Immunology, 1974, **27**, 195.

The Immunological Response of the Rat to Infection with *Taenia taeniaeformis*

I. IMMUNOGLOBULIN CLASSES INVOLVED IN PASSIVE TRANSFER OF RESISTANCE*

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(*Received* 30*th October* 1973; *accepted for publication* 14*th December* 1973)

Summary. Passive transfer of immunity to *Taenia taeniaeformis* infection 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 7Sγ2, 7Sγ1, 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. 7Sγ2a antibodies were able to confer passive protection when given alone, and probably contributed to the protective capacity of mixtures containing 7Sγ2 and 7Sγ1 immunoglobulins. A mechanism for specific acquired resistance to *T. taeniaeformis* is proposed based upon the recently established biological properties of 7Sγ2a.

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

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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 (Wilson, 1966; Ogilvie, 1970; Jones, Edwards and Ogilvie, 1970). 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*.

MATERIALS 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 and was 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, 2500 μg/ml of streptomycin, 333 u/ml of polymyxin B, and 1000 u/ml of penicillin G, and stored at 4°. 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 ten to fifteen 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 Laboratories, 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 acclimatized 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 μm 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 killed using carbon dioxide vapour and the total number of cysticerci in each liver was determined. The results were statistically analysed using a computerized programme for 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 were killed 28 days later using carbon dioxide vapour. 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° and left overnight at 4°. The serum was decanted, centrifuged and stored at −20° without preservatives until used.

Immunoelectrophoresis (IEP) and double immunodiffusion (DID)

Immunoelectrophoresis was performed following a slight modification of the method of Scheidegger (1955) in a Gelman apparatus (Gelman Instrument Company, Ann Arbor, Michigan) with a sodium barbital–HCl buffer, $\mu = 0{\cdot}038$, pH 8·2 (Williams and Chase, 1971). Two per cent Noble agar (Difco, Detroit, Michigan) was prepared with barbital buffer diluted 1:2 and contained 1:10,000 thiomersolate.

Double immunodiffusion was performed according to a micro-method modified from that described by Williams and Chase (1971). Two per cent Noble agar was prepared in a 0·1 M Tris–HCl buffer, pH 8·1 with a final concentration of thiomersolate 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 7Sγ2 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 siliconized 2·5 × 100 cm column of Sephadex G-200 (Pharmacia, Uppsala), equilibrated with 0·1 M 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-millimetre glass beads were siliconized, filling the bottom 2 cm of the column, and swollen Sephadex G-200 was poured over the bead layer. Samples were dialysed 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 Instrument Company, 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 × 30 cm siliconized glass columns. The cellulose was equilibrated against either 0·01 M phosphate buffer, pH 7·75 or 0·005 M phosphate buffer also at pH 7·75. The 0·005 M buffer was used initially where separation of the 7Sγ2a and 7Sγ2b immunoglobulins was required. Proteins were eluted in a stepwise manner using 0·01 M phosphate buffer followed by 0·05 M, pH 5·8, 0·1 M, pH 5·8 and finally 2 M NaCl. All phosphate buffers were made 0·015 M in NaCl and the samples were dialysed 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 Medical Electronics, Middleton, 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 Freund's complete adjuvant (FCA, Difco, Detroit). Rabbits received 0·5 ml by intramuscular (i.m.) inoculation in each hind leg and 0·1–0·2-ml portions were injected subcutaneously (s.c.) 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 10 days after the last injection.

Anti-rat γM (aγM) was prepared in both rabbits and sheep. Normal rat serum immunoglobulins were precipitated three times with 50 per cent saturated ammonium sulphate and passed through a Sephadex G-200 column. The ascending portion of the first peak was allowed to react in IEP with aWRS and the precipitin arcs corresponding to IgM were excised from eighteen slides. These agar slices were homogenized in a tissue grinder with a minimal volume of PBS and emulsified with an equal volume of FCA. Rabbits were inoculated i.m. with 0·5 ml of the emulsion and 0·2-ml portions were injected at several sites over the back. Twenty days later the animals were boosted with a similar preparation and bled out 10 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 were 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° 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 three times and taken up in 1 ml of PBS. One half of a millilitre of the suspension was mixed with an equal volume of FCA 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 IEP with γM and several other serum components. After absorption with foetal rat serum polymerized according to the method of Avrameas and Ternynck (1969) each antiserum recognized only one arc in IEP with normal rat serum, corresponding to γM (Fig. 1).

Anti-rat secretory γA (aSγA) 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 1–3-day-old rats were homogenized in a tissue grinder, stirred overnight at 4° and centrifuged at 20,000 *g* for 30 minutes. The supernatant was passed firstly through glass wool and then through a 0·45 μm filter. The whey was dialysed extensively against 0·1 M Tris–HCl and applied to a Sephadex G-200 gel filtration column. The pro-

tein in the first peak was concentrated to the original volume and emulsified with an equal volume of FCA and 1 ml was 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 IEP. The cathodic precipitin arc corresponding to secretory γA was excised from eighteen slides. The agar slices were ground in a tissue grinder with a minimal amount of PBS and emulsified with an equal volume of FCA. 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

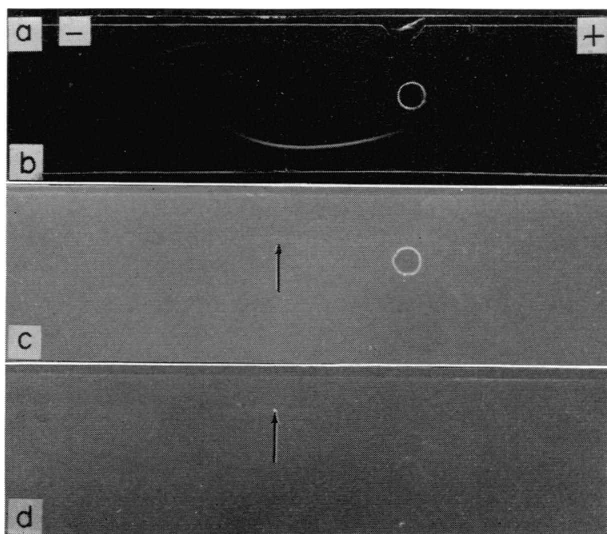

FIG. 1. Immunoelectrophoretic analysis of normal rat serum (NRS) *versus* (a) specific guinea-pig anti-7Sγ2, (b) guinea-pig anti-7Sγ1, (c) sheep anti-γM, and (d) anti-γA.

collected 10 days afterwards. Both rabbit and sheep anti-secretory γA reacted in IEP against normal rat serum to produce an arc recognizable as γA and several other minor arcs. After absorption with the foetal serum immunoadsorbent these antisera recognised 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 Sephadex G-200 gel filtration of normal rat globulin was dialysed against a 0·01 M phosphate buffer made 0·015 M in NaCl and applied to a DEAE–cellulose column equilibrated against the same buffer. The initial peak, containing only 7Sγ2a + b, was emulsified with an equal volume of FCA. Each guinea-pig received 30 μg of protein in 1 ml with 0·5 i.m. in each hind leg and the animals were bled out 20–25 days later. The antisera recognized only 7Sγ2 in IEP against normal rat serum (Fig. 1).

Anti-7Sγ_1 was also prepared in guinea-pigs by taking advantage of the method of Henney and Ishizaka (1969) to render the animals tolerant to 7Sγ_2 immunoglobulins. Guinea-pigs were each inoculated with 15 μg of total protein eluted from the DEAE–cellulose column with the 0·1 M phosphate buffer and emulsified with an equal volume of FCA. This preparation contained both 7Sγ2 and 7Sγ1 immunoglobulins as revealed by

IEP against aWRS. At the same time the guinea-pigs were given 5 mg of rat 7Sγ2 i.v. to suppress antibody formation to this immunoglobulin class. This antiserum produced only one arc in IEP 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 7Sγ2 immunoglobulin was purified by DEAE chromatography and 10 mg dialysed overnight against a 0·1 M phosphate buffer made 0·003 M in EDTA and 0·01 M in cysteine. Insoluble papain (20 mg) was added and digestion allowed to proceed for 3 hours while stirring at 37°. The reaction was stopped by centrifugation and the supernatant dialysed overnight against a 0·005 M 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 millilitre of the eluate containing 0·8 mg Fab was emulsified with an equal volume of FCA and injected i.m. into the hind limbs of a sheep. Thirty days later the sheep was bled. This antiserum reacted with γA, γM, γ2a+b and γ_1 in IEP 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 2–9-month-old infections were washed in distilled water several times and homogenized in a glass tissue grinder with a minimal volume of PBS. The suspension was stirred overnight at 4°, centrifuged at 17,000 *g* and stored at −20°. 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 homogenized 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 immunoadsorbent prepared by cross-linking the larval saline extract with glutaraldehyde (Avrameas and Ternynck, 1972). The mixture was stirred at 22–23° for 24 hours, followed by centrifugation and filtration through a 0·45 μm filter before passive transfer. In another experiment larval extract was coupled to polyacrylamide beads (P-300, P-400 mesh, BioRad) using the methods outlined by Ternynck and Avrameas (1972). Sensitized beads were allowed to react with immunoglobulins for 24 hours at 22–23° and were 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 per cent (v/v) and this was stirred for 24 hours at 22–23°. After incubation overnight at 4°, 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 DID tests against

concentrated larval extract. One millilitre 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 harboured 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

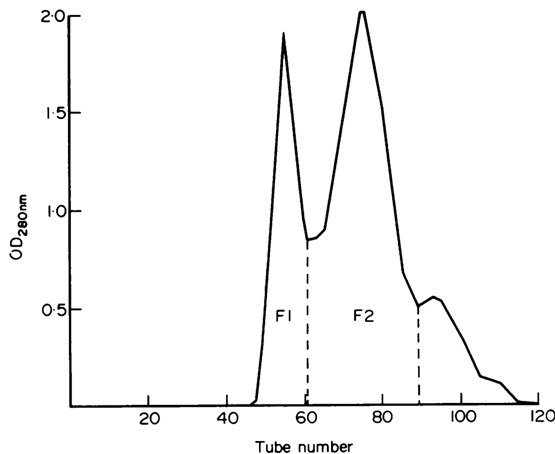

FIG. 2. Elution profile at 280 nm of the globulin fraction (50 per cent $(NH_4)_2SO_4$) of immune rat serum after gel filtration on Sephadex G-200. Fraction 1 (F1) was concentrated and tested for protective activity. Fraction 2 (F2) was further fractionated on DEAE–cellulose.

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 per cent $(NH_4)_2SO_4$ precipitation (×3) 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 per cent SAS three 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 Sephadex G-200 column, containing 7S immunoglobulin, was dialysed against a 0·01 M phosphate buffer

made 0·015 M in NaCl and applied to DEAE–cellulose equilibrated against the same buffer. Sequential stepwise elution was followed and the results are shown in Fig. 3. The presence of 7Sγ2 globulins was monitored in each fraction using guinea-pig antiserum specific for the Fc portion of each class. Both 7Sγ2a and 7Sγ2b were detected in the 0·01,

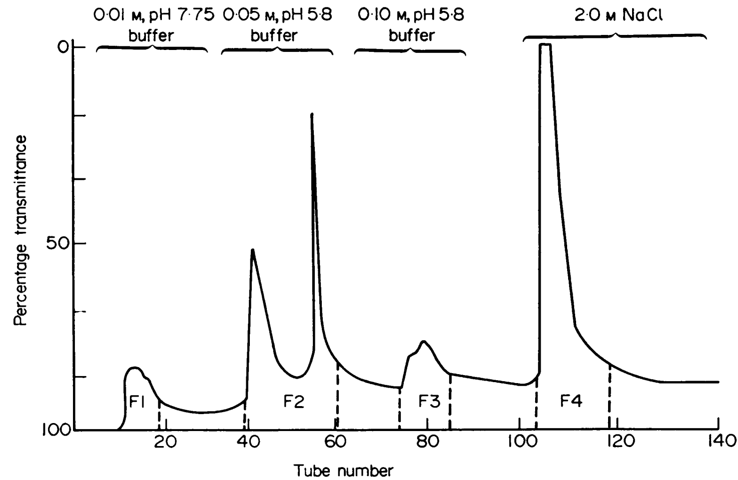

FIG. 3. Elution profile at 280 nm of the 7S globulin fraction (50 per cent $(NH_4)_2SO_4$) of immune rat serum fractionated on DEAE–cellulose with phosphate buffers and 2 M NaCl. All phosphate buffers were made 0·015 M in NaCl. Fractions 1, 2 and 3 (F1, F2 and F3) were tested for activity in passive transfer experiments.

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	n.s.*
Normal rat serum	66·83 ± 26·35	6	n.s.
19S fraction of immunoglobulin	48·66 ± 24·59	9	n.s.
0·01 M DEAE eluate of immunoglobulin	9·89 ± 6·45	9	< 0·001
0·05 M DEAE eluate of immunoglobulin	12·33 ± 13·65	9	< 0·001
0·10 M DEAE eluate of immunoglobulin	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 polymerized antigens of cysticerci	3·71 ± 8·98	7	< 0·001

* n.s. = Not significant.

0·05 and 0·10 M phosphate buffer eluates. 7Sγ1 was present only in the 0·10 M phosphate buffer eluate. Rat γA was detected with Fc-specific antiserum produced in sheep and rabbits and was present only in the 2 M NaCl eluate. Reaginic activity was limited to the 0·05 M 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 of larvae polymerized with glutaraldehyde.

Groups of 28-day-old female rats were given 300 eggs *per os* followed by an i.p. inocula-

tion of 0·8-ml quantities of each of the chromatographic fractions, PBS or normal 28-day serum. One millilitre portions of the absorbed solution and 0·4 ml of unfractionated immune serum and globulin were given. All groups were killed 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 polymerized larval extract did not reduce the protective quality of the globulin solutions. All three phosphate buffer eluates (0·01, 0·05 and 0·10 M) produced a highly significant reduction in parasite burdens ($P<0{\cdot}001$, 0·001 and 0·01, respectively) when compared to control animals. The levels of 7Sγ2 were determined in each of these phosphate buffer eluates and were as follows: 0·4 mg/ml for 0·01 M, 6·6 mg/ml for 0·05 M and <0·4 mg/ml for the 0·10 M.

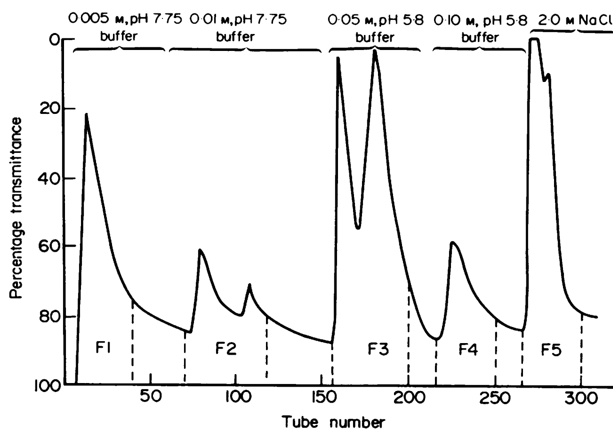

FIG. 4. Elution profile at 280 nm of the 7S globulin fraction (50 per cent $(NH_4)_2SO_4$) of immune rat serum fractionated on DEAE–cellulose with phosphate buffers and 2 M NaCl. All phosphate buffers were made 0·015 M in NaCl. Fractions 1 and 2 (F1 and F2) were tested for activity in passive transfer experiments.

In view of the fact that 7Sγ2a 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 7Sγ2a from the 7Sγ2b by lowering the molarity of the initial buffer in DEAE chromatography. In this case a 0·005 M phosphate buffer made 0·015 M 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 0·005 M phosphate buffer and applied to a DEAE–cellulose column. Again stepwise elution was followed (Fig. 4) and the 0·005 M and 0·01 M eluates were concentrated to the original serum volume and passed through a 0·45 μm filter before inoculation. In this passive transfer experiment further absorption procedures were attempted. Preliminary studies using immunoelectrophoresis and radioimmunoelectrophoresis (unpublished observations) had indicated antigen-binding activity in the γ2 immunoglobulins, and absorption procedures were therefore monitored by DID 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 millilitre

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° for 24 hours, after which the globulin solution was tested in DID against concentrated larval extract. All the precipitating antibody activity was not removed by this process and the solution was then absorbed with a freshly homogenized

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	n.s.‡
Immune rat globulin	32·11 ± 36·24	9	<0·01
19S fraction of immunoglobulin	85·50 ± 21·41	8	n.s.
0·005 M DEAE fraction of immunoglobulin	44·33 ± 35·32	9	<0·01
0·01 M DEAE fraction of immunoglobulin	93·89 ± 13·00	9	n.s.
Immune rat globulin absorbed*	52·67 ± 77·97	9	<0·2
Immune rat globulin absorbed†	19·86 ± 12·24	7	<0·001

* Absorbed with antigen-coated polyacrylamide beads, followed by larval suspension (10 per cent v/v).
† Absorbed with larval suspension (10 per cent v/v).
‡ n.s. = Not significant.

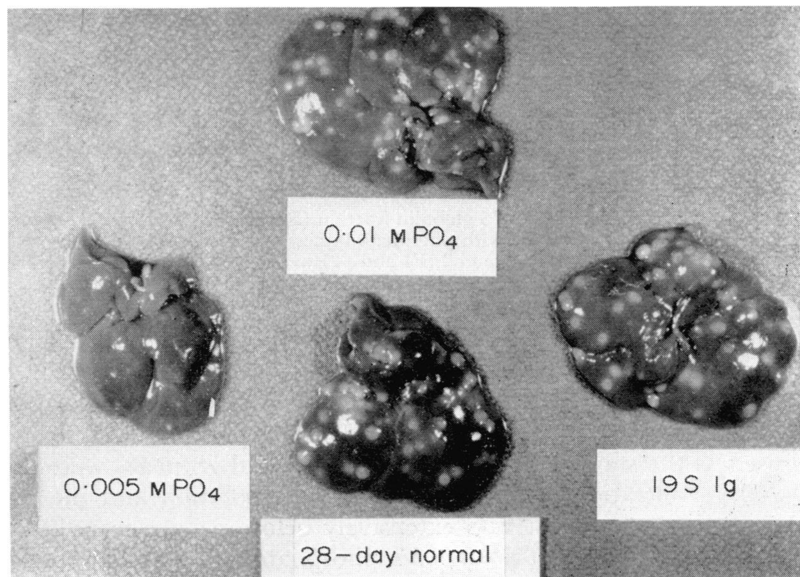

FIG. 5. Representative livers from groups of rats passively immunized with the DEAE fractions indicated. The animals were challenged *per os* with 600 eggs of *T. taeniaeformis* and killed 21 days later.

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 killed 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 0·005 M phosphate buffer resulted in highly significant protection in recipients ($P = 0·01$) while the 0·01 M did not. The 0·005 M eluate was tested in IEP with anti-whole rat serum and a single arc appeared, corresponding to 7Sγ2a (Fig. 6). It was also tested in DID against guinea-pig anti-γ2 and again only a single band formed.

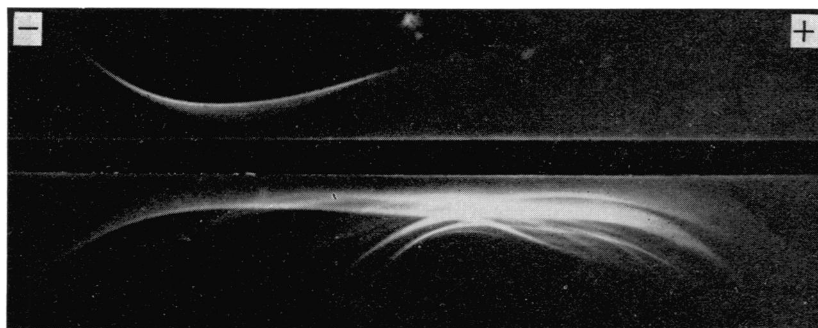

Fig. 6. Immunoelectrophoretic analysis of normal rat serum (bottom well) and fraction 1, 0·005 M eluate (upper well) from DEAE–cellulose fractionation (Fig. 4) *versus* rabbit anti-whole rat serum (aWRS).

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 7Sγ2a immunoglobulins contain the majority of protective antibodies to infection with *T. taeniaeformis*. We do not exclude the possible participation of 7Sγ2b, 7Sγ1 or γE in the successful passive transfer of resistance observed with fractions containing these immunoglobulin classes. However these DEAE eluates were contaminated with 7Sγ2a which was able to confer protection when given alone. Furthermore the eluate obtained with 0·1 M phosphate buffer contained less 7Sγ2a antibodies than the 0·01 M and 0·05 M fractions and conferred a lesser degree of protection. Enrichment with 7Sγ1-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 7Sγ2a, 7Sγ2b, 7Sγ1, γA, γM and possibly γE. This approach has recently been applied successfully by Saif, Bohl and Gupta (1972) in their 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 7Sγ2 antibodies, especially

7Sγ2a, 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.

7Sγ2 antibodies in the rat appear in response to a variety of artificial immunization procedures (Bloch, Morse and Austen, 1968; Jones, 1969) and were shown by Jones (1969) to be responsible for short-term homologous skin sensitization. Morse, Bloch and Austen (1968) demonstrated that 7Sγ2a antibodies were capable of preparing rat tissue for antigen-induced release of SRS-A, and 7Sγ2a antibodies inhibited histamine release from mast cells mediated by reaginic antibodies in rats infected with *Nippostrongylus brasiliensis* (Bach, Bloch and Austen, 1971). 7Sγ2 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 *et al.*, 1970). Passive protection was conferred predominantly by fractions enriched for 7Sγ1 immunoglobulins if serum was obtained from rats after primary infections. Preparations of 7Sγ2 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 7Sγ2a 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 sensitization at 2, 4 and 5 hours with 7Sγ2a antibodies against *T. taeniaeformis* (Leid and Williams, 1973), but this was readily achieved following artificial immunization (unpublished observations). Possibly those 7Sγ2a 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 sensitization 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 7Sγ2a 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 immobilization or destruction of the parasite. 7Sγ2a 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 important.

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 characterization 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.

ACKNOWLEDGMENTS

This work was supported in part by Grant AI-10842-01 and Training Grant GM-01911 from the United States Public Health Service. The senior author was supported by an N.I.H. pre-doctoral traineeship. The authors are grateful for the excellent technical assistance rendered by Mrs Anndy Whipple and the expert help of Dr A. J. Musoke in certain aspects of the work. His thoughtful and constructive criticism of the manuscript was very much appreciated.

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