Differential Effect of Hypophysectomy on Skin and Tracheal Cartilage Glycosaminoglycans

For many years, it has been known that hypophysectomy affects the glycosaminoglycans (GAG) of cartilage[1-3] and skin[4] of rats.

Methods recently developed for quantitative isolation and separation of GAG have now made it possible to compare the concentration of these substances in skin and tracheal cartilage of normal and hypophysectomized rats. Therefore this investigation was undertaken to determine whether hypophysectomy influences in a similar way the distribution of the GAG in both tissues.

Materials and methods. 24 male rats of the Wistar strain weighing approximately 250 g were used throughout these experiments. The animals were maintained on a standard laboratory diet (Forramez Lab, Argentina) and kept in a constant temperature room. One half of the group was hypophysectomized through the parapharyngeal approach. Completeness of hypophysectomy was checked at autopsy on each animal. All the determinations were done 5 months after hypophysectomy. The other half of the group was considered as controls. At autopsy, the whole trachea and the abdominal skin were 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 of constant weight. 5–500 mg samples of dry defatted tissue were suspended in 2–6 ml of phosphate buffer pH 7.2 and heated at 100 °C for 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 40 min. The activated papain was then added to the cooled samples (0.3–3.0 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 concentration of 5% to precipitate any residual protein. The precipitate was then washed with another volume of 10% TCA. 3 vol. of 5% potassium acetate in ethanol were then added to the combined TCA solutions. After standing 12 h at 3 °C the precipitated total crude GAG were centrifuged off and dissolved in 0.4–1.0 ml of water. Uronic acid was determined on an aliquot of the above by the method of BITTER and MUIR[5]. The crude GAG were then fractionated on cellulose microcolumns by the technique of SVEJCAR and ROBERTSON[6]. Uronic acid concentration was also determined on each GAG fraction. Recoveries of 150–3000 µg of chondroitin-4-sulphate alone or when added to about 50 mg of skin or 10 mg of trachea and carried through the entire procedure varied between 90 and 96%.

Results. The Table demonstrates the concentrations of all GAG fractions in skin and trachea of normal and hypophysectomized rats, expressed as µg of uronic acid/g of dry skin or trachea. Total uronic acids were 67% increased in skin and 28% decreased in tracheal cartilage of hypophysectomized rats when compared to normals. This fluctuation in opposite directions was due to a marked increase observed in the concentrations of both glycoproteins (2.85 times normal) and hyaluronic acid (2.17 times normal) in the skin coincident with a decrease of these compounds in trachea of hypophysectomized rats.

The other GAG fractions were lowered in both tissues of experimental rats between 5 and 40%. Chondroitin-6-sulphate, dermatan sulphate and heparin were the fractions most affected in skin, whereas heparitin sulphate, chondroitin-4-sulphate and chondroitin-6-sulphate were most affected in trachea.

These studies illustrate 2 things: (1) a differential effect of hypophysectomy on skin and tracheal cartilage regarding the metabolism of glycoproteins and hyaluronic acid; (2) a dissociation in the behaviour of hyaluronic acid and the sulphated GAG in skin following hypophysectomy. The former effect of pituitary deficiency has not been reported previously. The latter has been observed by us[7] in thyroidectomized rats and by SCHILLER et al.[4,8] in both hypophysectomized and propylthiouracil-treated rats, although these authors did not consider all the sulphated GAG fractions[9].

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Effect of hypophysectomy on concentration of GAG in rat skin and trachea

	Skin			Trachea		
	Normal rats	Hypophysectomized rats	Times normal	Normal rats	Hypophysectomized rats	Times normal
Uronic acids	1184.7	1985.2	1.67	30948.0	22416.0	0.72
Glycoproteins	48.3 ± 6.2	138.0 ± 21.4	2.85	3011.0 ± 271	2316.0 ± 193	0.76
Hyaluronic acid	528.2 ± 48.6	1161.0 ± 92.1	2.17	1671.0 ± 197	1421.0 ± 187	0.89
Heparitin sulphate	58.7 ± 7.3	56.3 ± 6.1	0.96	1716.0 ± 183	1316.0 ± 124	0.76
Chondroitin-4-sulphate	69.3 ± 9.0	63.7 ± 8.4	0.92	20063.0 ± 1297	14324.0 ± 938	0.71
Chondroitin-6-sulphate	68.5 ± 8.3	48.3 ± 5.4	0.70	1498.0 ± 111	872.0 ± 68	0.58
Dermatan sulphate	205.4 ± 26.0	148.0 ± 19.2	0.72	793.0 ± 62	668.0 ± 94	0.84
Heparin	71.4 ± 16.3	53.0 ± 13.6	0.74	–	–	–

Concentration expressed as µg uronic acid/g dry skin or µg uronic acid/g dry trachea.

Resumen. En 12 ratas normales y en 12 hipofisectomizadas 150 días antes se determinaron las concentraciones de ácidos urónicos, glicoproteínas, ácido hialurónico, heparitín sulfato, condroitín-4-sulfato, condroitín-6-sulfato, dermatán sulfato y heparina en piel y cartílago traqueal. Estos estudios demostraron dos hechos: (1) un efecto distinto de la hipofisectomía sobre piel y tráquea en relación con el metabolismo de las glicoproteínas y el ácido hialurónico, cuyas concentraciones aumentaron en la primera y descendieron en la segunda; (2) una disociación entre el comportamiento del ácido hialurónico y los glicosaminoglicanos sulfatados en piel, aumentando la concentración del primero y disminuyendo las correspondientes a los segundos en forma variable según la fracción considerada.

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Effect of Feeding Psoralen on the Copper Content of Different Organs in Albino Rats

Furocoumarins have been known to induce pigmentation in vitiliginous skin when administered along with longwave UV- or solar-irradiation[1-3]. The precise mechanism of this induction is at present largely unknown. MOFTY et al.[4] have reported that feeding of 8-methoxypsoralen to albino rats caused a marked rise in blood copper and a significant drop in its content in liver. However, 8-isoamyleneoxypsoralen was found to be ineffective. Hence it was considered worthwhile to investigate the effect of feeding psoralen for different periods and observing its effect on total copper of the different organs in albino rats. The result of such studies are reported in the present communication.

Male albino rats (100 g) were divided into 4 groups of 8 rats each. The composition of the feeding mixture was 250 mg gum tragaconth, 750 mg glucose, 125 mg psoralen, 2.5 ml ethyl alcohol and water to make 25 ml. In the normal feeding mixture psoralen was omitted. Each rat was fed 0.5 ml of the solution daily. After the requisite period of feeding the rats were killed, organs immediately removed, weighed and digested to a transparent colourless liquid. The digested samples of various organs containing concentrated sulphuric acid were polarographed using Lange's manual polarograph with a multiflex galvanometer for recording current. In all the samples, the halfwave potential, measured against Hume and Harris saturated calomel electrode at 37 °C, was exactly at 0 V, a potential reported for copper ions in this medium. The concentration of copper was calculated by measuring the diffusion current at -0.200 V. The effect of different concentrations of sulphuric acid, different ionic strength obtained by the additions of potassium chloride and potassium sulphate was also investigated and found to be negligible.

Before actually doing the copper content of different organs of normal and psoralen-fed albino rats, some recovery experiments were done by adding different amounts of copper to all the organs under study in order to know the error in the estimation. The error was found to vary from $\pm$ 2 to $\pm$ 5% in most of the organs and that is well within the limit of polarographic analysis[5].

The Table represents the total copper in different organs of psoralen-fed rats for 3, 7 and 15 days. The copper content of spleen indicated a rise of around 46.2% and liver on the other hand exhibited a decrease of about 42.87% after 3 days of psoralen administration. These changes were more or less maintained in both the organs even after 15 days of feeding. Skin was found to give a variable response at different periods. Initial feeding for 3 days indicated a rise of about 43.52%. Further feeding

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Total copper in different organs of normal and psoralen-fed albino rats

Groups	Copper content of various organs (in mg%)							
	Liver	Skin	Heart	Brain	Kidney	Lung	Spleen	Muscle
Normal	8.00 $\pm$ 3.27	3.35 $\pm$ 2.77	4.95 $\pm$ 1.32	1.49 $\pm$ 0.67	3.61 $\pm$ 2.41	5.25 $\pm$ 3.70	9.16 $\pm$ 4.64	2.07 $\pm$ 1.59
Psoralen-fed								
3 days	4.50 $\pm$ 3.27	4.81 $\pm$ 2.27	5.22 $\pm$ 4.26	1.58 $\pm$ 0.90	3.14 $\pm$ 1.16	5.27 $\pm$ 2.87	13.40 $\pm$ 2.58	2.29 $\pm$ 1.07
7 days	4.75 $\pm$ 1.45	1.40 $\pm$ 1.29	5.47 $\pm$ 3.38	1.45 $\pm$ 0.56	3.28 $\pm$ 2.47	4.77 $\pm$ 3.57	12.24 $\pm$ 8.05	2.02 $\pm$ 1.32
15 days	4.46 $\pm$ 1.67	3.25 $\pm$ 1.84	4.75 $\pm$ 2.46	1.98 $\pm$ 0.73	3.48 $\pm$ 0.75	5.43 $\pm$ 2.71	13.99 $\pm$ 5.10	2.79 $\pm$ 1.32

Values given are mean of 8 animals.