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Enzymatic distinction of rat intestinal cell brush border and endoplasmic reticular membranes

Separation of different subcellular fractions of the rat intestinal mucosal cell has been carried out for some years[1], but clean isolation of component organelle fractions still has been difficult to achieve. Intestinal epithelial cell brush borders may be isolated as a special organelle of this particular type of cell and are known to contain hydrolytic enzymes[2], but many of the synthetic activities of the gut cells are localized in the cell sap and endoplasmic reticular membranes. FORSTNER *et al.* found some glyceride synthetic activity in brush borders[3] but commented[4] on the difficulty of assessing whether brush border preparations were free of microsomal contamination. On the other hand, after tissue grinder homogenization of whole mucosal cells, brush border fragments are found in the "microsomal" fraction obtained by ultracentrifugation[5], or in the nuclear fraction[6]. In the various preparations available to date, it has been uncertain how much isolated brush borders have been admixed with microsomal membrane fragments and *vice versa*. Recently, enzymatic activity concerned with electron transport from NADPH to cytochrome *c* has been found localized in hepatic microsomal fractions[7,8]. We have found that the NADPH cytochrome *c* reductase (NADPH:cytochrome *c* oxidoreductase, EC 1.6.2.3) activity in the microsomal fraction of the intestinal mucosal cell may be used as a marker for the endoplasmic reticular fraction as distinct from membranes of the brush border portion of the cells.

Female rats of the Sprague–Dawley strain weighing 200–250 g were used. After the intestine was removed, the brush borders were isolated, first as a crude sediment, and then "purified" by filtration and further centrifugation[4]. The supernatant from the first low speed separation was then centrifuged at 22500 × *g* for 15 min to isolate a mitochondrial fraction; microsomes were isolated by centrifugation of the resultant supernatant at 140000 × *g* for 45 min.

Sucrase (β-D-fructofuranoside fructohydrolase, EC 3.2.1.26) was measured by the method of CARNIE AND PORTEOUS[9], and cytochrome oxidase (cytochrome c:O_2 oxidoreductase, EC 1.9.3.1) activity by the method of SMITH[10]. The NADPH cytochrome *c* reductase activity was assayed by the method of MASTERS, WILLIAMS AND KAMIN[11], using $3.6 \cdot 10^{-5}$ M cytochrome *c* and 10^{-5} M NADPH, both from Sigma Chemical Company, in 0.05 M phosphate buffer (pH 7.7) containing $1 \cdot 10^{-4}$ M EDTA (disodium salt) and $1 \cdot 10^{-3}$ M KCN in a final volume of 1 ml. The activity of the enzyme was measured in a Britton-Chance dual wavelength spectrophotometer, American Instrument Company, taking the difference in absorption at 545 and 550 mμ. Protein was measured by the method of LOWRY *et al.*[12].

For electron microscopy, pellets were fixed at room temperature in 3% glutaraldehyde (pH 7.2) for 2 h or overnight, then after rinsing were post-fixed in 2% osmium tetroxide for 2 h for subsequent dehydration and embedding in Epon 812, Shell Chemical Company. Silver sections were stained with uranyl acetate and lead citrate and viewed with an EM 6 electron microscope, Associated Electrical Industries, England.

The NADPH cytochrome *c* reductase showed high specific activities in the microsomal fraction of rat intestinal homogenates (Table I). The mitochondrial

fraction, which we made no attempt to purify, also showed high specific activity. In the effort to reduce contamination of the microsomal fraction with mitochondria, as shown by cytochrome oxidase determination, we chose centrifugation conditions which caused considerable sedimentation of the endoplasmic reticular fragments with the mitochondria. We found the intestinal microsomal specific activity of NADPH cytochrome *c* reductase to be approx. 60% that of activity in rat liver microsomes prepared in parallel. Inhibition of the intestinal microsomal fraction of rat intestinal

TABLE I

SPECIFIC ACTIVITIES OF MARKER ENZYMES IN FRACTIONS OBTAINED FROM RAT INTESTINAL EPITHELIAL CELLS

Values given as means ± one standard deviation. Number of observations in parentheses. $K = 60\,k$, where k (sec^{-1}) is the first order velocity constant for the oxidation of reduced cytochrome *c* under conditions defined by SMITH[10].

Fraction	*Sucrase (μmoles/min per mg protein)*	*Cytochrome c oxidase (K/min per mg protein)*	*NADPH cytochrome c reductase (nmoles/min per mg protein)*
Homogenate	0.27 ± 0.2 (15)	3.73 ± 1.14 (8)	1.93 ± 1.38 (4)
Mitochondria and debris	0.59 ± 0.6 (12)	11.3 ± 3.55 (7)	3.27 ± 0.47 (4)
Microsomal fraction	0.16 ± 0.1 (12)	0.94 ± 0.66 (7)	3.35 ± 0.85 (4)
Brush borders, purified	4.77 ± 2.0 (15)	0.11 ± 0.11 (7)	0.35 ± 0.26 (4)

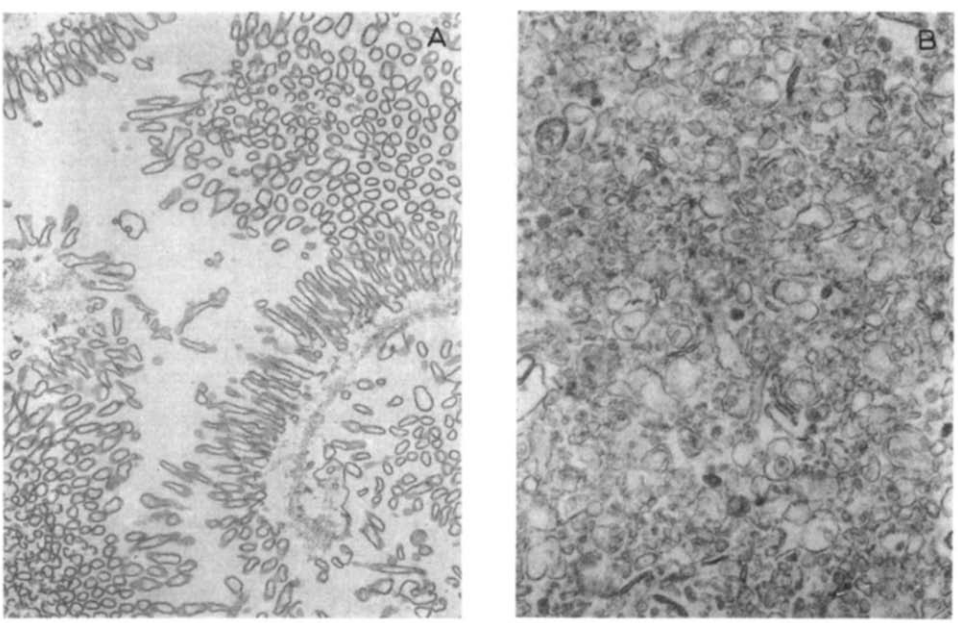

Fig. 1. A. Brush border preparation[4], showing microvilli cut both obliquely and in cross section. Bits of endoplasmic reticulum cling to the terminal web portion of the brush border. Magnification, × 8000. B. Microsomal fraction, after prior removal of brush borders. Magnification, × 8000.

enzyme incubated without cyanide but with $2 \cdot 10^{-5}$ M oxidized nicotinamide adenine dinucleotide phosphate was 75 % and with $2 \cdot 10^{-6}$ M *p*-hydroxymercuribenzoate was 67 %. Similar inhibition was described by WILLIAMS AND KAMIN[7] for hepatic microsomes. Our brush border preparations nearly always showed a small degree of NADPH cytochrome *c* reductase activity (Table I), which could be correlated with the small amount of endoplasmic reticulum seen attached to brush border fragments (Fig. 1). As the brush border fraction was purified, however, the specific activity of the NADPH cytochrome *c* reductase was reduced, as was that of cytochrome oxidase, while sucrase specific activity increased to about 30 times that of the microsomal fraction. Conversely, the NADPH cytochrome *c* reductase activity of the microsomal fraction was about 10 times that of the "purified" brush border fraction. In some brush border preparations we could demonstrate almost no NADPH cytochrome *c* reductase activity at all; however, we cannot exclude completely the possibility that a small amount of this enzyme might be present in the brush border membranes themselves rather than contained in contaminating endoplasmic reticular membranes. Glucose-6-phosphatase (D-glucose-6-phosphate phosphohydrolase, EC 3.1.3.9) had been proposed[5] as a possible marker for intestinal microsomes on the basis that it had been so used for liver microsomes, but confusing non-specific phosphatase activity in brush borders[4] has reduced the usefulness of this marker to distinguish intestinal endoplasmic reticular membranes from brush borders. This distinction now appears possible, using NADPH cytochrome *c* reductase as the marking enzyme activity. By using enzyme markers localized to separable types of membrane fractions, various specific functions of the brush border membranes on the surface and those of the endoplasmic reticulum within the intestinal cells may be defined.

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