The Acid Mucopolysaccharides of Furth's Mastocytoma in the Mouse

Lennart Rodén and Albert Dorfman

Wenner-Grens Institut, Stockholm, Sweden, and La Rabida-University of Chicago Institute, Chicago, U.S.A.

Mast cells, though widely distributed in mammalian connective tissues, are difficult to isolate in amounts large enough to permit a detailed study of the biosynthesis of one of their most important components, heparin. The recent development of two different transplantable mastocytomas in the mouse [1,2] therefore seems to offer considerable promise in this respect.

In naturally occurring mast cells isolated from peritoneal washings Schiller and Dorfman [3] found no other mucopolysaccharide than heparin. However, before using the malignant mast cells in biosynthetic studies it was considered advisable to carry out a more detailed characterization of their mucopolysaccharide fraction.

Tumours of the Furth type * were used in the present study. The mucopolysaccharides were prepared from a boiled water homogenate by the following procedure: 1) extraction with sodium hydroxide, 2) digestion of the dialyzed extract with trypsin, 3) precipitation of undigested material with trichloroacetic acid and dialysis of supernatant and 4) fractionation of the cetyl pyridinium complexes on a cellulose column according to Borelius, Gardell and Scott [4].

In this way two fractions were obtained in approximately equal amounts, which were eluted from the column with 1.2 N NaCl and 2.0 N NaCl, respectively. The 2.0 N fraction contained glucosamine, uronic acid and sulphate in molar ratios of 1:1:2, was resistant to digestion with testicular hyaluronidase and had an anticoagulant activity of 92 units per mg of the air-dried powder. The 1.2 N fraction contained hexosamine, uronic acid and sulphate in equimolar amounts, was digested to two thirds by hyaluronidase and had an anticoagulant activity of 15 units per mg. Both glucosamine and galactosamine were present in this fraction, as shown with the method of Stoffyn and Jeanloz [5].

On the basis of these observations it is suggested that the mast cell tumours contain, in addition to heparin, a fraction with low sulphur content similar to heparin monosulphuric acid, and chondroitin sulphuric acid A and/or C.

* Kindly provided by Dr. Jacob Furth.

1. Furth, J., Hagen, P. and Hirsch, E. I. *Proc. Soc. Exptl. Biol. Med.* **95** (1957) 824.
2. Dunn, T. M. and Potter, M. *J. Natl. Cancer Inst.* **18** (1957) 587.
3. Schiller, S. and Dorfman, A. *Biochim. et Biophys. Acta.* **31** (1959) 278.
4. Borelius, E., Gardell, S. and Scott, J. *To be published.*
5. Stoffyn, P. J. and Jeanloz, R. W. *Arch. Biochem. Biophys.* **52** (1954) 373.

Turbidimetric Analysis of Inorganic Sulfate in Serum, Plasma and Urine

Fredrik Berglund and Bo Sörbo

Department of Pharmacology, Karolinska Institutet, Stockholm, and Research Institute of National Defence, Dept. I, Sundbyberg, Sweden

The method is based on a precipitation of sulfate in the acidified sample by a barium chloride-gelatine reagent. The latter contains preformed barium sulfate (as commercial gelatine is contaminated with sulfate) and this accounts for the accuracy of the method [1]. The resulting turbidity is determined absorptiometrically in the ultraviolet region. Serum is analyzed after deproteinization with an equal volume of 8 % trichloroacetic acid and to a 4 ml aliquot is added 1 ml of the barium chloride-gelatine reagent. The latter is prepared by dissolving 2 g $BaCl_2.2H_2O$ in 400 ml of a 0.5 % solution of commercial gelatine "for microbiological work" (or of equivalent purity). 10–60 min later the absorbancy at 360 mμ is determined against a reagent blank. The standard curve is linear for amounts of sulfate up to 2 μmoles in the test. The method can also be applied to plasma, if citrate is used as anticoagulant, but heparin cannot be used as it strongly interferes in the test. Urine is analyzed by diluting a suitable aliquot with 4 % TCA and proceeding as above. The standard deviation of the method (in per cent of the mean) was obtained as 5 % in case of serum and 1 % in case of urine.

Gassner, K. and Friedel, H. *Z. anal. Chem.* **152** (1956) 420.