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Host Sex and Sex Hormones as a Factor Affecting *Trypanosoma lewisi* Population in White Rats

Sarojam K. MANKAU

Department of Biology, California State College at San Bernardino, California U.S.A.

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The influence of host sex differences on susceptibility to parasitic infections in natural populations and experimental animals, and of controlled studies, using gonadectomy and gonadal hormone induced sex changes has been reported in a number of studies. Male metazoan parasites have generally been found to be more susceptible to experimental infection than female hosts and their susceptibility could be altered by sex hormone treatment (Campbell and Melcher (1940), Lees and Bass (1960), Solomon (1966), Mankau and Hamilton (1972), etc.)

Host sex-parasite relationships have also been reported in infections with such protozoans as *Entamoeba histolytica*, *Leishmania donovoni*, *Trypanosoma equiperdum* and malarial parasial parasites (Culbertson, 1941). The studies of *Plasmodium* infections on various hosts have not shown a consistant pattern in host sex susceptibility. Zuckerman and Yoeli (1954) found no sex differences in *Plasmodium berghei* infections in normal or gonadectomized rats, whereas, Greenberg, *et al.* (1953) observed a higher mortality rate in C57 black males than in females when infected with the same parasite. But in subsequent studies on host sex-strain interrelationship of *P. bergei* in mice, Greenberg and Kendrick (1957 and 1959), found significant variations in susceptibility ranging from no sex related differences in some strains to increased susceptibility of males or females in other strains. Gobel, *et al.* (1955) also (1965) also reported lower parasite numbers in experimental malaria infections of human males than in females, but they observed that under carefully controlled conditions of host age, sex and weight, females demonstrated greater resistance than males.

Studies on the effect of host sex and sex hormones on trypanosome populations have also failed to show consistant patterns in host susceptibility. Andrews, *et al.* (1930) found that host sex had no effect on the infection of rats with *Trypanosoma equiperdum*. Raffel (1934) however reported that when low doses of *T. equiperdum* were given to rats, males developed acute infections and died earlier than females. But, Morrel, *et al.* (1937) observed that when exposed to an equally virulent dose, female rats died sooner than males and that both sexes contained the same number of trypanosomes. Although Hauschka (1947) found that male mice were more susceptible to *Trypanosoma cruzi*, Goble (1954) reported no significant difference in the development of *T. cruzi* in male and female dogs.

The effect of gonadectomy of trypanosome populations was studied by Perla and Mormortson (1930) who reported that the mean number of *T. lewisi* in bilaterally gonadectomized male rats was 3 times greater than in normal females. In a similar study on young rats, Taliafero, *et al.* (1931) could find no difference in numbers of *T. lewisi* in males and females. Lincicome and Emejuaiwe (1963) agreed with the latter's observation and also noted that when normal or gonadectomized males and females were treated with homologous sex hormones trypanosomes developed better in females.

These conflicting reports and observations prompted the undertaking of the present study to determine whether host sex influences the population of *T. lewisi* harboured by male and female rats and to what extent the parasite number can be modified by gonadectomy and heterologus gonadal hormone treatment.

Materials and Methods

Hooded rats (Var Sprague-Dawley) about 8 weeks old were used as experimental animals. Males weighed approximately 140 g and females 120 g. The rats were fed a commercially prepared diet (Ralston Purina Co.) and provided water, *ad libitum*. *Trypanosoma lewisi* were initially obtained from an infected rat supplied by Carolina Biological Supply Co. The inoculum was prepared by mixing a few drops of infected rat blood with a glucose solution. Each individual inoculum consisted of 0.5 ml of the above mixture containing approximately 250,000 trypanosomes.

A total of 48 rats were placed in three treatment groups of 8 males and 8 females each. Group I served as a control. Group II were normal rats in which the males received stilbesterol and the females testosterone. Group III were gonadectomized and the males injected with stilbesterol and females with testosterone. All rats were infected at the same time. Gonadectomy was performed 7 days before infetion, under sodium pentabarbitol anesthesia. Hormone preparations were made by suspending 0.6 mg. of testosterone propionate and 0.005 mg. of stilbesterol respectively, in 0.5 ml of peanut oil. The hormone dose was administered intraperitoneally for 14 consecutive days.

The first blood smears were made 5 days after infection and then every alternate day for 14 days, from the tip of the tail. Smears were fixed in methyl alcohol for one minute and then stained with Wright or Giemsa stain. Five random areas were marked on each slide and the number of flagellates and blood cells found within a microscopic field were counted. The total number of *T. lewisi* per millileter of blood was calculated using the formula

$$\frac{7\times10^6}{\text{number of R.B.C.}} \times \text{Number of trypanosomes.}$$

Results

Average trypanosome population (Fig. 1) were over three times higher in the blood of males than in females of control rats (Group I). Normal male rats injected with

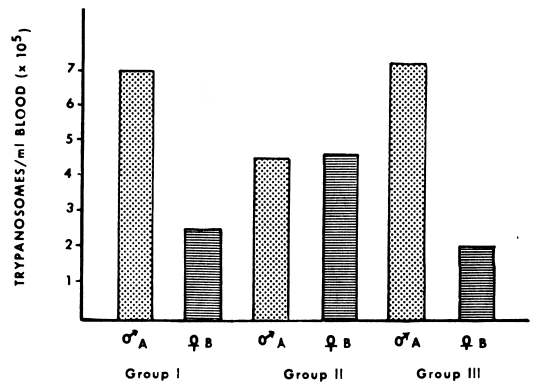

Fig. 1 Average number of trypahosomes found in the three groups of rats

stilbesterol and normal female rats injected with testosterone (Group II) had similar parasite counts. The hormone injections, therefore, appeared to have decreased the number of trypanosomes significantly in male rats and more than doubled the count in females. In the gonadectomized animals (Group III) which received heterologous hormone treatment, the trypanosome count in both males and females reached levels very similar to that of the control animals. The gonadectomized males exhibited the normal host-sex relationship to parasite numbers even under the influence of the female sex hormone, females similarly showed no effect of male hormone.

Fig. 2. shows the range of distribution of the number of trypanosomes found in the male and female rats of the 3 treatment groups. In all groups the highest parasitemia reached between 7 and 10 days after infection

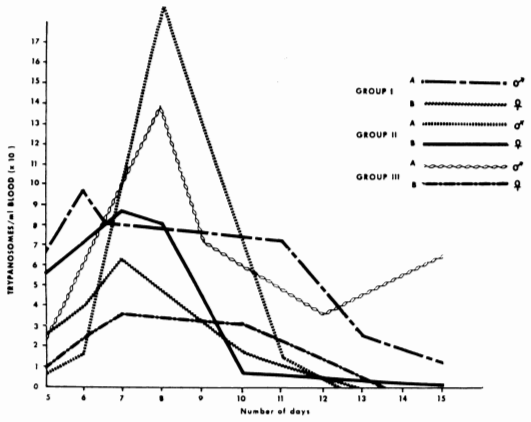

Fig. 2 Range of disribution of the number of trypanosomes

with the exception of testosterone treated males in Group I. In this group the number of trypanosomes increased from the 6th day of infection and peaked on the 8th day at a level higher than any other group and then dropped suddenly to very low levels, resulting in an average number of parasites well below that of males in Groups I and III. The males in the control group (Group I) maintained a consistently high level of parasitemia thereby reaching the highest average number for the period of infection, whereas gonadectomized females receiving testosterone treatment (Group III. B) showed a consistently low level of infection. A strikingly higher initial number of parasites are found in the female rats that received testosterone in Group II, compared with the female rats in the control group.

Discussion

The occurrence of three times as many *Trypanosoma lewisi* in the blood of male rats compared with female rats in the control group indicates a distinct female resistance of this parasite. Lincicome and Emejuaiwe (1963) also found the highest number of *T. lewisi* in experimentally infected young male rats compared with other groups. Similar sex differences were demonstrated in mice infected with *T. gambiense* and *T. congolense* when the inocula used were dilute enough not to overwhelm the natural immune mechanism (Goble 1954, *et al.* 1965). Hauschka (1947) found far more *T. cruzi* in experimentally infected male white mice then in females whereas, *T. cruzi* in dogs showed no significant differences in numbers between male and female hosts (Goble 1954) indicating susceptibility variations to the same parasite in different hosts. Higher resistance in female hosts was also exhibited by hamsters infected with *Leishmania donovoni* where edema resulting from the infection was found to be much more common in males than in females.

The infected rats treated with heterologous sex hormones (Group II), showed a significant decrease in the number of trypanosomes in males and an increase in the females compared with the control group. The resistance of the females to *T. lewisi* was therefore reduced by administration of testosterone and male susceptability was decreased when injected with stilbestrol. Sex hormones thus appear to have a distinct role in host resistance to *T. lewisi* infections. These results differ from an earlier report by Goble (1952) in his study of *T. cruzi* in mice where no sex difference was demonstrated on the course of infection in males and females even when treated with heterologous sex hormones. He therefore concluded that "substances other than the steroids of the adult gonads were involved". Goble *et al.* (1965) failed to get any significant effects by administering androgens and estrogens to mice infected with *Plasmodium berghei*. Trager (1948) had shown that female ducks with inactive ovaries and normal males had considerably heavier infections of *Plasmodium lophurae* compared with the female ducks at the time of egg laying, thus linking the level of parasitemia with ovarian activity. Shaw and Dusanic (1973) showed significant differences in the degree of resistance and level of parasitemia shown by female rats infected with *Trypanosoma lewisi* during the different stages of pregnancy. Rats infected early and late in the first week of pregnancy showed parasite counts similar to non-

pregnant females but half of the females in the latter group died before parturition. When female rats were infected during the mid-term of pregnancy, a majority died at the time of parturition without giving birth to the young. Also, the number of parasites were much higher than in non-pregnant females. But animals infected during the past week of pregnancy gave birth to normal litters and had very few parasites compared with non-pregnant rats. To what extent the fluctuation in parasite numbers is related to the changing levels of estrogen and progestrone as well as the gonadotropic hormones, needs further investigation.

The sex difference in susceptability to *Trypanosoma lewisi* infections demonstrated by Group I animals in this study was no longer evident when the male rats were treated with heterologous hormone treatment demonstrating that testosterooe injections can increase the susceptibility to parasitic infections not only in males but also in females, and that diethylstilbestrol can increase resistance to infection both in male and female animals. Mankau and Hamilton (1972) reversed the increased susceptability to *Trichinella spiralis* infection of male rats by the above method. Buschkiel (1954) made both male and female chicks more susceptible to *A. galli* infection with testosterone treatment, whereas diethylstilbestrol had no apparent effect on the worm burden. Ackert and Dewhirst (1950) also significantly increased the resistance of female chicks to *A. galli* infection by administering diethylstilbestrol.

Removal of gonads prior to injections with heterologous hormones, however, failed to change the susceptibility pattern and thus maintained the same level of parasitemia as in control animals. Lincicome and Emejuaiwe (1963) also reported that gonadectomy eliminated the sexual differences in susceptibility demonstrated by normal young rats to *Trypanosoma lewisi* infections. The interaction between artificially administered hormones and a hosts system of endocrine glands appear to be more complex than the simple effect of sex hormones, as gonadectomy demonstrated. The stress caused by gonadectomy may also interfere with the short period in which the hosts normal immune mechanisms eliminate parasites. Some other reports on the effect of gonadectomy and subsequent hormone treatment have been inconclusive (Berg 1953, 1957).

Robinson (1959) concluded that although hormonal changes within host mice may affect the survival of schistosomes, all the factors involved in this phenomenon are not known. Mankau and Hamilton (1972) observed that gonadectomy enhanced the effects of heterologous hormone treatments in rats with *Trichinella spiralis* infections and Campbell and Melchior (1940) obtained similor results in their work with *Taenia taeniformis* in rats. Many factors capable of influencing hosts-parasite relationships as listed in a review by Solomon (1969), but he also states "there is some evidence that estradiol decreases host susceptibility, but further studies are needed to clarify the situation". In the unique and complex microbiome of the parasite, host sex and sex hormones rppear to assume a significant role. Within all of the male rats used in the present study, the level of parasitemia was significantly higher when compared to females. There is a significant decrease in host susceptability in male animals injected with female sex hormones.

Summary

Male hooded rats infected with *Trypanosoma lewisi* had three times more flagellates in their blood than the females. Stilbestrol administered to normal male rats caused a marked decrease in the number of *T. lewisi* while testosterone administered to normal females resulted in a significant increase in the number of parasites. But when gonadectmized rats were given heterologous hormone injections, the sex difference in susceptibility tothe infection did not occur.

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ラットの *Trypanosoma lewisi* 感染濃度に及ぼす宿主の性および性ホルモンの影響

Sarojam K. Mankau
（カリフォルニア州立サン・ベルナルディノ大学）

自然集団および実験動物における宿主の性の違いとか，さらには生殖腺摘出術ないしは性ホルモン投与など性転換の，寄生虫感染の感受性に及ぼす影響については，多くの報告がある．本研究では，雌雄のラットに感染させた *Trypanosoma lewisi* の感染濃度に，宿主の性が影響を与えるかどうか，また生殖腺摘出術および異性の性ホルモン投与がどの程度寄生虫体数を変え得るかについて検討した．

48匹の8週令ラットを各群雌雄8匹ずつ3群に分け，第1群を対照とし，第2群を異性ホルモンで処置した．すなわち，雄にはスチルベステロール（0.005 mg）を，雌にはテントステロン（0.6 mg，以上いずれも0.5 mlのピーナッツオイルに混入）を14日間連日腹腔内に投与した．また第3群には，あらかじめ生殖腺摘出術を施した上，7日後に雌雄のラットにおのおのテストステロンあるいはスチルベステロールを第2群と同様に投与した．感染はホルモン投与初日に全てのラットに同時に行ない，血液塗抹標本を感染後5日目より14日間1日おきに作製した．血液1 ml中の *T. lewisi* 総数は，次の式により算出した．

$$\frac{7\times10^6}{\text{赤血球数}}\times\text{虫体数}$$

その結果，対照群雄ラットの平均虫体数は，同群雌のそれよりも3倍以上多かつたが，第2群でおのおの異性ホルモンを投与した場合には，雌雄の虫体数に差は認められなくなった．第3群では，第1群に極めて類似した結果が得られた．すなわち，たとえ異性ホルモンを投与しても，生殖腺摘出後の動物では虫体数に対するホルモンの影響はみられなかつた．

第2群の雄ラットを除き，全例において感染後7日目から10日目に血中虫体数は最高に達した．対照群の雄では，虫体数が高いレベルで維持された．第2群でテストステロンを投与された雌ラットにおいては，対照群の雌と比較し，

極めて多数の虫体が初期に出現した．血中虫体数のレベルは全ての雄ラットで著しく高く，また雌性ホルモンを注射した場合には雌雄ともに宿主感受性が著しく低下した．