Proceedings
of the
Society
for
Experimental Biology and Medicine

VOL. 96 OCTOBER, 1957 No. 1

# Effect of Cortisone on Natural Immunity to *Schistosoma mansoni* in Mice.* (23377)

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Investigators who have used mice(1) as experimental hosts in *Schistosoma mansoni* infections find that the average number of worms recoverable from matured infections does not usually approach 100% of the number of cercariae to which the mice are exposed. A 50% yield of adult worms is considered good. Some of the factors known to influence the degree of success of infection with a particular strain of mouse include variations in infective potential of different batches of cercariae(2) and variations in manner and route of exposure of the host to the parasite. Natural immunity factors undoubtedly are involved as well, but as yet there appears to be little information as to what the natural immunity factors are and when and how they operate(3). Previous work with other helminth parasite-host combinations(4,5) has demonstrated that cortisone provides a powerful tool with which to study the immunity relationships. Accordingly it was regarded as worthwhile to test the effect of cortisone upon an experimental *S. mansoni* infection. Because cortisone is known to have a limiting effect upon the lymphoid-macrophage system it was suspected *a priori* that the natural immunity of the mouse host would be weakened; however, the results of 3 separate experiments outlined below indicate that natural immunity was not weakened and, on the contrary, provide evidence that it may have been enhanced somewhat.

*Materials and methods.* Local strain white mice were used in Experiment 1, brown mice in Experiments 2 and 3. They were fed Purina dog checkers and water *ad libitum*. *S. mansoni* cercariae for infecting the mice were obtained from laboratory-reared snails (*Australorbis glabratus* of Puerto Rican origin) infected in the laboratory with miracidia hatched from eggs isolated from human stools. Exposure of the mice was by tail immersion, for one hour, to cercariae recently emerged from 7-22 snails, with the mice restrained in plastic holders(6). The numbers of cercariae were standardized and delivered into rain water in the infection tubes, using a semi-automatic pipette. Cortisone was cortisone acetate Merck ("Cortone") diluted with 0.85% NaCl to a strength of 10 mg per ml, given by the subcutaneous route in 0.5 mg

* This study was supported by a grant under contract with the Preventive Medicine Research Division, Department of the Army, Washington, D. C. The author is grateful to Dr. José Oliver-González for reviewing the manuscript and offering valued suggestions.

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TABLE I. Average Numbers of Worms Recovered from Treated and Control Mice, and Results of T-Tests of Mean Differences. NS = not significant.

| Exp. No. | Avg No. worms recovered Cortisone | Control | S.E. of mean diff. | Signif. level | Degrees of freedom |
|---|---|---|---|---|---|
| 1 | 10.1 | 17.1 | 2.414 | .05 | 12 |
| 2 A (♂) | 19.7 | 28.5 | 4.760 | NS | 6 |
| 2 B (♀) | 1.5 | 5.5 | 2.512 | NS | 6 |
| 3 | 16.9 | 23.9 | 2.067 | .01 | 8 or 18 |

doses. Seven or 8 weeks after exposure to infection each mouse was killed by cervical fracture, the viscera carefully removed and the worms teased out in saline for counting under a low power binocular dissecting microscope, recording their occurrence in mesenteric vessels, main portal vein and liver. Spleen, kidneys and lungs were examined occasionally but not routinely; in the cases where they were examined no worms were found in them.

*Experimental procedures.* In Experiment 1, 14 mice (8 males, 6 females), all 8 weeks old, were divided into 2 groups of 7 mice each (4M, 3F per group) and exposed simultaneously to approximately 40 cercariae per mouse. Mice of the experimental group were given a total of 12 injections of cortisone on the following days with reference to infection: –2, 0, 1, 2, 6, 10, 13, 17, 20, 23, 26 and 29. In Experiment 2, 17 mice 7 weeks old were divided into 2 groups of 9 and 8 mice respectively (cortisone: 5M, 4F; control: 4M, 4F) and exposed to approximately 55 cercariae per mouse. Inadvertently all males were exposed first and the females an hour later. The cortisone group mice were given a total of 9 injections on days: –1, 0, 1, 3, 5, 7, 9, 11 and 15. In Experiment 3, 20 mice 15 weeks old were divided into 2 groups of 11 and 9 mice respectively (cortisone: 7M, 4F; control: 6M, 3F) and exposed simultaneously to 50 cercariae each. The cortisone group mice received a total of 7 injections on days: –1, 0, 1, 3, 4, 5 and 7.

*Results.* All mice survived to necropsy except for 1 male cortisone-treated mouse in Experiment 2, which succumbed on the 20th day after infection. The cortisone-treated mice lost weight during cortisone treatment and appeared somewhat debilitated where the treatments were of extensive duration as in Experiment 1, but after treatments ceased they soon regained normal appearance and vigor comparable with the controls. A summary of average total worm counts in each experiment is given in Table I along with results of statistical tests of differences between the means. In all 3 experiments fewer worms were recovered from the cortisone-treated mice than from the controls. The differences between the means of treated and control groups were statistically significant in Experiments 1 and 3. In Experiment 2 far fewer worms were recovered from female mice of both groups than from the male mice. This does not represent a true sex difference, in view of the results of Experiment 3 and of another experiment not reported here, both of which utilized the same strain of brown mice. As previously indicated, the female mice were inadvertently exposed to infection at a later time than the males, although on the same day from the same pool of cercariae, and the cercariae apparently lost much of their infectivity even though this is not normally the case where the time between exposures is so short.

*Discussion.* These 3 experiments were closely but not exactly similar in design. In all of them cortisone treatments began prior to infection but the treatments were continued for progressively shorter periods of time, terminating on days 29, 15 and 7 respectively. The consistent trend of the results shows definitely that the regimens of cortisone treatment employed did not lessen the natural resistances of the treated mice to *S. mansoni* as measured by numbers of worms recovered from the hepatic portal tracts of the mice 7-8 weeks after infection. There is, moreover, evidence strongly suggestive of an opposite effect, since in all 3 cases fewer average num-

bers of worms were recovered from the cortisone-treated mice than from the untreated control mice. It is possible, but not likely, that worms may have ended up in other portions of the vascular system where they could have been overlooked; they did not accumulate in spleen, lungs or kidneys. This apparent strengthening of immunity stands in sharp contrast to findings with 2 nematode parasites, *Trichinella spiralis*(5,7) and *Nippostrongylus muris*(4), where cortisone treatment of the host weakens both natural and acquired resistances presumably because the treatment restrains the cellular phase of the immunity mechanism(8). By inference it is likely that in mice cellular defenses play little part in the initial phases of natural immunity to *S. mansoni*, that is during the period of the transient residence of the larva in the skin tissues. If the immunity strengthening effect observed here is a true one, then from the results of Experiment 3 where treatment ended on the 7th day after exposure to the parasite it would appear that the effect is exerted in the very early stage of infection and may be related to an inhibition of skin penetration and/or further migration by the larvae. Cercariae are known to exhibit hyaluronidase activity(9), and cortisone is known to inhibit hyaluronidase(10). According to Lewert and Lee the infective larvae of both *S. mansoni* and *Strongyloides ratti* possess collagenase activity, and cortisone treatment of the rat host seemed to inhibit this activity of *S. ratti* and to cause longer retention of the young worms in the skin(11). These facts may not necessarily be related to the present findings, but certainly further investigation is in order on the effects of cortisone in schistosomiasis.

*Summary.* In 3 separate experiments small groups of mice of 2 strains were exposed to infection with *Schistosoma mansoni* and half of them were treated with cortisone beginning shortly prior to infection and ending on the 29th, 15th and 7th days after infection, respectively. The various regimens of cortisone treatment employed did not increase susceptibility to the parasite, as judged by numbers of adult worms recovered from hepatic portal tracts of the mice 7-8 weeks after infection. The treatments may have enhanced the natural immunity slightly, since significantly fewer worms were recovered from the cortisone-treated mice.

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Received July 8, 1957. P.S.E.B.M., 1957, v96.

## Paper Electrophoresis of Muscle Fractions from Vitamin E Deficient Rabbits.* (23378)

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These studies were undertaken in an attempt to correlate vit. E avitaminosis with changes in muscle proteins and excretion of urinary nitrogen components, such as creatine, allantoin and amino acids. The easily soluble

* This investigation was supported by National Muscular Dystrophy Research Foundation Inc., and Muscular Dystrophy Associations of America, Inc.