

La-Roche, of Basle (through Drs. O. Isler, U. Gloor and J. Würsch), for this result, based on isotope dilution of ^{14}C -labelled acetate.

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Fractionation of Heparin on an Anion Exchanger

PREPARATIONS of heparin have been shown to be heterogeneous by a variety of techniques^{1,2}, but no method is available for the fractionation of bulk quantities of heparin or of other sulphomucopolysaccharides. These compounds carry a strong negative charge over a wide range of pH, and, therefore, it seemed possible that they could be purified on basic resins. Five anion-exchange resins ('Dowex-1', 'Dowex-2', 'Dowex-3', 'Amberlite CG-45', and 'Duolite A-4'), each buffered at pH 6.0, readily adsorbed heparin, which was easily eluted with strong salt solutions, but the eluates differed from the original material in having greater absorption at 400 m μ than at 535 m μ in the carbazole reaction³. However, 'Ecteola'^{4,5} (exchange capacity = 0.3 m.equiv./gm.) resolved a commercial preparation of bovine heparin (obtained from Nutritional Biochemicals Corp.) into four major and one minor carbazole-positive fractions (Fig. 1), each with maximum absorption at 535 m μ , and all showing metachromasia⁶. 90 per cent of the original material was recovered. Heparin-³⁵S₄, extracted from a mast-cell tumour of the mouse⁷, gave only one peak on chromatography on 'Ecteola' (Fig. 2), and only 50 per cent of the material was eluted.

Independently, Mozen (personal communication) has used 'Ecteola' for the resolution of heparin and found that those fractions that came off the column

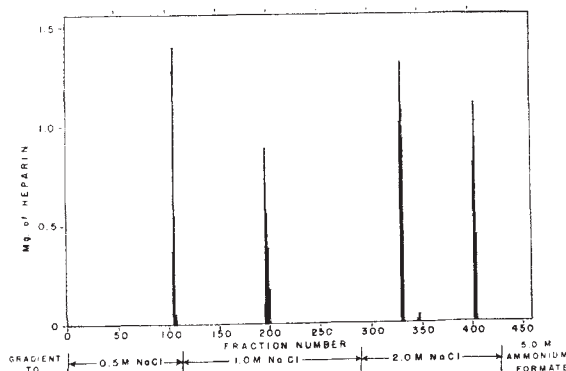


Fig. 1. Chromatography of 10 mgm. of heparin on 'Ecteola' (10 gm.; 2 x 9 cm.; flow-rate 4.0 ml./hr.). The heparin was applied in 100 ml. water. A gradient elution system was used, with a single mixing chamber containing 400 ml. water. Each fraction contained 5.5 ml.

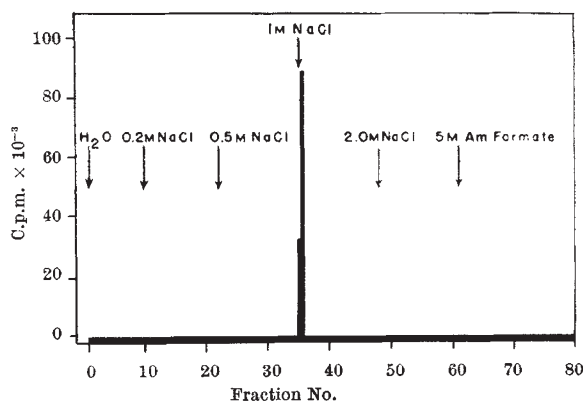


Fig. 2. Chromatography of mouse mast-cell heparin-³⁵S₄ on 'Ecteola'. Each fraction contained 10 ml.

latest had the highest anti-coagulant potency, as well as the highest sulphate content.

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Mammalian α -L-Fucosidase

ACCORDING to our surmise that the enzymes, β -N-acetylglucosaminidase, α -mannosidase and β -galactosidase, with their particularly high activity in the epididymis^{1,2}, are concerned in the breakdown of mucosubstances, they should be accompanied by an L-fucosidase. Serological evidence suggests that the L-fucose (6-deoxy-L-galactose) residue present in many mucopolysaccharides has the α -configuration^{3,4}. Rat epididymis has now been found to have an α -L-fucosidase activity of about 20,000 units per gm. moist weight, compared with activities of about 4,000 units per gm. in organs such as liver, where 1 unit of enzyme liberates 1 μ gm. *p*-nitrophenol in 1 hr. at 37° from *p*-nitrophenyl α -L-fucoside in an arbitrary concentration of 1 mM. The maximum activity is displayed at about pH 6. There is no α -D-fucosidase activity in mammalian tissues, but the limpet, *Patella vulgata*, with a high α -L-fucosidase activity, has a feeble effect on the related α -D-fucoside. This may well be a side-action of α -D-galactosidase.

Whereas β -glycosidases are always inhibited by the aldono-lactones of corresponding configuration, the α -glycosidases only sometimes display this pheno-