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A STUDY OF THE BIOLOGICAL PROPERTIES OF TRYPANOSOMA RHODESIENSE AND TRYPANOSOMA GAMBIENSE

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by

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ABSTRACT

A. Three enzymes, hexokinase, aldolase and phosphohexoisomerase, were partially purified by a combination of $(NH_4)_2SO_4$ fractionation, and DEAE-Sephadex column chromatography. These enzymes have been biologically and physically characterized. Since these enzymes are critical trypanosome enzymes, neutralizing rabbit antisera prepared against them have been tested for their ability to passively protect mice. No protection was observed.

In addition by similar techniques a protective antigen has been partially purified from crude extracts of *Trypanosoma gambiense*.

B. One of the most significant advances made by these studies has been the development of a working hypothesis to account for the death and pathology of *T. gambiense* infected animals. In synopsis, this hypothesis involved the presence of a trypanosome toxin, delayed-type hypersensitivity and auto-immunity.

FORWARD

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care" as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences-National Research Council.

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INTRODUCTION

The work to be discussed in this report can be divided into two major areas:

A. The isolation and characterization of various enzymes, other antigens, and cell fractions; and to determine if any of these materials might be used in the development of an effective vaccine.

B. To study the immunological response of the host to infection by the African Trypanosomes, with particular emphasis on the role that nonspecific inflammation, hypersensitivity, and auto-antibody, play in the pathology or clinical symptoms found in infected animals.

Although these two areas of work are not mutually exclusive, they will be discussed separately for simplicity.

A. The hypothesis that a critical enzyme might be used as a vaccine originated with A. C. Chandler (2). Some success has been obtained against migrating *Ascaris suum* larva by immunizing guinea pigs with malic dehydrogenase (presumably a critical enzyme) from *Ascaris suum*. Hexokinase, aldolase, and phosphohexoisomerase are critical trypanosome enzymes. This suggested that antibody to trypanosome hexokinase, aldolase, or phosphohexoisemerase might be capable of inhibiting enzyme activity and therefore trypanosome development. This report describes our effort to purify, characterize and determine if neutralizing antiserum to these enzymes would passively protect animals against a *T. gambiense*

infection. In addition, by techniques similar to those used in the purification of the trypanosomal enzymes, other antigens have been partially purified, i.e., a protective antigen, a vascular permeability-increasing factor, etc. The characteristics of these antigens will be described.

DISCUSSION

Three enz[illegible] from T. gambi[illegible], T. rhodesiense, T. [illegible], and T. equiperdum have been partially purified by a combination of $(NH_4)_2SO_4$ fractionation, and diethylaminoethyl (DEAE) Sephadex column chromatography. A summary of the purification procedures, and extent of purification are shown in tables 1, 2, and 3. These enzymes were characterized with respect to the pH optimum, temperature optimum, substrate specificity, etc. The partially purified enzymes were used to immunize rabbits and the serum tested for its ability to neutralize or precipitate the enzymes. Table 4 shows that antiserum to T. gambiense enzymes cross-reacted with the same enzymes from T. equiperdum. These results were confirmed by cross-absorption studies. The extensive antigenic cross-reactions between the glycolytic enzymes of T. gambiense, other species of African Trypanosomes, T. equiperdum and T. lewisi have lead to some very interes[illegible]ng taxonomic speculations. For example, this work supports the conclusions of Ormerod (4) and others that T. gambiense and T. rhodesiense are not separate species. In addition, these antisera have been tested for their ability to passively protect animals against a T. gambiense infection (Table 5). Note that a

Table 1. A summary of the purification procedures used to isolate hexokinase from Trypanosome extracts.[1]

STEP	PROCEDURE	Total mg of protein	Units of Hexokinase	Specific Activity	% Recovery of Activity
1	None (crude)	140	1380	1.0	100
2	Supernatant (sonicated)	99	1040	1.0	75
3	45-60% Saturated $(NH_4)_2SO_4$ cut.	9	345	3.83	25
4	Elute from DEAE-Sephadex Column	0.084	270	321.4	19

[1] Data taken from Ph. D. thesis submitted to Tulane University by Dr. Edward Risby.

[2] One unit of hexokinase equals one µmole of glucose phosphorylated.

Table 2. A summary of the purification procedures used to isolate aldolase from Trypanosome Extracts.[1]

STEP	PROCEDURE	Total mg of protein	Units of Aldolase[2]	Specific Activity	% Recovery of Activity
1	None (crude)	140	1440	1.0	100
2	Supernatant (sonicated)	99	800	0.85	56
3	45-60% saturated $(NH_4)_2SO_4$ cut.	9	471	8.0	33
4	Elute from DEAE-Sephadex Column	0.18	120	133.0	8.4

[1] Data taken from Ph. D. thesis submitted to Tulane University by Dr. Edward Risby.

[2] One unit of aldolase = 1μM of F-1, 6DiP Split.

TABLE 3. A summary of the purification procedures used to isolate phosphohexose isomerase from Trypanosome extracts.[1]

STEP	PROCEDURE	Total mg of protein	Unit of PHI[2]	Specific Activity
1	None (crude)	140	4300	3.0
2	Supernatant (sonicated)	99	2400	2.2
3	45-60% saturated $(NH_4)_2SO_4$ cut	6	900	15.2
4	Elute from DEAE-Sephadex Column	0.15	300	200.0

[1] Data taken from Ph. D. thesis submitted to Tulane University by Dr. Edward Risby.

[2] One phosphohexose isomerase unit = 1µg of F-6-P formed.

TABLE 4. The neutralization of T. equiperdum (T. eq.) enzymes by antisera to enzymes of T. gambiense (T.g.)[1,2]

Rabbit Antibody To[3]	Antigen (Enzyme)[3]	Neutralization Titer[4]
Hexokinase (T.g.)	Hexokinase (T.g.)	1/64
Hexokinase (T.g.)	Hexokinase (T.eq.)	1/64
Aldolase (T.g.)	Aldolase (T.g.)	1/64
Aldolase (T.g.)	Aldolase (T.eq.)	1/64
Phosphohexoisomerase (T.g.)	Phosphohexoisomerase (T.g.)	1/128
Phosphohexoisomerase (T.g.)	Phosphohexoisomerase (T.eq.)	1/128
Hexokinase (T.eq.)	Hexokinase (T.g.)	1/64
Hexokinase (T.eq.)	Hexokinase (T.eq.)	1/64
Aldolase (T.eq.)	Aldolase (T.g.)	1/32
Aldolase (T.eq.)	Aldolase (T.eq.)	1/32
Phosphohexoisomerase (T.eq.)	Phosphohexoisomerase (T.g.)	1/64
Phosphohexoisomerase (T.eq.)	Phosphohexoisomerase (T.eq.)	1/64

[1]Data taken from Ph.D. thesis submitted to Tulane University by Dr. Edward Risby.

[2]T. equiperdum and T. gambiense were antigenically different by the agglutination and the protection test.

[3]See Tables 1, 2 and 3 for summaries of the purification procedures for the various enzymes. Rabbits were immunized with the partially purified preparations and the enzymes used in the neutralization tests were also partially purified.

[4]Different enzyme preparations used in the neutralization test always contained equivalent amounts of enzyme activity; hexokinase, 5-6 units; aldolase, 3.0 - 3.5 units; and phosphohexoisomerase, 7.5 - 8.5 units.

mixture of antisera to 2 enzymes protected animals *in vivo* against a *T. gambiense* infection. However, this mixed antiserum should have also protected animals against a *T. equiperdum* infection. The enzymes from these two species appeared antigenically identical. Therefore, the protection observed would appear to be due to antibody formed against a contaminating antigen in the partially purified enzyme preparations. Contaminating antigens have been demonstrated in the best preparations by other criteria. In addition, antiserum (ER-41) which had good inhibitory activity to phosphohexoseisomerase failed to neutralize trypanosomes *in vitro* (Table 6). The injection of large amounts of glycerol, an energy source readily utilized by the trypanosomes, failed to significantly alter the survival times of mice. This also suggests that the protection observed was not due to antibody against hexokinase and phosphohexoisomerase. It has therefore been concluded that hexokinase, aldolase and phosphohexoisomerase cannot be used as a vaccine.

Animals can however be passively or actively protected against a challenge trypanosome infection by passive transfer of antibody to, or immunization with the homologous strain of trypanosomes (Table 5, serum S-4E). The antigen(s) responsible for the protection has been partially purified (15-45xs) from *T. gambiense* (Table 7). A summary of the purification procedure and the extent of purification is shown in Table 8. Figure 1 is a representative example of numerous elution patterns using DEAE-Sephadex Column Chromatography. The major protective activity appears to be in peaks C and D. Note that at present, these fractions

Table 5. Attempts to passively protect mice against a challenge trypanosome infection with rabbit anti-trypanosome enzyme serum.

Rabbit Serum	Antibody To[2]	Challenged with[4]	Average Survival Time (Days)[6]	Percent Survival[6]	Number of animals
ER-8A	------------	T. g.	0	0	10
ER-1I +ER-4J	Hexokinase (T. g.)[3] Phosphohexoisomerase (T. g.)	T. g.	11.0	60	10
RS-68D[1]	T. g.	T. g.	14.0	40	5
S-4E[1]	T. g.	T. g.	11.0	60	5
ER-1I +ER-4J	Hexokinase (T. g.) Phosphohexoisomerase (T. g.)	T. eq.[5]	0	0	5
S-4E	T. g.	T. eq.	0	0	5
ER-1I +ER-4J +glycerol	Hexokinase (T. g.) Phosphohexoisomerase (T. g.)	T. g.	12.7	75	4

[1]Antiserum obtained from <u>T. gambiense</u> infected rabbits.

[2]Injected 0.5 ml antibody on day of challenge and 0.5 ml on second day after challenge.

[3]T. g. <u>T. gambiense</u>.

[4]Mice challenged with 1.0×10^3 trypanosomes.

[5]T. eq. <u>T. equiperdum</u>

TABLE 6. Attempts to protect mice against a challenge trypanosome infection by prior treatment of trypanosomes *in vitro* with rabbit anti-trypanosome enzyme serum.[1]

Rabbit Serum	Antibody Dilution	Antibody To:	Challenge Species	Average Survival Time (Days)[3]	Percent Survival[3]	Number of Animals
RS-6A	1/8	-------	T. g.	0	0	5
ER-1I	1/8	hexokinase (T. g.)	T. g.	0	0	5
ER-4I	1/8	phosphohexoisomerase (T. g.)	T. g.	0	0	5
RS-63E[2]	1/8	T. g.	T. g.	13.6	60	5

[1]A washed suspension of trypanosomes (5.0 x 10^6 cells/ml) was incubated with an equal volume of antiserum for 1 hr. at 37°C, and then 0.1 ml of this mixture was injected into a mouse.

[2]Antiserum obtained from *T. gambiense* infected rabbit.

[3]Based on a 20 day observation period following challenge injection.

TABLE 7. The protection of mice against a challenge trypanosome infection by immunization with various fractions isolated from crude extracts of T. gambiense.

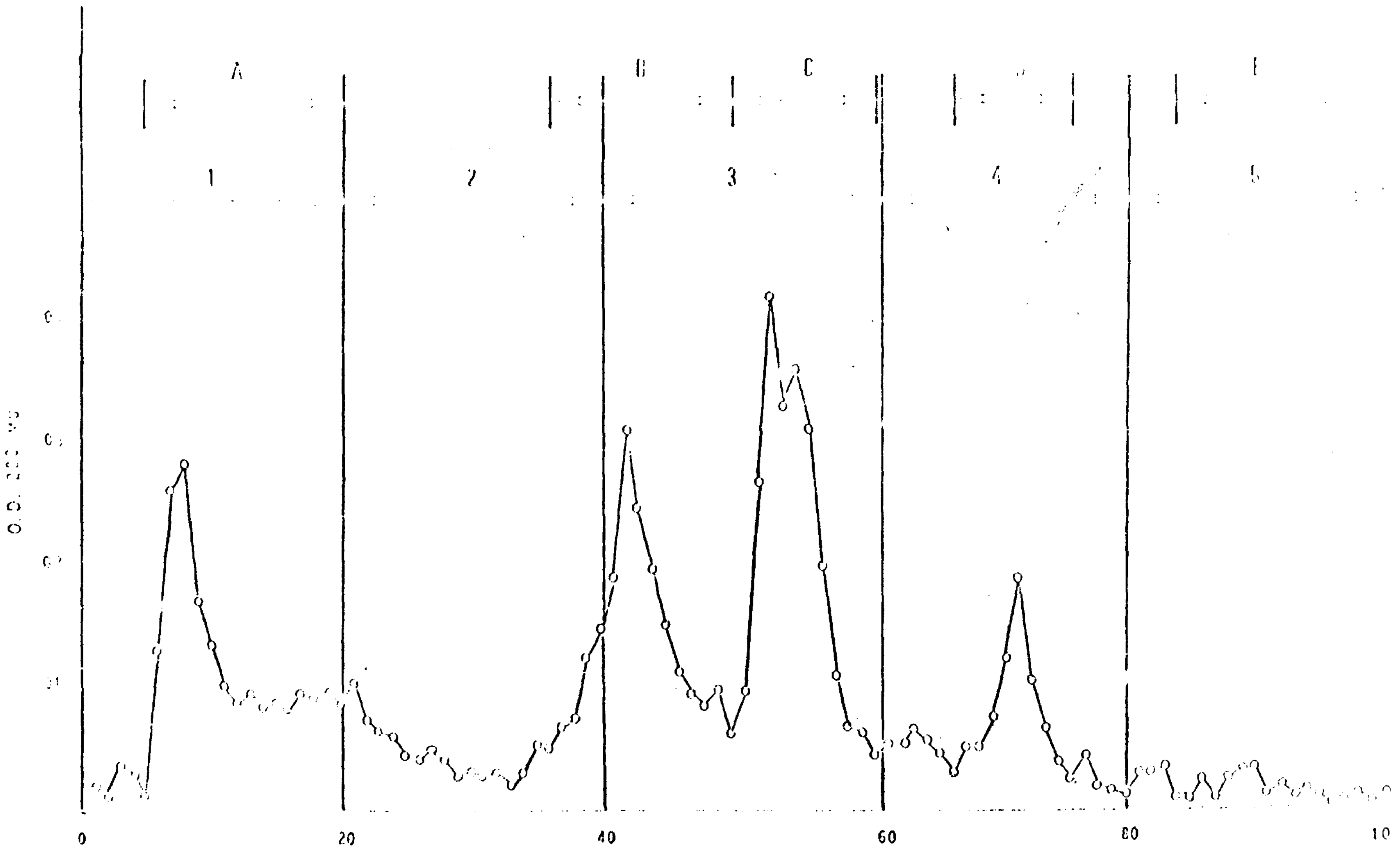
Fraction	mg protein / ml	Average Survival Time (Days)[1]	Units Protection[2]	Percent Survival[3]	Number of Animals
Crude	2.8	6.8	2.43	25	4
Supernatant	3.5	6.4	1.83	20	5
Washed Sediment	2.3	6.8	2.96	25	4
$(NH_4)_2SO_4$ Fraction					
0-25%	0.45	5.6	12.5	20	5
25-45%	2.70	3.2	1.19	0	5
45-65%	2.50	3.4	1.36	0	5
65-80%	1.65	1.6	0.97	0	5
DEAE-Sephadex Fraction (3 runs)					
A	0.225	1.6	7.1	0	5
B	0.200	7.0	35.0	40	5
C	0.215	8.0	37.2	40	5
D	0.042	4.6	109.0	20	5
A	0.372	0.7	1.88	0	4
B	0.298	5.2	17.4	20	5
C	0.246	8.8	35.7	40	5
D	0.079	2.6	33.0	0	5
A	0.175	0.6	3.4	0	5
B	0.180	3.6	20.0	20	5
C	0.155	6.6	42.5	20	5
D	0.125	15.0	120.0	0	5
Saline control	0	0	0	0	20

1. Based on a 20 day observation period following a challenge T. gambiense injection.
2. Unit Protection = Average survival time/mg protein.
3. Based on a 20 day observation period.

TABLE 8. A summary of the purification procedures used to isolate the protective antigen from <u>T. gambiense</u>.

STEP	PROCEDURE	Specific Activity
1	Crude	1.0
2	Supernatant	0.75
3.	0-25% Saturated $(NH_4)_2SO_4$ Fraction	5.1
4	DEAE-Sephadex Column Elute (Fraction C).	15.0
	DEAE-Sephadex Column Elute (Fraction D).	45.0

Units protection crude extract = 1.0



A
B
C
1
2
3
4
0
20
40
60
80
FRACTION NO.

Figure 1 - Legend

1 - Eluted with 0.0125m phosphate buffer, pH 6.5, plus 0.5% $MgCl_2$.

2 - Eluted with 0.0125m phosphate buffer, pH 6.5, plus 0.5% $MgCl_2$, plus 0.075m NaCl.

3 - Eluted with 0.0125m phosphate buffer, pH 6.5, plus 0.5% $MgCl_2$, plus 0.2m NaCl.

4 - Eluted with 0.0125m phosphate buffer, pH 6.5, plus 0.5% $MgCl_2$, plus 0.4m NaCl.

5 - Eluted with 0.0125m phosphate buffer, pH 6.5, plus 0.5% $MgCl_2$, plus 1.0m NaCl.

contain a number of different precipitating antigens. Also a second biological activity has been found in fraction D. A vascular-permeability-increasing-factor (V. P. I. F.) is eluted in this peak. The relationship of the V. P. I. F. to the protective antigen(s) is unknown. It should however be noted that this is the first report to convincingly demonstrate a toxic component in trypanosome extracts. Identical elution patterns have also been obtained using extracts of T. equiperdum. However, the protective antigen(s) for T. equiperdum has not yet been located in the elution pattern.

In summary, it is apparent that a protective antigen can be partially purified from T. gambiense. The use of this antigen in the preparation of a vaccine against trypanosomiasis is questionable because of the numerous different serotypic strains (relapse strains) of trypanosomes which are known (5). Each strain has its own specific protective antigen(s) (6). The large number of these antigenic strains makes a multivalent vaccine unlikely. However, it has been suggested that protection might be obtained in nature if during passage through the tsetse fly a common protective antigen(s) (or a limited number) is obtained (7). Gray (3) has shown that a limited number of serotypes are obtained after fly passage by the agglutination reaction. Therefore by developing techniques to purify the protective antigen it may be possible to isolate a similar antigen from the fly forms.

B. Numerous theories have been proposed to account for death of animals infected with African trypanosomes. Mechanical mechanisms such as blockage of small blood vessels by agglutinated blood trypanosomes (1) and also physiological disturbances of the host due to trypanosome

metabolism (9). However, none of the hypotheses are entirely satisfactory (9). This report describes our efforts to account for death of trypanosome infected animals and to develop a working hypothesis.

DISCUSSION

In the previous section (A), it was stated that a V. P. I. F. could be partially purified from trypanosome extracts by a combination of $(NH_4)_2SO_4$ fractionation and DEAE-Sephadex column chromatography. The intradermal injection of this factor into rabbits and guinea pigs causes a definite increase in vascular permeability as measured by Evans' Blue Concentrations at the site of injection (Table 9). Similar results have also been obtained with the intradermal (ID) injection of live suspensions of T. gambiense. It has been possible to show that the water content of the lesion is greatly increased. Also, the amount of extract necessary to induce increased vascular permeability is compatible with the number of trypanosomes found at the sites of the lesions. It is of interest to note that extracts of T. lewisi but not normal rat serum, or rat red blood cells contain high concentrations of this factor. T. lewisi, which is nonpathogenic to the rabbit, will produce a large inflammatory reaction after ID injection of live trypanosomes (Table 10). The lesion however disappears in 4-5 days, and the skin appears normal. In contrast, a T. gambiense lesion continues to enlarge and spread (Table 10). The character of the T. gambiense lesion does however change between the 4th and 5th day. Initially the lesion is highly inflammed, raised and hard. After the 5th day, the swelling is reduced, and the lesions begins to dry and show small areas of scab and crust. It is therefore concluded that

Table 9. The presence of a vascular-permeability-increasing-factor (V. P. I. F.) in extracts of *T. gambiense* and *T. lewisi*.

Number Animals Tested (Guinea Pigs)	Total Number of Sites Injected	Fraction	mg protein / ml	Average Size (mm)	Average Intensity[5]
2	3	T. gambiense[1]	2.0	11.0 x 12.3	2.7
2	2	T. lewisi[1]	2.0	11.5 x 12.5	3.0
2	3	IRS[1,2]	2.0	8.7 x 11.0	1.7
2q	3	NRS[1,3]	2.0	5.3 x 8.3	1.3
3	4	DEAE-Sephadex-D	0.5	12.8 x 15.0	3.0
2	7	NRS[1]	0.5	2.3 x 3.4	± -1.0
2	5	Rat RBC[1] s	0.5	1.0 x 1.6	± -1.0
2	2	Glucose-RP[4]	0	2.0 x 3.0	± -1.0
2	2	Saline	0	3.0 x 6.5	±

[1] A 0-50% $(NH_4)_2SO_4$ fraction of the crude material.

[2] IRS-Infected Rat Serum.

[3] NRS-Normal Rat Serum.

[4] Glucose-Ringer's Phosphate

[5] (-) no reaction; (±) questionable reaction; and (1^+ to 4^+) degrees of reaction (Bluing) with 4^+ maximum.

TABLE 10. A comparison of skin lesions in rabbits injected intradermally with either *T. lewisi* or *T. gambiense*.

Serial Number[1]	Injected with[2]	Skin lesions (mm)[3] 2 days	4 days	7 days	14 days
RS-137	T. gambiense	15 x 16	30 x 36	34 x 37	31 x 40
RS-138	T. gambiense	19 x 22	31 x 36	35 x 40	33 x 41
RS-162	T. lewisi	18	16 x 17	-[4]	-
RS-163	T. lewisi	31 x 34	30 x 31	±[5]	-

[1]All rabbits were injected intradermally at 3 sites (except RS-162 injected at only 2 sites) with 0.1 ml/site of a washed trypanosome suspension (5.0×10^7 cells/ml).

[2]Note (Table 11) controls with rabbits injected intradermally with equivalent numbers of normal rabbit spleen cells (RS-129, 130, 136).

[3]Average size of skin lesions.

[4]No obvious reaction or lesion.

[5]Slight discoloration of skin at injection site.

during the first 4 to 5 days after intradermal injection of *T. gambiense*, a trypanosome toxin (V. P. I. F.) causes increased vascular permeability and edema. The increased vascular permeability is believed to be responsible for the presence of extravascular trypanosomes at the sites of the skin lesions. Increased vascular-permeability and edema are believed to account for the early pathology of skin lesions.

After this first phase, a second stage involving delayed-type hypersensitivity is believed to play a major role in the pathology of skin lesions in rabbits. Table 11 shows that artificial lesions can be produced by the transfer of spleen cells from infected animals but not by spleen cells from normal animals. The passive transfer of precipitating antibody fails to elicite the skin reactions (Table 12). Although further work is necessary it suggests that delayed-type hypersensitivity plays a role in the pathology of skin lesions. The microscopic appearance of the skin lesions, the ability to observe trypanosomes at extravascular sites in the skin lesions, as well as the ability of anti-inflammatory drugs to greatly reduce the skin lesions are in agreement with this hypothesis.

The early delayed-hypersensitivity reactions lead to cell damage; the release of antigens not recognized as self; and the synthesis of auto-antibody. Previous work has demonstrated antibody in infected animals to antigens from normal rabbit tissues (8). This is the final factor believed to be involved in the death of trypanosome infected animals. However at present, there is no evidence to support the hypothesis that auto-antibody is involved in the pathology of infected animals.

TABLE 11. The passive transfer of white blood cells from chronic <u>T. gambiense</u> infected rabbits to a rabbit infected for 48 hrs. with <u>T. gambiense</u>.

Animal Number	Treatment	Skin lesions (mm)	
		4 days	7 days
RS-112	Infected WBC (1.1 x 10^7 cells)[1]	20	32 x 33
	Normal WBC (9 x 10^6 cells)	-[2]	±
RS-113	Infected WBC (1.1 x 10^7 cells)	13 x 16	12 x 14
	Normal WBC (1.1 x 10^7 cells)	-	-
RS-116	Infected WBC (1.6 x 10^7 cells)	14	32 x 37
RS-117	Infected WBC (1.6 x 10^7 cells)	15	16
RS-121	Infected WBC (1.8 x 10^7 cells)	9 x 12	28 x 31
	Normal WBC (15 x 10^7 cells)	-	-
RS-122	Infected WBC (1.8 x 10^7 cells)	±[3]	22 x 26
	Normal WBC (1.5 x 10^7 cells)	±	-
RS-124	Infected WBC (4.1 x 10^6 cells)	-	-
	Normal WBC (1.2 x 10^7 cells)	-	-
RS-125	Infected WBC (4.1 x 10^6 cells)	-	-
	Normal WBC (1.2 x 10^7 cells)	-	-
RS-129	Infected WBC (1.8 x 10^7 cells)	13	28 x 29
	Normal WBC (2.7 x 10^7 cells)	±	±
RS-130	Infected WBC (1.8 x 10^7 cells)	11	18 x 20
	Normal WBC (2.7 x 10^7 cells)	±	±
RS-135	Infected WBC (52 x 10^7 cells)	8 x 9	24 x 28
	Normal WBC (5.0 x 10^7 cells)	10	8 x 10
RS-136	Infected WBC (5.2 x 10^7 cells)	-	-
	Normal WBC (5.0 x 10^7 cells)	-	-

1. White blood cells obtained from 21 to 30 day infected rabbits showing definite skin lesions. The various suspensions contained a maximum of 1.5 x 10^3 blood trypanosomes. In most infected WBC preparations, no viable trypanosomes were observed. For example, in the WBC preparation injected into RS-135 and RS-136, no trypanosomes were observed in a minimum of 200 high power fields, and the survival times of mice injected with this preparation demonstrated that 0.2 ml contained approximately 50 blood trypanosomes.
2. No obvious reaction or lesion.
3. A small raised red area near injection site.

Table 12. The passive transfer of normal rabbit serum, precipitating antibody, and washed blood trypanosomes to a rabbit infected for 48 hrs. with *T. gambiense*.

Animal Number	Treatment	Skin lesions (mm) 4 days	7 days
RS-112	Normal Rabbit Serum (0.15 ml)	-[3]	-
	Precipitating-Ab(RS-59F)(0.15 ml)	-	-
RS-113	Normal Rabbit Serum	-	-
	Precipitating-Ab(RS-59F)(0.15 ml)	-	-
RS-116	Normal Rabbit Serum (0.2 ml)	-	-
	Washed blood trypanosomes (4.0×10^2 cells)	±[4]	-
RS-117	Normal Rabbit Serum (0.2 ml)	-	-
	Washed blood trypanosomes (4.0×10^2 cells)	-	-
RS-121	Precipitating-Ab(RS-51C)(0.2 ml)	-	-
	Washed blooded trypanosomes (1.1×10^4 cells)	16	13 x 18
RS-122	Precipitating-Ab(RS-51C)(0.2 ml)	-	-
	Washed blood trypanosomes (1.1×10^4 cells)	-	14 x 17
RS-124	Precipitating-Ab(RS-117C)(0.2 ml)	-	-
	Washed blood trypanosomes (1.0×10^5 cells)[2]	-	-
RS-125	Precipitating-Ab(RS-117C)(0.2 ml)	-	-
	Washed blood trypanosomes (1.0×10^5 cells)[2]	-	-
RS-129	Precipitating-Ab(RS-109T)(0.2 ml)	-	-
RS-130	Precipitating-Ab(RS-109T)(0.2 ml)	-	-
RS-135	Precipitating-Ab(RS-121C)(0.2 ml)	-	-
RS-136	Precipitating-Ab(RS-121C)(0.2 ml)	-	-

1. Sera obtained from *T. gambiense* infected rabbits. All antisera were examined for precipitating antibody by agar diffusion techniques. Serum (RS-59F) from a 177 day infected rabbit, showed a minimum of 2-precipitin bands; RS-51C from a 90 day infected rabbit showed a minimum of 3 precipitin bands; RS-117C from a 28 day infected rabbit showed a minimum of 5 precipitin bands; RS-109T from a 21 day infected rabbit showed a minimum of 2 precipitin bands; RS-121C from a 30 day infected rabbit showed a minimum of 5 precipitin bands.
2. No. of blood trypanosomes determined by survival times of infected mice.
3. No obvious reaction or lesion.
4. A small raised red area near the injection site.

TABLE 13. **A possible sequence of events in the pathology (skin lesions) and death of rabbits infected with T. gambiense.**

	I	II	III
Description	**Increased Vascular Permeability (a trypanosome toxin)**	**Delayed-type Hypersensitivity**	**Auto-Antibody**
Time Sequence (Days)	0	4	18 Death
Inhibited by Cortisone Treatment	+	+	-

In summary, it is suggested that a combination of 3 factors or events accounts for the pathology and eventual death of trypanosome infected animals: 1. a trypanosome toxin causing increased vascular permeability; 2. delayed-type hypersensitivity; and 3. auto-antibody (Table 13).

This work has lead to the publication of 9 papers, and 1 additional paper submitted for publication during the last year, and 1 in preparation

PUBLICATIONS

1966 Passive Immunity to Experimental Trypanosomiasis. (with A. A. Gam). J. Parasit. 52: 1134-1140.

1967 Characterization and localization of Acid Phosphatase Activity of <u>Trypanosoma gambiense</u>. (with J. Byram III and A. A. Gam) J. Protozool. 14: 117-125.

1967 Studies of the susceptibility and host parasite relationships of infections by Trypanosoma gambiense in the American opossum, Didelphis marsupialis. (with A. A. Gam) J. Parasit. 53: 651-652.

1967 The presence of antibody to a normal rabbit liver antigen in rabbits infected with Trypanosoma gambiense (with A. A. Gam) J. Parasit. 53: 946-960.

1968 Studies on frog Trypanosomiasis. A 24-hour cycle in the parasitemia level of Trypanosoma rotatorium in Rana clamitans from Louisiana (with C. Southworth and [illegible] Mason) J. Parasit. 54: 255-258.

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1968 Studies on frog Trypanosomiasis. II Seasonal variations in the parasitemia levels of Trypanosoma rotatorium in Rana clamitans from Louisiana (with R. Bollinger and A. A. Ham), Tulane Studies in Zoology (In press

1968 Purification and Properties of purified hexokinase from the African trypanosomes and Trypanosoma equiperdum (with E. Risby) J. Protozool. (In press

Submitted for Publication.

The presence of agglutinating antibody in the IgM immunoglobulin fraction of rabbit antiserum during experimental African trypanosomiasis (with R. Cornille, E. Risby, and A. A. Ham), Parasit. (In press).

Purification and properties of purified phosphohexose isomerase from the African trypanosomes and T. equiperdum. (with E. Risby) Submitted to Experimental Parasitology.

The effect of hydrocortisone on the development of skin lesions, agglutinating antibody titers, and parasitemia levels in rabbits infected with Trypanosoma gambiense (with H. Marcus and E. Risby) Experimental Parasitology (In press).

The presence of a vascular-permeability-increasing factor in extracts of Trypanosoma gambiense, and its possible role in the pathology of skin lesions in T. gambiense infected rabbits. (In preparation)

Personnel Receiving Contract Support.

Note that only consumable supplies were purchased for their research problems. Funds were not utilized for their personal support nor for the purchase of equipment necessary to their research problems.

NAME	THESIS TITLE	DEGREE	DATE OBTAINED
Edward Risby	A comparative study of the characteristics and serological properties of some trypanosomal enzymes	Ph. D.	Aug., 1968
Yolanda Marciacq	Biochemical and ultrastructural changes in guinea pigs infected with *Trypanosoma gambiense*.	M.S.	June, 1967
		Ph. D.	Expected June, 1970

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6 Seed, J.R., 1963. The characterization of antigens isolated from *Trypanosoma rhodesiense*. J. Protozool. 10 380-389.

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