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Leishmania braziliensis: Physical and Chemical Stress in Hamsters

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NOBLE, G. A. 1971. *Leishmania braziliensis:* Physical and chemical stress in hamsters. *Experimental Parasitology* **29**, 30–32. Eighty hamsters were inoculated via the skin of the nose with promastigotes of *Leishmania braziliensis*. Sixty, in groups of 10, were subjected to crowding, swimming, restraint, and to injections of dexamethasone and of ethylene glycol. Twenty were kept as controls. Ninety-five per cent of the injected animals that had nose ulcerations also developed large skin lesions on their backs. No control animals had back sores.

Cultivation of tissues showed dissemination of infection to viscera or skin in 13.3% of the stressed and injected hamsters. No dissemination occurred in control animal. Results showed that cortisone, ethylene glycol, and the stress of restraint significantly favored the development and spread of leishmanial infection.

INDEX DESCRIPTORS: *Leishmania braziliensis;* Stress; Hamster; Dexamethasone; Ethylene glycol; Drug effects.

Parasites are usually benefited when their hosts are subjected to more than moderate stress. This statement is based on the assumption that increase in parasite numbers and decrease in host resistance is beneficial to invading organisms. Most reports show that these phenomena occur when parasitized animals are stressed. The literature is repleat with reports on studies of stress, but few of them concern parasites and only a small percentage of these deal with parasites of man.

This report presents the results of a study of the effects of stress on hamsters after inoculation with promastigotes of a nonhuman strain of *Leishmania braziliensis*, the causative agent of American cutaneous leishmaniasis.

MATERIALS AND METHODS

Eighty young adult laboratory-raised golden hamsters (*Mesocricetus auratus*) were used. Each was inoculated intradermally in the nose with 15 million promastigotes from a culture of *Leishmania braziliensis*. This strain was originally found by Dr. Aristides Herrer in the spleen of a sloth in Panama and is maintained by him in a medium prepared as follows: To 1000 ml distilled water add 25.0 g Difco Bacto-beef. Boil and filter through filter paper. Add distilled water to bring the mixture to its original volume. Add 20.0 g Difco Bacto-peptone, 5.0 g NaCl, and 30.0 g Difco Bacto-agar. Adjust the pH to 7.2–7.4 and autoclave this stock medium. Add whole rabbit blood (no anticoagulant or defibrination) to the melted medium in the proportion of 10–13%. Tube and slant the medium (Herrer *et al.* 1966).

The inoculations consisted of 0.05 ml of saline-diluted culture. Sixty hamsters were subjected daily to stress, cortisone injections or control injections beginning with the first day after inoculation. Groups of 10 animals each were stressed for 1–2 hr by crowding (in a wired cage 20 cm square and 20 cm deep), swimming (in a mesh-covered metal tank containing water about 8 cm deep), and restraint (each in a tight plastic container). Ten animals were given injections of dexamethasone (a synthetic corticosteroid, Schering Corp.,

Bloomfield, N. J.), and two groups of 10 were injected with ethylene glycol, the carrier for the dexamethasone as described below. Twenty animals, 10 of each sex, were kept as controls. These control hamsters were caught and handled regularly with leather gloves in the same manner as were the other animals.

Fifty milligrams of dexamethasone was dissolved in 2 ml of 95% ethyl alcohol and added to 3 ml of ethylene glycol and water (50/50). The alcohol permitted dexamethasone to go into solution. This solution (0.05 ml), containing 0.5 mg dexamethasone, was used for each daily subcutaneous injection in the inner thigh region. This amount of drug was equivalent to 10 mg/kg of hamster weight. As controls for the corticosteroid group, 10 individuals were injected with 0.05 ml of the ethylene glycol, water, and alcohol solution and another 10 received only ethylene glycol and water. The average starting weight of all hamsters was 50 g.

The livers, spleens, and various superficial tissues of all animals that died were tested by cultivation or smear examination for possible dissemination of the infection. All of the remaining hamsters were sacrificed 5–8 months after infection and similarly tested. Those in which dissemination occurred are listed in Table I.

TABLE I

List of Hamsters That Showed Any Dissemination of Infection from the Point of Inoculation as Indicated by Visual Inspection, Smear Examination, and Tissue Culture

Animal identity	Days after nose infection	Tissue showing infection	Stress
3	6	Liver and spleen	Cortisone
17	190	Right hind foot	Ethylene glycol
26	167	Upper lip	Restraint
28	167	Upper lip	Restraint
30	167	Tail	Restraint
1217	68	Liver and spleen	Cortisone
1220	189	Right hind foot	Ethylene glycol
1227	259	Foot, liver, tail, spleen	Cortisone

The first and sixth animals died of undetermined causes, the others were sacrificed.

Results

Swelling of the nose in most of the 30 stressed animals started sooner and progressed more rapidly than it did in those of the control groups, but the difference was not statistically significant. Nose swelling started in the 10 dexamethasone and 10 ethylene glycol–alcohol groups on the third day. It developed rapidly to near maximum size within 3 weeks. In the 20 control animals (no injections or stress) and in the 10 animals injected with ethylene glycol and water without alcohol, the nose enlargement started on the fifth day and reached near maximum in 28 days.

Ulceration of the nose occurred primarily in hamsters injected with dexamethasone and in those injected with the ethylene glycol–alcohol solution. These lesions started as small, rough spots by the 10th day. They were white or brown in color. By the 20th day, 8 out of 9 dexamethasone-injected animals (one died earlier) and 9 out of 10 ethylene glycol hamsters had ulcer scabs. The group of animals injected with ethylene glycol and water without the alcohol showed normal nose swelling but only one developed an ulcer scab. Among the stressed groups of hamsters 36% developed nose ulcerations. These occurred as follows: in the restraint group 4 had minute scabs, crowding produced 4 with large ulcers, and in the swimming group only 1 had a scab. Among the 16 living control animals 3 developed nose ulcerations. This was 19% compared to 95% of those injected with cortisone or with ethylene glycol and alcohol.

Along with the nose involvement, skin lesions on the back in the shoulder area appeared in the dexamethasone-treated animals and their ethylene glycol controls. Seventeen of these 19 hamsters had the back lesions, starting approximately the same time as did the nose ulcers. The uniform location of back skin sores was remarkable. Only 2 of the other 50 animals developed back lesions, 1 each in the crowding group and the ethylene glycol group without alcohol. After 2.5 months many of the back lesions had apparently healed and many nose ulcers had lost their scabs and were healing.

Discussion

The characteristic response to leishmanial infection of the nose in hamsters is a swelling of the nose. In some animals, infected with certain species or strains of *Leishmania* (e.g., *L. mexicana* or Peruvian species), an ulcer may form on the nose. This development usually appears, if at all, after several months or even years. Dissemination to the viscera occurs with *L. braziliensis*, but with the strain of parasite used in this study it had not been demonstrated since the first hamster was inoculated at the laboratory 1.5 years previously. An increase in the normal rate or magnitude of the nose swelling, in addition to other reactions, may be considered as a rough measure of the effect of stress. Failure to swell, however, is not proof of absence of infection.

Repeated injections of dexamethasone in a solution of ethylene glycol, water, and alcohol, and the injection of ethylene glycol solution without dexamethasone provided sufficient stress to cause a significant development of cutaneous leishmaniasis in the golden hamster as judged by the appearance of nose ulcerations. Since injections without the alcohol produced little effect, the ulceration may have been initiated by alcohol rather than cortisone or ethylene glycol. There was no visible evidence of local alcohol irritation. The stresses of crowding, swimming, and restraint produced observable acceleration of leishmaniasis as indicated by nose swellings, but this acceleration was not significant. All smears and culture of nose tissue have been positive. The significance of almost 100% correlation between development of nose ulcers and appearance of large skin sores on the back has yet to be determined.

In 8 (13%) of the 60 stressed animals dissemination occurred. All of these had been injected with cortisone or the ethylene glycol solution, or were stressed by restraint. Those stressed by crowding or swimming did not show dissemination. In none of the control animals was there any dissemination. The metastasis to the liver and spleen in one animal (first one in the table) in 6 days was surprising, especially as other reports indicated that a year or more is usually required before evidence of spread of infection. The fact that the most extensive dissemination occurred in the animal that was kept alive the longest suggests that if the others were maintained longer they would also show heavier infection. Of the eight hamsters showing metastasis, back skin lesion and nose ulceration occurred in two of the cortisone-injected individuals. Neither of these developments occurred in the other six animals. The failure of metastasis to appear in any of the twenty control animals clearly suggests that early spread of the infection may be associated with stress.

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Reference

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