Role of Mucopolysaccharides in Pathogenesis of Experimental Exophthalmos.* (17604)

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In the course of investigations of hormonal effects on connective tissue in these laboratories, our attention was drawn to the problem of localized myxedema. As a result of our clinical impression that this lesion was often associated with progressive exophthalmos, the studies to be reported here were initiated. Recently, Curtis(1) has also pointed out that the two conditions occur frequently in the same patient, and have a very similar clinical course.

The primary pathological change in localized myxedema is an infiltration of the dermis by a mucinous intercellular and interfibrillar ground substance,(2,3) Watson and Pearce,(4) by direct chemical analysis of skin from patients with pretibial myxedema, demonstrated a marked increase in hyaluronic acid, chondroitin sulfuric acid, and water in the affected areas.

Experimental exophthalmos has been produced in guinea pigs by several investigators both by thyroidectomy and with extracts of the adenohypophysis containing the thyroid stimulating hormone (TSH)(5). Smelser(6,7) reported that the orbital tissues of such animals were edematous and contained a stainable amorphous material in the connective tissue. Subsequently, he demonstrated that the increase in weight of the orbital contents was due to an increase in water content, and suggested that edema of these tissues is responsible for the forward proptosis of the eye.

On the basis of these observations, it was decided to study the amounts of mucinous substances in the orbital tissues in experimental exophthalmos. In addition to histochemical studies, water and hexosamine contents were determined. Since mucopolysaccharides contain fixed quantities of hexosamines, the determination of these substances may be used as an index of the amount of mucopolysaccharide present.

Methods. Four sets of young guinea pigs (250 g) were studied: A) Intact controls, B) Intact animals treated with thyroid stimulating fraction from the adenohypophysis (TSH), C) Thyroidectomized controls, and D) Thyroidectomized animals treated with TSH. The animals were fed a stock diet of Purina Dog Chow with additional fresh greens, excluding cabbage. Those receiving TSH‡ were injected intraperitoneally with 25-30 Junkmann-Schoeller units per day. The degree of exophthalmos was estimated grossly. The animals were etherized and sacrificed by exsanguination at intervals up to 3 weeks after the start of the experiment. The entire orbital contents were removed and the eyes were carefully dissected free and discarded. The lacrimal and Harderian glands were separated from the remaining tissues, which consisted of the extraocular muscles, adipose tissue and loose connective tissue. The contents of one orbit were then fixed in formalin and absolute alcohol, while the other was saved for meas-

* Supported by a grant from The Life Insurance Medical Research Fund, and from the Ciba Pharmaceutical Products, Inc.

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1. Curtis, A. C., Cawley, E. P., and Johnwick, E., *Arch. Dermat. and Syph.*, 1949, v60, 318.

2. Pillsbury, D. M., and Stokes, J. H., *Arch. Dermat. and Syph.*, 1931, v24, 255.

3. O'Leary, P. A., *Arch. Dermat. and Syph.*, 1930, v21, 57.

4. Watson, E. M., and Pearce, R. H., *Am. J. Clin. Path.*, 1949, v19, 442.

5. Brunton, C., *Physiol. Rev.*, 1949, v29, 260.

6. Smelser, G. K., PROC. SOC. EXP. BIOL. AND MED., 1935, v35, 128.

7. Smelser, G. K., *Am. J. Physiol.*, 1943, v140, 308.

‡ The TSH used was Thyrotropic Hormone, Parke Davis and Co., R No. 099802, 6-8 Junkmann-Schoeller units per mg, obtained through the courtesy of Dr. D. A. McGinty.

urement of water and hexosamine content. The fixed material was sectioned at five micra and stained with hematoxylin and eosin, and toluidine blue. Sections of the material were digested in a solution of bull testis hyaluronidase§ containing 50 turbidity reducing units per ml. To estimate water content, the tissues were placed in tared watch glasses and weighed on an analytical balance immediately after removal from the orbit. They were then dried in an oven at 100°C for 12 hours and kept dry in a vacuum desiccator over $CaCl_2$ until reweighing. The hexosamine content was determined by an adaptation to whole tissues of West's(8) modification for serum of the method originally described by Elsom and Morgan.(9) The dried tissues were transferred to 10 ml volumetric flasks and hydrolyzed for 6 hours in a boiling water bath after the addition of 3 ml of 2N HCl. After cooling, the flasks were filled to the 10 ml mark with distilled water, and the contents filtered through a No. 5 Whatman filter. 0.5 ml aliquots of the filtrate were then carried through as described by West. The hexosamine values were expressed as mg per 100 mg of tissue (dry weight), using a glucosamine standard.

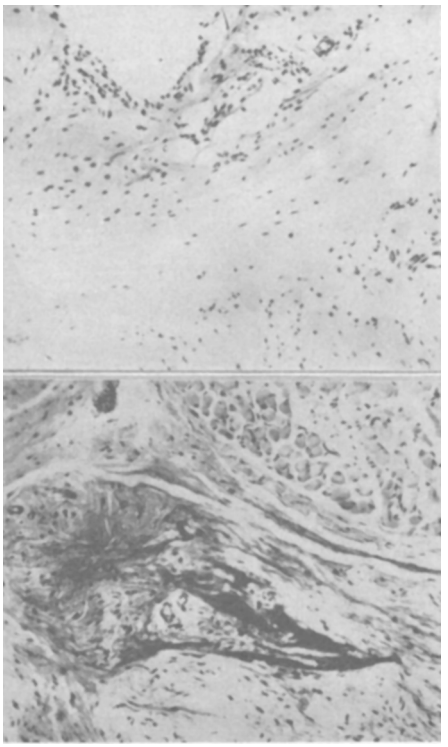

FIG. 1.
Section of extraocular muscle and connective tissue from a control animal, showing the faint metachromasia. Toluidine blue stain. × 125.

FIG. 2.
Section of similar area from thyroidectomized guinea pig treated with TSH. Note heavy deposition of intensely metachromatic material. Toluidine blue stain. × 125.

Results. None of the animals in the control group developed exophthalmos. Histologic examination revealed extremely small amounts of metachromatic material scattered throughout the connective tissue and in the capsule of the Harderian gland (Fig. 1).

Exophthalmos appeared in the intact animals receiving TSH (Group B) within 2 to 3 days after the onset of treatment, began to diminish within 10 to 12 days, and disappeared almost entirely after 3 weeks. The thyroidectomized control animals (Group C) developed moderate exophthalmos. All the thyroidectomized animals receiving TSH (Group D) developed marked exophthalmos. The orbital tissues of this group were distinctly mucoid and viscous on gross examination.

The histologic findings paralleled the macroscopic observations. All animals with exophthalmos showed large deposits of intensely metachromatic ground substance in the connective tissue, particularly in the fat tissue, the fasciae of the extra-ocular muscles, and the capsules of the Harderian and lacrimal glands. (Fig. 2 and 3). In Group D, where the extent of the changes was greatest, there were actual lakes of this material and metachromasia in the connective tissue between the muscle fibers and in the septa of the glands. Digestion with hyaluronidase resulted in almost complete removal of the metachromatic deposits. (Fig. 4) Lymphoid nodules in the

§ Bull testis hyaluronidase supplied by Dr. E. Seifter, of the Wyeth Research Laboratories, Philadelphia, Pa.

8. West, R., and Clarke, D. H., *J. Clin. Invest.*, 1938, v17, 173.

9. Elson, A. L., and Morgan, W. T. J., *Biochem. J.*, 1933, v27, 1824.

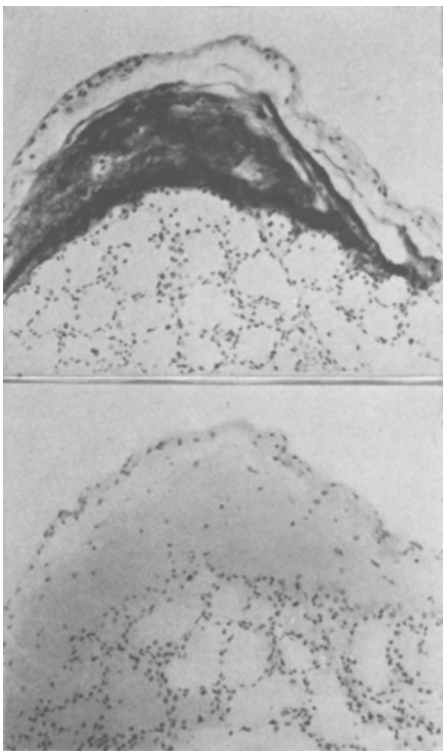

FIG. 3.
Section of Harderian gland from thyroidectomized guinea pig treated with TSH, with large amounts of mucopolysaccharides in the capsule. Toluidine blue stain. × 125.

FIG. 4.
Adjoining section from same animal after hyaluronidase digestion. Toluidine blue stain. × 125.

subconjunctival area, seen in the controls, were considerably enlarged in the other groups, but no lymphocytic infiltration of the connective tissue was observed. One section revealed a small focus of muscle degeneration.

Water and hexosamine determinations were carried out on all three components into which the retro-ocular tissues were divided, the lacrimal gland, Harderian gland, and the muscle-fat fraction. The changes were most striking and clear-cut in the latter fraction, because of the presence of a greater proportion of connective tissue. Accordingly, only the data obtained from this tissue will be presented now. The water contents of these tissues correlated well with the degree of metachromasia and the degree of exophthalmos. The most striking and significant increase occurred in the thyroidectomized group given TSH (Group D). The intact group receiving TSH (Group B) exhibited a moderate increase at first, followed by a fall to control levels. (Table I)

The hexosamine concentrations also varied directly with the degree of exophthalmos, metachromasia, and water content. (Table I) It, too, was most strikingly elevated in Group D and less so in the other groups. Group B exhibited an initial rise, but the subsequent fall was less striking than the secondary decreases in the other factors studied. The increase in water and hexosamine content in the treated animals cannot be ascribed to proliferation of the orbital tissues, since analysis of the total dry weights of the orbital contents in the four groups reveals an actual decrease in the weights of the retro-ocular tissues in Groups C and D in relation to the control group. (Table I) This indicates that in this experiment exophthalmos is independent of the growth of the orbital tissues.

Discussion. These results indicate that the pathologic changes observed in exophthalmos are similar to those of localized myxedema and consist essentially of the accumulation of interfibrillar ground substance in the orbital connective tissue. The response to hyaluronidase digestion indicates that a large proportion of the mucopolysaccharide moiety of this ground substance is hyaluronic acid. It is accepted that the mucopolysaccharides are extremely hydrophilic (10) and, when present, play a major role in the maintenance of tissue water content. The large amounts of these substances in the orbital tissues in experimental exophthalmos, therefore, must contribute significantly to the increase in water content reported by Smelser,(7) and confirmed by us. It would appear, therefore, that the increase in volume of the orbital contents in experimental exophthalmos is the result of both an accumulation of mucopolysaccharides and the resultant rise in water content. In the rigid confines of the orbit, the increased volume of the retro-orbital tissues is in turn

10. Ropes, M. W., Roberts, W. vB., Rossmeisl, E. C., Peabody, R. B., and Bauer, W., *Acta med. Scandinav.*, 1947, Suppl. 196, 700.

TABLE I.
Summary of Analyses of Orbital Contents of Guinea Pigs.

	Group	No. animals	Water content, %	Hexosamine content, mg/100 mg tissue (dry wt)	Total dry w t orbital content
A	Intact controls	12	80.6 ± .4*	.70 ± .03	145.5 ± 6.4
B	Intact + TSH (5 days)	4	82.7 ± .8	.97 ± .05	156.9 ± 7.3
B	Intact and TSH (14 and 21 days)	8	80.5 ± 4	.90 ± 09	
C	Thyroidectomized	5	83.1 ± .2	.92 ± .07	122.9 ± 15.5
D	Thyroidectomized + TSH	8	86.9 ± .5	1.27 ± .11	109.7 ± 8.9

* S.E. $= \sqrt{\frac{\Sigma\Delta^2}{n(n-1)}}$

responsible for the extrusion of the ocular globe.

Summary. The development of experimental exophthalmos in the guinea pig is dependent on the accumulation of large quantities of intercellular ground substance and water in the connective tissue of the orbital contents. This is evidenced by the presence of considerable amounts of metachromatic material, of which hyaluronic acid is an important component, and by an increase in the hexosamine and water content of these tissues.

This is similar to the changes observed in localized myxedema in man, and suggests a common mechanism for the two conditions.

Received December 22, 1949. P.S.E.B.M., 1950, v73.

Inhibition of Aerobic Respiration of Rat Brain by Desoxycorticosterone *in vitro*.* (17605)

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Selye's observation that large amounts of steroids induce anesthesia,(1) together with the relationships thought to exist between anesthetics and the carbohydrate metabolism of brain,(2) have provided a basis for studies concerning the effect of steroids on rat brain cell respiration. It has been found that these agents are capable of inhibiting the aerobic respiration of rat brain, presumably at the dehydrogenase level.(3) Our previous studies indicate that this phenomenon also operates in the intact organism since (a) deprivation of gonadal steroids by castration leads to an increased rate of glucose oxidation by rat brain,(4) (b) tissues from castrated male rats differ from normal tissues in their response to steroids added *in vitro*,(5) and (c) a parallelism can be shown between the *in vitro* inhibiting potency and *in vivo* anesthetic activity of the steroids.(3)

* Aided by grants from the National Institutes of Health, Bethesda, Md., and from Ciba Pharmaceutical Products, Inc., Summit, N.J.

1. Selye, H., *Endocrinology*, 1942, v30, 437.
2. Michaelis, M., and Quastel, J. H., *Biochem. J.*, 1941, v35, 518.
3. Gordan, G. S., and Elliott, H. W., *Endocrinology*, 1947, v41, 517.
4. Eisenberg, E., Gordan, G. S., and Elliott, H. W., *Science*, 1949, v109, 337.
5. Eisenberg, E., Gordan, G. S., and Elliott, H. W., *Endocrinology*, 1949, v45, 113.