THE ISOLATION OF PURIFIED BRUSH BORDERS FROM RAT SMALL INTESTINE

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Porteous and Clark [1] devised a method for the isolation of all the major subcellular organelles or rabbit intestinal epithelium; each isolated fraction was characterized microscopically, by chemical analysis and enzyme assay. Hübscher, West & Brindley [2] applied this method to rabbit, cat and guinea pig intestine and extended the observations in several important ways. Despite extensive efforts neither Porteous and Clark [1] nor Hübscher et al. [2] succeeded in separating nuclei from brush borders. Porteous [3] reviewed thirteen publications, in eleven of which claims were made that pure brush borders had been isolated from small intestine; in only one of these eleven publications (Eichholz [4]) is any quantitative analytical information available to permit the degree of contamination of the brush borders by other organelles to be assessed. It is probable that all brush border preparations available to date [3] contained nuclei or nuclear debris as major contaminants.

A method is presented for the isolation of purified brush borders essentially free of DNA and RNA but containing a large part of the intestinal invertase at high specific activity. The method is based on the original method of Miller and Crane [5] and on an unpublished observation (J.W.Porteous and Ann C.Paterson) that intestinal epithelial cell nuclei gave a flocculent sediment in phosphate-citrate-saline whereas brush borders failed to flocculate.

The intestine of an ether-anaesthetised male Wistar rat (200g) was washed out *in vivo* with ice-cold 0.9% NaCl, excised and washed inside and out with ice-cold 2.5 mM-EDTA (pH 7.0). Clean mucosa was expressed with a glass slide [1]. Subsequent steps in the procedure were carried out at 0 - 5°. Mucosa (2-3g) was either: (a) suspended in 60 ml 2.5 mM-EDTA (pH 7.0) with five strokes of a hand-operated Teflon/glass homogeniser [6] (working capacity 60 ml and a radial clearance of 0.004 in. between pestle and mortar), then homogenised in the same motor-driven homogeniser (2000 rev/min, 60 sec, 12 complete strokes) and diluted to 120 ml with 2.5 mM-EDTA (pH 7); or (b) homogenised in 120 ml 2.5 mM-EDTA (pH 7.0) in a top-drive macerator (Measuring and Scientific Instruments Ltd., London) operating at 4000 rev/min for 40 sec.

Homogenates were filtered through 100μ square-mesh nylon cloth [1], the filtrate centrifuged (1000 g, 10 min), the supernatant discarded and the sediment resuspended (hand-operated Teflon/glass homogeniser, 5 strokes) in the original volume of 2.5 mM-EDTA (pH 7.0). Sedimentation and resuspension of the sediment was repeated three or four times until the final suspension contained only brush borders and nuclei upon phase-contrast examination [3]. The procedure up to this stage was essentially that described by Miller and Crane [5] for the isolation of hamster intestine brush borders and was the method used in most of the publications reviewed [3].

Sedimented brush borders and nuclei were resuspended (hand-operated Teflon/glass homogeniser) in 2.5 mM-EDTA/50 mM-potassium phosphate/ 25 mM-potassium citrate/77 mM-potassium chloride (P/C/C), pH 7.0, and kept at 0° for 20 - 30 min. The turbid supernatant was decanted from a flocculant sediment through 25μ square-mesh nylon cloth and brush borders sedimented from the filtrate (1000 g, 10 min). The sediment, resuspended in 2.5 mM-EDTA, was free of intact nuclei when examined by phase-contrast microscopy. Electron microscopy showed that a little debris, probably of nuclear origin,

was still present amongst the brush borders but separate from them; the fine structure of the brush borders themselves was well preserved [3].

The filtered cell homogenate, the suspension of brush borders + nuclei (before P/C/C treatment) and the final suspension of purified brush borders (after P/C/C treatment) were each assayed [1] for invertase and succinate dehydrogenase activities and analysed [1] for protein, DNA and RNA. The brush border + nuclei preparation contained 16% of the protein, 100% of the DNA, 23% of the RNA, 64% of the invertase activity and none of the succinate dehydrogenase activity of the original filtered cell homogenate; the corresponding figures for the purified brush border preparation were: protein 3%, DNA 0.9%, RNA 1%, invertase 42%, succinate dehydrogenase nil. If it is assumed (i) that invertase activity is associated solely with brush borders and that the enzyme is not inactivated during isolation of the subcellular fractions, and (ii) that DNA is predominantly associated with nuclei but not with brush borders, then the analytical figures quoted suggest that the Miller and Crane [5] technique, at least when applied to rat intestine, preserves nuclei rather better than it does brush borders. On the same assumptions, the invertase activity/protein quotient should increase and reach a high plateau value as purification of brush borders proceeds, whereas the invertase activity/DNA and invertase activity/RNA quotients should simultaneously increase towards infinity.

Table 1 summarizes the only known analytical results which allow assessment of the purification of brush borders from nuclei. Several points are of interest: (i) the increase in the value of the quotients in columns (2) and (4) reflects a steady purification of the brush borders through stages (a), (b) and (c) of the present work; purification in respect to RNA greatly exceeds that in respect of protein. The value of the quotients in columns (1) and (3) show a marked *decrease* between stages (a) and (b), reflecting the relative conservation of nuclei and destruction of brush borders at this stage of purification. Between steps (b) and (c) the values of these two quotients show a sharp increase; that for invertase/DNA is much the greater of the two. Between steps (a) and (c) there is a fourteen-fold purification of brush borders in respect of protein and a forty-fold purification in respect of DNA and RNA. These results suggest that further purification from adventitious DNA and RNA should be possible or that these compounds are an integral part of the brush border of intestinal epithelial cells; the former view seems preferable until substantial evidence to the contrary is produced. These analytical results, together with the microscopic observations detailed elsewhere [3] and summarised above provide substantial quantitative support for the claim [5] that invertase activity is a specific attribute of the brush border of these cells; (ii) Forstner et al. [7] isolated pure rat intestinal brush borders by techniques very similar to those described here; they used somewhat different homogenisation conditions, removed a "large quantity of viscous contaminant" (probably nucleoprotein) selectively from the homogenate by treatment with 90 mM-NaCl/0.8 mM-EDTA, pH 7.4, and aided removal of this material by filtration through glass cloth. The quotients calculated from the results of Forstner et al. [7] are remarkably similar in magnitude to those quoted from the present work with but one exception; the value of the quotients in columns (1) and (3) do not decrease sharply between stages (a) and (b) because these authors found only 5% (instead of 100%) of the original DNA in their crude brush border fraction. This may be accounted for partly by the higher pH of their homogenisation medium [7,9], and partly by the more vigorous homogenisation conditions used by these authors, a supposition which would also account [6] for the lower yield of invertase activity (40% instead of 64%) at stage (b). The conditions of homogenisation have been more precisely defined in the present work than in most other work reviewed [3] and it is clear that these conditions may have a profound effect on the relative proportions of nuclei and brush borders isolated from a given species of intestine by the Miller and Crane [5] technique; (iii) at stage (c) Forstner et al. [7] achieved the same order of magnitude of purification of the brush borders in respect of protein (Column 2) but somewhat better purification in respect of DNA and RNA (columns 3 and 4) at the expense of some overall loss of invertase activity (25% recovery instead of 40% in the present work, column 5); (iv) Forstner et al. [7] prepared a membrane component of the purified brush borders thereby achieving further purification of the invertase activity relative to protein content (column 2) but a markedly

Table 1

A comparison of selected analytical results for various preparations from mammalian small intestinal epithelium; (a) cell homogenate, (b) crude brush borders (brush borders + nuclei), (c) purified brush borders, (d) brush border membrane. All results have been quoted to the nearest whole number. Where necessary units of invertase activity (U) have been recalculated to conform with the activity defined elsewhere [1].

Authors	Animal		(1) Protein / DNA (mg/mg)	(2) Invertase / protein (U/mg)	(3) Invertase / DNA (U/mg)	(4) Invertase / RNA (U/mg)	(5) Invertase (%)
Porteous & Clark [1]	rabbit	(a)	20	2	40	65	100
(table 2)		(b)	7	4	30	230	70
Hübscher et al. [2]	g. pig	(a)	18	1	60	-	100
(tables 4 and 5)		(b)	3	4	49	-	75
	rabbit	(a)	33	1	125	-	100
		(b)	5	5	84	-	60
Eichholz [4]	hamster	(b)	1	75	75*	-	-
(tables 1 and 2)		(d)	-	280	-	-	-
Porteous [3] – and	rat	(a)	24	5	120	66	100
present work	(wistar)	(b)	4	20	80	200	64
		(c)	70	70	4900	2400	40
Forstner et al. [7]	rat	(a)	30	3	90	90	100
(tables 1 and 3)	(Sprague-	(b)	25	30	750	1400	40
	Dawley)	(c)	60	60	7500	4300	25
		(d)	220**	90	20000	9000	20

* Calculated from results quoted in two preceding columns.
** Calculated from results quoted in two following columns.
*** DNA determined as DNA-P; recalculated here assuming DNA/P = 12 (ref. [10]).

greater purification relative to DNA and RNA content (columns 3 and 4); (v) the less extensive information available from Eichholz [4] does not accord as closely as might be expected with the corresponding values quoted from the present work and from Forstner et al. [7]; this may reflect differences between the hamster and the rat, or differences arising from the use [4] of a quite different technique for isolating brush border membranes directly from what was probably a mixture of brush borders and nuclei [3]. Eichholz's invertase/protein quotient for the membrane fraction is considerably greater than that obtained by Forstner et al.; unfortunately Eichholz does not give analytical results which would allow calculation of values for columns 1, 3, 4 and 5; (vi) the results quoted from the present work and from Forstner et al. [7] illustrate the utility of the three quotients (invertase/protein, invertase/DNA, invertase/RNA) in assessing the purification of intestinal brush borders from other organelles [3] on the basis of the assumptions made earlier in this paper. On the information available these two brush border preparations [3,7] and the membrane preparation of Forstner et al. [7] are probably the purest and certainly the best characterized preparations available to date; There is a pressing need for more critical quantitative analyses of isolated organelles and membrane preparations. It would be of considerable interest to compare results obtained in one laboratory with one animal species using all three methods [3,4,7] of isolation of brush borders.

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