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Concurrent Infection of TRYPANOSOMA MUSCULI Kendell, 1906, and SCHISTOSOMA MANSONI Sambon, 1907 in Mice

Berhane Andeberhan

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Abstract

CONCURRENT INFECTION OF TRYPANOSOMA MUSCULI KENDELL, 1906 AND SCHISTOSOMA MANSONI SAMBON, 1907 IN MICE

by Berhane Andeberhan

The possible effects of ablastin on Schistosoma mansoni were investigated by (1) establishing a concurrent infection of Trypanosoma musculi and Schistosoma mansoni in mice, and (2) by obtaining Trypanosoma lewisi immune rat serum (ablastin) and injecting it into S. mansoni infected mice. These procedures were followed on days 4, 14 and 28 of S. mansoni infection. For the groups that were given immune rat serum, controls were kept into which normal rat serum was injected.

Ablastin does not have any effect on the development or morphology of the S. mansoni worms as tested in this study. Although ablastin inhibits glycolytic pathway enzymes in young trypanosomes, it does not seem to affect those of the blood fluke. Effects of schistosome membrane function must also be ruled out as no reduction in worm burden and no morphological abnormalities were observed. Both immune and normal rat serum caused an increase in the number of worms recovered. This is most probably due to a serum sickness type reaction in the mice, caused by the foreign rat protein. This is

supported by preliminary experiments which showed that mice given immune rat serum every third day died of anaphylactic shock on the twelfth day, a few minutes after the fifth injection was given.

Starting both T. musculi and S. mansoni infections at the same time as well as establishing a high titer of ablastin by introducing the T. musculi infection 8 to 10 days prior to the schistosome infection, would establish the effect of ablastin on the penetration of S. mansoni cercariae.

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Graduate School

CONCURRENT INFECTION OF TRYPANOSOMA MUSCULI KENDELL, 1906 AND SCHISTOSOMA MANSONI SAMBON, 1907 IN MICE

by

Berhane Andeberhan

A Thesis in partial Fulfillment of the

Requirements for the Degree Master of Science

in the Field of Microbiology

June 1976

Each person whose signature appears below certifies that he has read this thesis and that in his opinion it is adequate, in scope and quality, as a thesis for the degree of Master of Science.

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INTRODUCTION

Mixed parasitic infections have been used extensively in experimental investigations using Schistosoma mansoni and various other parasites. In such mixed or concurrent infections the parasites are introduced into a host so that both are present at various times during the concurrent infection. The parasites may have no effect on each other, and thus the host is not affected differently than if each parasite were present in a separate infection. Or, the parasites in mixed infections may tend to enhance the course of infection, and in this manner produce a greater harmful effect upon the host. This is known as synergistic effect. Finally, the parasites in a mixed infection may have a negative, or antagonistic effect upon each other, and the host experiences a lighter infection than if either one were present independently.

Yoeli (1956) reported that when field voles were exposed to Plasmodium berghei from 4 to 7 weeks after S. mansoni infection, a mild course of parasitemia resulted. Jachowski (1961) found that a reduced worm burden of S. mansoni resulted in the rat host when heavy infections of S. mansoni were given after a prior Trichinella spiralis infection. Hunter et al (1961) reported that mice that were immunized to S. mansoni by repeated infections and were then challenged with Schistosomatium douthitti, had fewer S. douthitti worms

than controls. The possibility that Trypanosoma cruzi infection may have a protective effect against S. mansoni was suggested by Abath et al (1966). (These examples represent antagonistic concurrent infections.)

A synergistic effect in a mixed infection was demonstrated by Weinman (1960) who reported that mice became more susceptible to S. mansoni infection when the mice had prior infection with T. spiralis. Also, Ee-siriporn and Wagner (1969) noted a synergistic effect when mice were concurrently infected with S. mansoni and T. equiperdum. The mice that were infected for 5, 7 and 9 weeks did not survive as long as controls infected only with T. equiperdum.

The hemoflagellate, T. musculi (Syn. T. duttoni Thiroux, 1905) has also been used in concurrent infections. Galliard, as quoted by D'Alesandro (1970) used a relatively avirulent strain of T. gambiense that caused death in mice in 11 months. When mice were infected simultaneously with T. gambiense and T. musculi death occurred in 7 days. Parasitemia due to T. musculi was increased, whereas T. gambiense was present in low numbers. Death was attributed to T. musculi. Singer et al (1964) studied T. musculi infection in mice treated with bacterial endotoxin. Mice that were made endotoxin-tolerant before infection had decreased parasitemias.

T. lewisi, a rat parasite of the subgenus Herpetosoma (Hoare, 1972) is the most studied member of the subgenus and has been used

in several concurrent infection studies which show effects that range from lethal to beneficial. Taliaferro (1938) demonstrated cross immunity of both ablastic and trypanosomicidal antibodies between T. lewisi and T. musculi. Marmorston-Gottesman et al (1930) found high mortality of rats infected with both T. lewisi and Bartonella, although either parasite by itself does not cause death. Mixed infections with T. lewisi and the pathogenic species of trypanosomes have been studied by several workers as reported by D'Alesandro (1970), and in every case each species seems to have no effect on the development of the other; the outcome being always fatal. It is of interest here to mention that in a limited pilot project done in connection with this study, it was observed that when T. equiperdum was injected into mice immunized with T. musculi, the survival time in the immunized mice was significantly longer than in the control mice.

Except in very young rats, or in very high dosage innocula, neither T. lewisi nor Plasmodium berghei is considered to be lethal to the host. However, in dual infections with these two parasites a 50% mortality was reported by Hughes and Tatum (1956), and they found that the parasitemias were significantly increased. Thus, the effect of P. berghei resembles that of Bartonella. Jackson (1959) also found that although these two parasites did not alter each other's course of infection, there was a high rate of mortality (68%) which

he attributed to severe anemia.

Ashley (1962) studied the effects of concurrent T. lewisi and Nippostrongylus brasiliensis infection in rats and found there was no significant effect on the course of infection. Tate (1951) observed an antagonistic action by Spirillum minus toward T. lewisi. He found that when rats were infected with T. lewisi two to four weeks after inoculation with S. minus, the development of T. lewisi was suppressed, and only slight or subpatent infections resulted.

To study possible effects of the antibody ablastin on the development of the blood fluke, S. mansoni in mice, an experiment was designed to test the ablastin on the parasite in two different approaches. These are (1) a concurrent infection of T. musculi (which during the course of infection in mice induces ablastin production) and the blood fluke, S. mansoni; and (2) to utilize serum of rats infected with T. lewisi as a heterologous source of ablastin, and inject this immune serum into the mice which have been infected with S. mansoni. The tests are referred to as (1) Concurrent T. musculi and S. mansoni infections, and (2) Effects of immune (ablastic) rat serum on S. mansoni in mice.

The purpose of the tests was to determine the effects of T. musculi on the S. mansoni infections in mice and to see if any of these are mediated by the antibody ablastin as produced directly against T. musculi in the mouse host harboring the blood fluke, and more

indirectly by the ablastin produced against T. lewisi in the rat host; and transferred by intraperitoneal injection into the mice harboring the blood fluke, S. mansoni.

MATERIALS AND METHODS

Maintenance of T. musculi

Lincicome strain T. musculi (ATCC #30182) was obtained from American Type Culture Collection in diphasic blood agar medium, and maintained by serial passage from mouse to mouse (0.1 ml of 1:1 blood/saline mixture). The parasitemias were checked by making wet film preparations of blood from tail snip. Parasitemia levels were indicated by -, +, ++, +++, and ++++, indicating negative, low, medium, high and very high parasitemia, according to the following quantitative schedule:

+	at least one parasite per 22 x 22 mm coverslip preparation
++	2-5 trypanosomes per high power field (450x)
+++	5-20 trypanosomes per high power field
++++	Too numerous to count

When the parasitemia reached a ++++ level, trypanosome counts were performed by using a hemacytometer to determine the number of trypanosomes injected into each mouse in the study.

Schistosoma mansoni

Young 16-20 gm HA (ICR) Swiss albino female mice were obtained by the University of Michigan from Spartan Research Animals Inc., Haslett, Michigan and were injected with the Puerto Rican strain of S. mansoni cercariae. This was done by the Mollusk Division, Museum of Zoology, under the direction of Dr. H. D. Blankespor.

The route of infection was intraperitoneal and approximately 80 cercariae were injected per mouse. Materials used in this study were provided by the U.S.-Japan Cooperation Medical Science Program - NIAID.

Trypanosoma lewisi immune rat serum

Frozen T. lewisi cultures were obtained from Dr. E. Platzer of the University of California, Riverside. Three rats were injected with 0.2 ml each and 7 rats with 0.15 ml each of the stock. In 5 days the parasitemias in all rats were very high and by the tenth day the parasitemia was still high but some reduction was obvious. Eight rats were sacrificed on the eleventh day and their blood was collected. After being allowed to clot each aliquot of blood was ringed using an applicator stick and centrifuged at 2000 rpm for 30 minutes. The serum was pooled and frozen in aliquots of 5 ml each. Normal serum was similarly collected from noninfected rats and frozen at the same time.

Experimental procedure

A total of 155 S. mansoni infected and 15 noninfected mice was divided into 13 groups as follows (with code given in parenthesis):

A. Twenty S. mansoni infected mice were designated as controls and set aside. (C)

B. On day 4 of S. mansoni infection:

1. Twenty infected mice were injected intraperitoneally with 16,000 T. musculi each. (4M)

2. Five noninfected mice were also injected with 16,000 T. musculi each to serve as controls. These mice and 5 of the mice in Number 1 were followed for parasitemia starting the second day of infection. (4MC)

3. Twenty S. mansoni infected mice were given 0.2 ml each of T. lewisi immune rat serum intraperitoneally and injections were continued every other day for a total of 3 injections per mouse. (4A)

4. Five S. mansoni infected mice were given 0.2 ml each of normal rat serum intraperitoneally every other day for a total of 3 injections per mouse. These served as rat serum controls. (4AC)

C. The procedures in "B" were repeated with additional mice starting on day 14, and again on day 28 of S. mansoni infection.

D. Starting on week 5 of the S. mansoni infection, weekly checks for eggs were made of 5 numbered mice from each group. One or two fecal pellets were mixed in about 1 ml of saline and a coverslip preparation made and examined under the low magnification of a compound microscope. The number of eggs per coverslip preparation was recorded and it was noted if any of the eggs were nonviable.

E. On week 8 of S. mansoni infection the mice were killed by injecting 0.5 ml (i.p.) followed by up to 0.3 ml (i.v., as needed) of a mixture of Sodium Nembutal, Heparin and Saline (in proportions of 5:1:15 respectively). Schistosomula were recovered by flushing the peritoneal cavity with saline. The adult worms were recovered by perfusion using normal saline (Roth and Heidtke, 1966). The livers of all mice were crushed to recover worms that were not perfused out. A careful visual check was made of the mesenteric veins and any worms left were picked out using forceps and probes.

F. 1. The schistosomula were counted, sexed and directly put into vials with AFA (85% Alcohol, 10% Formalin, 5% Glacial Acetic Acid).

2. The adults were fixed in AFA and flattened under glass coverslips for about 15 minutes and then transferred into vials. Samples from each group were stained using Mayers Carmine and counterstained using Fast Green for examination under the compound microscope to check for any gonad alterations or abnormalities. Worm counts and sexing of all stained and unstained worms was performed using dissecting microscopes. The experimental design is outlined below.

The experimental design:

	No. of Mice	Code
Experimental		
S. mansoni and *T. musculi*		
4-day *S. mansoni* infection	20	4M
14-day *S. mansoni* infection	20	14M
28-day *S. mansoni* infection	20	28M
S. mansoni and Ablastin (transfer in rat serum - *T. lewisi*)		
4-day *S. mansoni* infection	20	4A
14-day *S. mansoni* infection	20	14A
28-day *S. mansoni* infection	20	28A
Controls		
S. mansoni	20	C
S. mansoni and normal rat serum		
4-day infection	5	4AC
14-day infection	5	14AC
28-day infection	5	28AC
T. musculi only		
4-day infection time	5	4MC
14-day infection time	5	14MC
28-day infection time	5	28MC

RESULTS

Trypanosoma musculi

In the concurrent infections T. musculi was well established in all the mice. The checks using 5 mice from each concurrent infection group and comparing them to mice infected with T. musculi only, showed that there was no obvious change in the course of the parasitemia. All mice (except Mouse 3 of group 14MC which apparently did not get infected) were positive on day 4 with the parasitemia staying at a high level between days 6 and 12, then rapidly declined so that most mice were negative on day 14, and all on day 16 (see Table I).

Schistosoma mansoni and Trypanosoma musculi

The mean number of S. mansoni adults recovered per mouse in the control mice was 18.71 which signified a 23.38% of worm recovery rate. This compares appreciably with the reported recovery rate of 19% for S. mansoni in mice infected intraperitoneally (Cram et al, 1947). The mean number of S. mansoni adults per mouse in the mice with concurrent T. musculi and S. mansoni infections and percent worm recovery rates are listed in Table II.

Although more worms (on the average) were recovered from the mice with concurrent infections with T. musculi, this was found to be

insignificant by performing a t-test using a 95 percent confidence level. T-values were obtained by using the # BSTATTTEST on the "IRIS-A" computer.

Schistosoma mansoni and Ablastic Serum

More worms than in controls were recovered from the group of mice receiving immune rat serum starting day 4 of S. mansoni infection, but the increase is not statistically significant. However, statistically significant increases in worm recoveries were found in the mice that were given immune rat serum starting on days 14 and 28 of S. mansoni infection.

Schistosoma mansoni and Normal Rat Serum

More worms were also recovered from the S. mansoni infected mice receiving normal rat serum starting on days 4, 14 and 28. However, because of the low number of animals used in these control groups no statistical significance could be established between any of these groups and the S. mansoni controls. Similarly, no significance was observed between the corresponding ablastin containing vs. normal rat serum groups, namely between 4A and 4AC, 14A and 14AC, as well as 28A and 28AC.

Schistosoma mansoni

a. In all groups more males were found than females (ranging 58.6 - 67.7%), the pattern being that there would be a number of paired couples and a few single males (see Table II). Stained samples of each group were examined under the microscope and all appear to have normal gonads.

b. Eggs

Starting on week 5 a weekly check of fecal pellets for S. mansoni indicated only some of the mice were positive for eggs. The vast majority of eggs seen was normal with live developed miracidia.

c. Schistosomula

Most of the immature worms flushed out of the peritoneal cavity were males (73.3 - 100%). Several of them were completely encapsulated and one partially. Quite unusually, two sets of male and female pairs were found coupled together. One pair was found in a 4A mouse, another in a 28A mouse. The mean number of immature worms found in each group of mice ranged from 0.8 to 2.9 schistosomula per mouse (see Table III).

Table I. Levels of parasitemia in mice infected with Trypanosoma musculi. Five mice from each control and experimental group were tested by tail snip. Negative = -; positive levels are represented by 1+ to 4+. M = T. musculi infection; MC = T. musculi control.

Group	Mouse No.	Day of T. musculi infection							
		2	4	6	8	10	12	14	16
4M	1	-	+	++	++++	+++	++	+	-
	2	-	+	++	++++	++++	++++	++	-
	3	-	+	++	+++	+++	+	-	-
	4	-	+	++	++++	+++	++	-	-
	5	+	+	+++	++++	+++	+	-	-
4MC	1	-	+	+++	++++	++++	++++	-	-
	2	-	+	+++	++++	++++	++++	+	-
	3	-	+	+++	++++	++++	+	-	-
	4	-	+	+++	++++	++++	++++	+++	-
	5	-	+	+++	++++	++++	+	-	-
14M	1	-	+	+++	++++	++	+	-	
	2	-	+	++	++++	++++	+++	-	
	3	-	+	++++	++++	++	+	-	
	4	-	+	+++	+++	+++	+	+	
	5	-	+	+++	+++	++	+	-	
14MC	1	-	+	++++	++++	++++	++	+	
	2	-	+	++	++++	++++	+++	-	
	3	-	-	-	-	-	-	-	
	4	-	-	++	++++	++++	+++	-	
	5	-	+	++++	++++	++++	+++	-	
28M	1	-	+	++	++++	+++	+	-	
	2	-	+	+++	+++	++	-	-	
	3	-	+	+++	+++	++	-	-	
	4	-	+	+++	++++	++	-	-	
	5	-	+	+++	++++	++++	++	-	
28MC	1	-	+	+++	++++	+++	-	-	
	2	-	+	+++	++++	+++	-	-	
	3	-	+	+++	++++	++++	-	-	
	4	-	+	+++	++++	+++	-	-	

Table II. Adult Schistosoma mansoni recovered by perfusion following 8 weeks of infection (1) concurrently with Trypanosoma musculi, (2) in mice which received immune rat serum, (3) normal rat serum and as controls.

		WORMS					
Group	No. of Mice	Total	% Males	Mean (worms/M)	Variance	S.D.	% Worm Recovery
C	17	318	62.3	18.71	175.97	13.27	23.38
4M	14	337	61.7	24.07	174.99	13.22	30.09
14M	19	369	60.2	19.42	275.04	16.58	24.28
28M	18	440	62.3	24.44	355.56	18.86	30.55
4A	20	546	61.5	27.3	235.91	15.36	34.13
14A	20	575	58.6	28.75*	306.30	17.50	35.94
28A	19	534	62.9	28.11*	227.21	15.07	35.14
4AC	4	133	67.7	33.25	112.916	10.63	41.56
14AC	5	126	62.7	25.2	199.7	14.13	31.5
28AC	5	132	59.1	26.4	564.3	23.75	33.0

* Found to be significantly higher than control mean by t-test.

Code: C = Control; M = Musculi; A = Ablastin in immune serum; AC = Normal rat serum (Ablastin Control).

Table III. Schistosoma mansoni schistosomula recovered from the peritoneal cavity of Control and Experimental mice.

Group	No. Mice	No. Schistosomula	% Males	Mean No. Schistosomula per mouse
C	17	20	100	1.2
4M	14	41	85.4	2.9
14M	19	21	95.2	1.1
28M	18	15	73.3	0.8
4A	20	16	93.8	0.8
14A	20	36	88.9	1.8
28A	19	36	91.7	1.9
4AC	4	6	100	1.5
14AC	5	9	100	1.8
28AC	5	5	100	1.0

Code: C = Control (S. mansoni); M = T. musculi concurrent infection; A = Ablastin in immune rat serum; AC = Ablastin Control (normal rat serum).

DISCUSSION

The peak parasitemia levels of T. musculi were found to be between days 6 and 16. This agrees with findings by Taliaferro (1938) and Viens and Targett (1971). The S. mansoni infected mice were available on day 4 of infection and this was the earliest stage when T. musculi infection could be introduced. At this stage the S. mansoni are most probably developed to schistosomula in the lungs (Cheng, 1968, pg. 263).

On day 15 the worms are in the liver where they feed on portal blood and grow rapidly. By the twenty-third day young adults immigrate to the mesenteric veins where sexual maturation and pairing take place.

Thus, in the 4M group the S. mansoni are exposed to ablastin when they are in the lungs and later in the liver. In the 14M group ablastin is present when the worms are in the liver and later in the mesenteric veins. In the 28M group ablastin is present during sexual maturation and the early part of egg production. The results indicate that ablastin had no effect on the development of S. mansoni.

Schistosoma mansoni depend on the glycolytic pathway for carbohydrate metabolism (Bueding 1949, von Brand 1966, Cheng 1968). One of the known effects of ablastin on division forms of T. lewisi is the reduction of enzyme levels in the glycolytic pathway. Ablastin causes

reduction of protein synthesis and almost complete cessation of nucleic acid synthesis in the trypanosomes (Taliaferro and Pizzi 1960). However, the results of this experiment seem to indicate that ablastin had no effect on the growth and development of S. mansoni. No significant change was observed between the mean number of worms recovered from control mice or those with concurrent infections. This was the case with groups in which T. musculi infection was introduced on days 4, 14, and 28 of S. mansoni infection. Morphologically, there was no observable difference between the worms recovered from the control and experimental mice.

Rigby and Chobotar (1966) found that T. lewisi inhibited the growth of H. diminuta in concurrently infected rats, and proposed that such inhibition could be through an indirect immunological factor. Any such factor does not seem to be effective in this experiment. Whether this factor is ablastin, and whether a corresponding factor is produced by T. musculi is not known.

Patton (1975) reports that several lines of evidence have been found indicating that ablastin may act by affecting membrane function. The normal development of S. mansoni in the concurrent infection indicates that no such impairment of membrane function occurred in the schistosomes. However, it must be noted that the worms were not exposed to ablastin until about day 10 in this experiment since the S. mansoni infected mice were available to us on day 4 of the

infection. It would be of interest to start the infections on the same day, as well as infect with T. musculi about 10 days before S. mansoni in order to have a high level of ablastin at the time the cercariae are injected. This would show if ablastin has any effect on cercarial penetration.

In the groups where immune rat serum was transferred into S. mansoni infected mice starting on days 14 and 28, a slight but statistically significant increase in the number of worms was found. When the normal rat serum controls were compared with these there was no significant difference. However, when the normal rat serum controls from each group were compared with the corresponding group with immune serum transferred, no significant difference was observed. This must be due to the small sample size (5 mice) of the normal rat serum controls. It must be concluded that there is a slight increase in worm burden in the mice given immune as well as normal rat serum. The foreign rat protein seems to be responsible by causing a condition resembling serum sickness (Davis et al 1973). In a pilot study performed in preparation for this project, S. mansoni infected mice were given 0.2 ml each of ablastic rat serum every third day. When the fifth injection was given (12 days after the first one) the mice died of anaphylactic shock within a few minutes. This was why the decision was made prior to this project to inject immune serum every other day and give only 3 injections in all to minimize the possibility of

anaphylaxis. However, the immune system must have been somewhat compromised even with the 3 injections, the S. mansoni encountering a lowered resistance as a result. Another possible explanation for the slight increase in the number of worms is that rat serum may supply some materials that might enhance the growth and development of the parasite. This could also explain the unusual finding of coupled pairs of worms in the intraperitoneal cavities of two of the mice that had received rat serum by intraperitoneal injections. Some nutrients in the rat serum may have enhanced the tendency toward maturation of the schistosomula.

Ablastin produced against T. musculi in concurrent infections as well as transferred in T. lewisi immune rat serum had no effect on the growth and development of S. mansoni. As indicated above, this may be due to the fact that the ablastin was present at the earliest about 10 days after the cercaraie of S. mansoni were injected into the mice, and therefore it is not known whether or not ablastin might have had an effect if the antibody would have been present prior to infection.

SUMMARY

Ablastin produced by T. musculi in a concurrent infection with S. mansoni has no effect on the development of the blood fluke infection when the T. musculi parasite is introduced on days 4, 14 and 28 after the S. mansoni cercariae are introduced in the mice. Since it takes from 8 to 10 days for ablastin to be produced in the mice, the schistosome infection was about 2 weeks established at the earliest trial time (4 days) before any effect might have been produced on the worms. Ablastin inhibits protein and nucleic acid synthesis in dividing forms of the trypanosomes. Some evidence also exists which suggests that ablastin might act by altering membrane function. However, in the concurrent infections no significant difference was found between the mean number of worms recovered from the mice and those from the controls.

In another test, ablastin was introduced into groups of S. mansoni infected mice by transferring T. lewisi immune rat serum into the mice starting on days 4, 14 and 28 (each mouse receiving a total of three 0.2 ml injections i.p.). No adverse effects were noted on the worms. The slight increase in the mean number of worms recovered in the mice receiving immune rat serum and those receiving normal rat serum must be attributed to a compromising of the immune system of the mice due to the repeated injections of large amounts of

foreign protein.

It is suggested that valuable information might be gained from a study in which the experimental mice would be given the T. musculi injections 10 and 5 days before, and at day zero of schistosome infections. Introducing T. musculi 10 days prior to the schistosome injection would ensure a high titre of ablastin when cercariae are introduced. It is possible that ablastin has an effect on the early stages of infection, including cercarial penetration.

REFERENCES

Abath, G.M., E. Coutinho-Abath, and J.M. Barbosa. 1966., Histopathology of skeletal muscle in experimental Chagas' disease. II. Alterations in late phase and in combined infection with Schistosoma mansoni. Am. J. Trop. Med. Hyg. 15:141-145.

Ashley, W., Jr. 1962. The effect of a concurrent infection with Trypanosoma lewisi on the development and maintenance of acquired immunity to Nippostrongylus brasiliensis in rats.

Brand, T. von. 1966. Biochemistry of Parasites. Academic Press. N.Y. and London, pp. 429.

Bueding, E. 1949. Metabolism of Parasitic Helminths, Physiol. Rev. 29: (No. 3) 195-217.

Cheng, T.C. 1968. The Biology of Animal Parasites, Saunders Co. Philadelphia and London, pp. 727.

Cram, E.B. and W.B. Figgat 1947. Experimental Mammalian Infection with the Schistosomes of Man. II. Comparative study of Schistosoma mansoni and Schistosoma japonicum infections produced by immersion and by intraperitoneal injection. Studies in Schistosomiasis. National Inst. Health Bulletin No. 189, pp. 106-108.

D'Alesandro, P.A. 1970. "Nonpathogenic Trypanosomes of Rodents" in Immunity to Parasitic Animals, Vol. II. Appleton-Century-Crofts, N.Y., pp. 1217.

Davis, B.D., R. Dulbecco, H.N. Eisen, H.S. Ginsberg and W.B. Wood, 1973. Microbiology, Harper and Row, Hagerstown, Md., New York, Evanston, San Francisco, London, pp. 1562.

Ee-siriporn, V., and E.D. Wagner. 1969. The effect of Trypanosoma equiperdum on mice infected with Schistosoma mansoni. Japanese J. Parasit. 18:66-70.

Hoare, C.A. 1972. The Trypanosomes of Mammals, Blackwell Scientific Publications, Oxford and Edinburgh, pp. 749.

Hunter, G.W., III, C.J. Weinmann, and R.G. Hoffmann. 1961. Studies on schistosomiasis. XVII. Non-reciprocal acquired resistance between Schistosoma mansoni and Schistosomatium douthitti in mice. Exp. Parasit. 11:133-140.

Hughes, F.W., and A.L. Tatum. 1956. Effects of hypoxia and intercurrent infections on infections by Plasmodium berghei in rats. J. Infect. Dis. 99:38-43.

Jachowski, L.A., Jr. 1961. Influence of Trichinosis on Schistosoma mansoni in mice. J. Parasit. 47:719.

Jackson, G.J. 1959. Simultaneous infections with Plasmodium berghei and Trypanosoma lewisi in the rat. J. Parasit. 45:94.

Krampitz, H.E. 1969. Verbreitung, Wirt-Parasit-Beziehungen und Vermehrung sizilianischer Stamme von Trypanosoma (Herpetosoma) duttoni Thiroux, 1905 (Protozoa, Trypanosomatidae). Zeitschrift fur Parasitenkunde 32:297-315.

Marmorston-Gottesman, J., and D. Perla. 1930. Studies on Bartonella muris anemia of albino rats. I. Trypanosoma lewisi infection in normal albino rats associated with Bartonella muris anemia. II. Latent infection in adult normal rats. J. Exp. Med. 52:121-129.

Patton, C.L. 1975. The Ablastin Phenomenon: Inhibition of Membrane Function. Exp. Parasit. 38:357-369.

Rigby, D.W., and B. Chobotar. 1966. The effects of Trypanosoma lewisi on the development of Hymenolepis diminuta in concurrently infected white rats. J. Parasit. 52:389-394.

Roth, A.A., and H.E. Heidtke. 1966. Removal of schistosomes from hosts with minimal physiological disturbance to the parasite. Tr. Am. Micr. Soc. 85:422-426.

Singer, I., E.T. Kimble III, and R.E. Ritts, Jr. 1964. Alterations in the host parasite relationship by administration of endotoxin to mice with infections of trypanosomes. J. Infect. Dis. 114:243-248.

Taliaferro, W.H. 1938. Ablastic and trypanocidal antibodies against Trypanosoma duttoni. J. Immun. 35:303-328.

_____, and T. Pizzi. 1960. The inhibition of nucleic acid and protein synthesis in Trypanosoma lewisi by the antibody ablastin. Proc. Nat. Acad. Sci. U.S.A. 46:733-745.

Tate, P. 1951. Antagonism of spirillum minus infection in rats towards Trypanosoma lewisi and T. equinum. Parasitology 41:117-127.

Viens, P., and G.A.T. Targett. 1971. Trypanosoma musculi infection in intact and thymectomized CBA mice. Trans. Roy. Soc. Trop. Med. & Hyg. 65:424.

Weinmann, C.J. 1960. Studies on Schistosomiasis. XV. Resistance to Schistosoma mansoni in mice immunized with Trichinella spiralis. J. Parasit. 46 (suppl.):37.

Yoeli, M. 1956. Some aspects of concomitant infections of plasmodia and schistosomes. 1. The effect of Schistosoma mansoni on the course of infection of Plasmodium berghei in the field vole (Microtus guentheri). Am. J. Trop. Med. Hyg. 5:988-999.

APPENDIX

```
NO. OF OBS. IN GROUP 1 ? 17 C
NO. OF OBS. IN GROUP 2 ? 14 4M
IS DATA IN DATA FILE ? ? N      INPUT DATA FOR EACH GROUP.

GROUP 1 : 16,39,32,0,\? 0
          27,37,26,28,18
          6,27,27,1,21
          1,12
GROUP 2 : 28,23,0,45,\? 35
          16,0,30,42,27
          29,25,15,22

ARE INTERMEDIATE RESULTS DESIRED ? Y

                                GROUP 1          GROUP 2

SAMPLE SIZE . . . . . .          17               14
SUM OF OBSERVATIONS . . . .      318              337
SUM OF SQUARES . . . . . .       8764             10387
MEAN . . . . . .                 18.7058          24.0714
VARIANCE . . . . . .             175.97           174.994
STANDARD DEVIATION . . . . .     13.265368445693                  13.228529
                                                  (ST. DEV.)
POOLED VARIANCE . . . . . .      175.532          13.248849006611
VARIANCE OF MEAN DIFFERENCE      22.8507          4.7802405797198

                                T-VALUE          D.F.             P-VALUE

EQUAL   VARIANCES . . . . .     -1.12214         29               .271023
UNEQUAL VARIANCES . . . . .     -1.12245         28               .271224
```

```
NO. OF OBS. IN GROUP 1 ? 17 C
NO. OF OBS. IN GROUP 2 ? 19  14M
IS DATA IN DATA FILE ? ? N      INPUT DATA FOR EACH GROUP.

GROUP 1 : 16,39,32,0,0
          27,37,26,28,18
          6,27,27,1,21
          1,12
GROUP 2 : 0,1,0,37,30
          21,26,0,1,0
          0,19,\? 29,46,33
          44,19,27,36

ARE INTERMEDIATE RESULTS DESIRED ? Y

                                GROUP 1          GROUP 2

SAMPLE SIZE . . . . . .          17               19
SUM OF OBSERVATIONS . . . .      318              369
SUM OF SQUARES . . . . . .       8764             12117
MEAN . . . . . .                 18.7058          19.421
VARIANCE . . . . . .             175.97           275.035
STANDARD DEVIATION . . . . .     13.265368445693                  16.58417
                                                  (ST. DEV.)
POOLED VARIANCE . . . . . .      228.416          15.113437729385
VARIANCE OF MEAN DIFFERENCE      24.8267          4.9826398625628

                                T-VALUE          D.F.          P-VALUE

EQUAL   VARIANCES . . . . .     -.141747         34            .888119
UNEQUAL VARIANCES . . . . .     -.143538         34            .886716
```

```
NO. OF OBS. IN GROUP 1 ? 17
NO. OF OBS. IN GROUP 2 ? 18
IS DATA IN DATA FILE ? ? N      INPUT DATA FOR EACH GROUP.

GROUP 1 : 16,39,32,0,0
          27,37,26,28,18
          6,27,27,1,21
          1,12
GROUP 2 : 38,22,28,1,0
          0,40,9,13,46
          43,0,27,35,52
          42,43,1

ARE INTERMEDIATE RESULTS DESIRED ? Y

                                  GROUP 1          GROUP 2

SAMPLE SIZE . . . . . .           17               18
SUM OF OBSERVATIONS . . . .       318              440
SUM OF SQUARES . . . . . .        8764             16800
MEAN . . . . . .                  18.7058          24.4444
VARIANCE . . . . . .              175.97           355.555
STANDARD DEVIATION . . . . .      13.265368445693                  18.856
                                                   (ST. DEV.)
POOLED VARIANCE . . . . . .       268.483          16.385450863494
VARIANCE OF MEAN DIFFERENCE       30.1042          5.4867294447603

                                  T-VALUE          D.F.             P-VALUE

EQUAL   VARIANCES . . . . .      -1.03555         33               .307956
UNEQUAL VARIANCES . . . . .       -1.0459          31               .30371
```

```
NO. OF OBS. IN GROUP 1 ? 17 C
O. OF OBS. IN GROUP 2 ? 20 CA
S DATA IN DATA FILE ? ? N      INPUT DATA FOR EACH GROUP.

GROUP 1 : 16,39,32,0,0
          27,37,26,28,18
          6,27,27,1,21
            1,12
OUP RS : \? 0,24,33,18,66
          28,33,15,14,16
          29,34,31,46,37
          0,41,22,21,38

ARE INTERMEDIATE RESULTS DESIRED ? Y

                                GROUP 1          GROUP 2

SAMPLE SIZE . . . . . .          17               20
SUM OF OBSERVATIONS . . . .      318              546
SM OF SQUARES . . . . . .        8764             19388
EAN . . . . . .                  18.7058          27.3
ARIANCE . . . . . .              175.97           235.905
TANDARD DEVIATION . . . . .      13.265368445693                   15.359199
                                                  (ST. DEV.)
OLED VARIANCE . . . . . .        208.506          14.439736839707
ARIANCE OF MEAN DIFFERENCE       22.1464          4.705996175094

                                T-VALUE          D.F.             P-VALUE

EQUAL   VARIANCES . . . . .     -1.8042          35               7.98322E-02
UNEQUAL VARIANCES . . . . .     -1.82622         35               7.63781E-02
```

```
NO. OF OBS. IN GROUP 1 ? 17  C
NO. OF OBS. IN GROUP 2 ? 20  14 A
IS DATA IN DATA FILE ? ? N      INPUT DATA FOR EACH GROUP.

GROUP 1 : 16,39,32,0,0
          27,37,26,28,18
          6,27,27,1,21
          1,12
GROUP 2 : 31,63,23,37,32
          21,30,0,48,28
          43,0,47,39,37
          27,37,0,32,0

ARE INTERMEDIATE RESULTS DESIRED ? Y

                                  GROUP 1          GROUP 2

SAMPLE SIZE . . . . . .           17               20
SUM OF OBSERVATIONS . . . .       318              575
SUM OF SQUARES . . . . . .        8764             22351
MEAN . . . . . .                  18.7058          28.75
VARIANCE . . . . . .              175.97           306.302
STANDARD DEVIATION . . . . .      13.265368445693                  17.5014
                                                   (ST. DEV.)
POOLED VARIANCE . . . . . .       246.721          15.707354965111
VARIANCE OF MEAN DIFFERENCE       25.6662          5.066181994362

                                  T-VALUE          D.F.          P-VALUE

EQUAL   VARIANCES . . . . .      -1.93843         35            6.06893E-02
UNEQUAL VARIANCES . . . . .      -1.98259         35            5.53267E-02
```

```
NO. OF OBS. IN GROUP 1 ? 17  C
NO. OF OBS. IN GROUP 2 ? 19  28 A
IS DATA IN DATA FILE ? ? N      INPUT DATA FOR EACH GROUP.

GROUP 1 : 16,39,32,0,0
          27,37,26,28,18
          6,27,27,1,21
          1,12
GROUP 2 : 31,42,12,0,51
          51,27,24,15,33
          31,38,1,27,48
          35,32,24,12

ARE INTERMEDIATE RESULTS DESIRED ? Y

                                  GROUP 1          GROUP 2

SAMPLE SIZE . . . . . .           17               19
SUM OF OBSERVATIONS . . . .       318              534
SUM OF SQUARES . . . . . .        8764             19098
MEAN . . . . . .                  18.7058          28.1052
VARIANCE . . . . . .              175.97           227.21
STANDARD DEVIATION . . . . .      13.265368445693                  15.07348
                                                   (ST. DEV.)
POOLED VARIANCE . . . . . .       203.097          14.251210474903
VARIANCE OF MEAN DIFFERENCE       22.3095          4.7232933425735

                                  T-VALUE          D.F.          P-VALUE

EQUAL   VARIANCES . . . . .      -1.97559         34            5.63954E-02
UNEQUAL VARIANCES . . . . .      -1.99            34            5.47216E-02

DO YOU WISH TO MAKE ANOTHER RUN  ? Y
```

```
NO. OF OBS. IN GROUP 1 ? 17
NO. OF OBS. IN GROUP 2 ? 4
IS DATA IN DATA FILE ? ? N      INPUT DATA FOR EACH GROUP.

GROUP 1 : 16,39,32,0,0
          27,37,26,28,18
          6,27,27,1,21
          1,12
GROUP 2 : 18,41,40,34

ARE INTERMEDIATE RESULTS DESIRED ? Y

                                  GROUP 1          GROUP 2

SAMPLE SIZE . . . . . .           17               4
SUM OF OBSERVATIONS . . . .       318              133
SUM OF SQUARES . . . . . .        8764             4761
MEAN . . . . . .                  18.7058          33.25
VARIANCE . . . . . .              175.97           112.916
STANDARD DEVIATION . . . . .      13.265368445693                   10.62619
                                                   (ST. DEV.)
POOLED VARIANCE . . . . . .       166.014          12.884642020638
VARIANCE OF MEAN DIFFERENCE       38.5801          6.211288111173

                                  T-VALUE          D.F.             P-VALUE

EQUAL   VARIANCES . . . . .      -2.03124         19               5.64707E-02
UNEQUAL VARIANCES . . . . .      -2.34157         5                6.62511E-02

DO YOU WISH TO MAKE ANOTHER RUN  ? Y
```

```
NO. OF OBS. IN GROUP 1 ? 17 C
NO. OF OBS. IN GROUP 2 ? 5  14AC
IS DATA IN DATA FILE ? ? N      INPUT DATA FOR EACH GROUP.

GROUP 1 : 16,39,32,0,0
          27,37,26,28,18
          6,27,27,1,21
          1,2212
GROUP 2 : 30,0,33,32,31

ARE INTERMEDIATE RESULTS DESIRED ? Y

                                  GROUP 1          GROUP 2

SAMPLE SIZE . . . . . .           17               5
SUM OF OBSERVATIONS . . . .       318              126
SUM OF SQUARES . . . . . .        8764             3974
MEAN . . . . . .                  18.7058          25.2
VARIANCE . . . . . .              175.97           199.7
STANDARD DEVIATION . . . . .      13.265368445693                  14.1315
                                                   (ST. DEV.)
POOLED VARIANCE . . . . . .       180.716          13.443065126674
VARIANCE OF MEAN DIFFERENCE       50.2911          7.0916218173278

                                  T-VALUE          D.F.             P-VALUE

EQUAL   VARIANCES . . . . .      -.949566          20               .353678
UNEQUAL VARIANCES . . . . .      -.915756          6                .395114
```

```
NO. OF OBS. IN GROUP 1 ? 17
NO. OF OBS. IN GROUP 2 ? 5
IS DATA IN DATA FILE ? ? N      INPUT DATA FOR EACH GROUP.

GROUP 1 : 16,39,32,0,0
          27,37,26,28,18
          6,27,27,1,12
          1,21
GROUP 2 : 7,58,0,\? 27,40

ARE INTERMEDIATE RESULTS DESIRED ? Y

                                GROUP 1          GROUP 2

SAMPLE SIZE . . . . . .          17               5
SUM OF OBSERVATIONS . . . .      318              132
SUM OF SQUARES . . . . . .       8764             5742
MEAN . . . . . .                 18.7058          26.4
VARIANCE . . . . . .             175.97           564.3
STANDARD DEVIATION . . . . .     13.265368445693                  23.7549
                                                  (ST. DEV.)
POOLED VARIANCE . . . . . .      253.636          15.925953660613
VARIANCE OF MEAN DIFFERENCE      123.211          11.100045044953

                                T-VALUE          D.F.             P-VALUE

EQUAL   VARIANCES . . . . .     -.949633         20               .353646
UNEQUAL VARIANCES . . . . .     -.693168         5                .519078
```

```
NO. OF OBS. IN GROUP 1 ? 20 4A
NO. OF OBS. IN GROUP 2 ? 4  4AC
IS DATA IN DATA FILE ? ? N      INPUT DATA FOR EACH GROUP.

GROUP 1 : 0,24,33,18,66
          28,33,15,14,16
          29,34,31,46,37
          0,41,22,21,38
GROUP 2 : 18,41,40,34

ARE INTERMEDIATE RESULTS DESIRED ? Y

                                  GROUP 1          GROUP 2

SAMPLE SIZE . . . . . .           20               4
SUM OF OBSERVATIONS . . . .       546              133
SUM OF SQUARES. . . . . .         19388            4761
MEAN . . . . . .                  27.3             33.25
VARIANCE . . . . . .              235.905          112.916
STANDARD DEVIATION . . . . .      15.359199197874                   10.6261
                                                   (ST. DEV.)
POOLED VARIANCE . . . . . .       219.133          14.803141558466
VARIANCE OF MEAN DIFFERENCE       40.0242          6.3264682090404

                                  T-VALUE          D.F.             P-VALUE

EQUAL   VARIANCES . . . . .      -.733841         22               .470804
UNEQUAL VARIANCES . . . . .      -.940493         6                .383276
```

```
NO. OF OBS. IN GROUP 1 ? 20 14A
NO. OF OBS. IN GROUP 2 ? 5  14AC
IS DATA IN DATA FILE ? ? N      INPUT DATA FOR EACH GROUP.

GROUP 1 : 31,63,23,37,32
          21,30,0,48,28
          43,0,47,39,37
          27,37,0,32,0
GROUP 2 : 30,0,33,32,31

ARE INTERMEDIATE RESULTS DESIRED ? Y

                                GROUP 1          GROUP 2

SAMPLE SIZE . . . . . .          20               5
SUM OF OBSERVATIONS . . . .      575              126
SUM OF SQUARES . . . . . .       22351            3974
MEAN . . . . . .                 28.75            25.2
VARIANCE . . . . . .             306.302          199.7
STANDARD DEVIATION . . . . .     17.501485651224                  14.1315
                                                  (ST. DEV.)
POOLED VARIANCE . . . . . .      287.762          16.963549156942
VARIANCE OF MEAN DIFFERENCE      55.2551          7.4333774288677

                                T-VALUE          D.F.             P-VALUE

EQUAL   VARIANCES . . . . .      .418544          23               .679437
UNEQUAL VARIANCES . . . . .      .477575          7                .647507
```

```
NO. OF OBS. IN GROUP 1 ? 19  28A
NO. OF OBS. IN GROUP 2 ? 5   28AC
IS DATA IN DATA FILE ? ? N     INPUT DATA FOR EACH GROUP.

GROUP 1 : 31,42,12,0,51
          51,27,24,15,33
          31,38,1,27,48
          35,32,24,2212
GROUP 2 : 7,58,\? 0,27,40

ARE INTERMEDIATE RESULTS DESIRED ? Y

                                GROUP 1          GROUP 2

SAMPLE SIZE . . . . . .          19               5
SUM OF OBSERVATIONS . . . .      534              132
SUM OF SQUARES . . . . . .       19098            5742
MEAN . . . . . .                 28.1052          26.4
VARIANCE . . . . . .             227.21           564.3
STANDARD DEVIATION . . . . .     15.073486657041                  23.75499
                                                  (ST. DEV.)
POOLED VARIANCE . . . . . .      288.499          16.985258314197
VARIANCE OF MEAN DIFFERENCE      124.818          11.172197635201

                                T-VALUE          D.F.          P-VALUE

EQUAL   VARIANCES . . . . .     .199737          22            .843524
UNEQUAL VARIANCES . . . . .     .152628          5             .884659
```

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