PHYSIOLOGY

Effects of Cold Stress on Serum Protein Fractions of the Prairie Meadow Mouse

THE reaction of an animal to cold stress varies with the species and with the degree of stress imposed on the animal. Shields, Platner and Neubeiser¹ have shown that the albino laboratory rat reacts to a cold stress ($4.0^{\circ} \pm 2^{\circ} \text{C}$ for 20 days) with a marked decrease in serum albumin. They regarded catabolism of albumin as the prime factor responsible for its fall in serum.

The present investigation was undertaken to test the effects of cold stress on the distribution of serum protein fractions of a wild species of rodent, the prairie meadow mouse, *Microtus ochrogaster*, and compare its response with that of the rat. The meadow mouse, common throughout Missouri, was obtained from a stock raised in the laboratory. These animals do well in captivity and can be obtained in large numbers.

Twenty-four virgin females of the same age were divided into two equal groups. The control group was held at 22°C room temperature while the experimental group was placed in a cold room with a temperature of $5^{\circ} \text{C} \pm 1.5^{\circ} \text{C}$. Both rooms were on a timed 12-h light cycle. Each mouse was individually caged with water and feed *ad libitum*. The food was 'Purina' laboratory rat chow, identical to that used by Shields *et al.*¹ in their experiments on the rat.

After 20 days, the mice were bled by heart puncture. The blood was allowed to clot and the serum obtained was stripped on electrophoresis paper and migrated in the 'Beckman/Spinco Model R' cell with barbiturate (Veronal) buffer, pH 8.6, ionic strength 0.075. The paper strips were stained with bromophenol blue dye and scanned on the 'Beckman/Spinco Analytrol' equipped with the B-3 cam. Total protein was measured with a Goldberg refractometer (American Optical Co.).

Table 1. MEAN VALUES AS PERCENTAGE OF SERUM PROTEIN FRACTIONS OF MICE BEFORE AND AFTER EXPOSURE TO COLD

	Controls	Cold-exposed
Weight change (g)	$+3.7 \pm 0.78$ (12)	$+2.8 \pm 0.69$ (12)
Total protein (g)	5.2 ± 0.53 (7)	5.2 ± 0.18 (8)
Albumin	67.1 ± 1.50 (11)	$70.7 \pm 0.57^*$ (10)
α -1 globulin	4.4 ± 0.47 (11)	4.0 ± 0.29 (10)
α -2 globulin	10.2 ± 0.30 (11)	9.0 ± 0.63 (10)
β globulin	15.0 ± 1.03 (11)	13.4 ± 0.57 (10)
γ globulin	2.7 ± 0.23 (11)	$2.1 \pm 0.16^{\dagger}$ (10)

* $P < 0.02$.

$\dagger P < 0.05$.

Number of animals shown in parentheses.

The results are shown in Table 1. The gain in weight was not significantly different between the two groups. Nor was the total serum protein changed by the cold exposure. This observation agrees with previous reports³. The constituent serum proteins did, however, display a significant change by a rise in serum albumin and a drop in the serum gamma globulin fraction in the cold-exposed group. Such a rise in the albumin fraction of the prairie meadow mouse is not consistent with the findings reported for the laboratory rat. Apparently this species of mouse is able to meet its energy needs without resorting to catabolism of albumin. Its energy needs are apparently supplied adequately from non-protein sources. The significantly elevated albumin levels further imply an increased osmotic effect associated with serum proteins. A fall in serum γ -globulin is suggestive of an increased rate of antibody turnover and a decreased rate of antibody production by animals exposed to cold. Trapani⁴ found similar results with rabbits exposed to cold. A comparison of control serum protein fractions of the meadow mouse shows that they are of the same order of magnitude as the data reported by Moore² for the rat, but they disagree with Shields's rat data. As total protein was not measured by the latter authors, the low control albumin values reported (31 per cent) raise a question regarding the general health of their rats.

Additional similar investigations should be performed on other wild species to ascertain their response to cold stress. It is possible that the laboratory rat is unique in using albumin as an energy source when exposed to cold.

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¹ Shields, J. L., Platner, W. S., and Neubeiser, R. E., *Amer. J. Physiol.*, **199**, 942 (1960).

² Moore, D. H., *J. Biol. Chem.*, **161**, 21 (1945).

³ Baker, D. G., and Sellers, E. A., *Canad. J. Biochem. Physiol.*, **35**, 631 (1957).

⁴ Trapani, I. L., *Fed. Proc.*, **19**, Suppl. No. 5, 109 (1960).

Decreased Lysosome Fragility in Response to Wounding in the Skin and Oral Mucosa of the Mouse

DURING a comparative histochemical investigation of repair in the skin and oral mucosa of the mouse, a marked change in the distribution of acid phosphatase activity in response to wounding was noted in the epithelial cells.

The Gomori lead nitrate technique, with all solutions made isotonic, was used to demonstrate enzyme activity in unfixed cold microtome sections. In the epithelium of normal skin and oral mucosa after incubation for 1.5 h, sites of enzyme activity were marked by particulate deposits of lead sulphide and by a diffuse cytoplasmic staining in the cells of the basal layers. Within 5 min of wounding (a single incision) the diffuse cytoplasmic staining was lost not only from the epithelial cells bordering the wound, but also from those some distance away from the wound margin (Fig. 1). The particulate deposits of lead sulphide persisted. Re-epithelialization occurred after 4 days, but the diffuse cytoplasmic staining did not re-appear until 7 days after wounding.

It is now generally agreed that sites of acid phosphatase activity in cells are confined to lysosomes or lysosome-like structures. Bitensky¹ has shown that the lysosome membrane has a given degree of fragility in the Gomori incubating medium, and that this varies not only for different tissues but also for the same tissue under differing experimental conditions. The diffuse and particulate deposition of lead sulphide obtained in this investigation indicates over-incubation and this was confirmed when a section of normal tissue was incubated for 1 h. In this case only a particulate deposition of lead sulphide marked sites of enzyme activity. However, as all the experimental

Fig. 1. Section through the skin surface of mouse lower lip 15 min after wounding. The wound margin is at the top left-hand corner of the photomicrograph. The epithelium adjoining the wound shows no diffuse deposition of lead sulphide. Gomori lead nitrate method. Incubation period 1.5 h.