

EFFECTS OF COLD AND HIBERNATION ON THE HISTOLOGY AND HISTOCHEMISTRY OF THE HAMSTER SUBMAXILLARY GLAND

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ABSTRACT.—A marked atrophy of the submaxillary glands of hamsters resulted during hibernation and exposure to cold (5°C) for a period of three months. This study utilized both histological and histochemical techniques to characterize these changes. The acinar cells were markedly reduced in size in the atrophic glands. Histometric analyses confirmed the visual impressions and indicated a significant reduction in tubular diameter in the atrophic glands of hibernating hamsters. The atrophic acinar cells of hibernating hamsters were almost devoid of RNA although the accumulation and distribution of RNA was not altered in the cold exposed animals. The concentration and distribution of PAS-positive material in the acinar cells, tubules and ducts in glands of cold exposed and hibernating hamsters was not changed.

Alterations in size, histology and histochemistry of digestive glands during hibernation when very little food is ingested and digested have been reported (Mayer and Bernick, 1957; Troyer, 1959). Mayer (1960) suggests that the collapse of acini of submaxillary glands of hibernating arctic ground squirrels might indicate a withdrawal of protein from these inactive cells for utilization in essential metabolic processes. Hamsters, unlike ground squirrels, have frequent periods of awakening in the course of hibernation in which they feed. This study was under-

taken, therefore, to study the effect of cold and hibernation on the morphology and histochemistry of submaxillary glands of hamsters. The histochemical tests were utilized to further characterize any morphological changes that may occur.

MATERIALS AND METHODS

Ten adult male hamsters (*Mesocricetus auratus*) were exposed to a continuous cold environment of 5°C for 12 weeks. Five of these hamsters entered hibernation during this cold exposure period; the others remained active and showed no signs of becoming torpid. The torpid animals had hibernated for a period of one to three days before autopsy. Ten control hamsters were maintained at ambient temperature (24° ± 1°C).

The animals were killed by a sharp blow to the head, submaxillary glands were removed and dissected free of extraneous connective tissue. The right submaxillary glands were then weighed. Tissues were then bisected and fixed in neutral formalin. After routine paraffin embedding, the tissues were sectioned at six and eight microns.

Hematoxylin and eosin staining of tissues was used for morphologic study and histometric methods. Additional technical details for histometric measurements are supplied under Results.

The periodic acid-Schiff (PAS) technique was employed for the demonstration of 1:2 glycol linkages (McManus, 1948). Control slides were incubated for one hour in a 0.1 per cent aqueous malt diastase solution at 40°C in order to eliminate glycogen.

TABLE 1.—Effect of Cold Exposure (3 months at 5°C) and Hibernation on Body Weight, Right Submaxillary Gland Weight, Counts of Acinar Cell Nuclei and Tubular Diameter¹.

Treatment	No. of Hamsters	Final Body Weight ² (gm)	Gland Weight (mg)	Acinar Cell Nuclei ³	Tubular Diameter ⁴ (μ)
Control.....	10	121±3.0	266±5	578±23	51.1±0.8
Cold-exposed.....	5	112±6.1	245±9	744±34	47.9±1.1
Hibernating.....	5	97±5.2	213±6	827±34	40.8±0.4

¹ All values are means ± standard error

² Initial body weight 105 ± 2.3 gm

³ Counts/10 high power fields

⁴ Twenty measurements/gland

For the demonstration of RNA, sections were incubated for one hour at 37°C in glass distilled water. Parallel control slides were incubated for one hour at 37°C in crystalline ribonuclease (Worthington Laboratories) solution prepared with glass distilled water (1 mg/ml). All sections were then stained by the methyl green-pyronin technique of Brachet (Pearse, 1960, p. 825).

RESULTS

Results obtained for body weights, submaxillary gland weights and histometric measurements are shown in TABLE 1. Cold exposure affected the gain in body weight. Cold-exposed animals gained significantly ($P<.05$) less than the controls while the hibernating animals lost a significant ($P<.01$) amount of weight.

Cold exposed and hibernating animals exhibited a significant ($P<.001$) decrease in the weight of the submaxillary glands. This decrease was more pronounced among the hibernating animals. Examination of the acinar cells of the atrophied glands revealed that these cells were smaller than the acinar cells of control glands. Confirmation of this visual impression of reduced acinar cell size was made by use of histometric measurements. The cell population density of the glands was determined by counting the number of acinar cell nuclei with the aid of an ocular grid. Utilizing sections cut from the glands of each of the ten experimental and con-

trol hamsters, ten random high power fields from each gland were selected and counted. For the cold-exposed hamsters there was a significant ($P<.01$) increase of approximately 29 per cent in the number of acinar nuclei per high power field. Hibernating animals showed a significant ($P<.001$) increase of approximately 43 per cent. The increase in number of nuclei indicated that there were more cells to count in a high power field because each of the cells had reduced in size.

The diameter of the granular tubules of the submaxillary glands was measured under high power with an ocular micrometer. Twenty random tubular diameters were chosen from a number of sections from the glands of each of the ten experimental and control animals. The results in TABLE 1 indicate that there was no significant alteration in the diameter of the granular tubules of the cold-exposed animals. There was, however, a significant ($P<.001$) reduction among the hibernating animals. The lumina of granular tubules among hibernators appeared narrow and the nuclei of tubule cells were observed to be crowded (FIG. 1, A and B).

Pyronin staining material that was digested by ribonuclease was interpreted as ribonucleic acid (RNA). In the control glands RNA appeared to be concentrated in the basilar and perinuclear regions of the acinar cells (FIG. 1, C). RNA was dispersed as fine granules in the remaining cytoplasm. In the acinar cells of cold-exposed animals, the perinuclear and basilar regions contained

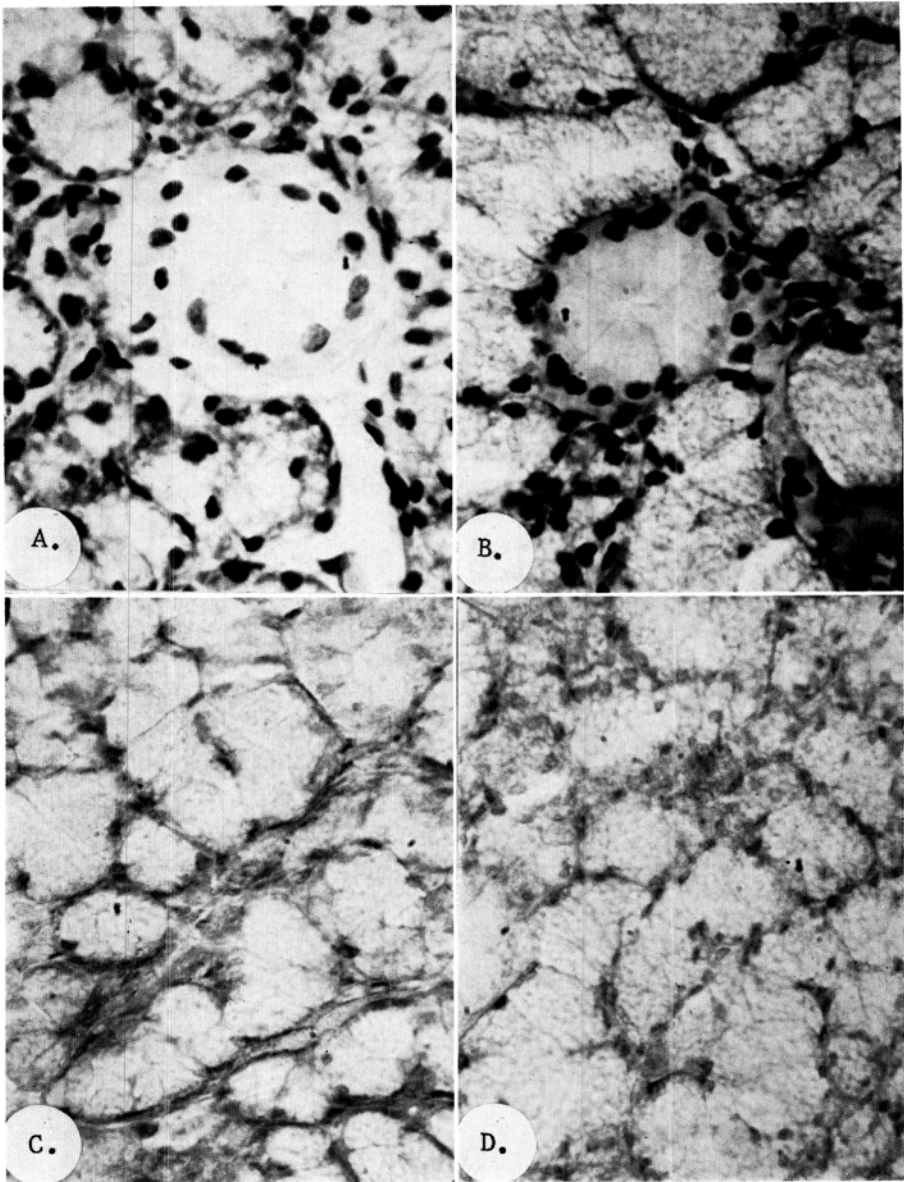


FIGURE 1. Sections of submaxillary glands of male hamsters. X400. (A) control, granular tubule, H&E (B) hibernating, granular tubule, H&E (C) control, RNA in basilar regions of the acinar cells, methyl green-pyronin (D) hibernating, small amount of RNA in the acinar cells, methyl green-pyronin.

material which stained as intensely as the controls. Hibernation resulted in a marked decrease in the RNA content of the acinar cells (FIG. 1, D).

In the acini, ducts and tubules of the submaxillary glands of control animals there was a relatively high concentration of PAS-positive granules. Many acinar cells were more intensely positive than others. A similar staining pattern was observed in the glands of cold-exposed and hibernating animals. Malt diastase had no effect upon the PAS reaction in any of the groups.

DISCUSSION

The morphologic and histometric data indicate that the decrease in weight of the submaxillary glands that follows during cold exposure and hibernation is due to a decrease in acinar cell size. The reduction in size in the granular tubules of glands of hibernating animals may account for part of this reduction in glandular weight.

In his description of the histological changes that occur in the submaxillary gland of the hibernating arctic ground squirrel, Mayer (1960) noted a decrease in the size and a disorganized appearance of acinar cells. The present data for hibernating hamsters indicate a similar response for acinar cell size. The disorganized appearance of acini among the experimentals in this study was not apparent and may be due to the fact that the animals were hibernating for a very brief period.

The decrease in RNA content of the atrophic cells of hibernating an-

imals is indicative of a decreased protein synthesis.

The present data with PAS stain indicates that the apparent concentration of PAS-positive substances in the atrophied glands was not significantly different from controls. It appears likely that the change in weight of the glands resulting from cold exposure and hibernation is not due to excessive loss of PAS-positive substances such as mucoproteins and mucopolysaccharides. The chemical nature of the PAS-positive material has not been determined, however, the malt diastase controls indicated that the material present was not glycogen.

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