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Autoimmunity in Trypanosome Infections

I. TISSUE AUTOANTIBODIES IN *TRYPANOSOMA* (TRYPANOZOON) *BRUCEI* INFECTIONS OF THE RABBIT

A. R. MacKenzie* and P. F. L. Boreham

Imperial College Field Station, Silwood Park, Ascot, Berkshire

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Summary. Ten rabbits infected with *Trypanosoma* (Trypanozoon) *brucei* showed a substantial increase in a natural anti-tissue autoantibody and Wassermann antibody. Absorptions suggest that the liver and Wassermann antibodies are distinct. The liver antibody reacts equally well with homologous and autologous liver. Absorption with trypanosomes and liver show that cross-reacting trypanosomal antibodies are not responsible for the liver activity. These antibodies will contribute to the raised IgM levels of rabbit trypanosomiasis and may be important in this respect but the precise extent of the contribution is not known.

It is suggested that a depression of certain T-cell functions may release antibody secreting B-cell descendants from T-cell control resulting in elevated IgM.

INTRODUCTION

In common with many other chronic parasitic diseases it is difficult to identify the exact cause of death in African trypanosomiasis. While it seems likely that infected rabbits finally die in a state of severe vascular shock (Goodwin and Hook, 1968), the events leading up to this are poorly understood.

Immune complexes and subsequent release of kinins and other pharmacologically active substances has been suggested by Boreham (1968a) as possibly contributing to the state of shock. In addition immune complexes may have a direct effect similar to an Arthus Type III reaction on the glomeruli of the kidney (Boreham and Kimber, 1970). These pathological mechanisms may be thought of as examples of the immune response becoming out of control. Seed and Gam (1967) however have suggested that autoimmunity may contribute to the pathogenesis of the disease. They described an anti-liver autoantibody in *Trypanosoma* (Trypanozoon) *gambiense* infections of rabbits which reached a peak around day 18 of the infection and then slowly declined. Seed and Gam used the complement fixation test for most of their work and considered titres less than 1/8 as negative. Precipitin tests showed a single line against the liver antigen for post-infection sera and none for pre-infection sera.

The rabbit, however, has been shown to possess natural antibodies to many different antigens, amonst them an autoantigen present in many tissues including the liver (Kidd and Friedewald, 1942a, b). The antigen sedimented at high gravity forces and was labile

* Present address and correspondence: Dr A. R. MacKenzie, Pathology Department, Allen & Hanburys Ltd, Ware, Hertfordshire.

at 65°. The antibody appears to be present in all rabbits older than 1 month (Kidd and Friedewald, 1942a) although the titres they found were not high.

Muschel, Simonton, Wells and Fife (1961) also reported increases in the anti-tissue antibody in a number of disease states including *T.* (T.) *gambiense* and *T.* (T.) *rhodesiense* infections of rabbits. More recently Mansfield and Kreier (1972) made a study of auto-immunity to tissue antigens in *T.* (N.) *congolense*-infected rabbits. They looked for both autoantibody production and evidence for cell-mediated immunity. Their results show that complement fixing and precipitating autoantibodies occur which react with brain, liver, heart and kidney. Delayed hypersensitivity skin reactions, macrophage inhibition factor (MIF) production and skin reactive factor (SRF) could not be demonstrated. Antibodies to Wassermann antigens could not be consistently found by any of the three groups of workers.

Tissue autoantibodies have been found in rats following the administration of carbon tetrachloride (Weir, 1963; Pinckard and Weir, 1966) and Asherson and Dumonde (1962, 1964) have shown that increased titres of anti-tissue autoantibody in rabbits may be produced by immunization with heterologous liver and, to some extent, homologous liver. Asherson and Rose (1963) on the other hand have demonstrated a natural increase in anti-liver titres due to coccidial infection.

The present work investigates the increase in anti-tissue antibody during *T.* (T.) *brucei* infections of the rabbit and considers how it may contribute to the pathogenesis of African trypanosomiasis.

MATERIALS AND METHODS

Animals

Adult New Zealand White rabbits of either sex weighing 2·5–4·0 kg were obtained from A. E. Moss, White Cloud Farm, Tring, Herts.

Trypanosomes

Two laboratory strains of *T.* (T.) *brucei*, S42 and 427 were used, both of which were obtained from the Lister Institute of Preventive Medicine and subsequently maintained in this laboratory by blood passage through mice every 2–3 days. Strain S42 kills rabbits in 2–4 weeks while strain 427 normally kills in 4–6 weeks although occasional rabbits become asymptomatic after 4 or 5 weeks and survive for much longer periods.

Rabbits were infected by subcutaneous injection of 5×10^7 to 2×10^8 trypanosomes either separated from mouse blood and washed in buffer or in whole mouse blood.

Sera

Samples of blood were collected from the marginal ear vein of the rabbits and allowed to clot at 37° for 3–4 hours. The serum was then removed and centrifuged. Sera were stored in 2·0 ml plastic tubes at −20° until required. Before use in the complement fixation test, sera were heated at 56° for 30 minutes to destroy complement.

Antigens

Rabbit livers were perfused for 5 minutes with cold normal saline to remove most of the blood and were frozen at −20°. When required 1·0 g wet weight of liver was homogenized in a ground glass homogenizer in 4·0 ml of saline or complement fixation diluent

(CFT diluent, Oxoid) i.e. a 1/5 homogenate. After centrifugation at 32,000 ***g*** for 15 minutes the supernatant was removed and stored at −20°. Rabbit kidney antigen was prepared in a similar way.

Trypanosomal antigens were prepared by freezing and thawing a suspension of washed separated trypanosomes containing 1×10^9 trypanosomes/ml in CFT diluent. This preparation was diluted 1/20 for use in the complement fixation test (CFT).

Wassermann antigen was obtained from Wellcome Reagents Ltd. It consisted of an alcoholic extract of sheep heart with added cholesterol. A 1/150 dilution in CFT diluent was used in the CFT.

Agar gel double diffusion test

Tests were carried out as described by Boreham (1968a) and the plates normally read after 72 hours.

Complement fixation test (CFT)

The CFT was performed using Microtitre plates and 25 μl-microdiluters. The method of Bradstreet and Taylor (1962) was used with the exception that an arbitrary dilution of antigen (1/20) was employed. Sheep red blood cells, preserved guinea-pig serum and horse haemolytic serum were obtained from Wellcome Reagents Ltd. A 4 per cent suspension of the indicator cells was sensitized with an equal volume of 1/100 haemolytic serum. Three CH_{50} of complement were used and fixation was allowed to occur for 30 minutes at 37°. Haemolysis occurred for 30 minutes at 37° and the cells were allowed to settle for 1 hour at 4° before the plates were read.

Titres were taken as the reciprocal of the highest dilution of serum showing 50 per cent haemolysis in a system comprising equal volumes of serum, antigen, complement and 2 per cent sensitized sheep red blood cells. Unless otherwise stated normal liver was used as the antigen in the tests relating to anti-liver activity.

Gel filtration

Two-millilitre samples of serum were separated on Sephadex G-200 using an 0·1 M Tris/HCl buffer, pH 8·0 with 1·0 M NaCl. A pressure of 150 mm was maintained and the flow rate was approximately 40 ml/hour. Three or 5 ml fractions of eluate were collected and the absorbance at 280 nm measured in a Beckman DB spectrophotometer. The fractions were pooled according to the major peaks and concentrated by lyophilization after overnight dialysis against cold 0·15 M phosphate buffer, pH 7·3.

Inactivation of IgM

Heat inactivation. Sera were heated at 65° for 30 minutes.

2-*Mercaptoethanol inactivation.* Sera, diluted 1/5 with 0·15 M phosphate buffer, pH 7·3, were treated with an equal volume of 0·2 M 2-mercaptoethanol in buffer, for 3 hours at room temperature. This was followed by overnight dialysis against cold 0·02 M monoiodoacetic acid in 0·15 M phosphate buffer, after which a further 3 hours dialysis against 0·015 M phosphate buffer, pH 7·3, was allowed before freeze drying and reconstitution to the original volume of the undiluted serum with distilled water.

A control was included for each serum in which buffer was substituted for 2-mercaptoethanol. The rest of the treatments were identical.

Absorptions

Trypanosomes. $2{\cdot}5 \times 10^9$ trypanosomes (*T.* (T.) *brucei* strain 427) were frozen to $-20°$ and then thawed to disrupt the trypanosomes. After centrifuging at 38,000 ***g*** for 30 minutes the supernatant was discarded and 1 ml of serum was mixed with the sediment and incubated at 37° for 1 hour, followed by 1 hour at 4°. At the end of this period the mixture was centrifuged at 38,000 ***g*** for 30 minutes and the absorbed serum recovered.

Alternatively an equal volume of whole trypanosome homogenate containing 1×10^9 trypanosomes/ml was added to the undiluted serum. After 1 hour at 37° and 1 hour at 4° the mixture was centrifuged at 38,000 ***g*** for 20 minutes and the supernatant retained. The process was repeated twice on the supernatant giving a final dilution of 1/8. A control was taken through each step substituting CFT diluent for trypanosomes.

Liver. Sera diluted 1/4 with CFT diluent were mixed with an equal volume of a 1/5 liver homogenate in CFT diluent and incubated for 1 hour at 37° followed by 1 hour at 4°. A control consisting of an equal volume of CFT diluent and 1/4 serum was treated similarly. The mixture was centrifuged at 38,000 ***g*** for 20 minutes and the supernatant retained. The supernatant was diluted to the same volume as the control with CFT diluent, to maintain a known dilution. This procedure was carried out a second time giving a final dilution of 1/16 for both absorbed and control sera.

Kidney. Sera diluted 1/4 with CFT diluent were treated in the same way as the liver absorption, but substituting kidney homogenate for liver.

Wassermann. Wellcome alcoholic sheep heart preparation was evaporated to dryness and the solids resuspended in CFT diluent. After any absorption the sera were heated to 56° for 30 minutes and centrifuged at 2000 ***g*** for 15 minutes.

Serum aminotransferase levels

Aspartate aminotransferase (ASAT; GOT) and alanine aminotransferase (ALAT; GPT) serum levels were measured by Biochemica Test Combinations colorimetric method (Boehringer Corporation). The results are expressed as mu/ml.

RESULTS

ANTI-LIVER TITRES

Ten rabbits were examined, six infected with the 427 strain (S163, S164, S184, S185, S203 and S216) and four with the S42 strain (S176, S177, S193, and S199). Sera from several additional rabbits were used in some absorption and separation experiments. The antibody levels were also measured in six normal rabbits of various ages.

Complement fixation tests for anti-liver activity were performed on sera collected from the rabbits at intervals during the infection. The anti-complementary titre was also measured to ensure the validity of the anti-liver titres. The anti-liver titre was considered significant only if it was at least 2 $\log_2$ units greater than the anti-complementary titre. The significance of differences between anti-liver titres of different sera was similarly assessed. The results are shown graphically in Fig. 1.

AUTOIMMUNE NATURE OF THE ANTIBODY

Rabbit S216 was also treated for anti-liver activity against it own liver homogenate

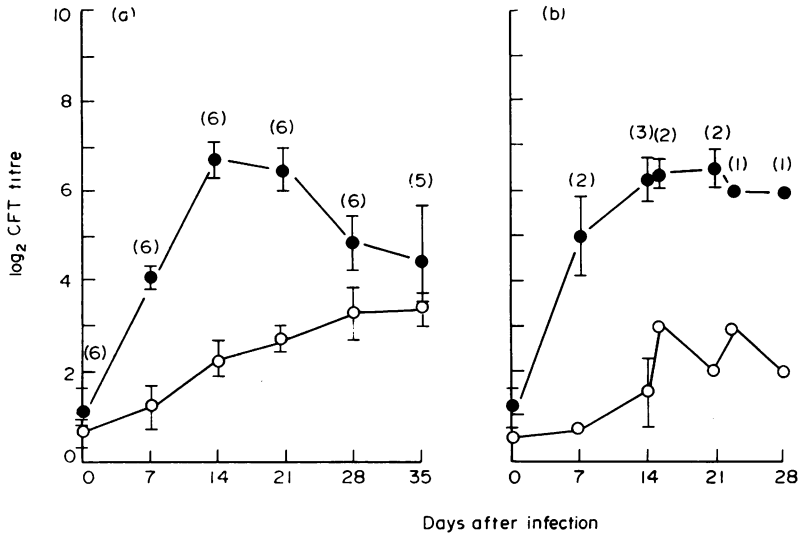

FIG. 1. (a) Strain 427. (b) Strain S42. (●) Anti-liver and (○) anti-complementary complement fixation titres of rabbits infected with *T.* (T.) *brucei*. Numbers in parentheses refer to the number of observations for each point. Sera collected from a further six control rabbits had the following titres: 1/1; 1/2; 1/4; 1/4; 1/4; and 1/16.

to show that the antibody was indeed autodirected. The titres obtained were comparable with those using normal liver homogenate as shown in Table 1.

ANTI-WASSERMANN ANTIBODY

Serial samples of serum from two rabbits S203 and S216 were examined for antibodies to Wassermann antigen by the CFT. Fig. 2 shows these results indicating a strong reaction with a similar time course to the anti-liver response. The possibility therefore existed that the anti-liver activity was in fact due to anti-Wassermann antibodies reacting with cardiolipin in liver. To learn something of the specificity of the antibodies absorption studies were made.

ABSORPTION STUDIES

Trypanosomes

Rabbit S203 day 38 serum and rabbit S216 day 22 serum were absorbed with trypanosomes and then tested by CFT against liver antigen and trypanosome antigen. The results are displayed in Table 2. A significant reduction in titre occurs only with the

TABLE 1

COMPARISON OF ANTI-LIVER COMPLEMENT FIXATION TITRES OF RABBIT S216 SERA INFECTED WITH *T.* (T). *brucei* 427 AGAINST HOMOLOGOUS AND AUTOLOGOUS LIVER HOMOGENATE

	Day						
Antigen	0	2	7	14	22	28	33
Homologous	1	4	16	128	128	64	32
Autologous	1	4	16	128	128	64	64

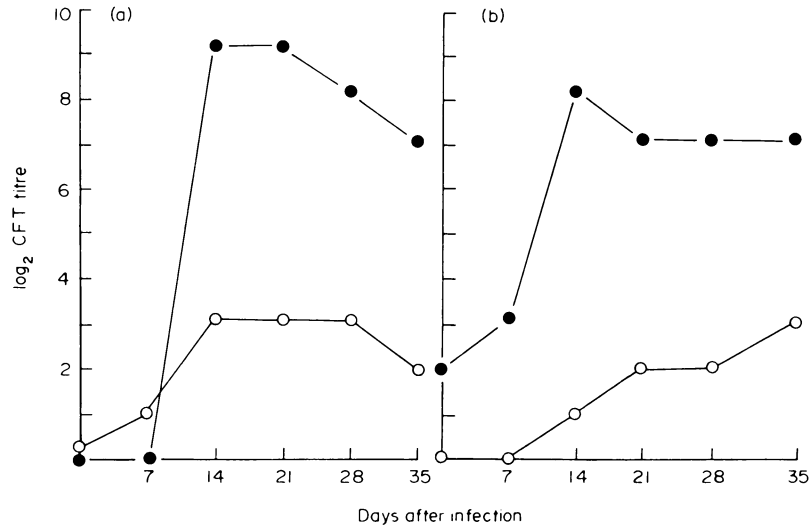

FIG. 2. (a) Rabbit S203. (b) Rabbit S216. (●) Anti-Wassermann and (○) anti-complementary complement fixation titres in two rabbits infected with *T.* (T.) *brucei* 427.

TABLE 2

EFFECT OF ABSORPTION WITH TRYPANOSOME HOMOGENATE ON ANTI-LIVER AND ANTI-TRYPANOSOME COMPLEMENT FIXATION TITRES OF TWO RABBITS INFECTED WITH *T.* (T.) *brucei* 427

	S203 Day 38		S216 Day 22	
	Absorbed	Control	Absorbed	Control
Anti-trypanosome titre	32	512	32	512
Anti-liver titre	32	64	128	256
Anti-complementary complement-fixation titre	16	16	32	16

TABLE 3

EFFECT OF ABSORPTION WITH LIVER HOMOGENATE ON ANTI-LIVER AND ANTI-TRYPANOSOME COMPLEMENT FIXATION TITRES

	Serum							
Antigen	S163 Absorbed	D. 21 Control	S176 Absorbed	D. 21 Control	S177 Absorbed	D. 14 Control	S216 Absorbed	D. 22 Control
Anti-liver titre	32	256	32	128	32	128	32	256
Anti-trypanosome titre	64	128	16	16	16	16	128	128
Anti-complementary complement-fixation titre	16	16	16	16	< 16	16	< 16	16

trypanosome antigen, suggesting that the anti-liver activity is not due to cross-reacting anti-trypanosomal antibody.

Liver

Sera absorbed with liver were found to lack anti-liver activity but to retain most of their anti-trypanosome activity. Table 3 shows the results of these experiments. The selective absorption of the anti-liver activity further supports the view that it is a true autoantibody.

TABLE 4

EFFECT OF ABSORPTION WITH LIVER AND KIDNEY HOMOGENATES ON THE COMPLEMENT FIXATION TITRES OF INFECTED SERA TO LIVER AND KIDNEY ANTIGENS

Absorbent	Serum	Complement fixation titres		
		Anti-liver	Anti-kidney	Anti-complementary complement-fixation titre
Liver	S192 Day 11	16 (4)*	32 (4)	16
Control	S192 Day 11	256	512	64
Liver	S185 Day 14	128 (2)	16 (2)	8
Control	S185 Day 14	512	64	16
Kidney	S192 Day 15	64 (2)	128 (2)	64
Control	S192 Day 15	256	512	64

* Numbers in parentheses are the reduction in titre expressed as $\log_2$ units.

TABLE 5

RESULTS OF ABSORPTION EXPERIMENTS WITH LIVER HOMOGENATE AND CARDIOLIPIN S290 = DAY 35 INFECTED SERUM; S291 = DAY 28 CONTROL SERUM; S292 = DAY 35 INFECTED SERUM

Absorbent	Serum	Complement-fixation titres		
		Anti-liver	Anti-Wassermann	Anti-complementary complement-fixation titre
Liver	S290	32 (3)	256 (1)	16
Cardiolipin	S290	256 (0)	1024 (0)	64
Control	S290	256	512	32
Liver	S292	32 (4)	512 (1)	32
Cardiolipin	S292	128 (2)	256 (2)	32
Control	S292	512	1024	64
Liver	S291	8 (1)	16 (0)	8
Cardiolipin	S291	16 (0)	16 (0)	8
Control	S291	16	16	4

* Numbers in parentheses are the reductions in titre expressed as $\log_2$ units.

Kidney

Absorption with kidney homogenate also removed anti-liver activity (Table 4) suggesting that the antigen is not organ-specific.

Wassermann

Table 5 shows the effect of absorption with cardiolipin and liver antigen on liver and Wassermann titres. While the liver appeared to be quite specific absorbing out liver antibody but leaving the Wassermann titre intact, evaporated Wassermann antigen seemed to be a rather poor absorbent. The results, however, suggest that the antigens are distinct.

TITRATION OF LIVER ANTIGEN

Doubling dilutions of the antigen were prepared and the complement fixation titre with S216 day 22 serum measured at a sensitivity of 3 C^1H_{50}'s. The serum was used at 1/64, i.e. the highest dilution showing complete fixation with excess antigen. A titre of 1/1280 was obtained with 1/640 being the last well showing complete fixation. Therefore the antibody in 1 volume of 1/64 was completely fixed by 1 volume of 1/640 antigen, while the antibody in 1 volume of neat serum would be completely fixed by 1 volume of 1/10 antigen or approximately 1 ml of serum to 0·1 gram of liver.

If the weight of a rabbit liver is taken as 150 grams then it is capable of absorbing 1500 ml of serum taken at the peak of the antibody response. Since the plasma volume of an adult rabbit infected with *T.* (T.) *brucei* is between 140 and 200 ml at day 10 (Boreham, 1968b) the absorptive capacity of the liver is nearly one order of magnitude in excess of antibody. This is undoubtedly an underestimate since not all of the antigen in the liver is extracted during the preparation of the antigen.

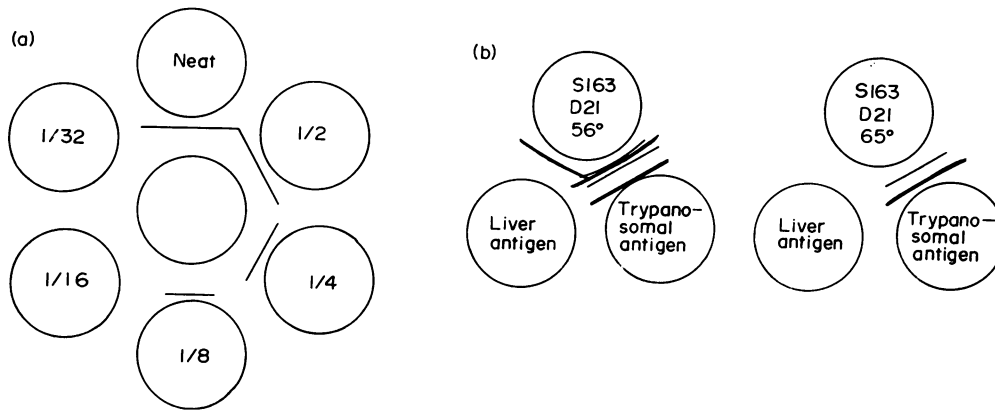

FIG. 3. (a) Drawing of a double diffusion test with serial dilutions of day 21 serum from rabbit S163 (outer wells) against a saline extract of normal rabbit liver (centre well). (b) The effect of heating to 56° and 65° on the precipitating antibodies to liver antigen and trypanosomal antigen.

PRECIPITIN TEST

Agar gel double diffusion tests were performed on the sera from rabbits S163, S164, S176 and S177. In most cases a single band, close to the serum well, developed against a liver antigen. On a few rare occasions a second band was detected. The band was distinct in all preinfection sera and in sera collected during the infection. When sera taken before the infection were diluted more than half the band did not develop. Sera collected between day 14 and day 21 however developed a band at $\frac{1}{8}$ dilution. Dilution of the serum within this range made little difference to the position of the band. Each serum tested gave a similar line irrespective of whether homologous or autologous antigen was used. Fig. 3 shows a typical plate.

MACROGLOBULIN NATURE OF THE ANTIBODIES

The position of the precipitin band and its apparent independence of the antibody concentration suggests it is retarded by the gel and may therefore be a macroglobulin. This is supported by the following experiments.

Gel filtration

When both normal and infected sera from two additional rabbits were separated on Sephadex G-200 the anti-liver and anti-Wassermann activity appeared only in the macroglobulin fraction except in the case of the Wassermann activity of the infected serum. In this case a small proportion of the activity was found between the γ-globulin and albumin peaks. The results of the separation are shown in Fig. 4.

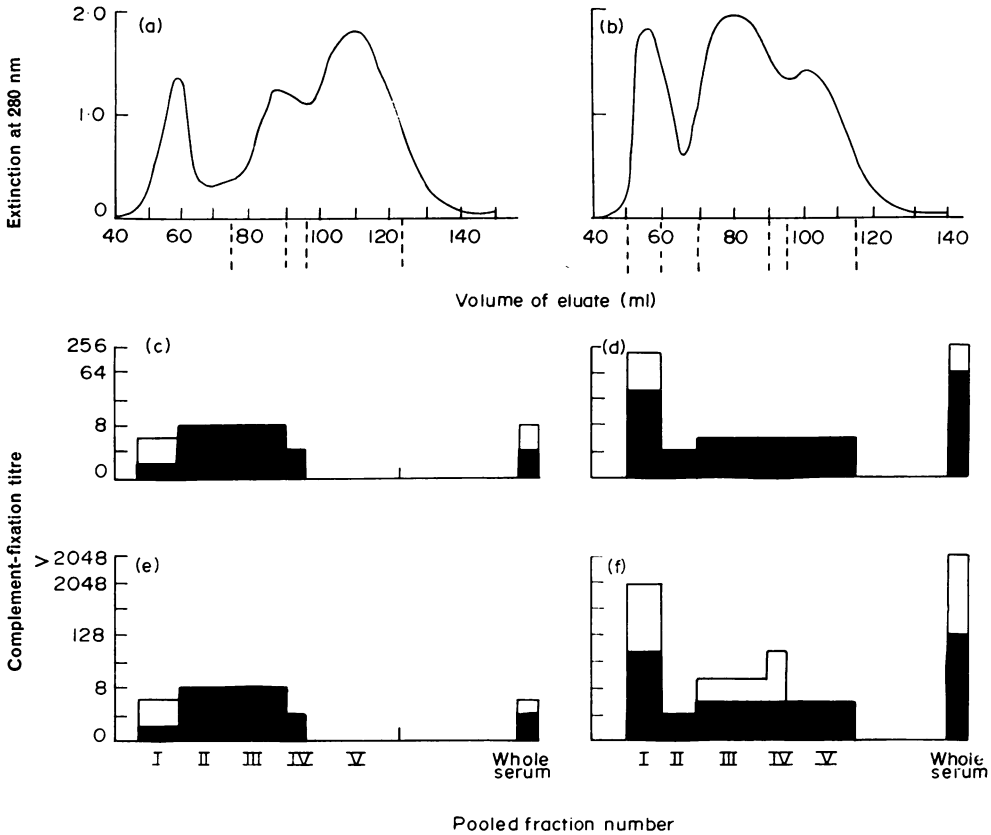

FIG. 4. Sephadex G-200 separation of normal rabbit serum and serum from an infected littermate at day 35. (a) Protein distribution of normal rabbit serum, as measured by u.v. absorbance at 280 nm. (b) Protein distribution of infected serum, as measured by u.v. absorbance at 280 nm. (c) Anti-liver activity of normal rabbit serum, as measured by CFT. (d) Anti-liver activity of infected serum, as measured by CFT. (e) Anti-Wassermann activity of normal rabbit serum, as measured by CFT. (f) Anti-Wassermann activity of infected serum, as measured by CFT. In (c)–(f)(■) shows the level of anticomplementary activity in the fractions and (□) shows the specific activity.

Heat lability.

Heating sera to 65° for 30 minutes destroyed all anti-liver activity of both preinfection and postinfection sera whilst only partially reducing the anti-trypanosome titre. Both precipitin results (Fig. 3) and CFT results (Table 6) support this finding.

2-*Mercaptoethanol reduction and alkylation*

The serum from rabbit S216 lost all its activity to liver and Wassermann antigen in the CFT after mild reduction and alkylation strongly suggesting that the antibody is IgM. The results are shown in Table 7.

TABLE 6

COMPLEMENT FIXATION TITRES OF INFECTED AND CONTROL (DAY 0) SERUM AFTER HEATING TO 56° OR 65° FOR 30 MINUTES

Days infected	Complement fixation titres							
	S163 Anti-liver		S164 Anti-liver		S203			
					Anti-liver		Anti-trypanosome	
	56°	65°	56°	65°	56°	65°	56°	65°
0	4 (2)*	<1	2 (<1)	0	1 (0)	0 (0)	2 (0)	0 (0)
7	16 (4)	<1	16 (2)	0	16 (1)	0 (0)	16 (1)	1 (0)
14	64 (4)	1	64 (8)	<1	128 (8)	1 (0)	512 (8)	32 (0)
21	256 (4)	2	64 (8)	1	64 (8)	1 (1)	64 (8)	32 (1)
28	128 (4)	2	32 (16)	1	16 (8)	1 (1)	128 (8)	128 (1)
35	128 (8)	1	32 (16)	<1	16 (8)	2 (1)	256 (8)	256 (1)

* Numbers in parentheses are the corresponding anti-complementary titres.

TABLE 7

EFFECT OF REDUCTION AND ALKYLATION ON THE ANTI-LIVER, ANTI-WASSERMANN, ANTI-TRYPANOSOME AND ANTI-COMPLEMENTARY COMPLEMENT FIXATION TITRES OF SERA FROM RABBIT S216 INFECTED WITH *T.* (T.) *brucie* 427

Day	2-ME and alkylation treatment				Alkylation only control treatment			
	Anti-liver titre	Anti-Wassermann titre	Anti-trypanosome titre	Anti-complementary titre	Anti-liver titre	Anti-Wassermann titre	Anti-trypanosome titre	Anti-complementary titre
0	2	2	2	2	4	4	2	<2
7	<2	<2	<2	<2	16	8	8	2
14	2	4	<2	2	64	128	128	4
22	<2	4	NT	<2	64	64	NT	8
28	2	4	NT	<2	32	64	NT	4
33	2	8	8	2	16	64	32	8

* NT = not tested.

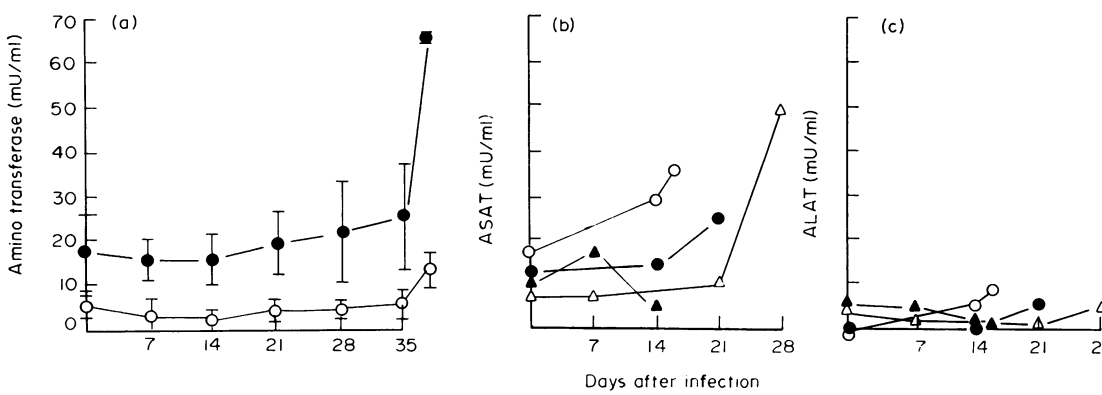

FIG. 5. Serum aminotransferase activity in rabbits infected with *T.* (T.) *brucei*. (a) Strain 427. (○) Alanine aminotransferase. (●) Aspartate aminotransferase. (b) Strain S42, aspartate aminotransferase. (○) S176. (●) S177. (▲) S193. (△) S199. (c) Strain S42, alanine aminotransferase. (○) S176. (●) S177. (▲) S193. (△) S199.

AMINOTRANSFERASE LEVELS

In an attempt to discover whether or not the anti-liver antibody caused any damage to the liver, serum ASAT and ALAT levels were measured. Fig. 5 shows that although there is a marked rise in ASAT levels in most cases near the terminal stages of the disease, the rather modest increase in ALAT suggests that little of the former is derived from the liver since the ratio ASAT/ALAT is less than unity in liver diseases except carcinoma and active or progressive Laennec cirrhosis (Sunderman and Sunderman, 1968). The low normal values for ALAT however make the results difficult to interpret, but the indications are that very little damage occurs to the liver except in the terminal stages.

DISCUSSION

The normal levels of anti-liver autoantibody and Wassermann antibody found in rabbits are raised during a chronic *T.* (T.) *brucei* infection by 4 or 5 $\log_2$ units. Since both infected autologous and uninfected homologous liver antigen gave similar results it may be deduced that the effective antigen is not trypanosomal material lying in the liver and that the antigen is a true autoantigen.

Cross-reaction of a liver component and trypanosomes is excluded by the absorption studies with liver and trypanosomes. The autoantibody to liver and Wassermann antibody appear to be separate entities since liver homogenate was capable of absorbing out the former but not the latter.

The pattern of the response is similar to that observed by Seed and Gam (1967) during *T.* (T.) *gambiense* infections. An important difference however is that Seed and Gam were unable to demonstrate the natural antibody by precipitin test before infection. The CFT titres they observed before infection were however up to $\frac{1}{8}$ but these were considered negative because of anti-complementary activity at lower dilutions. A possible explanation of this discrepancy could lie in the breed of rabbit used. The time course in the present experiments is however different from that described by Mansfield and Kreier (1972) using *T.* (N.) *congolense* infections of the rabbit, in which the peak was reached at 28 days.

The position of the precipitin lines, behaviour on Sephadex G-200, heat lability and 2-mercaptoethanol sensitivity of sera throughout the infection suggest that only IgM antibody is involved at all stages of the infection. This is in contrast to the auto-antibody increase due to immunization with rat or rabbit liver (Asherson and Dumonde, 1962, 1964) where the anti-liver activity after immunization could be divided into stable and labile components at 65°. The natural autoantibody, however, normally consists entirely of the 65° labile component, although some rabbits appear to possess heat-stable natural autoantibody (Asherson and Dumonde, 1964). It would be of interest to observe the effect of a trypanosome infection in such rabbits as the results could shed some light on the reasons for the apparent inability of the rabbits used in the present work to switch to IgG synthesis with respect to anti-liver antibody. Although this could reflect a shortcoming of the tests, e.g. antibodies belonging to a subclass of IgG which do not fix complement well, this is not thought to be the case since two different tests gave similar results. It is perhaps worth noting that in Mansfield and Kreier's work (1972) and in the present work a second line was sometimes seen in the precipitin tests against liver antigen, but it is not known whether it represents a second antigen or a different class of antibody.

It has been realized for some time that only a small proportion of the immunoglobulin found in the sera of infected animals is specific for trypanosomes and recently Freeman, Smithers, Targett and Walker (1970) have shown in the Rhesus monkey that the specific IgG may be as little as 5 per cent. Similarly the very high IgM levels found in trypanosomiasis of man, cattle, monkey and rabbits cannot be accounted for by trypanosome-specific antibody alone. The anti-tissue and Wassermann autoantibodies appear to account for a part of the non-trypanosome-specific IgM although probably a very small proportion of the whole.

Other antibodies of defined specificity apart from anti-trypanosomal antibodies are known which probably account for more of the elevated IgM. Cold agglutinins have been reported by Yorke (1911) to occur in trypanosome infections of man and several other animals including rabbits. High titres of anti-Forssman-like antibodies and rheumatoid factor-like globulins are also seen in man and Rhesus monkeys (Houba and Allison, 1966; Houba, Brown and Allison, 1969).

Conflicting evidence appears in the literature regarding the occurrence of anti-Wassermann antibodies during trypanosome infections. Rabbits infected with 'Nagana' trypanosomes developed a raised antibody titre (Browning and McKenzie, 1911). It is not clear whether this refers to *T.* (N.) *congolense* or *T.* (D.) *vivax* infections, although the possibility that *T.* (T.) *brucei* was also involved cannot be excluded. Using alcoholic extracts of *T. equiperdum* Landsteiner and Van der Scheer (1927) were able to induce anti-Wassermann antibody in rabbits. The different methods of measuring the antibody may account for the discrepancy.

Some of the anti-complementary activity is associated with the IgM peak on Sephadex G-200 and could therefore be immune complexes. Most of it however appears in the γ-globulin peak. It is possible that the activity is due to an immunoconglutinin shown to occur in *T.* (T.) *brucei* infections of the rabbit by Ingram and Soltys (1960). Alternatively an increase in a heterophile antibody to a component of guinea-pig serum (the complement source) may be fixing complement.

The polyclonal macroglobulinaemia which develops in trypanosomiasis appears to consist partly of trypanosomal antibody and partly of increased levels of natural IgM antibodies of which anti-liver and anti-Wassermann antibodies are a part.

The reason for the increased synthesis of natural antibody during a trypanosome infection is not known but several possibilities exist. Minor tissue damage may result in the release of liver and Wassermann antigens. This however is considered unlikely since the injection of homologous liver into rabbits by Asherson and Dumonde (1964) gave only a slight, somewhat equivocal rise in anti-liver antibody. In the rat, however, Weir (1963) was able to produce liver autoantibodies by inducing liver damage with carbon tetrachloride. This possibility must therefore remain open. The absorption studies appear to rule out the existence of shared antigenic determinants. The trypanosomes may act as carriers for host haptens particularly in the case of cardiolipin. A major objection to this mechanism is that it implies that a T cell-dependent response takes place which would lead to IgG synthesis, something not found experimentally.

Non-specific antibody occurs to some extent even when immunization to a defined antigen is induced (Askonas and Humphrey, 1958). In trypanosomiasis the successive waves of antigenically different variants may intensify the non-specific reaction. While this provides an analogy it still does not provide a mechanism.

A general 'adjuvant' effect of the trypanosomes seems possible. If this is so, the adjuvant

effect is a very special kind not operating through the T-cell system as appears to occur with Freund's complete adjuvant (Allison and Davies, 1971). A preferential B-cell mitogen, like Pokeweed mitogen (Parkhouse, Janossy and Greaves, 1972) is possible but certainly not demonstrated to date. Mansfield and Kreier (1972) suggest that a thymic malfunction may allow the B-cells to escape from a T cell-induced control system. Controlled low levels of liver and Wassermann antibodies are thereby allowed to rise. In support of a thymic deficiency is the observation that in experimental *T.* (T.) *brucei* infections of young rabbits a runting syndrome develops with severe involution of the thymus (MacKenzie, unpublished).

In mice, however, strictly cell-mediated functions of the T-cell system appear intact in chronic *T.* (T.) *brucei* (Murray, Urquhart, Murray and Jennings, 1973; Freeman, Hudson, Longstaffe and Terry, 1973. This is not to say that co-operative and controlling functions are intact. A thymic deficiency of this kind would account well for the lack of a switch to IgG synthesis.

The contribution, if any, of the anti-liver autoantibody to the pathogenesis of trypanosomiasis appears to be slight. The antibody is present in normal rabbits where it presumably does no harm and there is no reason to believe it can do otherwise in the infected animal. Further, it has been shown that liver contains sufficient antigen to absorb about 1500 ml of serum taken at the peak of the anti-liver response. If all the antigen were available to the circulation it should not be possible to detect antibody. Johnson, Asherson, Kaklamanis and Dumonde (1963) have shown by the fluorescent antibody technique that no *in vivo* fixation of anti-tissue antibody occurs in rabbits immunized with rat liver. The serum transferase levels suggest that no gross liver damage occurs since the ASAT levels are much higher than the ALAT levels and this is not typical of liver damage. Furthermore there is a complete lack of correlation between ASAT and ALAT levels, particularly in rabbits S163 and S164 in which no rise in the transferase levels occurred but which, nevertheless, showed a typical anti-liver response. If the antibody caused damage, significant serum transferase levels would be expected before the antibody peak. It is likely that the ASAT derives either from the trypanosomes themselves (Godfrey and Kilgour, 1973) or more probably from the heart since myocarditis has been reported in human sleeping sickness by Bertand, Baudin, Vacher, Sentillies, Ducasse and Veyret (1967). This is supported by histological evidence where no gross liver damage can be seen but to date no electron microscopical study has been undertaken.

Indirectly the liver and Wassermann autoantibodies may have some pathological effects. First, they contribute to the raised IgM content of the serum which is likely to lead to an increased erythrocyte sedimentation rate and to increased viscosity of the serum, causing considerable circulatory embarrassment. Haemodilution may counteract the latter effect (Fiennes, 1954; Boreham, 1967). Second, immune complexes may form leading to release of pharmacologically active substances locally or at a distance resulting in an inevitable vicious circle (Boreham, 1968a).

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