

Isolation of Chondroitinsulfuric Acid from the Cock's Comb.* (22396)

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Since both chondroitinsulfuric acid (CSA) and hyaluronic acid (HA) exist concomitantly in the skin of man and animals it seemed reasonable to suspect that the ground substance of the cock's comb also contains both substances. The isolation of HA has been reported by Boas(1) but the possible presence of sulfated mucopolysaccharides in the cock's comb appears to be unexplored. The present paper concerns the isolation and characterization of CSA from cock's combs.

Methods. Fresh combs[†] were freed of the outer corny and epithelial layers by means of a scalpel and ground in an electric grinder. A total of 293 g of ground comb was defatted with acetone and dried at room temperature, yielding 35 g of acetone-defatted dry material. The mucopolysaccharides were extracted by a method previously described for skin(2). The crude polysaccharide mixture which weighed 2.4 g gave values of 2.85 and 26.6%, respectively, for nitrogen and hexosamine. This mixture was subjected to zone electrophoresis on a slab of Celite filter-aid for the separation of HA and CSA fractions (2).

Results. From the slower migrating fraction, 1.5 g of HA was isolated. Chemical analyses demonstrated nitrogen, uronic acid, and hexosamine in a molecular ratio of 1.16, 1.00, and 0.93 respectively.

The faster moving fraction proved to be analytically identical with the CSA isolated from rabbit skin(2). A total of 105 mg was isolated. Nitrogen, hexosamine, uronic acid (as determined by the carbazole reaction of Dische(3)), and sulfur analyses indicated molecular ratios of 1.02, 1.00, 0.67, and 1.00, respectively. The CSA fraction of the cock's comb, like that of rabbit skin(2), is partially

resistant to hydrolysis by testicular hyaluronidase as measured by a reduction in turbidity(4), since after incubation with the enzyme the optical density was decreased by only 26%.

In neutral solutions, the $[\alpha]_D^{24} = -56^\circ$ for the CSA fraction of the cock's comb. This value agrees with that of the corresponding fraction from rabbit(2) and from pig and calf skins(5).

The hexosamine of the CSA fraction was identified as galactosamine by the method of Stoffyn and Jeanloz(6).

Discussion. Since a considerable portion of the cock's comb is connective tissue it may prove of value as a tool for metabolic studies. A high percentage, 4.7%, of the dry weight was actually isolated as HA and CSA. The relative proportions of the isolated mucopolysaccharides are quite different from those found in rat skin(7). The preponderant mucopolysaccharide in the comb is HA, thus confirming the observation of Boas(1) regarding the presence of large quantities of HA in the cock's comb. While CSA represents only a small fraction of the total mucopolysaccharides, larger quantities were isolated from the cock's comb than were found in the rat skin, using an isotope dilution technic(7). In view of the partial hydrolysis of the CSA fraction by testicular hyaluronidase and the low carbazole values for uronic acid, the CSA fraction in rooster combs appears to be a mixture similar to that in rabbit skin(2).

Summary. 1. Hyaluronic acid (HA) and chondroitinsulfuric acid (CSA) were isolated from the combs of roosters in a ratio of 15 to 1. 2. The CSA fraction from combs appears to be similar to that isolated from rabbit skin.

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† Combs from assorted breeds of roosters were supplied by Palo Products Co., Chicago.

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Comparative Effects of Periodic Bleedings on Serum Glycoprotein Concentrations in Guinea Pig and Rat.* (22397)

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Increased concentrations of the carbohydrate-containing proteins of serum have been reported for a wide variety of physiologic and pathologic states(1). Precise information relative to the physiologic mechanisms involved in their formation or function is, however, still not available(2). Inasmuch as serial determinations are necessary in many experimental studies, the present investigation was undertaken to determine the effect of repeated bleedings, at weekly intervals on the serum concentrations of total glycoprotein, seromuroid and total protein, and on the hematocrit and hemoglobin values of blood in normal guinea pigs and rats.

Materials and methods. *Guinea pigs.* Adult, male, hybrid guinea pigs were housed 2 per cage and were maintained on a diet of Purina rabbit pellets, supplemented with lettuce, and tap water, *ad lib.* *Rats.* Adult, male, Sprague-Dawley rats were housed 2 per cage and were maintained on a diet of Purina laboratory chow and tap water, *ad lib.* *Bleeding.* The animals were bled by cardiac puncture, under light ether anesthesia, at weekly intervals for a total of 5 bleedings. At each bleeding 1.3 ml of blood per 100 g of body weight were removed, the sample representing approximately 20% of the total blood volume(3). Several animals died from the ef-

fects of the repeated hemorrhages. Data are presented for those that survived the experimental period. *Chemical analyses.* Total serum glycoprotein, seromuroid, total serum protein, hemoglobin, and hematocrit values were determined by methods previously reported(4,5). *Statistical analyses.* The mean, standard error of the mean, t, and probability values were determined by standard statistical procedures(6).

Results. Summaries of the results of the chemical analyses and hematologic data for the guinea pig and for the rat are presented in Tables I and II respectively.

The animals of both species continued to gain weight throughout the course of the experiment. A slight stimulation of growth followed the first bleeding; however, the average weekly weight increment gradually decreased.

No statistically significant increases occurred in the concentration of the serum components in comparison with base line values. In the guinea pig, a significant decrease was observed in the total serum glycoprotein at the third bleeding. Significant decreases in seromuroid levels occurred in the rat at the third and fourth bleedings. The diminished concentrations of seromuroid were reflected in significantly lower seromuroid-total glycoprotein ratios. Although a significant hypoproteinemia was exhibited by the rat at the second bleeding, total serum protein values

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