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Purification of β-glucuronidase from the female-rat preputial gland on Sephadex

In specific activity, no other β-glucuronidase preparation (β-D-glucuronide glucuronohydrolase, EC 3.2.1.31) approaches the one described by LEVVY, MCALLAN AND MARSH[1] (for review, see LEVVY AND CONCHIE[2]). The enzyme in a cell-free extract from female-rat preputial gland was almost completely precipitated with $(NH_4)_2SO_4$ between the limits of 20 and 80% saturation. This removed more than half the protein from the preparation. It was then put through a three-stage fractionation, using ethanol, $(NH_4)_2SO_4$ and ethanol, to give a recovery of 26% of the enzyme. A specific activity of 455 000 glucuronidase units/mg protein (as defined below) was reported, but slightly higher figures have since been obtained by the same procedure[3]. The final product was stable in buffered solution at 0° for several years.

It has been found possible to replace the last three stages of fractionation of the enzyme by passage through a column of Sephadex G-100, as illustrated in Fig. 1. Glands (3.1 g) from 36 rats were suspended in water with an Ultra-Turrax TP 18/2 N disintegrator, taken to Stage 3 in the method of LEVVY, MCALLAN AND MARSH[1]

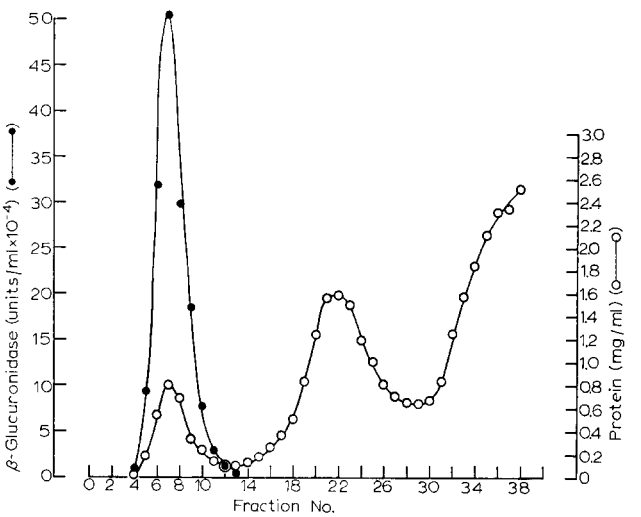

Fig. 1. Elution of β-glucuronidase from a column of Sephadex G-100, 60 cm × 3 cm, with 0.05 M acetate buffer (pH 4.5), containing 0.1 M NaCl; 100 ml was run to waste and 5-ml fractions were collected; ●—●, enzyme; ○—○, protein.

and dialyzed. It was important to maintain a high protein concentration (about 25 mg/ml) when loading the column, and to add NaCl to the eluting fluid. The column was run at 4°. Results are expressed in glucuronidase units, where 1 unit liberates 1 μg of phenolphthalein in 1 h from 0.63 mM phenolphthalein β-glucuronide at 37° and pH 4.5 in 0.05 M acetate buffer containing 0.01% albumin. Protein was determined by the method of LOWRY *et al.*[4], using bovine albumin as standard.

An average specific activity of 510 000, with more than 80% recovery of the

enzyme activity, was obtained in Fractions 5 to 10 from the column. These fractions contain 6% of the protein in the column load (2.5% of the tissue protein). There was no further separation of inactive protein when the fractions were pooled and passed through Sephadex again, nor on fractionation with ethanol. Material put through the whole of the original purification procedure[1] underwent no change on Sephadex. The purified enzyme could not be induced to crystallize and the activity in solution was destroyed by freezing. At the peak in Fig. 1, the specific activity was 620 000, and we consider this to be a limiting value for the enzyme in preputial gland.

The revised procedure for female-rat preputial gland affords a simple and convenient source of pure β-glucuronidase for use as a hydrolytic agent in the steroid and other fields, with a remarkably high recovery of the enzyme.

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Hepatic glycolytic and gluconeogenic enzymes of the obese-hyperglycemic mouse

The obese-hyperglycemic mouse (Jackson Laboratory C57BL/6J-ob) exhibits certain features of maturity-onset diabetes. These include significant hyperglycemia[1], greater than normal levels of plasma insulin during normal alimentation[2], relative insensitivity of adipose tissue to insulin[3], development of nodular glomerular renal lesions[4] and greater than normal susceptibility to skin infections. The fat metabolism of these mice has been studied extensively, but relatively little work has been done on their hepatic carbohydrate metabolism.

During alloxan diabetes in rats, hepatic glucokinase (ATP:D-glucose 6-phosphotransferase, EC 2.7.1.2), phosphofructokinase (ATP:D-fructose-6-phosphate 1-phosphotransferase, EC 2.7.1.11) and pyruvate kinase (ATP:pyruvate phosphotransferase, EC 2.7.1.40) activities fall significantly and insulin administration restores these activities to normal[5–7]. Conversely, an elevation of the hepatic gluconeogenic enzymes pyruvate carboxylase (pyruvate:CO_2 ligase (ADP), EC 6.4.1.1), phosphoenolpyruvate carboxykinase (GTP:oxaloacetate carboxy-lyase (transphosphorylating), EC 4.1.1.32), fructose-1,6-diphosphatase (D-fructose-1,6-diphosphate 1-phosphohydrolase, EC 3.1.3.11) and glucose-6-phosphatase (D-glucose-6-phosphate phos-