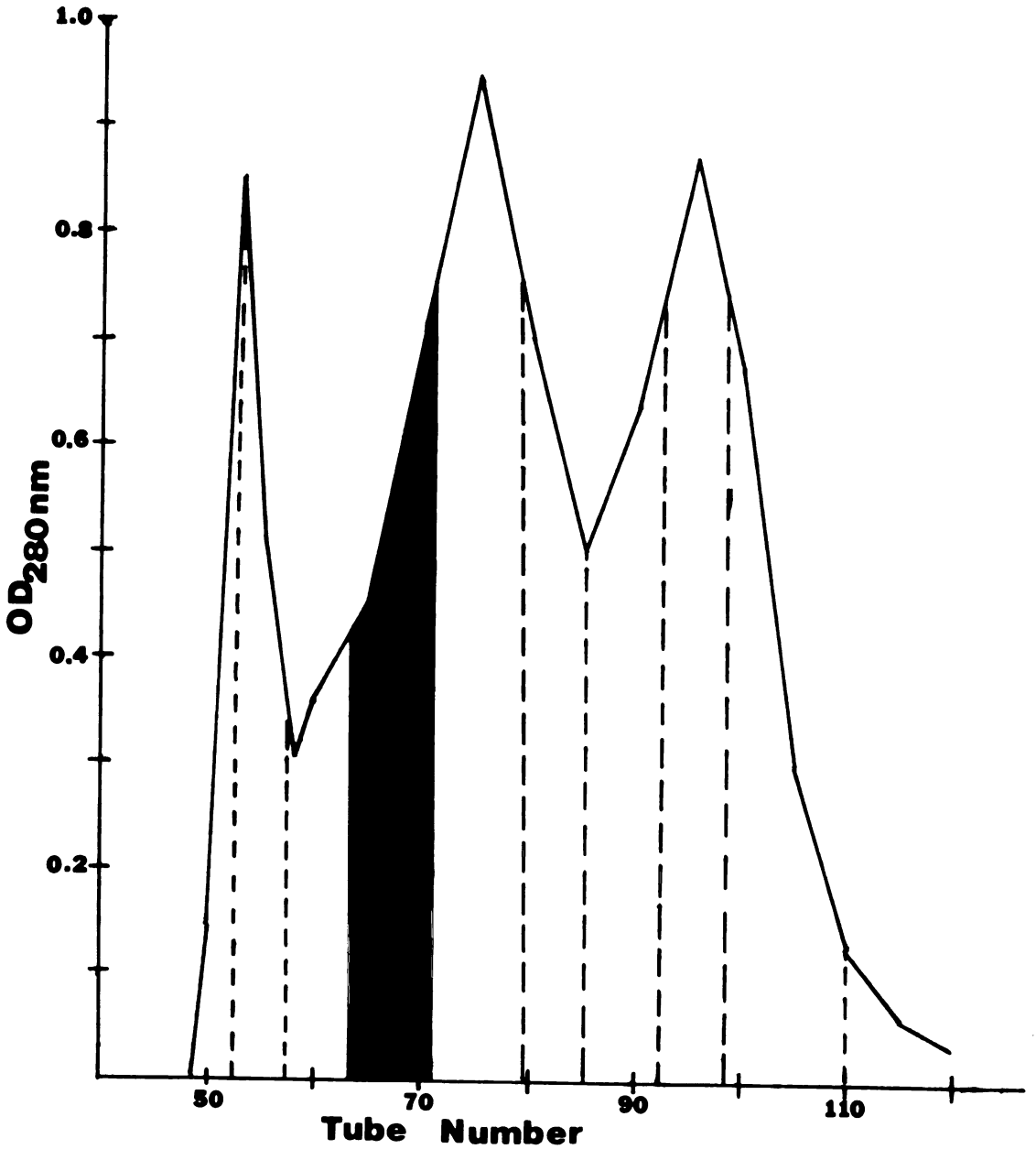

Figure 2

The serum was also subjected to anion exchange chromatography on DEAE cellulose and the elution profile is illustrated in FIG. 3. Fraction 4, eluted with the 0.05M phosphate buffer, pH 5.8, contained all the detectable skin fixing activity. Absorption with an antiserum prepared in sheep specific for the Fab portion of rat immunoglobulin eliminated PCA reactivity.

In view of the fact that protective antibody activity had been associated with $7S\gamma_{2a}$ (Leid and Williams, 1973) attempts were made to demonstrate short term skin sensitisation with serum and immunoglobulin fractions. Latent periods of 2, 4, 5 and 6 hours were used and positive reactions were detectable by 2 hours. However, the physico-chemical characteristics of this antibody in gel filtration, anion exchange chromatography, heat lability and susceptibility to reduction and alkylation were identical to those described for rat reagin. In no instance were we able to demonstrate reactivity in the $7S\gamma_{2a}$ eluate from reaginic serum, taken at any time during infection.

An experiment was performed to determine if maternal transfer of reaginic antibodies occurred. Attempts were made to demonstrate skin sensitising activity in the colostrum of infected rats whose serum had been shown positive in PCA and which had been given booster doses of eggs. Colostral samples were obtained daily over the first 21 days of lactation by either milking the mother rats using a vacuum tube method (McBurney, Meier and Hoag, 1964) or from the stomachs of sacrificed offspring. Colostral whey was prepared as described previously (Leid and Williams, 1973). Pooled serum samples from newborn rats and from those suckling infected mothers were obtained at daily intervals over the 3 week period. Neither colostrum nor serum from neonatal rats gave positive reactions in PCA in repeated trials.

FIG. 3. Elution profile at 280 nm of rat serum obtained 35 days after infection with *T. taeniaeformis* and subjected to DEAE cellulose chromatography. All phosphate buffers were made 0.015M in NaCl. Fractions indicated were pooled and tested in PCA. Reactivity was limited to the blacked out areas.

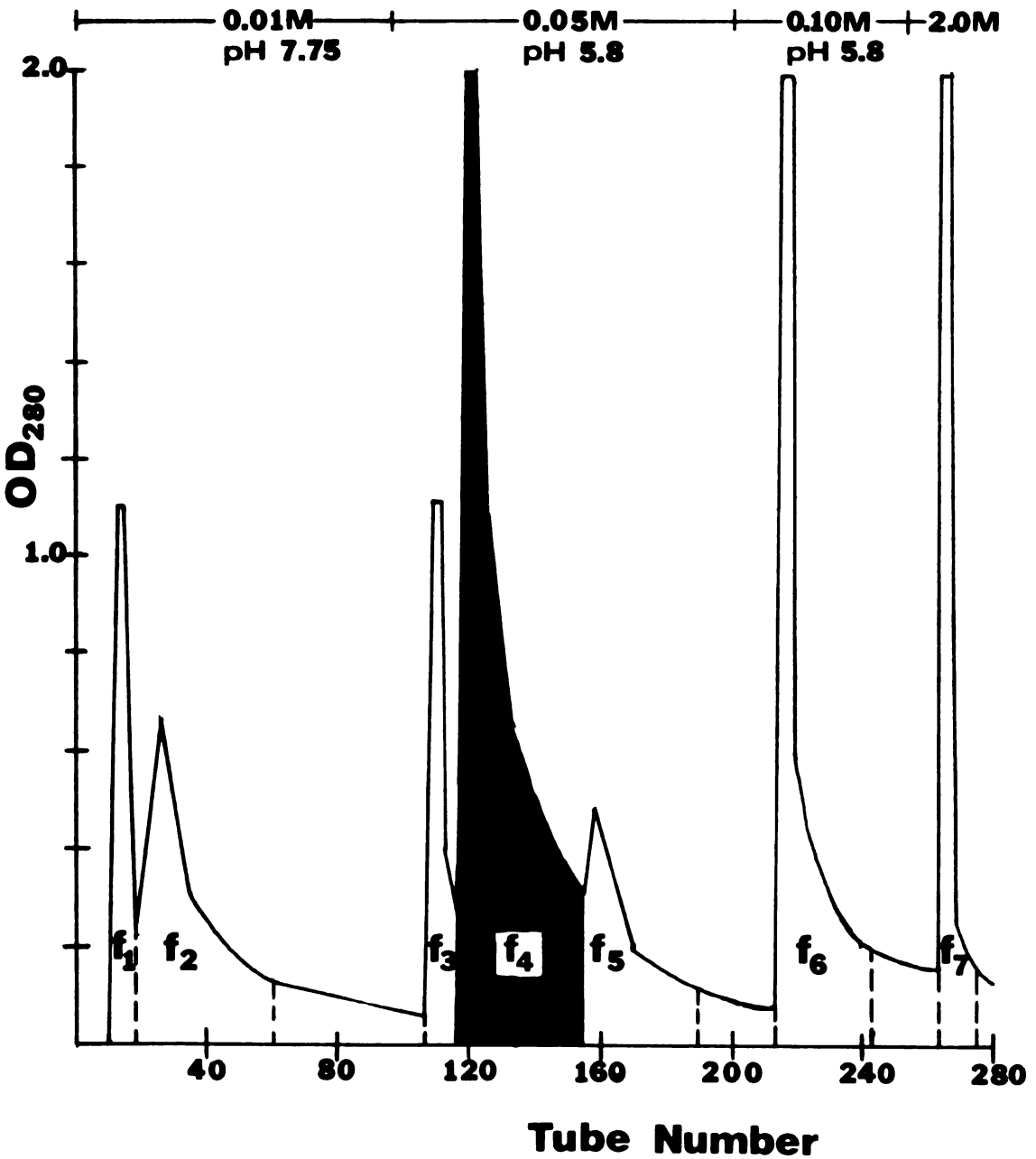
0.01M
pH 7.75
0.05M
pH 5.8
0.10M
pH 5.8
2.0M
2.0
1.0
OD280
f1
f2
f3
f4
f5
f6
f7
40
80
120
160
200
240
280
Tube Number

Figure 3

PCA reactions could be provoked with extracts of both larvae and adults of *T. taeniaeformis* and with the *in vitro* culture products. Since the adult tapeworm represents a population of progressively maturing individual segments we attempted to demonstrate allergen enrichment associated with segment development. Anterior, middle and posterior regions of the adult strobila were homogenised and extracts prepared and tested in PCA. We were not able to detect any differences in reactivity in these preparations.

It appeared that there was no advantage in using extracts of adult worms or the *in vitro* culture products so further investigations on the purification of allergen were made using extracts of the more conveniently obtained cysticerci.

Initially an extract of cysticerci of *T. taeniaeformis* was treated with trichloroacetic acid and PCA activity was completely removed, suggesting the presence of proteinaceous allergens. The extract was then subjected to $(NH_4)_2SO_4$ precipitation at 20, 40, 60 and 80% saturation. These fractions and the supernatant remaining from the 80% precipitation were all tested in PCA. Positive reactions were obtained from the 40, 60 and 80% fractions and it did not appear that localisation of allergenic activity would be possible with this method. Alternative procedures such as gel filtration, ion exchange chromatography, preparative and polyacrylamide gel electrophoresis were therefore used. In the first instance the extract was dialysed against 0.1M Tris-HCl buffer, pH 8.0, and passed through a Sephadex G-200 column equilibrated against the same buffer. Gel filtration studies produced irregular and unpredictable results. In some instances aggregation apparently occurred and all PCA reactivity was eluted in the void volume of the column, while in other separations allergenic activity extended over a

wide range of eluted fractions. We therefore felt that chromatographic procedures involving separation based upon charge differences might be more useful.

Anion exchange chromatography of the extract on DEAE cellulose resulted in several elution peaks and some resolution of biological activity (FIG. 4). The fractions indicated were concentrated to the original sample volume and tested in ACA. A strongly positive reaction was observed with the 2M NaCl eluate but slight or questionable reactions occurred with the other fractions. However, activity in PCA was limited to the 2M NaCl eluate and when tested in I.E.P. with aTLE several anodal precipitin arcs appeared. Since the allergen or allergens involved were negatively charged, CM-Sephadex cation exchange chromatography was employed (FIG. 5). The fractions indicated in the diagram were concentrated to the original sample volume and tested in ACA. Fraction 1, the 0.0175M phosphate buffer eluate, was the most reactive, although slight activity was again observed with the remaining fractions. All the eluates were tested in PCA and a positive reaction was found with the first peak only. In I.E.P. against aTLE this fraction produced several anodal precipitin arcs. An attempt was then made to purify the negatively charged allergen by preparative block electrophoresis on Pevikon. The fractions were eluted from 1 cm strips of the block at the end of the separation and the protein content of each eluate was determined (FIG. 6). Each fraction was examined in PK tests in order to obtain preliminary information on the location of allergenic activity. PK reactivity was limited to eluates 16-20. Fractions containing similar precipitin arcs in I.E.P. were pooled and the samples were used to challenge sensitised rats in PCA. The rabbit aTLE antiserum recognised eleven

FIG. 4. Elution profile at 280 nm of extract prepared from cysticerci of *T. taeniaeformis* and subjected to DEAE cellulose chromatography. All buffers were made 0.015M in NaCl. Fractions indicated were pooled and tested for their ability to provoke PCA in sensitised rats. Positive reactivity is indicated by blacked out areas.

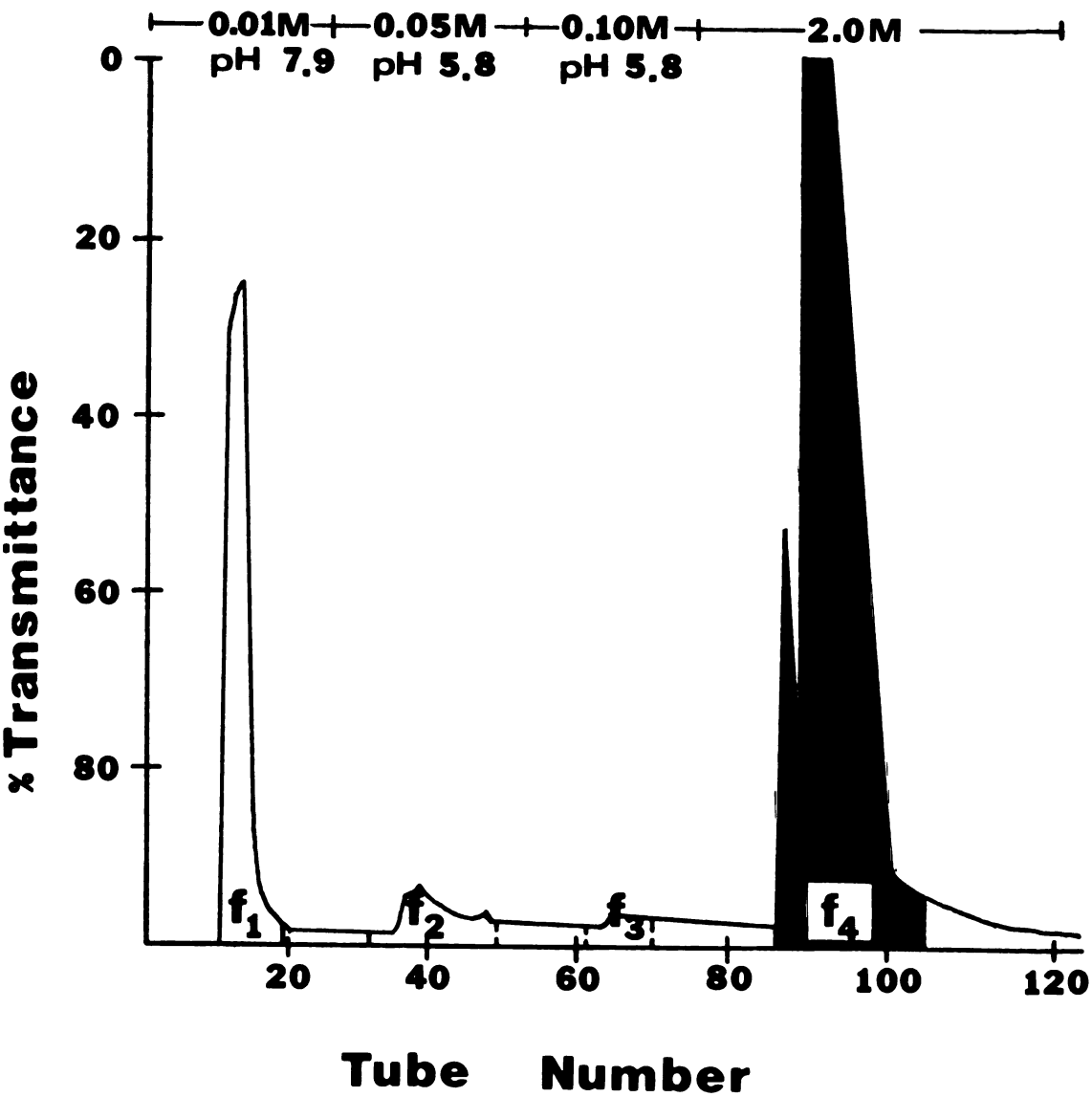
0.01M
pH 7.9
0.05M
pH 5.8
0.10M
pH 5.8
2.0M
0
20
40
60
80
% Transmittance
f1
f2
f3
f4
20
40
60
80
100
120
Tube Number

Figure 4

FIG. 5. Elution profile at 280 nm of extract prepared from cysticerci of *T. taeniaeformis* and subjected to CM Sephadex chromatography. Fractions indicated were pooled and tested for their ability to provoke PCA in sensitised rats. Positive reactivity is indicated by blacked out areas.

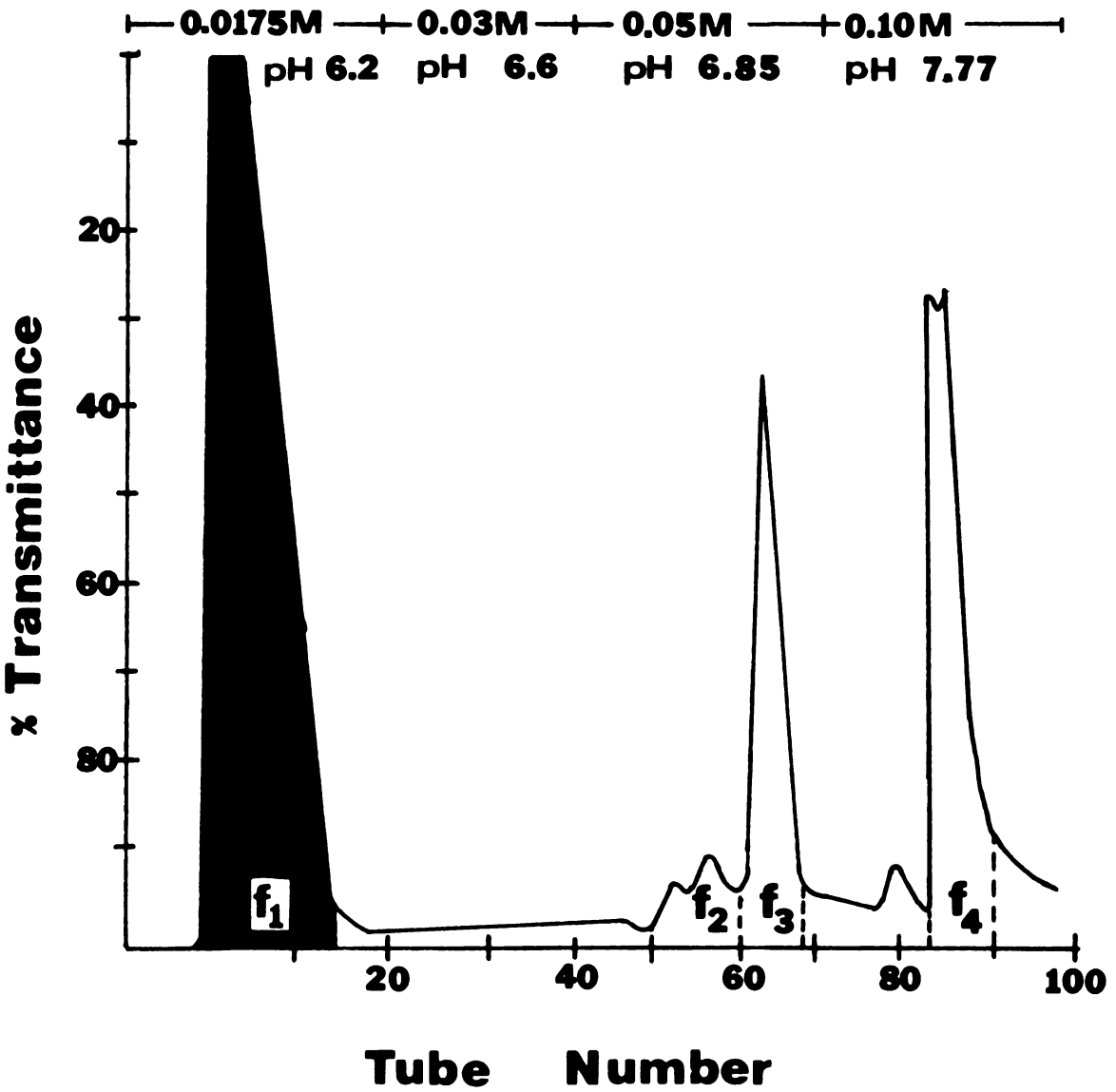
0.0175M
0.03M
0.05M
0.10M
pH 6.2
pH 6.6
pH 6.85
pH 7.77
% Transmittance
20
40
60
80
f1
f2
f3
f4
20
40
60
80
100
Tube Number

Figure 5

FIG. 6. Elution profile of an extract prepared from cysticerci of *T. taeniaeformis* expressed as protein content of 1 cm fractions from Pevikon block electrophoresis. Fractions were pooled as indicated and tested for their ability to provoke PCA in sensitised rats. Reactivity was demonstrable in pools 4-10 although the most intense reactions were seen with 7-10.

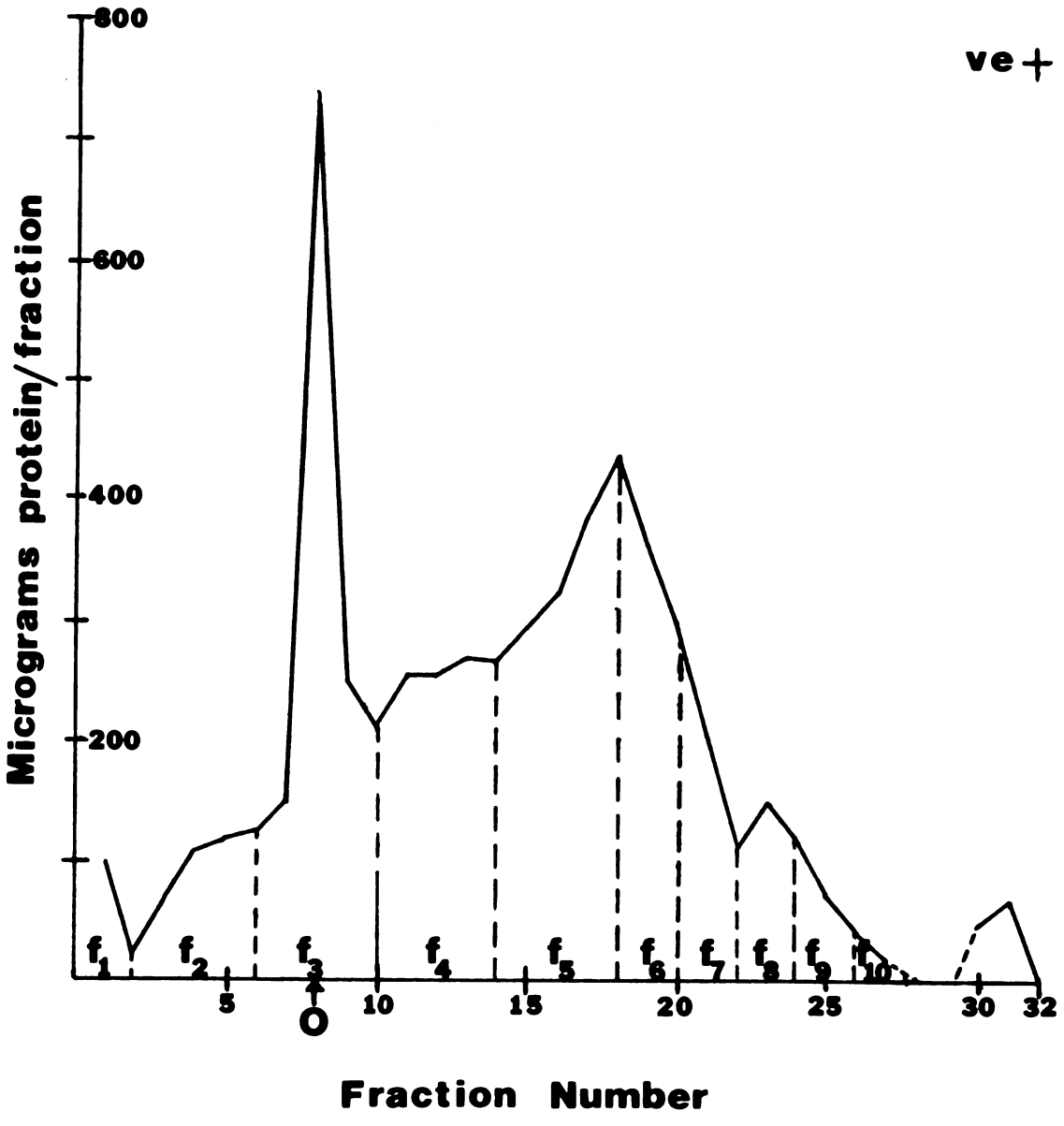
800
600
400
200
ve +
Micrograms protein/fraction
f1
f2
f3
f4
f5
f6
f7
f8
f9
f10
5
O
10
15
20
25
30
32
Fraction Number

Figure 6

precipitin arcs in I.E.P. The most intense PCA activity was present in pools 7-10 although some activity was seen in 4-6. All pools were subjected to PAGE using 5% gels (FIG. 7) with a single protein band detected in pool 9. Pools 7-10 were also electrophoresed on 10% gels and again only a single band was stained in pool 9. Pool 9 was again separated in PAGE (5%) and stained for both lipid and carbohydrate (FIG. 8). The periodic acid-Schiff (PAS) reaction was positive, indicating the presence of a carbohydrate moiety but the test for lipid was negative. As little as 5.4 µg of this purified allergen were sufficient to provoke PCA reactions in sensitised rats.

Since pool 9 had produced only a single arc in I.E.P. against aTLE, 100 µg of protein from this pool were used to immunise a sheep and a monospecific antiserum was raised (FIG. 9). This antiserum was then used in experiments on the absorption of allergenic activity from extracts of larvae and adults and also from *in vitro* culture concentrates. Allergic reactivity was completely removed from the larval extract but only slightly reduced in the adult antigenic preparations. Activity remained unaffected by absorption of the *in vitro* concentrate. This antiserum reacted slightly in I.E.P. against adult extract (FIG. 9) but no precipitin arc developed with the *in vitro* preparation. Extracts of larvae and adults in addition to the *in vitro* culture concentrates were used to immunise rabbits and cross reacting proteins were present in all three extracts in I.E.P. However, a band comparable to that for PAGE pool 9 was not detectable in the *in vitro* preparation.

Extracts from other members of the family *Taeniidae* and two dissimilar helminths were tested for their ability to provoke PCA reactions in rats sensitised with 35 day serum (Table 3). Several of the extracts of taeniid parasites at both the metacestode and adult

FIG. 7. Five percent polyacrylamide gel electrophoresis of pools 1-10 from preparative electrophoresis of larval extract. Fractions were stained with a 1% solution of aniline blue black.

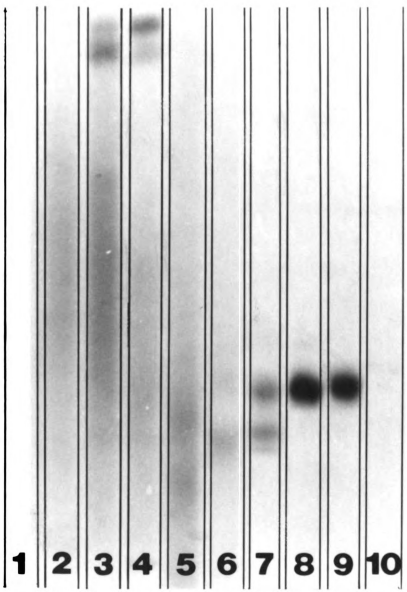

Figure 7

FIG. 8. Pool 9 from preparative block electrophoresis subjected to 5% polyacrylamide gel electrophoresis and stained for protein (1), carbohydrate (2) and lipid (3).

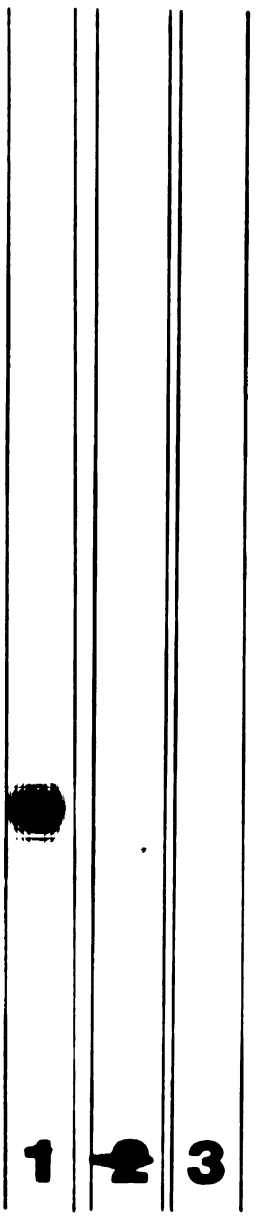

Figure 8

FIG. 9. Immunoelectrophoretic analysis using sheep antiserum against an allergen from cysticerci of *T. taeniaeformis* in the troughs. Antigens in the wells from top to bottom were as follows: adult extract, larval extract, larval extract and *in vitro* culture concentrate.

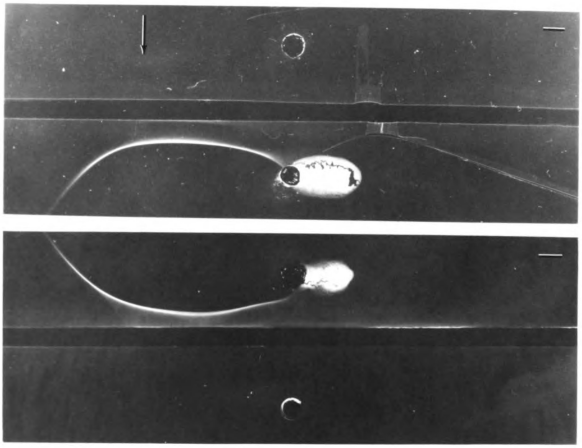

Figure 9

TABLE 3. Cross reactions in rats sensitised with reaginic serum from infections with *T. taeniaeformis* and challenged with extracts prepared from various taeniid parasites, *Schistosoma mansoni* and *Fasciola hepatica*. PCA lesions were graded from negative to +++ according to the criteria described in the Materials and Methods.

TABLE 3

CROSS REACTIVITY OF VARIOUS HELMINTH EXTRACTS

Adult	Larval	
+++	+++	*Taenia taeniaeformis*
+++	nt	*Taenia pisiformis*
nt	+++	*Taenia crassiceps*
nt	+++	*Taenia hydatigena*
nt	+++	*Echinococcus multilocularis*
neg	nt	*Fasciola hepatica*
neg	nt	*Schistosoma mansoni*

stage were able to provoke PCA. Extracts of *E. multilocularis* protoscolices gave positive reactions but preparations of *S. mansoni* and *F. hepatica* were ineffective.

DISCUSSION

The results presented in this paper demonstrate the appearance of a long term skin sensitising antibody or reagin in the serum of rats infected with *T. taeniaeformis*. The antibody was first detected during the third week of infection, peaked during the fifth week and thereafter declined. Skin sensitising activity was abolished by heat treatment and by reduction followed by alkylation. Activity eluted slightly ahead of the 7S immunoglobulins when serum was subjected to gel filtration and PCA reactivity was limited to the 0.05M phosphate buffer eluate in DEAE cellulose chromatography. These physico-chemical and biological properties are consistent with those of the rat immunoglobulin designated as γE by Stechschulte and Austen (1970). In addition the rat reagin to *T. taeniaeformis* was found to share with the γE of the rabbit a susceptibility to precipitation with $(NH_4)_2SO_4$ at 50% but not at 33% saturation (Zvaifler and Robinson, 1970). In preliminary experiments we have been able to achieve a reduction in PCA reactivity by absorption with an antiserum raised in sheep against rat mast cells sensitised with reagin. This antiserum did not cross react with any of the other immunoglobulin classes of the rat or their light chains in I.E.P. or D.I.D.

Optimal sensitisation of rat skin occurred in 24-72 hours and persisted for at least 9 days. However, some PCA reactivity was evident as early as 2 hours after sensitisation and our evidence indicates that this is due wholly to fixation by γE. We were unable to associate skin sensitising activity with $7S\gamma_{2a}$ in this infection, although antibodies

of this type produced in response to artificial immunisation were strongly positive in short term PCA tests (unpublished observations).

A study of the pattern of reagin production indicated that it was possible to boost serum titres with challenge doses of eggs. The biphasic response curve seen after boosting is difficult to interpret, since the embryos in the challenge dose certainly would not have survived more than a transient period of penetration of the gut mucosa in these highly immune rats. However, the observations of Moguillansky (1972) on human patients with hydatid disease may have some bearing on this finding. Increased vascularisation was consistently seen around liver cysts in radiographs after intradermal inoculation of antigens of *Echinococcus granulosus*. This might result from an allergic reaction around the cyst and in the *T. taeniaeformis* system a similar phenomenon could have occurred. The initial rise in titre after the booster egg dose is suggestive of an immediate allergenic stimulation while the secondary peak may have resulted from damage to some of the cysticerci associated with release of allergen molecules and an increase in serum levels of reagin.

Reaginic antibodies to *T. taeniaeformis* were not detectable in the serum of newborn rats from mothers with circulating reagins and did not appear in colostrum or in the serum of suckling rats over a 21 day period. This finding is largely in accord with the observations of Jones and Ogilvie (1967) on maternal transfer of reagins to *Nippostrongylus brasiliensis*, although they did detect reagin in the milk of some rats. Their results and our own are in sharp contrast to the successful colostral transmittance of anaphylactic antibody to DNP-BGG in the rat shown by Binaghi, Oettgen and Benacerraf (1966). Possible explanations for this difference have been discussed by Jones and

Ogilvie (1967). We did not attempt to monitor the titres of reagins in maternal serum during the course of our experiments although parallel groups of rats similarly boosted had sustained PCA titres of 1:32 or greater. Titration did not appear to be crucial to the attempt to demonstrate maternal transfer since Jones and Ogilvie (1967) had shown no correlation between serum reagin levels and PCA titres of milk.

Nevertheless reaginig antibodies formed a prominent feature of infection with *T. taeniaeformis* and appeared at the time when passive transfer of immunity with serum became possible (Leid and Williams, 1973). Chromatographic fractions enriched for reagin conferred a highly significant degree of resistance in recipient rats and the potential involvement of reagin in acquired protective responses must be considered. However, we were able to protect recipient rats with fractions devoid of reagin (Leid and Williams, 1973) and normal newborn rats receiving colostrum from immune mothers which did not contain reagins were resistant to challenge (Williams, Leid, Musoke, and Williams, in prep.). The role which reagins or γE antibodies may play in acquired resistance to helminthic infections is at present a highly controversial issue, but Murray (1972) has summarised the evidence implicating this immunoglobulin in the protective responses of the host *in vivo*. He proposes that combination of allergen and γE antibody at mucosal surfaces would result in release of vasoactive amines and increased vascular permeability around the parasite. This reaction would bring about the accumulation of humoral and cellular elements and culminate in an immunological attack on the invading organism. Possibly the reagin produced in response to *T. taeniaeformis* infection functions in a similar manner, potentiating the effects of $7S\gamma_{2a}$ mediated antigen induced release of vasoactive amines postulated by

Leid and Williams (1973). There is some evidence to support the notion of an intestinal immune mechanism in experimental cysticercosis in the rabbit and Leonard and Leonard (1941) suggested that there might exist an immunological "intestinal barrier." The participation of γE in such a mechanism seems probable in view of the large numbers of γE secreting cells which occur in the intestinal mucosa (Tada and Ishizaka, 1970).

The presence of accumulations of mast cells around the cysticerci of *T. taeniaeformis in situ* (Varute, 1971) points to another possible role for reagin in this infection. Mast cells and circulating basophils are known to bind γE (Ishizaka and Ishizaka, 1971) and conceivably an immunological attack on the parasite in the tissue could be mediated by processes similar to those described by Murray (1972). This might contribute to the rapid and intense inflammatory reaction around degenerating larvae which characterises the pathogenesis of both cysticercosis and hydatidosis in humans (Smyth and Heath, 1970). Once again the possibility of γE and γ_{2a} acting in concert in the tissue reaction of the rat cannot be excluded.

The allergens responsible for stimulating the appearance of reaginic antibody in *T. taeniaeformis* infections are complex and evidence suggestive of the presence of at least 2 distinct components resulted from our study. One of these was purified to homogeneity from extracts of cysticerci. Allergenic activity was eluted with 2M NaCl in DEAE cellulose chromatography and with 0.0175M phosphate buffer in CM-Sephadex fractionation but there was a broad distribution of the allergen in fractions derived from preparative block electrophoresis. However, sufficient separation occurred to allow us to harvest from several anodal fractions a pure preparation of an

allergen, approximately 5 µg of which would provoke PCA. This reactivity was removed by precipitation with trichloroacetic acid and in polyacrylamide gel electrophoresis the single band stained positively in tests for protein and carbohydrate but not for lipid. Furthermore, a monospecific antiserum was prepared against this glycoprotein which abolished PCA activity in larval extracts used for absorption. Under the conditions used for 10% polyacrylamide gel electrophoresis the migration characteristic of the band is indicative of a protein of low molecular weight (50,000 or less).

Our preliminary attempts to resolve the allergenic activity by salting out procedures and gel filtration were unsuccessful. The lack of reproducibility in separations on Sephadex G-200 was surprising considering the extent to which this technique has been applied in the purification of allergens of nematodes (Ambler, Doe, Gemmell, Roberts and Orr, 1972; Jones and Ogilvie, 1967; Hussain, Strejan and Campbell, 1972; Hussain, Bradbury and Strejan, 1973). However, both Ambler *et al.* (1972) and Hussain *et al.* (1973) have suggested that the range of activity seen in some of their Sephadex separations of crude extracts of *Ascaris* is due to aggregation of allergen subunits. Perhaps similar interactions may account for our results, which could have been accentuated by the selection of Tris-HCl as eluting buffer. The results from the combination of preparative and polyacrylamide gel electrophoresis certainly suggest that under alternative buffering conditions aggregation is much less pronounced.

The broad distribution of activity in fractions resulting from the spectrum of physico-chemical techniques employed was indicative of the participation of more than one allergen in PCA tests. The results of experiments on absorption with monospecific antiserum

confirmed this view. While the reactivity of larval extracts was abolished, *in vitro* culture concentrates were unaffected by this treatment. Reaginic antibodies to the allergens in the latter preparation were not detected before the third week of infection, coincidental with the onset of reaginic antibodies to the pure allergen. Absorption of the adult worm extracts resulted in only partial reduction in PCA activity and without more experimentation it is not possible to comment more definitively on the existence of a third allergen which could have been responsible for this finding. Evidence regarding the multiplicity of allergens in other helminth extracts has recently come to light. Ambler *et al.* (1972) have demonstrated the presence of at least 2 distinct allergens in adult *Ascaris suum* and Ford (1971) was able to detect allergenic activity in fractions of extracts from *Trichostrongylus retortaeformis* separated by isoelectric focusing. These findings and our own suggest that the occurrence of multiple allergenic components in helminthic extracts is not exceptional but should be anticipated. This possibility ought not to be ignored in the interpretation of experiments on anaphylaxis in helminth infections.

Allergenic activity was detected throughout the length of the strobilate adult worm and our results therefore shed no light on the suggestion of Smyth (1969) that the secretory granules of the taeniid scolex are the source of antigenic stimulation in the intestine. However, contact between the scolex and the mucosal surface is intimate and in intestinal infections with *T. saginata* (Machnicka-Roguska and Zweirz, 1970) and *E. granulosus* (Williams and Perez Esandi, 1971) anaphylactic antibody responses are known to occur.

Extracts of a variety of other taeniid parasites were able to provoke PCA reactions in rats sensitised with serum containing reagins to *T. taeniaeformis*, indicating sharing of allergenic components between members of this family. This is borne out by immunodiagnostic procedures in the field where antigenic preparations of most *Taenia* spp. can be used satisfactorily for intradermal tests in cattle infected with *T. saginata* (Froyd, 1963). Extracts of *S. mansoni* or *F. hepatica* did not give positive reactions in our tests although these parasites are known to cross react in cases of clinical taeniasis. Froyd (1963) showed that cattle infected with *T. saginata* react to intradermal inoculations of *F. hepatica* and Kagan (1968) in his review of serological cross reactions in hydatidosis points out that *S. mansoni* and *E. granulosus* share antigenic determinants. Our data provide no information on the specificity of the allergen which we have purified from *T. taeniaeformis*. However, the simplicity of the isolation procedure and the homogeneity of the preparation represent a significant advancement in our understanding of the nature of taeniid allergens. Future efforts to obtain specific reagents for clinical and antemortem diagnosis in human and animal cysticercosis should take into account this finding.

This is the first time that a cestode allergen has been purified to the degree of homogeneity achieved recently by Hussain *et al.* (1973) with *Ascaris suum*. Further characterisation of the antigen might contribute to the development of a more precise appreciation of the molecular basis of allergenicity and can be expected to clarify the allergic facets of acquired resistance to *T. taeniaeformis*. Studies along these lines are being actively pursued in our laboratory.

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