Effect of Castration in Male Mice on *Schistosoma mansoni.* (20272)

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Schistosoma mansoni (Sambon, 1907) has received extensive study both clinically and in the laboratory, but only recently has attention been focused on the physiological aspects of the parasite. Investigators(1-3) have noted the variations which occur with unisexual and bisexual infections obtained by introducing different ratios of male and female cercariae. Niyamasena(4), Gönnert(5), and Vogel(6,7) have studied the genetic phases and the histological development of the male and female worms. Variations have been detected in the sexual apparatus of the parasite by Vogel(7), Short(8,9), Gönnert(5), Najim(10) and Buttner(11). In this respect Buttner has found changes in different hosts. Thus the question of the possible influence of hormones, both from the parasite and the host standpoint is implicated. Addis(12) made interesting studies on several sex hormones and their effect on the growth of certain cestodes in rats. In his results he indicated that testosterone was necessary for the growth of these parasites, in male rats. Since cestodes and trematodes are both in the same phylum (platyhelminthes), it was thought by the writer that a similar relationship might exist between this hormone and *S. mansoni.*

An attempt has been made to see if any morphological change occurs, specifically that of the sexual apparatus of *S. mansoni,* in castrated male albino mice. After careful study it may be stated that morphologically the male and female worms recovered appeared to show normal sexual development. The females produced normal eggs while the males produced normal spermatozoa. However, there was a marked difference in the number of worms recovered from the castrated as compared to the uncastrated control mice. Further investigations on the cause of this phenomenon are now being carried on.

Materials and methods. Male albino mice (Rockland RAP strain) were employed. Fifty-five mice were surgically totally castrated at 5-6 weeks of age. Fifteen uncastrated mice of the same age group were used as controls. The average weight of the animals was 17.2 g at the time of castration. They were maintained throughout their life in white porcelain pans 12 x 8 x 5 inches with a wire screen top containing a depressed feeder. Each pan contained 7 mice which were fed exclusively on Purina Laboratory Chow, and each pan contained a watering bottle. The castration was done in the following way: The mice were anesthetized with sodium pentobarbital (Abbott), the dosage being 0.1 mg per gram body weight. The animal was then strapped to a dissecting board and taped across the abdomen to prevent the testis from reverting up the inguinal canals. A vertical incision 1-2 mm was made medially through the scrotal septum and the tunicas, thus exposing both testes. Each was clamped, ligated and excised at the epididymis level resulting in total bilateral castration. The scrotum was then clamped and sutured using two stitches. Four weeks after recovery, the castrated and uncastrated control mice were infected with approximately 140 pooled cercariae per mouse, at The Tropical Disease Laboratory, National Institutes of Health, Bethesda, Maryland, through the courtesy of Dr. Willard H. Wright. The source of the cercariae was laboratory bred snails of the species *Australorbis glabratus* (Say). The method of infection used was that of Olivier and Stirewalt(13) in modification. *Eggs* in the feces of the mice were first observed on the 45th day after infection. Previous examination revealed no visible eggs. The methods of stool examination employed were by direct smear in 1% eosin solution and by the Mathieson and Stoll Acid-Ether Method(14). Both the experimental and control animals were sacrificed at random 63-68 days after infection. They were killed with ether and the worms were perfused from the liver and intestine by the method of Yolles

TABLE I. *Schistosoma mansoni*, Total Adult Count by Sex Recovered from Castrated and Uncastrated Control Mice 63-68 Days after Infection.

	Castrated mice (43)	Uncastrated control mice (13)
Total worms	999	442
Male	671	321
Female	328	121

TABLE II. *Schistosoma mansoni*, Mean Adult Count by Sex from Castrated and Uncastrated Control Male Mice 63-68 Days after Infection.

	Castrated mice (43)	Uncastrated control mice (13)
Total worms	23.23	34.00
Male	15.61	24.69
Female	7.63	9.31

et al.(15). The tissues were then carefully teased to see whether any worms remained embedded therein. The schistosomes were then separated by sex and counted with the aid of a dissecting microscope.

Results. A total of 1442 *S. mansoni* adults were collected from both groups of mice with 999 from 43 castrated mice as compared with 442 from 13 uncastrated controls. For the total male and female worm population in the castrated mice a mean of 23.23 worms per mouse resulted, while in the uncastrated control group a mean of 34.00 per mouse resulted, indicating a difference of 10.37. The median and mode corresponded closely and the statistical analysis was based upon a normal probability curve. The Student "t" test for significance indicated that this difference was highly significant, the t value being 4.14 at the 5% level.

What is most interesting is that further analysis of individual sex indicated a significant difference between the male worms only.

A total of 671 *males* were collected from the castrated group while the uncastrated controls produced 321. The mean adult count per mouse was 15.61 for the former group and 24.69 for the latter indicating a reduction of 9.09 schistosomes. As analysed by the Student test, the "t" value of 4.32 for this difference was, in fact, more significant than the value for the total worm difference. The *female* schistosomes numbered 328 from the castrated mice and 121 from the uncastrated controls. The mean adult count was 7.63 and 9.31 respectively. Although a reduction of 1.68 worms occurred, statistical analysis indicated that this difference was within the realm of chance, the "t" value being 1.54 at the 5% level.

Discussion. An important observation from the results of this investigation is that in male mice, in which the sexual hormones of the testis were altered by castration, the male schistosomes were in some way affected whereby significantly fewer adult male worms occurred than in the animals with normally functioning testis. Thus, it may be assumed that male *S. mansoni* are influenced directly or indirectly by the sex hormones in the male albino mouse.

A factor which warrants attention is that the inactivation of testosterone takes place in the liver(16-21). It is in the liver and mesenteric venules that the parasites are normally found. In this respect a direct relationship may exist whereby testosterone is necessary for normal metabolism of male schistosomes. Of course, since the liver in itself is such a vital organ with so many activities simultaneously occurring, various other factors may be considered.

One of these which may be important to the parasite is the glycogenic and glycogenolytic activity which takes place in that organ(22). Bueding(23) in significant metabolic studies of *S. mansoni in vitro* has indicated the importance of glucose intake and glycolysis to the parasite. Thus any alteration of these processes may be harmful. Another point worthy of mention is the indication that the male worms undergo a greater rate of glycolysis than do the females. They also contain more than twice as much glycogen than the females in body weight(24).

There may be many intermediate and indirect factors involved in the mechanism of the experimental phenomena presented. Further research on these problems is being undertaken, and will be reported on at a future time. It is quite feasible that new information on host-parasite relationships may be forthcoming by the study of hormonal interaction between the two.

Summary. A group of castrated male albino mice and an uncastrated control group were equally infected with *Schistosoma mansoni.* Nine weeks later the animals were sacrificed and the adult parasites collected, separated according to sex and counted. The mean adult parasite count per mouse, for each group, showed a significant reduction in the male schistosomes of the castrated animals. This difference indicates a relationship between male sex hormones and the male parasite.

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Effect of Somatotrophic Hormone and Streptomycin on Mice Exposed to Total Body X-Irradiation.* (20273)

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Selye, Salgado and Procopio(1) have reported that rats subjected to whole body x-radiation (671 r total dose in 2 exposures 6 days apart) and treated twice daily with subcutaneous injections of 1.5 mg of somatotrophic hormone (STH) gained weight, whereas untreated controls lost weight during the 2nd and 3rd weeks post-irradiation. This treatment did not, however, reduce the mortality. Since loss of weight is a well recognized effect of radiation injury(2,3), their observation suggested that treatment with STH might have exerted some beneficial effect which was not reflected in the survival rates because of the occurrence of post-irradiation infection. In fact, they noted that some of the STH treated rats at autopsy showed lesions suggestive of infection.

Infection has been shown to play a significant role in the death of mice exposed to moderate doses of x-radiation(4). It seemed possible, therefore, that any therapeutic effect of STH in the treatment of radiation injury might be more clearly demonstrable in animals protected against infection by concomitant treatment with streptomycin. Accordingly, the following experiments were made to

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