Regulation of Connective Tissue Hexosamine Levels by the Anterior Pituitary and Thyroid Glands. (21295)

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Hexosamines* are important constituents of connective tissue ground substance and are distributed largely in the mucopolysaccharides (1) and the plasma proteins of connective tissue(2). The concentration of hexosamines is increased in the orbital connective tissue of guinea pigs rendered myxedematous by either thyroidectomy alone, or by the administration of thyrotropic hormone to the thyroidectomized animals(3). In order to study further the physiological factors concerned with the production of these hexosamine changes, similar studies have been made in the rat. Although the response of the rat is shown to be less sensitive than that of the guinea pig, the levels of hexosamines in rat orbital connective tissue may be also controlled by the thyroid and the anterior pituitary glands.

Methods. Male Sprague-Dawley rats were individually caged, fed a stock diet(4), and permitted to drink water *ad libitum*. Food intake was not measured since it has been shown that wide variations in food intake will not influence the concentration of connective tissue hexosamine(5). Animals of approximately the same weight were used for each experiment. All operations were performed under ether anesthesia. The rats were hypophysectomized by the parapharyngeal approach. The hypophysectomized animals and their controls received daily subcutaneous injections of 4000 units of penicillin. All hormone preparations were administered subcutaneously in single daily doses. The thyrotropic hormone preparation (TSH)† was formulated in corn oil with 2% aluminum monostearate. Thyroxin was made up in water adjusted to pH 9, and the growth hormone (GH)‡ preparation was dissolved in water. All the animals were killed by exsanguination under ether anesthesia by cutting a carotid artery. Completeness of hypophysectomy was checked post-mortem by examining the sella turcica under magnification. Completeness of thyroidectomy was checked in one group by the radioautographic technic of Reinhardt(6).§ In this paper, if the mean area of the images on the two roentgen films was less than 14 mm^2, the rat was considered to be completely thyroidectomized or to have a trace. If the mean area was greater than 14 mm^2 the rat was considered to be incompletely thyroidectomized. *Orbital connective tissue* was obtained after removing the eyelids flush with the orbital rims and separating the eyeballs from the conjunctiva, extra-ocular muscles and optic nerves. The remaining orbital tissues were removed; the Harderian gland was separated, leaving a mass which consisted of loose orbital connective tissue, the extra-ocular muscles, and a small amount of scleral conjunctiva. We have referred to this entire mass as orbital connective tissue since the conjunctiva and muscles make up only a small fraction of the total mass. For chemical analyses, the connective tissue of both orbits was pooled. After weighing, all the samples were placed in an oven at 101-104° C for 18 hours, re-weighed, then extracted in a Soxhlet apparatus for 3 hours with petroleum ether (B.P. 60-70° C). The dry, fat-free samples were weighed and the water and fat contents calculated. Water is expressed as percent $\frac{\text{(Wet Wt-Dry Wt) x 100}}{\text{Wet Wt}}$. The fat content of orbital connective tissue rarely exceeded a range of 1-3% and was not influenced by any of the physiological situations studied.

* The naturally occurring hexosamines are glucosamine and galactosamine. Method used here does not distinguish between these amino sugars.

† Armour thyrotropic hormone, Lot No. 129-31PRR3 was kindly supplied by Dr. Irby Bunding, Armour Laboratories, Chicago.

‡ Growth hormone, No. C_5I_5, E. R. Squibb & Co., was kindly supplied by Dr. Robert W. Bates. On bio-assay, this preparation at a dose level of 160 μg/day, induced a weight gain of 1 g/day in 100 g hypophysectomized rats.

§ We are indebted to Dr. Robert O. Scow for preparing the radioautographs.

These data are therefore not included. *Hexosamine* determinations were made on the dry defatted tissues. After a preliminary hydrolysis in 4 N hydrochloric acid for 15 hours, hexosamines were separated on Dowex-50, and determined by a modification of the method of Elson and Morgan(7). Hexosamine values are expressed as micrograms present in total orbital connective tissue and as milligrams per 100 g dry defatted tissue. All values are reported as Mean ± Standard Error.

Results. Thyroidectomy. To study the effect of thyroidectomy on the composition of orbital connective tissue, 3 groups of rats were used, 1) a control group (9 rats) sacrificed at 28 days of age, 2) a control group (10 rats) sacrificed at 76 days of age, and 3) a group (8 rats) completely thyroidectomized at 28 days of age and sacrificed at 76 days of age. The thyroidectomized group had a marked retardation of body growth (94 ± 5 g vs. 234 ±6 g for the 76-day, and 60 ± 2 g for the 28-day controls). The relative amount of connective tissue (mg/100 g Body Wt) did not change following thyroidectomy (73 ± 4 vs. 70 ± 1 for the 28-day controls). Whereas in the growing controls this ratio decreased (54 ± 2). Following thyroidectomy, the concentration of water (79.2 ± .3%) and hexosamine (494 ± 12 mg/100 g dry wt) remained the same as the 28-day controls (79.6 ± .4% and 493 ± 14 mg/100 g), whereas in the growing controls the concentrations of water (77.3 ± .3%) and hexosamine (384 ± 5 mg/100 g) decreased significantly.

In order to examine the hormonal factors responsible for this inhibition of connective tissue growth and development, 22 rats, weighing 110-130 g were used. They were divided into 5 groups, 1) an intact control group, 2) an uninjected thyroidectomized group, and thyroidectomized group receiving, 3) 3 μg of thyroxin, 4) 3 μg of thyroxin plus 500 μg of growth hormone and 5) 500 μg of growth hormone. The growth hormone preparation (C_5I_5) contained thyrotropic activity. All the animals were treated daily for 7 days from the day of operation. The differences in these data are not as striking as in the previous experiment, since the time elapsed was shorter and the rats were not screened for "completeness" of thyroidectomy. The concentration of hexosamine (mg/100 g dry wt) was again higher in the uninjected thyroidectomized group (448 ± 1) than in the control group (392 ± 10). This inability of thyroidectomized rats to develop the decrease in hexosamine concentration seen with normal growing rats was overcome by the administration of thyroxin, with (384 ± 1) or without growth hormone (393 ± 17) but not with growth hormone alone (458 ± 13). The weight of the connective tissue or its water content was not significantly altered in any group.

Hypophysectomy. In order to interpret the changes seen following thyroidectomy, the effects of growth hormone, TSH and thyroxin were studied in hypophysectomized rats. In the first experiment 3 groups of rats, with 6 rats in each group, were used, 1) an intact control group, and 2 groups of hypophysectomized rats, 2) uninjected and 3) injected daily with 2.5 μg of thyroxin from the day of operation. All were sacrificed 7 days from the day of operation. In comparison with the intact controls, the hypophysectomized rats had a significant retardation of body growth (99 ± 4 g vs. 135 ± 6 g) and connective tissue growth (62 ± 2 mg vs. 74 ± 4 mg) but no change in water (79.8 ± .3% vs. 79.6 ± .4%), or the concentration of hexosamine (406 ± 10 vs. 407 ± 16 mg/100 g dry wt). Thyroxin in the small maintenance dose given had no effect on the composition of connective tissue.

In the second experiment (Table I) groups of hypophysectomized rats with 3 or 4 rats in each group received daily injections of, 1) 24 μg of thyroxin, 2) 2 mg of thyrotropic hormone, and 3) 2 mg of growth hormone, known to contain thyrotropic activity. All rats were treated daily for 7 days following the day of operation and sacrificed on the 8th day. Thyroxin, even in the toxic doses administered, failed to produce any significant alterations in the weight, water, or hexosamine content of connective tissue. Both pituitary preparations produced significant thyroid gland hypertrophy. Thyrotropic hormone had no significant effect on the amount or concentration of connective tissue hexosamine or water. Growth hormone, on the other hand, stimu-

TABLE I. Effect of Administration of Thyroxin and Pituitary Hormones on Orbital Connective Tissue of Hypophysectomized Rats.

			Orbital connective tissue			
					Hexosamine	
Group	Body wt (g)	Thyroid (mg)	Wet wt (mg)	mg/100 g body wt	Total μg	mg/100 g dry wt
Control	100 ± 3	$6.2 \pm .1$	50 ± 2	50 ± 2	41 ± 3	441 ± 18
Thyroxin	96 ± 5	—	52 ± 1	54 ± 3	42 ± 1	413 ± 17
TSH	104 ± 8	11.1 ± 1.0	58 ± 4	56 ± 3	48 ± 2	433 ± 6
GH	135 ± 2	10.9 ± 1.2	74 ± 2	54 ± 2	61 ± 2	457 ± 4

lated body growth with a proportionate increase in the weight of orbit connective tissue and the total amount of hexosamine. The concentration of hexosamine was not significantly increased. No group showed any significant alteration in the concentration of water.

Discussion. It has been demonstrated in the guinea pig that thyroidectomy produces orbital myxedema with an increase in the concentration of connective tissue hexosamine (3). An even greater increase results when thyroidectomized guinea pigs are given pituitary extract (crude thyrotropin).

In the growing rat the concentration of orbital connective tissue hexosamine gradually decreases. Similar observations on age changes have been made on subcutaneous tissue of the rat(5) and in rat skin where the decrease in the concentration of hexosamine is shown to result primarily from a relative increase in collagen(8). The normal decrease seen with growth in the concentration of orbital connective tissue hexosamine is prevented by thyroidectomy, but can be restored by the administration of thyroxin in small doses. On the other hand, the normal decrease in the concentration of hexosamine with growth is not abolished by hypophysectomy, and thyroxin in small or large doses will not influence the concentration of hexosamine. Growth hormone, however, when administered to the hypophysectomized rat, in addition to increasing body weight, will increase the weight of connective tissue and the total hexosamine proportionately. Thyrotropic hormone had no effect on the amount or concentration of hexosamine in the hypophysectomized rat.

Rat orbital connective tissue is less sensitive than that of the guinea pig(3) to the effects of thyroidectomy and administration of pituitary hormones. It is apparent, however, that even rat orbital connective tissue can be influenced by the thyroid and by the anterior pituitary gland. A balance must exist between the production of growth hormone and thyroid hormone which determines the amount of hexosamine and connective tissue present in the orbit.

Summary. 1. A decrease in the concentration of hexosamine in orbital connective tissue is observed with increasing age in normal rats but is abolished following thyroidectomy. It can be reversed, however, by the administration of thyroxin. The concentration of connective tissue hexosamine in hypophysectomized rats is similar to controls of the same age and is unaffected by either small or large doses of thyroxin. Growth hormone, on the other hand, produces an increase in the amounts of connective tissue and hexosamine with no change in the concentration of hexosamine. 2. It is suggested that the concentration and amount of hexosamine in orbital connective tissue in the rat is determined by a balance between the production of hormones of thyroid and anterior pituitary (growth hormone). 3. The response of rat orbital connective tissue to thyroidectomy or to the administration of pituitary extract appears to be less sensitive than that in the guinea pig.

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