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The effects of *Trypanosoma equiperdum* on mice infected with *Schistosoma mansoni*

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Numerous studies on the effects of concurrent infections in laborattory animals have been reported in the literature. Only those reports dealing with mixed infections of schistosomes and one other parasite will be mentioned here. Yoeli (1956) observed that field voles had a mild course of malaria with low parasitemia when they were infected with *Plasmodium berghei* 4 to 7 weeks after *Schistosoma mansoni* infection. Weinmann (1960) postulated that *Trichinella spiralis* caused mice to become more susceptible to subsequent infection with *S. mansoni*, but Jachowski (1961) stated that prior trichina infections reduced the number of schistosomes when the mice were heavily infected with *S. mansoni*. Hunter *et al.* (1961) found that mice infected repeatedly with *S. mansoni* and challenged with *Schistosomatium douthitti* had fewer adult *S. douthitti* in comparison to challenge controls. Abath *et al.* (1966)suggested that *Trypanosoma cruzi* infection may have a protective effect against *Schistosoma mansoni* infection.

Double infections of *Schistosoma mansoni* in conjunction with *Fasciola hepatica*, and *S. iaponicum* with *Paragonimus westermani* have been reported in man, but no evaluations were made (Rodriguez-Molina & Hoffman, 1938; *Monserrat* & Buenaventura, 1959).

The purpose of this study is to determine whether a synergistic or antagonistic effect, or no effect is produced by inoculation of *Trypanosoma equiperdum* at varying periods of *Schistosoma mansoni* infection in white mice.

Materials and Methods

Female CF_1 mice weighing 14 to 15 grams were injected intraperitoneally with 100 *Schistosoma mansoni* cercariae each. The same number of mice of the same sex, age and weight but not infected with schistosomes were used as controls. The mice, divided into 5 series (numbered 1 through 5) of 25 mice each for experimental and control, were inoculated intraperitoneally with approximately 1.5×10^5 *Trypanosoma equiperdum* (NIH strain) when the duration of the schistosome infection was 1, 3, 5, 7, and 9 weeks. The time interval between removal of the trypanosomes from the donor rat and inoculation of the organisms into mice was within 1.5 hours. In all the tests the inocula were maintained at approximately 25 C.

The hours of survival of the mice after the *T. equiperdum* infection were noted. At death, the mice with schistosome infections were necropsied on a perfusion board (Roth & Heidtke, 1966), the peritoneal schistosomes were flushed out from the peritoneal cavity, and schistosomes within the hepatic-portal vein,

Table I Effects of *T. equiperdum* on the survival of mice infected with *S. mansoni*

Series no.	Sample size	Mouse age (weeks)	Wks of worm infect.	Hours of survival: Range	Mean	S.D.	S.E.	Difference of means	95% confidence intervals	*t* value
1										
Exptl.	25	5	1	77.5–114.0	86.72	7.87	1.57	−1.56	±3.45	−0.91
Control	25	5		82.0– 95.5	85.16	3.41	0.69			
2										
Exptl.	25	7	3	75.5–105.5	84.48	9.21	1.84	0.26	±4.65	0.11
Control	25	7		78.0–102.0	84.74	7.00	1.40			
3										
Exptl.	24	9	5	77.0– 84.5	81.04	1.85	0.38	7.00	±4.20	3.27*
Control	25	9		77.5–115.5	88.04	10.33	2.07			
4										
Exptl.	25	11	7	71.0– 92.0	77.02	4.73	0.95	13.00	±4.30	6.07*
Control	25	11		80.0–108.5	90.02	9.60	1.92			
5										
Exptl.	24	13	9	78.5–119.0	86.92	9.22	1.88	11.00	±5.00	4.47*
Control	25	13		84.5–124.0	97.92	8.18	1.64			

* Significant at the 0.01 level.

Table II Mean number of schistosomes recovered at necropsy

Series no.	No. of mice	Weeks of infection	Peritoneal cavity	Liver; hepatic-portal & mesenteric veins	Total no.	S. D.	S. E.
1	24	1	—	6.88(?)	—		
2	25	3	—	31.08	31.08	15.68	3.13
3	24	5	8.71	53.54	62.25	14.23	2.92
4	25	7	21.64	35.80	57.44	12.74	2.54
5	24	9	5.70	45.33	51.03	15.34	3.13

Because of their minute size and stage within the life cycle, the total number of schistosomes could not be ascertained in Series 1 and Series 2.

Table III Correlation between the number of schistosomes recovered at necropsy and the survival hours of mice infected with *S. mansoni* and *T. equiperdum*.

Series no.	No. of mice	Mean no. of adult schistosomes	Mean hours of survival	Correlation coefficient	*t* value
2	25	31.08	84.48	0.24	0.18
3	24	53.54	81.04	−0.19	0.89
4	25	35.80	77.02	−0.16	0.78
5	24	45.33	86.92	−0.40	2.07

Series 1 not done.

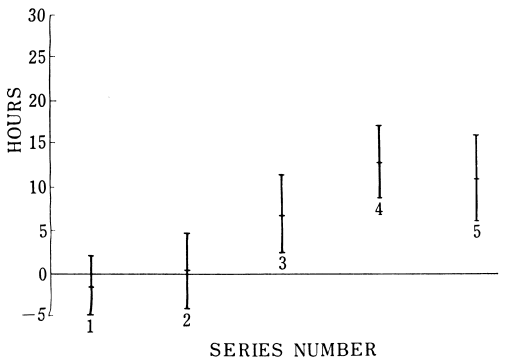

Fig. 1. Confidence intervals at 95% level for differences between the mean survival hours of control mice and mice infected with *T. equiperdum* at 1, 3, 5, 7, and 9 weeks after *S. mansoni* infection. (See Table I)

mesenteric veins and liver were manually recovered.

Results

The results of this study are shown in Tables I, II and III, and in Figure 1.

In Series 1, there was no significant difference between the survival hours of mice receiving *Trypanosoma equiperdum* one week after the *Schistosoma mansoni* infection (ave. =86.72 hrs.) and control mice (ave. =85.16 hrs.). Because of the relatively short period of development (1 week) the total number of schistosomulae could not be ascertained; however, an average of 6.88 immature schistosomes per mouse was found when livers were crushed between glass plates.

In Series 2 there was also no significant difference between the survival of mice receiving trypanosomes 3 weeks after the schistosome infection (ave. = 84.48 hrs. vs. control mice, ave. = 84.74 hrs.). An average number of 31.08 schistosomes was found within the liver, hepatic-portal vein, and mesenteric veins. Due to their minute size, it was difficult to determine the number of immature schistosomes within the peritoneal cavity.

In Series 3, 4, and 5, the differences between the survival hours of the experimental versus the control groups were significant. The results in Series 3 (schistosome infection at 5 wks.) show a difference of 7.0 hours between the means of the experimental (81.04 hrs.) and control (88.04 hrs.). The experimental group had a mean number of 53.54 adult schistosomes. In Series 4 (schistosome infection at 7 wks.), the difference between the average survival hours was 13.0 hours (experimental=77.02 hrs., control=90.02 hrs.), and the mean number of adult schistosomes recovered was 35.8 in the experimental group. The difference between the hours of survival in Series 5 (schistosome infection at 9 wks.) was 11.0 hours, and the experimental group had an average of 45.33 adult schistosomes.

As can be noted in Table III, there is no significant correlation between the mean number of mature schistosomes and hours of survival in mice infected with both schistosomes and trypanosomes.

Discussion

The results indicate that there were no significant differences between the means of the survival hours of control mice and mice infected with *Trypanosoma equiperdum* after 1 and 3 weeks of *Schistosoma mansoni* infection. Figure 1 and Table I show that the differences between the averages of the survival hours of the control and experimental groups at 5, 7, and 9 weeks of schistosome infection are significant by t test. In order to test whether the difference in survival time increases with the duration of the schistosome infection, a model involving regression coefficients β_1 and β_2 of the length of survival on the age of schistosome infection for the experimental and control groups was assumed. The hypothesis, Ho: $\beta_1=\beta_2$ was tested using the computer program BMDX64 of the X-series which is a supplement to the BMD Manual (Dixon, 1967). The F-ratio obtained, 30.49, with 1 and 242 degrees of freedom for the numerator and denominator, is highly significant ($P \ll .0005$).

Moore & Meleney (1955) reported that mice infected with *Schistosoma mansoni* by the intraperitoneal method could retain worms in the peritoneal cavity for as long as 24 weeks. In

bisexual infections, development of the worms in both the peritoneal cavity and within the organs was essentially the same during the first two weeks of infection; however, after this period, the worms within the liver, hepatic-portal vein and mesenteric veins reached maturity by 6 weeks, the peritoneal males by 8 weeks, and the peritoneal females between 10 to 12 weeks. Because few female worms in copula were inseminated, they postulated that blood may contain an essential nutritional requirement and therefore the peritoneal cavity would not be satisfactory for the development of schistosomes.

Frick *et al.* (1965) found that mice subjected to multiple, lowgrade cercarial exposures administered intraperitoneally acquired higher levels of resistance to *S. mansoni* than when the percutaneous method was utilized, and suggested that this could be due to prolonged immunological stimulation of the tissue phases in the lungs and liver caused by delayed passage of schistosomulae from the peritoneal cavity. In the present study, peritoneal forms of schistosomes were recovered at 5, 7, and 9 weeks of infection (Table II). Due to their minute size, it was difficult to detect the schistosomulae in the peritoneal cavity at 1 and 3 weeks of schistosome infection. The schistosomes within the liver and hepatic-portal vein were recovered, but the lungs and other organs were not examined for schistosomulae. Table III shows that there is no significant correlation between the survival hours of mice and the number of schistosomes found within the organs; however, in Series 5 (schistosome infection at 9 wks.), the t value is almost significant. Additional investigation is needed to clarify the results before an interpretation can be made.

Yoeli (1956) stated that the course of *Plasmodium berghei* in concurrent infection with *Schistosoma mansoni* was dependent on the period of schistosome infection as well as on the tissue reaction in field voles. He observed that plasmodial infections during the later stages of schistosomiasis were not as severe as plasmodial infections during the early stages of schistosomiasis. In the present investigation, it should be noted that the effects of *Trypanosoma equiperdum* was also dependent on the duration of the schistosome infection.

It was found that inoculation of *Trypanosoma equiperdum* into mice during the early stages of *S. mansoni* infection killed the mice in approximately the same length of time as mice infected with only *T. equiperdum*. Trypanosomes injected during the 5th, 7th, and 9th weeks of schistosome infection killed the mice within a shorter period than their corresponding controls. According to Moore & Meleney (1955) schistosomes within the liver, hepatic-portal vein and mesenteric veins are sexually mature at 6 weeks. Moore & Sandground (1956) reported that a single female *S. mansoni* in hamsters produced an average of 300 eggs per day, of which 78% remained embedded in the tissues. Eighteen per cent of the eggs remained in the wall of the large intestine, 32% in the small intestine, 26% in the liver, and 2% in the mesenteries and adjacent lymph nodes. A review of the pathogenicity and pathology of schistosomiasis in animals is given by Belding (1965). DeWitt & Warren (1959) observed that a syndrome resembling hepatosplenic schistosomiasis in man developed in mice infected with *S. mansoni*, and suggested that the syndrome was related to ova which caused granulomatous reactions in the liver and obstructed the portal blood flow. They also observed a marked anemia in some of the infected mice. Meleney *et al.* (1953) found that living worms did not stimulate tissue reaction, but dead worms in blood vessels incited thrombosis, perivascular reaction, scar formation and recanalization of the vessels.

With the maturation of the schistosomes between the 5th and 6th weeks of infection, it is apparent that the mice would have to adjust to the demands of the worm burden, including the pathological changes caused by the schistosome ova. Superimposed infection with *Trypanosoma equiperdum* may produce an increased stress and thus kill the mice within a shorter period of time than mice not infected with schistosomes. It is postulated that a synergistic

effect is produced in mixed infections of *Schistosoma mansoni* and *Trypanosoma equiperdum*.

Summary

In the study of concurrent infections using *Schistosoma mansoni* and *Trypanosoma equiperdum*, the mice were divided into experimental and control groups. The mice in the experimental group were injected intraperitoneally with 100 *Schistosoma mansoni* cercariae. Both groups were divided into 5 series each and inoculated intraperitoneally with 1.5×10^5 *Trypanosoma equiperdum* when the duration of the schistosome infection was 1, 3, 5, 7, and 9 weeks. The hours of survival of the control mice and schistosome-infected experimental mice were noted.

There is no difference between the means of the hours of survival in control and experimental groups when the trypanosomes are administered during the 1st and 3rd week of schistosome infection. However, the results show significant differences of survival hours between the means of the experimentals and their corresponding controls when the schistosome infection is at 5, 7, and 9 weeks of duration. The mice infected with schistosomes do not survive as long as the controls. It is proposed that a synergistic effect is produced in the mixed infections of *S. mansoni* and *T. equiperdum*.

The correlation between the hours of survival and the number of adult schistosomes recovered is not significant.

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