captured in April, 1967, and maintained at 20 °C for 8 weeks prior to hypophysectomy in late June. After hypophysectomy, the fish were kept at 20 °C for 15 weeks and half were then gradually transferred to water at −1.5 °C after acclimation to intermediate temperatures for various lengths of time (Table I). The remaining half were kept at 20 °C until the experiment was terminated. Control groups of intact fish were also maintained at 20 °C and −1.5 °C. At the time of autopsy, no hypophysectomized fish had increased significantly in length since the date of hypophysectomy and none showed any significant trace of nuptial coloration. These indications, together with an overall pallor and complete sexual regression, provided adequate evidence of the absence of a pituitary remnant. Serum glucose was determined the day following autopsy using an ultramicro adaptation of the Glucostat (Worthington Biochemical Corporation) enzymatic method, following the procedure of SAIFER and GERSTENFELD[16]. The results are presented in Table II.

At 20 °C, serum glucose levels were significantly lower in hypophysectomized fish than in intact controls. However, after living for 10 days at −1.5 °C, hyperglycemia developed in both intact and hypophysectomized killifish. These results indicate that even though the pituitary is involved in serum glucose regulation at 20 °C, the pituitary and the glands under its direct control are not responsible for eliciting the hyperglycemia observed at subzero temperatures.

Since the pituitary and the glands under its direct control are not responsible for the cold-induced hyperglycemia in killifish, further investigations are needed to establish the hormonal control of this response. Endocrine glands not under pituitary control that might be involved are the pancreatic islets and chromaffin tissue. Of these, the pancreatic islets seem to offer the most promise as controlling agents in the cold-induced hyperglycemic response since seasonal cycles in their histology are known. For example, PALLOT[17] examined the islet tissue from 8 species of fish to find increased numbers of α-cells during the winter; in the spring, the number of α-cells had diminished. In addition, SAID and AL-HUSSAINI[18] found that in the islets of *Tilapia* and *Mugil* there was almost no insulin present in the autumn and winter, but it was abundant in the summer; glucagon was present at all seasons without much difference. Either a decrease in insulin production or an increase in glucagon production, as reported for other fish, could account for the cold-induced hyperglycemia in *Fundulus heteroclitus*[19].

Table I. Period of acclimation of *Fundulus heteroclitus* to various temperatures

Group	Final temperature (°C)	Time at each temperature for acclimation 20 °C	10 °C	4 °C	−1.5 °C
Intact	20	51 weeks	–	–	–
	−1.5	23 weeks	22 weeks	4 weeks	10 days
Hypophysectomized	20	50 weeks	–	–	–
	−1.5	23 weeks	22 weeks	4 weeks	10 days

Table II. Serum glucose concentrations (mg/100 ml) of intact and hypophysectomized male *Fundulus heteroclitus* acclimated to various temperatures[a]

Group	Acclimation temperature 20 °C		−1.5 °C	
Intact	69.6 ± 3.2	(7)	230.4 ± 41.6	(5)[c]
Hypophysectomized	55.6 ± 4.0	(7)[b]	396.7 ± 51.4	(4)[b,c]

[a] Data expressed as mean ± standard error (sample size). [b] Significantly different from intact controls ($p < 0.05$) using Student's t-test. [c] Significantly different from 20 °C controls ($p < 0.05$) using Student's t-test.

Zusammenfassung. Glukose-Steigerung im Serum von *Fundulus heteroclitus* nach Akklimatisation an −1,5 °C wird nicht durch die Hypophyse verursacht. Hingegen ist die Hypophyse notwendig, um die Serumglukose bei 20 °C bei normaler Konzentration zu halten. Hypophysektomie bei 20 °C hat eine Abnahme der Serumglukose zur Folge.

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The Acidic Glycosaminoglycans in the Skin of Athyroid Rats: the Effects of L-Tri-Iodothyronine

Since the work of WATSON and PEARCE[1] it has been known that subnormal thyroid activity affects the glycosaminoglycans of the skin. The changes reported were an increse in the hyaluronic acid and a decrease in the dermatan sulfate content of the skin[2,3]. These results seem to be independent of thyrotrophin because the same changes are noted after hypophysectomy as in hypothyroidism[2].

This report presents data on the glycosaminoglycan content of the skin of normal, athyroid and athyroid-treated rats with L-tri-iodothyronine (L-T3), using the techniques now available for the quantitation of glycosaminoglycans in order to get further insight into the

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Effect of thyroidectomy and L-T3 on glycosaminoglycans concentration in skin of rats

	Normal rats	Athyroid rats	Athyroid + 1 μg L-T3	Athyroid + 1 μg L-T3
Total uronic acids	1088.3	1150.8	1102.7	1094.9
Glycoproteins	46.3 ± 5.7	49.8 ± 5.1	47.3 ± 7.8	45.2 ± 9.2
Hyaluronic acid	563.2 ± 48.3	696.2 ± 41.4	602.4 ± 47.3	569.4 ± 43.7
Chondroitin-4-sulfate	68.7 ± 9.0	60.6 ± 11.3	57.1 ± 9.1	59.7 ± 8.7
Chondroitin-6-sulfate	66.4 ± 7.1	61.7 ± 7.4	64.3 ± 8.4	69.5 ± 5.3
Heparitin sulfate	52.6 ± 7.4	57.9 ± 8.5	55.2 ± 8.9	62.8 ± 9.8
Dermatan sulfate	219.8 ± 21.4	157.4 ± 18.7	206.4 ± 27.3	226.8 ± 24.2
Heparin	71.3 ± 11.4	67.2 ± 9.8	69.8 ± 8.3	61.3 ± 11.4

Concentration of glucosaminoglycans expressed as μg uronic acids/g dry skin.

relationship between the thyroid gland and the metabolism of connective tissue.

Materials and methods. 40 Wistar male rats with an average weight of 231.8 ± 2.6 g were used throughout these experiments. The animals were maintained on a standard laboratory diet (Forramez Lab., Argentina) in pellet form with water ad libitum and kept in a constant temperature room. The animals were divided into 4 groups. Thyroid destruction was accomplished in 3 of them by administration of 700 μC of I^{131}. They were used after 3 months of radio-active iodine administration. Group 1 acted as untreated normal controls; group 2 was considered as athyroid rats and groups 3 and 4 were athyroid rats injected daily with 0.1 and 1 μg respectively of L-T3 (Sigma Chemical Co., USA) for 20 days. Completeness of thyroid destruction was checked at autopsy on each animal.

The abdominal skin of each animal was extracted and freed of blood and extraneous materials. The tissues were then defatted and dehydrated for 36 h with 2 changes of ether-acetone (1:1 v/v) and then dried at 80 °C for 5 h until constant weight. 300 mg samples of dry defatted tissue were suspended in 6 ml of phosphate buffer pH 7.2 and heated at 100 °C during 20 min. 15 mg of papain were activated in 20 ml of the same buffer with the addition of 0.005 *M* EDTA and 0.005 *M* cysteine HCl at 58 °C for 30 min. The activated papain was then added to the cooled samples (2 ml/sample) and the mixture was incubated for 24 h at 58 °C; after 12 h another aliquot of the enzyme was added. Following digestion, 10% TCA was added to reach a final concentration of 5% to precipitate any residual protein. The precipitate was then washed with another volume of 10% TCA. 3 volumes of 5% potassium acetate in ethanol were then added to the combined TCA solutions. After standing for 12 h at 3 °C, the precipitated total crude glycosaminoglycans were centrifuged off and dissolved in 1 ml of $3.5^0/_{00}$ NaCl solution. Uronic acid was determined on an aliquot of the above by the method of Bitter and Muir[4]. The crude glycosaminoglycans were then fractionated on cellulose microcolumns by the technique of Svejcar and Robertson[5]. Uronic acid concentration was also determined on each glycosaminoglycan fraction. Recoveries of 150–3000 μg of chondroitin-4-sulfate alone, or when added to 50–300 mg of dry skin and carried through the entire procedure, varied between 89–96%.

Results. The Table demonstrates the concentration of all glycosaminoglycan fractions in skin of normal, athyroid and athyroid-treated rats, expressed as μg of uronic acids/g dry defatted skin. The same table shows that the concentration of hyaluronic acid was increased 20% and that of dermatan-sulfate decreased 28% in the athyroid rats when compared with controls, whereas the other fractions did not show statistically significant differences. The administration of both doses of L-T3 reversed the effects of thyroidectomy. The findings of increased hyaluronic acid and decreased dermatan-sulfate with no significant differences among the other sulfated fractions in the skin of athyroid rats are similar to those found in cartilage[6]. Apparently thyroid hormone deficiency affects both tissues in a similar way. Treatment of thyroidectomized rats with the same doses of L-T3, increased heat production by 30% in excess of normal, whereas administration of D-T3 produced a lesser increase which was maintained throughout the injection period and then returned to the thyroidectomized level[7]. Whether L-T3 exerts a direct effect on cartilage and skin glycosaminoglycans, or whether the results noted are secondary to the caloric effect of the hormone, remains as an open question[8].

Resumen. Este estudio demuestra que en piel de ratas atiroideas, en comparación con las normales, el ácido hialurónico aumenta un 20% mientras que el dermatán sulfato disminuye un 28%, no encontrándose diferencias significativas en las demás fracciones de glycosaminoglycanos. Este efecto diferencial es similar al ya encontrado por nosotros en cartílago.

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