DISACCHARIDASE ACTIVITY OF RENAL TISSUE

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There are many examples of similarity of the enzymatic constitution of renal tissue and small intestine; brush borders of both tissues contain most of the alkaline phosphatase and much of the peptidase activity of both tissues. Also, both types of brush borders are high in content of adenosine triphosphatase, pyrophosphatase, and glucose-6-phosphatase. Intestinal brush borders are also high in content of various disaccharidases. However, disaccharides other than maltose are not utilized by mammalian tissues and many tissues other than kidney contain maltases. Thus, it might be anticipated, as this report confirms, that renal brush borders are devoid of or are very low in disaccharidase activities.

Recently, it became possible to isolate renal brush border particulates directly from renal tissue by isopycnic zonal centrifugation of homogenates treated with deoxycholate (submitted for publication). These fragments appear to be derived from the membranes of the brush borders and contain most of the alkaline phosphatase, essentially all of the pyrophosphatase, glucose-6-phosphatase, and adenosine triphosphatase activity (other than mitochondrial) of renal tissue. These particles were tested directly for disaccharidase activity.

Materials and methods. The brush border particulates were isolated from a linear gradient of sucrose, 17 to 50 per cent (w/w). Up to

20 gm fresh rat kidney was homogenized in 0.25 M sucrose containing 0.005 M $MgCl_2$, 0.01 M Tris buffer, pH 8.0, and 1 per cent deoxycholate (3 ml per gm fresh tissue) and placed on 1500 ml linear sucrose gradient with a cushion of 150 ml 55 per cent sucrose and with an appropriate amount of overlay of 0.01 M Tris buffer, pH 8.0, with 0.005 M $MgCl_2$. Centrifugation was for 2 hr in the B-IV rotor in the Spinco L-4 centrifuge at 20,000 rpm. Samples of 13 ml each were collected. The brush border particulates banded sharply at a concentration of sucrose of 38 per cent (w/w). The solubilized material remained at or near the sample zone. Brush border particulates were recovered by centrifugation of the diluted samples and were washed several times and dialyzed against water for 48 hr. The yield of brush border particles was 6-8 mg (dry weight) per gm of fresh renal tissue (wet weight). Thus, the concentration factor for the brush border enzymes was about 30-40 fold. Samples from the solubilized material were dialyzed thoroughly to remove sucrose. The assay for disaccharidases was by the method of Dahlqvist (1964); the other assays were by modifications of standard procedures.

Results. Only maltase activity was detected in the preparations of brush border particles; there was no activity in the hydrolysis of sucrose, lactose, trehalose, or cellobiose in homogenates of renal tissue of the rat or in fractions prepared from the homogenates. The distribution of the maltase activity, together with some other renal enzymes, is summarized in Table I.

Thus, the maltase does not appear to be an activity of the brush border particles. The activity in the particles appears to be identical with that in the soluble fraction in that the pH optimum is identical (about 6.2) and both activities are equally labile to heat (essentially total loss at 60° for 10 min). Prolonged dialysis was without effect upon the activity of either fraction and neither activity was increased by calcium or magnesium

Table I: Distribution of Enzymatic Activities in Renal Tissue

Fraction	Alkaline Ptase	Glucose-6-Ptase	Acylase I	Cytochrome C Oxidase	Acid Ptase	Maltase
Brush Border Particle	80	95	0	0	0	2
DOC Soluble	20	5	100	100	100	98

Acylase I is a soluble enzyme, Cytochrome C oxidase is in the mitochondrial fraction, and the acid phosphatase is found in the lysosomal fraction. The activities of the brush border particles normally distribute with the microsomal fraction but the distribution is not influenced by treatment with deoxycholate.

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ions. The renal activity appears to be very similar to that of liver tissue (Stetten, 1959).

Since there is little or no trehalase in renal tissue of the rat, this enzyme cannot be involved in renal transport (Sacktor, 1968) and, since the renal maltase is not associated with the particles derived from the brush borders, it is unlikely that it is involved with transport. At the present time there is no convincing evidence for a role of the disaccharidases in renal transport mechanisms.

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