THE HOST-PARASITE RELATIONSHIP BETWEEN THE SAUDI ARABIAN *SCHISTOSOMA MANSONI* AND ITS INTERMEDIATE AND DEFINITIVE HOSTS. 2. EFFECTS OF TEMPERATURE, SALINITY AND pH ON THE INFECTION OF MICE BY *S. MANSONI* CERCARIAE

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INTRODUCTION

The infectivity of schistosome cercariae may be influenced by physico-chemical factors, such as temperature (DeWitt, 1965; Stirewalt and Fregeau, 1965; Purnell, 1966; Upatham *et al.*, 1984), salinity (Stirewalt and Fregeau, 1965; Upatham *et al.*, 1984) and pH (Upatham *et al.*, 1984). The optimum temperature for infection of mice as judged by the percentage of infection and worm burden differred between the *Schistosoma mansoni* from Tanzania (Purnel, 1966), Puerto Rico (Stirewalt and Fregeau, 1965) and that studied by DeWitt (1965) who did not indicate its source.

Stirewalt and Fregeau (1965) also determined the effect of salinity on the infectivity of the Puerto Rican *S. mansoni* cercariae, while Upatham *et al.*, (1984) of *S. mekongi* and *S. japonicum* in mice. The findings were not consistent in the three schistostosome species studied.

The effect of pH on the infection of mice by *S. mekongi* and *S. japonicum* cercariae was investigated by Upatham *et al.*, (1984). There is hardly any information in the literature on the effect of pH on the infectivity of *S. mansoni* cercariae.

In Saudi Arabia where tropical desert climatic conditions prevail, the effects of physico-chemical factors on the penetration of schistosome cercariae into their hosts may be more pronounced.

This paper reports findings on the effects of temperature, salinity and pH on the infection of Swiss albino mice by the Saudi Arabian *S. mansoni* cercariae.

MATERIALS AND METHODS

The experiments were carried out as follows:

(1) The effect of temperature on the percentage of infection and worm burden in mice exposed to cercariae of *S. mansoni:*

Seventy Swiss albino mice were divided into 7 groups of 10 each. Every group was exposed to cercariae which had been acclimatized to temperatures ranging from 4° to 40°C at intervals of 6°C. Refrigerators were used to set temperatures of 4°, 10°, 16° and 22°C, while waterbaths were used for 34° and 40°C. The temperature of 28°C was that of water in a room without air-condition and served as a control. The exposure of mice to cercariae was done at room temperature (28°C). The tail immersion method was used (Viyanant *et al.*, 1977). Eighty cercariae were pipetted into each test tube, acclimatized to the exposure temperature for 2h, and then kept on the wooden rack. Immobilized mice in restraining chambers were exposed by inserting their tails in the test tubes for 30 min. After exposure, the mice were transferred to

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cages and reared for 8 weeks in the animal house. At the end of the eighth week, they were perfused (Smithers and Terry, 1965) and worms in positive mouse were recovered and counted.

(2) The effect of salinity on the percentage of infection and worm burden in mice exposed to cercariae of *S. mansoni:*

Solutions of NaCl ranging from 100, 200, 400, 800, 1,600, 3,200, 6,400 to 12,800 mg/l were prepared. Dechlorinated tap water with a salinity of 0.5 mg/l was used as a control. Ten mice were exposed to cercariae under each salinity. Thus, 10 test tubes were filled two-thirds full with solution of a particular salinity, then 80 cercariae were pipetted into each of them and left to acclimatize for 2 hours. After acclimatization, mice tails were introduced into the test tubes containing cercariae as described by Viyanant *et al.*, (1977). Exposure time was 30 min. The mice were reared in cages for 8 weeks. Thereafter, they were sacrificed and the worms were recovered by perfusion (Smithers and Terry, 1965) and counted under a dissecting microscope.

(3) The effect of hydrogen-ion concentration on the percentage of infection and worm burden in mice exposed to cercariae of *S. mansoni:*

Solutions of pH 4.4, 5.4, 6.4, 8.4 and 9.4 were prepared using 0.5 M KH_2 PO_4 and 0.5 M Na_2 HPO_4 solutions in distilled water. Distilled water with a pH of 7.4 was used as a control. Test tubes were filled with a solution of each pH and 80 cercariae were pipetted into each of them. The cercariae were left to acclimatize for 2 h to various pH values. Meanwhile, a group of 10 mice per pH value were immobilized by inserting them in restraining chambers and their tails were introduced into the cercarial suspension as described by Viyanant *et al.* (1977). Exposure time was 30 min. Then, the mice were kept in cages and reared for 8 weeks. At the end of the eighth week, the mice were sacrificed, and their worms were recovered by perfusion (Smithers and Terry, 1965) and counted.

RESULTS

The effects of water temperature during exposure on the infection of mice with cercariae of *S. mansoni* as determined by the percentage of infection and worm burdens are shown in Fig. 1 The percentage infection rate rose to 100% in mice infected by cercariae previously exposed to temperatures of 10°, 16°, 22°, 28° and 34°C. At 4° and 40°C, the percentage of infection was very low.

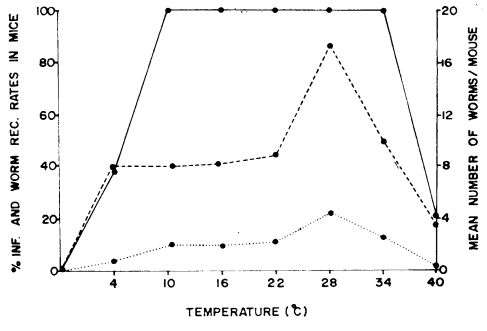

Fig. 1. Effect of water temperature on the infection of mice by cercariae of *S. mansoni* from Saudi Arabia. •.....• = % worm recovery rate; •---------• = worm burden; •———• = % infection rate.

The worm burden and worm recovery rate were highest in mice infected by cercariae previously acclimatized to a temperature of 28°C. Both the worm burden and worm recovery rate decreased as the temperature increased or decreased. The Anova test showed that there was a significant differences in the mean number of worms per mouse in different temperature regimes ($p < 0.01$). Thus, temperature had an effect on the worm burdens in mice exposed to the Saudi Arabian *S. mansoni* cercariae.

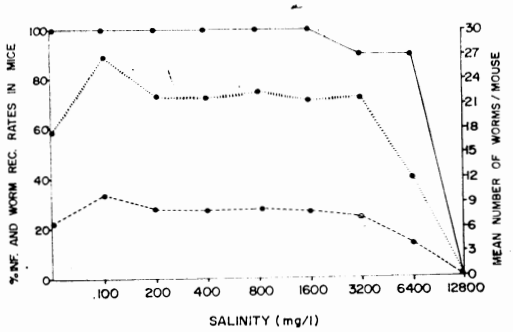

Fig. 2. Effect of salinity on the infection of mice with *S. mansoni* cercariae •.....• = worm burden; •----------• = % worm recovery rate; •———• = % infection rate.

The effects of salinity on the infection of mice by cercariae of a Saudi Arabian *S. mansoni* are illustrated in Fig. 2. The percentage infection rate was highest in mice infected by cercariae at salinities of 0.5 to 1,600 mg/l NaCl. There was no infection of mice at a salinity of 12,800 mg/l.

The worm burden and worm recovery rates were highest in mice infected by cercariae at salinity of 100 mg/l, thereafter both parameters decreased as the salinity increased. The analysis of variance showed a significant difference in the mean number of worms per mouse in different salinity levels ($p<0.05$). Therefore, salinity had an influence on the worm burden in mice.

Fig. 3 shows the effects of hydrogen-ion concentration (pH) on the infection of mice by cercariae of *S. mansoni* from Saudi Arabia. The percentage infection rate was highest in mice infected by cercariae at pH range of 6.4 to 8.4. Both the worm burden and worm recovery rate was highest in mice exposed to cercariae at pH 5.4. Mice exposed to cercariae at pH 4.4 did not develop any infection. The analysis of variance showed that there was a significant difference in mean number of worms per mouse in different pH levels ($p < 0.05$). Thus, pH had an influence in the infection and worm development in mice.

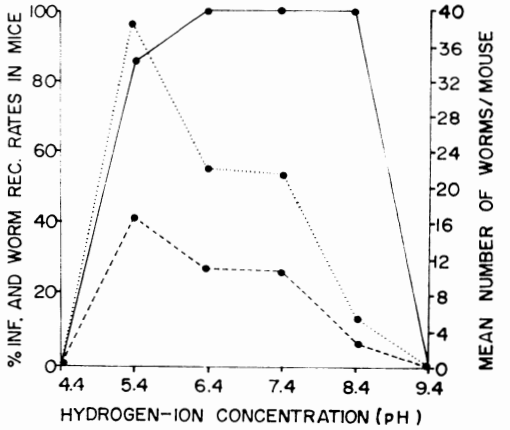

Fig. 3. Effect of pH on the infection of mice by *S. mansoni* from Saudi Arabia. •.....• = worm burden, •---------• = %worm recovery rate, •———• = % infection rate.

DISCUSSION

Temperature, salinity and hydrogen-ion concentration of water are some of the environmental factors which may influence the infection of definitive hosts by schistosome cercariae through their effects on the initial penetration process (Stirewalt and Fregeau, 1965; DeWitt, 1965; Purnell, 1966; Upatham *et al.*, 1984).

Temperature: Purnell (1966) investigated the effect of temperature on the infection of mice by a Tanzanian strain of *S. mansoni* and recorded the highest percentage of infection and worm recovery rates at 24°C. While Stirewalt and Fregeau (1965) observed the highest penetration and maturation levels in mice exposed to cercariae of a Puerto Rican strain of *S. mansoni* at 27° to 28°C. DeWitt (1965) found the highest worm burden at temperatures ranging from 30° to 35°C with a strain of *S. mansoni* maintained in the laboratory for a number of years, but he did not

mention its source. In the present study with the Saudi Arabian isolate of *S. mansoni*, both the worm burden and worm recovery rate were highest in mice infected with cercariae previously exposed to a temperature of 28°C. Nevertheless, the percentage infection rate remained high over a wide range of temperature (i.e. from 10°to30°C), a phenomenon also observed by DeWitt (1965). In *S. mekongi* and *S. japonicum* investigated by Upatham *et al.* (1984), the highest number of worms recovered occurred in mice infected by cercariae previously exposed to temperatures of 30° and 20° to 30°C, respectively.

These findings suggest that the optimum temperature for the infection of mice by cercariae of schistosomes differ from species to species and from strains of the same species, apparently depending on the ambient temperatures of a locality where the parasite was originally collected. Furthermore, temperatures other than the optimum tend to hinder the penetration of cercariae in mice resulting in lower maturation rates. This is particularly important in Saudi Arabia where ambient temperatures vary from one extreme to another between seasons, with the optimum temperature occurring at certain times of the year. This may impose a seasonality in the transmission of schistosomiasis mansoni.

Salinity: The infection of mice with schistosome cercariae has been found to occur under a wide range of salinities (Upatham *et al.*, 1984). The authors recorded the infection of mice with *S. mekongi* and *S. japonicum* cercariae at salinities ranging from 0.5 to 3,200 mg/l and 0.5 to 6,400 mg/l, respectively. However, the highest percentage of infection and worm recovery rates occurred at salinities of 200 mg/l for the former and 0.5, 100 and 200 mg/l for the later species. With the Saudi Arabian *S. mansoni* studied, infection of mice occurred at salinities ranging from 0.5 to 6,400 mg/l. The worm burden and worm recovery rates remained high in mice infected with cercariae at salinities of between 0.5 and 3,200 mg/l. It seems that the Saudi Arabian *S. mansoni* cercariae are more tolerant to a wide range of salinities and can produce high worm burdens and recovery rates even at higher salinity levels than *S. mekongi* and *S. japonicum* studied by Upatham *et al.* (1984). This might explain the existence of *S. mansoni* in places like Najran and Khayber which are very close to the sea and might have higher levels of NaCl in the fresh water habitats. It can be speculated that the Saudi Arabian *S. mansoni* might have acclimatized itself to high levels of salinity such that its transmission is no longer limited by it, unless the salinity level is very high (i.e. $\geqq$ 12,800 mg/l).

Hydrogen-ion concentration: In the study reported here with the Saudi Arabian *S. mansoni* isolate, it was shown that pH of water during exposure does influence the penetration of cercariae in mice resulting in varied percentages of infection and worm maturation rates. The highest percentages of infection occurred in mice infected with cercariae at pH range of 6.4 to 8.4, while the worm burden and recovery rate was highest in mice infected at pH 5.4. However, the worm burden and worm recovery rate remained high in mice infected at pH of between 5.4 to 7.4.

Upatham *et al.* (1984) investigated the effect of pH on the infection of mice with *S. mekongi* and *S. japonicum* cercariae and recorded the highest percentages of infection at pH 6.4 and 4.4 to 7.4, respectively. The worm recovery rate was highest at pH 4.4 to 7.4 for *S. mekongi* and pH 5.4 to 7.4 for *S. japonicum*.

Judging from these results, it can be said that the Saudi Arabian *S. mansoni* cercariae prefers neutral to slightly acidic conditions and does poorly in alkaline environments.

SUMMARY

The infectivity of cercariae of the Saudi Arabian isolate of *S. mansoni* was found to be influenced by factors such as water temperature, salinity and pH.

The optimum exposure temperature which resulted into the highest worm burdens and worm recovery rates in mice was 28°C. However, the percentage infection rate was highest at a temperature range of 10° to 34°C.

Mice were successfully infected with cercariae of *S. mansoni* at salinities of 0.5 to 6,400 mg/l. The highest worm burden and worm recovery rate occurred in mice infected by cercariae at a salinity of 100 mg/l, while the percentage infection rate was highest at a salinity range of 0.5 to 1,600 mg/l.

Mice exposed to cercariae at the pH of 4.4 and 9.4 did not develop any infection. The percentage infection rate was highest in mice exposed to cercariae at a pH range of 6.4 to 8.4. However, both the worm burden and worm recovery rates were highest in mice at pH 5.4.

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