11239 P

Mucopolysaccharide Acid of Cornea and Possible Relation to the "Spreading Factor."*

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The nature of the mucopolysaccharide of the cornea has been much debated. A number of investigators have considered it to be a mucoitin sulfuric acid,[1] while others have found no sulfuric acid.[2] It was reported recently, on the basis of colorimetric analysis,[3] that the carbohydrate contained an amino sugar and sulfuric acid, but galactose instead of uronic acid. Most of the reports have been based on analyses of protein complexes ("mucoids") or of digests prepared by strong alkali.

In the past we have prepared this mucopolysaccharide acid in high yields both by alkaline digestion and by more gentle methods which avoid the use of strong alkali.[4] Our preparations contained one mol each of hexosamine, acetyl, uronic acid, and sulfuric acid, and had the same composition and general properties as those of the mucoitin-sulfuric acid obtained from gastric mucosa.[5] Good yields of glucosamine were isolated from both acids. In several important respects, however, the two compounds were found to differ: (1) the acid of cornea always formed quite viscous aqueous solutions, while that of gastric mucosa did not; (2) the specific rotation of the acid from cornea was about $-50°$, while that of the gastric mucosa was around 0 ($+2°$ to $-8°$); and (3) the acid from gastric mucosa was completely refractory to the specific enzyme from pneumococcus,[6] while the polysaccharide from cornea was hydrolyzed by this enzyme at

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[1] Levene, P. A., and López-Suárez, J., *J. Biol. Chem.*, 1918, **36**, 105; Meyer, K., and Palmer, J. W., *J. Biol. Chem.*, 1936, **114**, 689.

[2] Mörner, C. T., *Z. Physiol. Chem.*, 1893, **18**, 213; Karlberg, O., *Z. Physiol. Chem.*, 1936, **240**, 55.

[3] Suzuki, M., *J. Biochem.* (Japan), 1939, **30**, 185.

[4] Meyer, K., *Cold Spring Harbor Symposia on Quantitative Biology*, 1938, VI, 91.

[5] Meyer, K., Smyth, E. M., and Palmer, J. W., *J. Biol. Chem.*, 1937, **119**, 73.

[6] Meyer, K., Hobby, G. L., Chaffee, E., and Dawson, M. H., *J. Exp. Med.*, 1940, **71**, 137.

about half the rate of hyaluronic acid. From this latter finding and from the fact that the rotation of hyaluronic acid is similar to that of the cornea polysaccharide, we conclude that cornea polysaccharide is the naturally-occurring mono-sulfuric acid ester of hyaluronic acid.

This conclusion would seem to be significant in view of the recent report by Chain and Duthie[7] on the "spreading factor" found in testis and bacterial filtrates. According to these workers the "spreading factor" of testis by hydrolysis reduces the viscosity of synovial fluid and vitreous humor. These fluids were shown in our laboratory to contain hyaluronic acid as the viscous component, which is hydrolyzed by a specific enzyme obtained from pneumococci, streptococci, *Cl. welchii,* and spleen. The explanation of the "spreading" action in skin suggested by the work of the English authors is that the "spreading factor" acts on a "mucin" present as interfibrillar substance in the skin.

We were able to confirm the findings of Chain and Duthie and found further that testis extracts contain an enzyme which hydrolyzes pure hyaluronic acid as well as the polysaccharide acid of the cornea, while the mucoitin sulfuric acid from gastric mucosa was found to be completely refractory. The concentration of the enzyme in testis was inferior to that of the similar enzyme found in pneumococcus and group A hemolytic streptococcus. A further distinction is the pH optimum which for the bacterial enzymes was found to be 5.8 and for the testis enzyme 4.3, with hyaluronic acid as substrate.

We believe, however, that the substrate in the skin on which the "spreading factor" exerts its effect is not hyaluronic acid itself but its sulfuric acid ester. Evidence in support of this belief is furnished by the fact that such sulfuric acid esters stain meta-chromatically with Toluidine blue while hyaluronic acid does not. The substantia propria of the cornea can be stained with Toluidine blue and so also are the fibrils of the corium layer of the skin. (For a beautiful illustration of the meta-chromatic staining of the cornea, see [8].) Furthermore the protein complexes of such sulfuric acid esters are very much more stable than those of hyaluronic acid. The latter are easily brought into solution in neutral medium by dilute salt solutions; the former require concentrated salt solutions, a more alkaline reaction, and often as in the case of cornea a peptizing agent such as concentrated urea. The stability of these protein complexes is apparently

[7] Chain, E., and Duthie, E. S., *Nature,* 1939, **144,** 977.

[8] Jorpes, E., Holmgren, H., and Wilander, O., *Z. Mikrosk.-Anatom. Forsch.,* 1937, **42,** 279.

responsible for the failure of some authors to obtain the cornea polysaccharide acid. By extraction with dilute salt solution, no hyaluronic acid was extracted from cattle skin. Finally, a sulfuric acid containing polysaccharide has been obtained from skin as a protein compound giving viscous solutions by Van Lier.[9]

Since the sclera is similar chemically and histologically to the substantia propria of the cornea, except for the absence of this polysaccharide, it becomes a problem to determine whether this polysaccharide is concerned with corneal transparency.

11240

Can Strophanthin Maintain Adrenalectomized Mice?*

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In a recent report by Zwemer and Lowenstein it was suggested that adrenalectomized cats can be maintained in good condition by the administration of strophanthin.[1] This substance was administered daily in the concentration of 15 μg per kg of body weight.

We have attempted to extend these findings to the mouse. For these experiments 21-day-old mice were bilaterally adrenalectomized. A total of 140 operated animals were employed, 40 serving as uninjected controls, while the remaining 100 animals received various concentrations of strophanthin. The strophanthin was dissolved in olive oil so that the daily dose was contained in 0.1 cc of oil. Injections were made subcutaneously, beginning 24 hours after the operation and continued for 8 days.

Table I summarizes the results of the administration of strophanthin in daily concentrations of 0.02, 0.2, 2.0, 5.0, 10.0, 20.0, 50.0, and 100.0 μg, respectively. Of 40 uninjected control animals, only 4 were alive on the 10th day after adrenalectomy. The average survival of the remaining 36 animals was 4.7 days. The administration of

9 Van Lier, E. H. B., *Z. Physiol. Chem.*, 1909, **61**, 177.

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1 Zwemer, R. L., and Lowenstein, B. E., *Science*, 1940, **91**, 75.