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Mast Cell Changes in Animals Exposed to Cold. (23440)

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A marked increase in number of mast cells of the lungs and intestine of hedgehogs during hibernation was recently reported(1). Since mast cells are known to be an abundant source of heparin, the authors concluded that the increase of these cells was an important factor in preventing blood coagulation and thrombosis and in decreasing the work of the heart during hibernation. More recently a great deal of evidence has accumulated which strongly favors the opinion that mast cells are also an important source of histamine(2,3,4, 5).

We planned this study on mast cell changes in the cold for the following reasons. First, knowing that corticosteroids cause a degranulation of mast cells(6), it was suspected that during a long exposure to cold, when definite changes in the adrenal activity have been shown to occur(7), some changes in number or structure of these cells would take place. Secondly, measurements of histamine content in laboratory animals(8) and indirect evidence in humans(9) have shown that local application of a cold stress releases histamine from the stimulated tissues. Finally, the cold induced vasodilation normally observed after exposure to low temperatures, was associated with histamine release from the skin(9,10).

Methods. Experiments were conducted at 2 ± 1°C and at 6 ± 1°C; work to be reported elsewhere has shown that histological changes induced by cold are different at these 2 temperatures. White male rats, weighing approximately 300 g were used. In Exp. A, where animals were exposed to 2 ± 1°C, three groups of 12 rats were used. The first group, kept at 23°C, was the control group, whereas the second and third were respectively exposed to cold for 2 and 4 weeks. In Exp. B the same procedure was used with the exception of the temperature which was 6 ± 1°C instead of 2 ± 1°C.

The following procedure was used for all

TABLE I. Variations in Number of Mast Cells in Mesentery and in Skin of the Ear and Abdomen of Rats Maintained at 2 and 6°C for Periods of 2 and 4 Weeks.

Tissue	Normal (No./3 mm²)	6°C		2°C	
		2 wk	4 wk	2 wk	4 wk
Skin (ear)	92 ± 18*	70 ± 18	64 ± 12	36 ± 18	26 ± 22
Skin (abdomen)	252 ± 60	402 ± 36	—	372 ± 18	—
Mesentery (intervascular)	300 ± 86	192 ± 91	260 ± 89	140 ± 62	294 ± 100
Mesentery (perivascular)	1†	3	3	4	3

* Stand. dev. † Estimated intensity (0 to 5 scale).

animals to prepare tissues for mast cell examination. Light ether anesthesia was induced prior to excising 5 mm² sample of the ear, top of hind paw and mid abdomen. Two 25 mm² portions of the mesentery were also obtained. The mesentery samples which were taken as close as possible to the stomach, were mounted on 2 glass concentric rings having inside diameters of 25 and 30 mm. These samples were immediately fixed in alcohol 100% for 5 minutes, then in alcohol 80% and 60% for 2 minutes, washed in water for 2 minutes, stained with Pugh solution* for 3 minutes, washed for 2 minutes and taken up to alcohol 100%. After quick examination under a dissecting microscope, the specimens were mounted permanently on slides with "Permount." The skin specimens after being fixed with absolute methyl alcohol for 24 hours were also stained with toluidine blue. Alcohol was preferred to formalin as a fixative because the mast cell granules are water soluble. In the mesentery a whole 3 mm² field was counted whereas in the skin 6 fields of 0.5 mm² were examined.

Results. Mesentery. Table I shows a large increase in concentration of mast cells in the perivascular region and a significant decrease in the intervascular areas after 2 weeks in the cold at both 2 and 6°C. (Typical preparations are shown in Fig. 1 and 2). The difference in the cell counts in the intervascular region of the mesentery between the animals exposed for 2 weeks at 2°C and those exposed for the same period at 6°C, (Table I) is not statistically significant. After 4 weeks in the cold the number of mast cells in the perivascular areas returns almost to the control value.

Skin. Table I shows a significant increase in number of mast cells in the skin of the abdomen after 2- and 4-week exposures at both 2 and 6°C. However, in the skin of the ear there is a decrease in the number of these cells, a decrease significantly greater at 2°C than at 6°C under all conditions tested. In contrast, the changes after 2 weeks in the cold in the skin of the abdomen and in the mesentery were essentially the same at 2°C and at 6°C.

Discussion. Numerous studies have shown

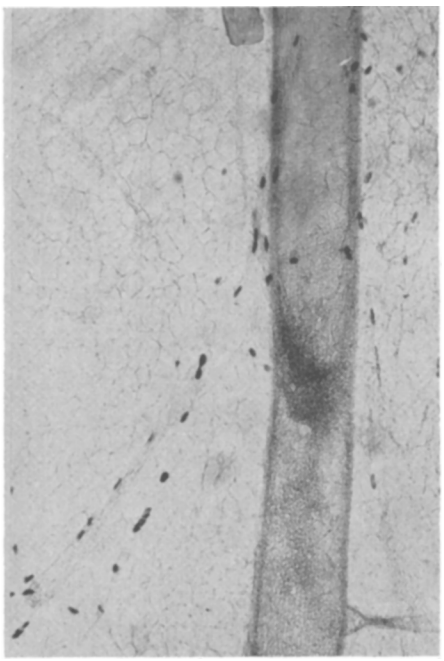

FIG. 1. Mast cells in perivascular region of mesentery of normal animals (× 80). Stained with toluidine blue.

* Pugh solution is made by dissolving 0.4 g of toluidine blue in 4 ml ethyl alcohol, 86 ml distilled water and 10 ml glacial acetic acid.

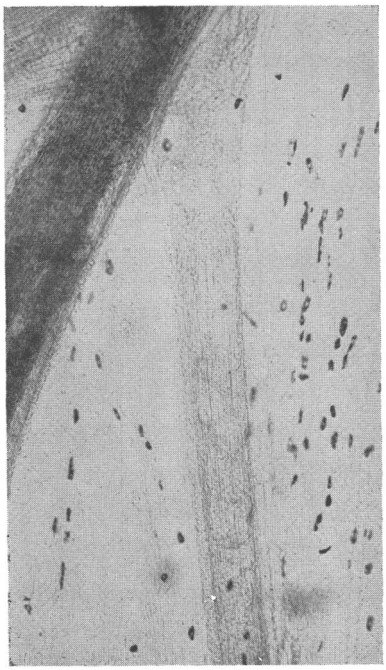

FIG. 2. Mast cells in perivascular region of mesentery of animals exposed to cold for 4 wk at 6°C (× 80). Stained with toluidine blue. A large increase in No. of mast cells is observed. This increase is also noted after 2 wk at 6°C and after 2 and 4 wk at 2°C.

that systemic acclimatization to low temperatures is associated with ability to maintain a sustained increase in heat production. This modification is made possible by the relatively slow process of gradually increased endocrine activity. On the other hand fewer studies have been made on local adaptation or on the interrelationships between local and systemic acclimatization. We have recently described a local and systemic adaptation to a locally applied cold stimulus in humans(9). Generally a larger number of studies are being carried out on systemic adaptation in laboratory animals than in humans, whereas the reverse is true for local adaptation studies. The present study was designed to distinguish between the local and systemic effect of cold on mast cells. Indeed changes in the number and in the structure of these cells in the extremities and ears, which are directly exposed to cold and chilled to temperatures nearing those of the environment, could be different from the changes taking place in parts of the body which are covered by hair or in internal organs such as the mesentery. The systemic effect of cold on number of mast cells results in an increase as evidenced by changes in the perivascular areas of the mesentery and in skin of the abdomen. Furthermore, this increase is the same whether exposure temperature was 2 or 6°C. This last effect was expected to some extent since the systemic response to these 2 environmental temperatures, which are so close to one another, could not be too dissimilar. On the other hand the local effect of cold on number of mast cells is contrary to the systemic effect since we have observed a decrease of these cells in the skin of the ear. Of additional interest is the fact that this decrease is much larger at 2° than it is at 6°C. Work being completed at our laboratories shows a marked difference in the histological response to these two temperatures. For instance, the edema is much more pronounced at 2° than it is at 6°C.

The question of primary importance is to find out if the increase in mast cells of the perivascular region of the mesentery and of the skin of the abdomen is an indication of increased production or decreased utilization of the substances known to be present in the granules of mast cells. Recently Fawcett(4) described an "active amoeboid migration" of mast cells from the perivascular region of the mesentery into the avascular areas, while Mergenthaler and Paff(11) gave some evidence for actual "shelling out" of these cells from the mesentery. Consequently, in the present study the increase in the perivascular mast cells may be interpreted as an increased production whereas the decrease in the avascular region of the mesentery would indicate an increased utilization. The final result of these changes should be a larger amount of heparin or histamine in the peritoneal cavity or probably even better in the circulation. This same suggestion was made by Harma, *et al.*(1) who also found an increase in the number of mast cells in the lungs and intestines of hedgehogs in hibernation. The final proof would of course be obtained by determination in the blood of substances known to result from mast cell degranulation, such as histamine or

heparin.

The cause for these local and systemic reactions is incompletely understood although different studies bring sufficient evidence to support some speculative views on this question. Pellerat and Murat(8) have shown that chilling human skin causes a decrease of histamine in the skin and an increase in the blood. We obtained indirect evident to this effect in humans(9). Consequently, the decrease in number of mast cells in the ear of rat exposed to cold is a local effect of this stress. The reason for the systemic changes observed is not obvious. However, the fact that degranulation of the mast cells in the intervascular region of the mesentery observed after 2 weeks in the cold is not evidenced at 4 weeks could indicate that these systemic changes are controlled by some mechanism which is less intense after 4 weeks in the cold than after 2 weeks. The adrenal cortex secretion in the cold has been shown by Heroux and Hart to follow this general pattern(7). These authors observed greater activity of the adrenal cortex at 2 weeks than at 4 weeks in animals exposed to cold.

These changes in mast cells in the cold will be understood only when their significance can be estimated in terms of our present knowledge of local and systemic acclimatization to cold.

Summary. In the mesentery the number of mast cells increased in the perivascular region after exposing the animals for 2 and 4 weeks at 6°C; in the intervascular region the number decreased after 2 weeks but returned to the initial value after 4 weeks. The same changes were observed in animals exposed at 2°C. In the skin of the abdomen an increase was observed at both temperatures after 2 weeks. In the skin of the ear, which is much more exposed to the environmental temperature than the abdomen, a significant drop was observed after 2- and 4-week exposures to 2 and 6°C. However, the decrease was significantly larger at 2°C than at 6°C. The increases in number of mast cells in abdominal skin and the changes in the mesentery are considered to be systemic effects of cold; the decreases in the skin of the ear are considered to be local effects.

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