REAGINIC ANTIBODY RESPONSE IN RABBITS INFECTED WITH
TAENIA PISIFORMIS

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antibodies were detected in the sera of rabbits infected with Taenia pisiformis. They were demonstrated
in the sera of some rabbits as early as 8 days after first exposure and peaked at 30 days post-infection
before declining over a period of 1 month. A second challenge dose of eggs boosted the titers markedly.
The physico-chemical characteristics of the antibody in gel-filtration and anion-exchange chromatog-
raphy, and in response to heat treatment and reduction and alkylation, conform to those previously
described for reagin or IgE. PCA reactions first became positive 4 h after sensitization, and were
maximal at 48 h, although positive reactions occurred at sensitized sites when challenge was delayed
for 8 weeks. The significance of this reaginic antibody response is discussed in relation to its potential
role in the mechanism of acquired resistance to experimental cysticercosis.

INDEX KEY WORDS: Taenia pisiformis; rabbits; reagin; immune mechanism.

INTRODUCTION

KERR (1935) showed that rabbits infected with the
cysticerci of Taenia pisiformis were immune to
challenge infection and that furthermore this
acquired immunity could be passively transferred
with serum. This was later confirmed by Campbell
(1938) and Leonard & Leonard (1941), but until
very recently there had been very little study of the
characteristics of the antibody response to this
infection.

Heath (1973a) has now shown that antibodies
which have a lethal effect on larvae in vitro appear
in the serum of rabbits as early as 12 days after
first exposure, and Nemeth (1973) demonstrated by
means of immunoadsorption techniques that the
primary response consisted exclusively of antibodies
of the IgG class. IgM antibodies were only detected
after secondary infection. However Wharton (1931)
had observed immediate hypersensitivity to intra-
dermally inoculated antigens in rabbits only 10
days after experimental infection. In view of the
fact that reaginic antibodies are known to be
able to mediate this type of reaction we have
extended this observation and report here on the
pattern of reaginic antibody production in the rabbit
after primary and secondary exposure to T. pisi-
formis.

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MATERIALS AND METHODS

Infective eggs

Gravid segments of T. pisiformis were derived from
infections in dogs autopsied at the Michigan State
University College of Veterinary Medicine. Eggs were
routinely teased from proglottids into saline containing
50 µg/ml amphotericin; 2500 µg/ml streptomycin;
333 U/ml of polymyxin B; and 1000 U/ml of penicillin G
and stored at 4°C. Egg doses were quantitated by a
simple dilution method and administered to rabbits by
stomach tube.

Experimental animals

Weanling New Zealand white rabbits were purchased
from local suppliers and given proprietary brand food
and water ad libitum. No natural infections with T.
pisiformis were observed in any animals from these
sources.

Collection of sera

Rabbits were infected per os with doses varying from
250 to 50,000 eggs of T. pisiformis and serial bleedings
were obtained at intervals as described below. Whole
blood was taken from the lateral ear vein, allowed to
clot at 22–23°C for 2–3 h and remained overnight at 4°C
before the serum was decanted, centrifuged and stored
at −20°C.

Measurement of protein concentration

Protein concentrations in antigen extracts were
determined by the method of Lowry, Rosebrough, Farr
& Randall (1951). In the case of immunoglobulin
solutions molar extinction values were used to determine
concentrations from the optical densities at 280 nm
(Williams & Chase, 1970).
Chromatography

Descending flow gel-filtration chromatography was performed on siliconized 2.5 x 100 cm columns of Sephadex G-200 (Pharmacia, Uppsala) equilibrated with 0.1 M Tris-HCl buffer pH 8.0 with 0.02 per cent azide added as a preservative. A modification of the method of Sachs & Painter (1972) was introduced in order to maintain satisfactory flow rates (25 30 ml/h) through repeated use of the columns. Six mm glass beads were siliconized 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 measured at 280 nm in a Beckman Spectrophotometer (Beckman Instrument Co., Fullerton, California). Immunoglobulin fractions were pooled and concentrated to original sample volume. These pools were dialyzed overnight against phosphate buffered saline (PBS) at 4°C and tested for their activity in passive cutaneous anaphylaxis (PCA).

The procedure for ion-exchange chromatography of immunoglobulins was as outlined by Leid & Williams (1974a). DEAE-cellulose (Cellex D., Biorad, Richmond, California) was prepared according to the directions of the manufacturer and poured into 1.5 x 30 cm siliconized glass columns. The cellulose was equilibrated against 0.01 m phosphate buffer, pH 7.7.5. Proteins were eluted in a stepwise manner using the 0.01 m phosphate buffer followed by 0.05 m phosphate, pH 5.8; 0.1 m phosphate, pH 5.8 and finally 2 m NaCl. All phosphate buffers were made 0.015 m in NaCl and the samples were dialyzed extensively against the starting 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 sample volume. These pools were dialyzed against PBS overnight at 4°C and tested for their ability to provoke PCA in sensitized rabbits.

Molecular weight determination

The approximate molecular weight of reaginic antibody was determined by the method of Andrews (1964). The Sephadex G-200 columns were calibrated using reference markers of known molecular weight (crystalline bovine serum albumin, chromatographically purified rabbit 7S immunoglobulin, chymotrypsinogen and ribonuclease).

Reduction and alkylation

Reaginic serum samples were dialyzed against 0.15 M 2-mercaptoethanol at 22°C for 2 h followed by dialysis against either 0.02 M iodoacetamide or PBS at 4°C overnight. Treated samples and similarly treated negative controls were titrated in homologous PCA tests.

Homologous passive cutaneous anaphylaxis (PCA)

The procedure for homologous PCA tests was a slight modification of that described by Zwaifler & Becker (1966). Rabbits were shaved and 0.2 ml quantities of serum or chromatographic fractions were injected intradermally (i.d.) on the back. Twenty-four to 72 h later rabbits were challenged intravenously (i.v.) with 0.8 ml of the parasite antigen preparation to be tested plus 2.2 ml of a 1 per cent solution of brilliant blue R (Biorad, Richmond, California). Reactions were read 15-60 min after challenge and graded on a scale from 0 to ++ +. Positive reactions varied from small areas of intense bluing several millimeters in diameter up to circular zones 2.5 cm or greater, which were classified ++ +. Positive and negative control sera were included in each recipient and at least 2 and usually 3 rabbits were used for each sample tested. Whenever doubtful responses had occurred the skin was reflected and viewed from the underside. Previous work in the rat—T. taeniaeformis system had indicated the superiority of brilliant blue R over Evans Blue because of its rapid clearing and the elimination of background bluing (Leid & Williams, 1974a).

Parasite extracts

Larvae of T. pisiformis from 2 to 9-month-old infections were washed in distilled water several times and homogenized in a glass tissue grinder in a minimal volume of PBS. The suspension was stirred overnight at 4°C, centrifuged at 17,000 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. Extracts were similarly prepared from adult specimens of T. pisiformis from dogs, and also from adults of T. taeniaeformis, cysticeri of T. taeniaeformis and T. crassiceps and protoscolices of Echinococcus multilocularis. Parasite cyst fluid was obtained from cysticeeri of T. hydatigena and hydatid cysts of E. granulosus. These parasites were either maintained in our laboratory according to established procedures or were acquired from natural infections at necropsy.

RESULTS

The reaginic antibody response during infection with T. pisiformis was initially detected in individual rabbits by testing in PCA sera obtained at 10 day intervals after infection. PCA reactivity was present in the first sample and continued until 50 days post-infection at which time the rabbits were sacrificed. Positive serum samples were used for studies on the physico-chemical characteristics of the antibody.

A more detailed analysis of the pattern of reagin production in a primary infection was obtained by serial bleedings of individuals at 48 h intervals during the first 10 days when reagin production was expected to begin, and also following a second, booster dose of eggs. Representative results are shown in Fig. 1. Skin fixing antibody first appeared on day 8, reached a titre of 1 : 128 on day 30 and then declined with activity persisting at 1 : 8 until a second dose of eggs was given. Variation in initial egg dosage within the range 250-50,000 appeared not to influence the time of appearance of reagin or the titres obtained. Eighty-one days after primary infection the rabbits were given 25,000 eggs of T. pisiformis per os and within 2 days a peak titre of 1 : 64 was reached which thereafter started to decline.

Approximately 50 per cent of all rabbits in these experiments produced reagins. At necropsy parasite burdens varied from hundreds of cysticeeri, to a few, and in some instances there was little or no
Fig. 1. Pattern of reaginic antibody response in rabbits infected with *T. pisiformis*. A second dose of eggs was given on day 81 (arrowed). Homologous PCA titrations were carried out with a latent period of 24–72 h.

evidence of fibrous tracts in the liver. The degree of infection did not seem to be related to the appearance of reagin and some rabbits with no detectable infection developed high titres of reagin in their serum.

The time period for optimal sensitization of the skin of recipients was determined by challenging rabbits at 4, 24, 48 and 72 h after i.d. inoculation of serial 2-fold dilutions of reaginic serum. The results are shown in Table 1. Reactivity was observed at 4 h with maximal reactivity by 48 h, although at 24 and 72 h titres were less by only a single dilution. Subsequent PCA tests were therefore performed with latent periods of 24–72 h. Recipient rabbits were also sensitized at 59, 31, 24, 17, 7 and 3 days before challenge. These results are also shown in Table 1. Titres of 1 : 32 were still evident 59 days after sensitization, and a titre of 1 : 128 was maintained over the 3–24 day period.

Heat susceptibility of reaginic antibody was established by titration of reactivity after incubation of positive serum for 1 or 2 h at 56°C. The results are presented in Table 2. Reagin activity was markedly reduced after only 1 h and was detectable only when undiluted after 2 h. The positive control titre was 1 : 256. Reactivity of reaginic serum after reduction with or without alkylation was determined by titration and the results are also shown in Table 2. Reduction with 2-mercaptoethanol alone reduced the titre to a point where only undiluted serum gave a positive reaction. Reduction followed by alkylation had a similar effect.

After ammonium sulfate fractionation of reaginic serum reactivity was absent from the globulin fraction precipitated at 33 per cent saturation but was precipitable at the 50 per cent level. Gel-filtration of the 50 per cent ammonium sulfate fraction 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. Maximum reactivity (titre 1 : 64) was seen at the beginning of the ascending portion of the second peak, indicating a molecular size slightly larger than that of the rabbit 7S immunoglobulins. An assessment of the molecular weight of reaginic antibody by gel-filtration on calibrated columns provided a value of approx. 190,000.

### Table 1—Relationship between the time of challenge after homologous sensitization with rabbit reagin and the titre of PCA activity

<table>
<thead>
<tr>
<th>PCA titre</th>
<th>4 h</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
<th>10 days</th>
<th>17 days</th>
<th>24 days</th>
<th>31 days</th>
<th>59 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neat</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>1 : 8</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>1 : 16</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
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<tr>
<td>1 : 32</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1 : 64</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1 : 128</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1 : 256</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*NT—not tested.

### Table 2—Sensitivity of rabbit reaginic antibody to *T. pisiformis* to heat and reduction and alkylation

<table>
<thead>
<tr>
<th>PCA titre</th>
<th>Control</th>
<th>56°C 1 h</th>
<th>56°C 2 h</th>
<th>Reduction</th>
<th>Reduction and alkylation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neat</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>1 : 8</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1 : 16</td>
<td>++</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>1 : 32</td>
<td>++</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>1 : 64</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>1 : 128</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>1 : 256</td>
<td>+</td>
<td>—</td>
<td>—</td>
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</tr>
</tbody>
</table>
Anion-exchange chromatography of the immuno-globulin fraction of reaginic serum on DEAE-cellulose produced an elution profile as shown in Fig. 3. The fractions indicated were pooled and concentrated to original sample volume and tested in PCA. Maximum reactivity (titre 1:16) was seen in the second peak eluted with the 0.1 M phosphate buffer, pH 5.8. However some residual reactivity was eluted with 2 M NaCl in this and duplicate experiments.

Extracts of cysticerci and adult stages of *T. pisiformis* were equally effective in provoking PCA reactions at all concentrations tested. Recipient rabbits were also sensitized with reagin and challenged with extracts prepared from other members of the family Taeniidae (Table 3). Several of these heterologous extracts provoked strongly positive PCA reactions. However it was not possible to produce PCA reactions in sensitized animals with hydatid cyst fluid of *E. granulosus* although extracts from protoscolices of *E. multilocularis* were effective.

### Table 3—Reactivity of Antigens of Taeniidae in Homologous PCA Tests with Reagin from Rabbits Infected with *T. pisiformis*

<table>
<thead>
<tr>
<th>Species</th>
<th>Adult</th>
<th>Metacystode</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. pisiformis</em></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>T. crassiceps</em></td>
<td>NT</td>
<td>+</td>
</tr>
<tr>
<td><em>T. hydatigena</em></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>T. taeniaeformis</em></td>
<td>NT</td>
<td>+</td>
</tr>
<tr>
<td><em>E. granulosus</em></td>
<td>NT</td>
<td>+</td>
</tr>
<tr>
<td><em>E. multilocularis</em></td>
<td>NT</td>
<td>+</td>
</tr>
</tbody>
</table>

* NT—not tested.
The allergenic activity in all extracts was eliminated after treatment with 10 per cent trichloroacetic acid.

DISCUSSION

Our results demonstrate the appearance of a long term skin sensitizing antibody, or reagin, in the serum of rabbits infected with larvae of *T. pisiformis*. This antibody was initially detected 8 days after primary exposure, reached peak titres during the fifth week of infection and steadily declined thereafter. Skin fixing activity of reaginic antibody was markedly decreased by heat treatment and by reduction alone or reduction followed by alkylation. Fixed antibody was evident at sensitized skin sites after only 4 h and remained there in detectable amounts for at least 8 weeks. Reactivity was eluted slightly ahead of the 7S immunoglobulins in gel-filtration, had an approx. molecular weight of 190,000 and was limited to the 0.1 M phosphate buffer and 2 M NaCl eluates in anion-exchange chromatography. In addition reaginic activity was precipitated by (NH₄)₂SO₄ at 50 per cent saturation but not at the 33 per cent level. All these data on the physico-chemical and biologic properties of this homologous skin sensitizing antibody are consistent with those described for antibodies of the rabbit immunoglobulin class designated IgE by Zvaifler & Robinson (1969), Ishizaka, Ishizaka & Hornbrook (1970) and Kravis & Zvaifler (1974). Thus cysticercosis must be added to the list of natural parasitic infections of the rabbit in which reaginic antibodies are produced (Sadun, Duxbury, Gore & Stechschulte, 1967; Ford, 1971).

We found no evidence for the presence of skin sensitizing antibodies of the IgG type described by Henson & Cochrane (1969), and the primary antibody response in our rabbits was not exclusively of the IgG type, as suggested by Nemeth (1973). Under these circumstances it seems reasonable to propose that the immediate hypersensitivity reactions observed by Wharton (1931) in infected rabbits were mediated by reaginic antibodies. However, our results indicate that the allergen or allergens which provoke this response are shared with other members of the family Taeniidae, whereas Wharton found his skin tests to be specific for *T. pisiformis*. Possibly this may have resulted from differences in the extraction procedures which we used to prepare antigens from heterologous species. Positive reactions were obtained with extracts of both adult and larval stages of several taenid cestodes and, while this is indicative of sharing of antigens between developmental phases, it does not preclude the possible occurrence of several allergenic substances. At least two and possibly three allergens are known to occur in *T. taeniaeformis* (Leid & Williams, 1974a).

The brisk reaginic antibody production which we detected early in infection must be taken into account in the interpretation of the immunologic response of the rabbit to the migrating oncosphere and the initial post-oncospheral reorganization. This phase is considered by Heath (1973b) to be most important in the development of acquired resistance. He was able to show that some rabbits developed a solid immunity by 9 days after infection and it seems likely that their sera may have contained IgE antibodies by this time. In the case of *T. taeniaeformis* in the rat we have been able to transfer resistance with immunoglobulin fractions devoid of IgG and containing antibodies exclusively of the IgG₂ type (Leid & Williams, 1974b). In the mouse passive transfer of resistance can be achieved with fractions containing IgG₁ antibodies but lacking in IgE (Musoke & Williams, In press). In both the rat and mouse reaginic antibodies appear in the serum during the first several weeks of infection, and direct immediate hypersensitivity skin tests become positive before circulating reagins appear. Possibly, although not crucial to the success of the protective response, tissue-fixed IgE antibodies, when present, act in concert with other immunoglobulin types to enhance resistance. In this regard it would be interesting to know which immunoglobulin types are associated with acquired resistance to *T. pisiformis* in the rabbit.

The observations of Heath (1970) are particularly significant in relation to the latter point. He found that oncospheres failed to penetrate the intestinal epithelium in immune rabbits, raising the possibility that antibodies present in the intestinal lumen could be responsible for protection. In our studies immune rabbits exposed to challenge doses of eggs of *T. pisiformis* rapidly responded with increased production of reagin and we feel that this may have been derived from the stimulation of IgE production at the intestinal mucosal level. IgE producing cells are generally present in large numbers at mucosal surfaces (Tada & Ishizaka, 1970), and this fact, combined with other evidence implicating the intestine as the site of resistance to cysticercosis (Leonard & Leonard, 1941), suggests that IgE may indeed participate in the mechanism of immunity to *T. pisiformis*. We visualize that its role may be ancillary to the primary and specific effector mechanisms involving other immunoglobulin types. However its function as a mediator of antigen-induced amplification systems could be of considerable importance in the overall success of the acquired resistance which characterizes cysticercosis in laboratory animals.

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REFERENCES


