## Chondroitin Sulfate and Hyaluronic Acid in Syphilomas of Cortisone-Treated Rabbits

In a recent publication (*1*) Turner and Hollander cited certain of my conclusions regarding the identity and relative concentration of chondroitin sulfate and hyaluronic acid in rabbit syphilomas. I want to report here the experimental evidence on which these conclusions were based (*2*).

The sulfated and nonsulfated polysaccharides were extracted from the syphilomas (*3*) of cortisone-treated and untreated animals and separated by a modification of the method of Pearce and Watson (*4*). The properties of the polysaccharides were then compared with those of the corresponding polysaccharides from normal skin.

The syphilomas were excised from the back of the rabbit (*1*), freed from hair and apparently normal skin, ground with sand in a mortar, and extracted with 10 ml of 2.5*N* sodium hydroxide for 24 hours at 35°C. After centrifugation the supernatant fluid was filtered through a 1-cm layer of Celite on a fritted glass funnel. The insoluble portion was reextracted four times with 10-ml quantities of distilled water and separated by filtration through Celite on a fritted glass funnel. The combined alkali and aqueous extracts were neutralized with glacial acetic acid (*p*H 7), treated with Lloyds reagent, filtered, and treated with an amyl alcohol-chloroform mixture to remove protein (*5*), and the polysaccharides were precipitated from the aqueous phase by the addition of 20 vol of ethanol. The precipitate was washed with ethanol and with ether, dried under a high vacuum at room temperature, and then redissolved in distilled water containing excess barium acetate. The solution was filtered and fractionated into sulfated and nonsulfated polysaccharides by the addition of ethanol to a concentration of 20 and 80 percent (*4*), respectively. The precipitate was collected by centrifugation, washed with ethanol and with ether, and dried under a high vacuum over phosphorus pentoxide.

Table 1 shows that, analytically, the sulfated polysaccharide of rabbit syphilomas is indistinguishable from chondroitin sulfate. The optical rotation indicates that it is the chondroitin sulfate C of Meyer and Rapport (*6*). The nonsulfated fraction is analytically the same as hyaluronic acid.

If we consider the quantities of chondroitin sulfate and hyaluronic acid isolated (Table 2), it is apparent that, although there is no marked difference in the relative concentrations in the syphiloma compared with normal skin, there is a threefold decrease in the chondroitin sulfate as compared with the hyaluronic acid after cortisone treatment. Although it is unlikely that the polysaccharides were quantitatively isolated, the relative amounts found should directly reflect the relative concentrations of the polysaccharides in the tissues.

Hyaluronic acid is frequently found in greatly hydrated gels and is apparently involved in water binding (*7*); hence, it is not too surprising to find an increase in the hyaluronic acid content of the soft hydrated syphilomas that are produced in the cortisone-treated animal as contrasted with the relatively water-free lesions of the untreated animal.

Various diseases and experimental conditions give rise to an increase in the concentration of mucopolysaccharide material. Such an increase is found in rheumatoid arthritis (*8*), in the Aschoff bodies of rheumatic fever, in myocarditis, in acute rheumatic carditis (*9*), and in the skin or coxcomb of experimental animals after the application of sex (*10*) or pituitary (*11*) hormones. The formation of dense collagen fibers, as shown by investigations on human skin (*12*), is accompanied by a relative drop in the ratio of hyaluronic acid to chondroitin sulfate. Layton has demonstrated (*13*) that cortisone inhibits sulfated-polysaccharide synthesis.

Table 1. Identity of the polysaccharide fractions that were isolated from the syphilomas of cortisone-treated and nontreated animals with chondroitin sulfate and hyaluronic acid.

| Item | Chondroitin sulfate* | | | | Hyaluronic acid | | | |
|---|---|---|---|---|---|---|---|---|
| | Hexosamine (*14*) | Nitrogen | Sulfur | $[\alpha]^{20}_D$ | Hexosamine (*4*) | Nitrogen | Sulfur | $[\alpha]^{20}_D$ |
| Cortisone-treated | 25.4 | 3.96 | 3.4 | −18° | 28.1 | 3.3 | 0.0 | −65° |
| Untreated | 25.6 | 3.94 | 3.1 | −18° | 28.5 | 3.4 | 0.0 | −65° |
| Normal skin | 25.7 | 3.9 | 3.1 | −18° | 28.5 | 3.3 | 0.0 | −64° |
| | 29(*15*) | 3(*15*) | | −19°(*16*) | | | | |
| Reported | 31(*17*) | 5(*16*) | 2–4(*16*) | −20°(*5*) | 30–40(*18*) | 3–3.5(*19*) | 0.0 | −70°(*5*) |

* Analyses obtained on the barium salt were recalculated for the potassium salt.

Table 2. Weight of chondroitin sulfate and hyaluronic acid isolated.

| Source | Weight of tissue (g) | Chondroitin sulfate (mg) | Hyaluronic acid (mg) | Chondroitin sulfate/hyaluronic acid |
|---|---|---|---|---|
| Cortisone-treated* | 20.1 | 205 | 532 | 0.4 |
| | 10.7 | 111 | 218 | 0.5 |
| Untreated | 4.5 | 100 | 79 | 1.3 |
| | 2.1 | 50 | 41 | 1.2 |
| Normal skin | 8.1 | 14.4 | 10 | 1.4 |
| | 10.0 | 16.4 | 10 | 1.6 |

* Six syphilomas excised from the back of one rabbit (*1*).

I suggest that the action of cortisone on the developing syphiloma is to inhibit the incorporation of sulfate into certain hyaluronic acid or hyaluronic acidlike polysaccharides produced by the animal tissue in response to the mechanical or biochemical stimuli of the treponeme. As a consequence, the relative concentration of nonsulfated polysaccharides or hyaluronic acid is increased; this, in turn, leads to a retention of water in the syphiloma.

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### References and Notes

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