ISOLATION OF MICROVILLI FROM MAMMALIAN CELLS

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1. Introduction

Microvilli have a variety of functions in animal cells. On epithelial cells of the renal tubule or small intestine, they increase the absorptive capacity of the cell, by increasing its surface area. In this instance, microvilli are concentrated at one end of the cells, in the form of 'brush border', and may be isolated as such and their composition studied [1–3].

On mammalian cells in culture, microvilli also increase the surface area (e.g. [4]). In this case, their function is to allow morphological changes such as 'spreading' [5] and cytokinesis [6] to take place without recourse to new membrane synthesis [7]. Since microvilli are not localized at any particular part of the surface of such cells, it is more difficult to remove them without disrupting the entire cell. Here we describe a method by which this can be achieved. It is based on sonication of cells lightly fixed with glutaraldehyde.

2. Materials and methods

Lettree cells (10^8/ml, grown ascitically in mice) were exposed to 0.1% glutaraldehyde ('EM' grade from Taab Laboratories, dissolved in pH 7.4 phosphate-buffered isotonic saline) for 2 min at room temperature (20–22°C). Lower temperatures lead to losses of intracellular components; at lower concentrations of glutaraldehyde, cell breakage occurs during the subsequent sonication; higher concentrations are without advantage. Serum albumin (final conc. 10 mg/ml) was added to stop the reaction and cells washed twice in Tris-buffered saline (pH 7.4). A chilled suspension (10^8/ml) was sonicated at 50 W for 30 s in a Braunsonic 1510 machine and spun for 5 min at 300 × *g*. The protein [8], 5′-nucleotidase (EC 3.1.3.5) [9] and acid phosphatase (EC 3.1.3.2.) [10] content of the supernatant and pellet was determined. Glutaraldehyde does not appreciably affect the activity of either of these two enzymes (85% of control in the case of 5′-nucleotidase; 77% of control in the case of acid phosphatase; cf 73% of control in the case of acid phosphatase by others [10a]).

The lipids of the supernatant and pellet were extracted [11] and the phosphorus [12] and cholesterol [13] content assayed. The lipid extracts were fractioned by two-dimensional thin-layer chromatography [14] and the sphingomyelin and phosphatidylcholine areas scraped off and analysed separately. In some experiments mice bearing Lettree cells were injected with inorganic [^{32}P]phosphate or [*Me*-^{3}H]-choline 15–20 h prior to removal of cells. In each case, the ratio of sphingomyelin to phosphatidylcholine in supernatant and pellet confirmed the values obtained by chemical analysis.

3. Results and discussion

Table 1 shows that approx. 50% 5′-nucleotidase activity, and 30–35% cholesterol and sphingomyelin content of cells is released by sonication. Since this material contains only approx. 20% protein, phospholipid, RNA or acid phosphatase activity of the cell, it is clearly enriched in plasma membrane-derived material. The ratios of cholesterol/phospholipid and sphingomyelin/phosphatidylcholine confirm this conclusion (table 1). Moreover 50% of the radioactivity

Table 1
Composition of supernatant material released from glutaraldehyde-treated cells by sonication

Fraction	Protein (mg)	Phospholipid[a] (μg)	RNA (μg)	DNA (μg)	Cholesterol (μg)	Cholesterol/phospholipid (ratio)
Supernatant	3.0	121	79	<3	88	0.72
Pellet	10.3	586	331	290	192	0.33

	Sphingomyelin (μg)	Sphingomyelin/phosphatidylcholine (ratio)	5′ Nucleotidase (units)	5′ Nucleotidase (spec. act.)	Acid phosphatase (units)	Acid phosphatase (spec. act.)
Supernatant	5	0.53	190	63	1440	480
Pellet	9	0.20	196	19	5400	520

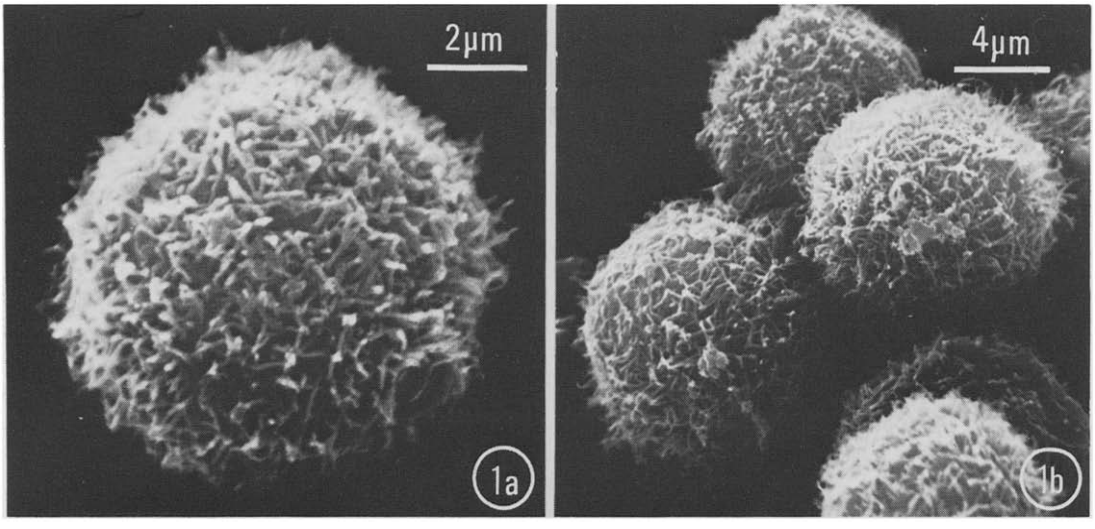
[a] assuming mol. wt 760

All values, which are means of 3 or more experiments, refer to 10^8 Lettree cells. Units of enzyme activity are in μmol substrate reacted/h; specific activity is μmol substrate reacted/h mg protein

that becomes associated with cells when these are labelled by the [^{125}I]iodide–lactoperoxidase technique [15] is present in the supernatant fraction. When cells that have not been treated with glutaraldehyde are sonicated, the supernatant material contains a mixture of most of the cellular constituents; there is no enrichment of any membrane material.

That the supernatant material is derived from microvilli that are sheared off from cells during sonication, is shown by scanning electron microscopy (fig.1). The effect is not striking because many microvilli are sheared off not at the base, but some may along their length, so that the appearance of the cell remains villated. Also, cells are not uniformly villated to begin with (e.g. [6,7]). Nevertheless, examination of a large number of cells shows that the effect of sonication is to shear microvilli off cells. During sonication the cell number assessed by light microscopy, falls by <10%. The reason why microvilli are sheared off in this manner is not clear. The possibility that it is due to the insertion of newly-synthesized membrane is being investigated.

The supernatant material does not represent pure microvilli, as judged by the contamination with RNA and acid phosphatase (table 1); these substances are presumably released at the moment of microvillar



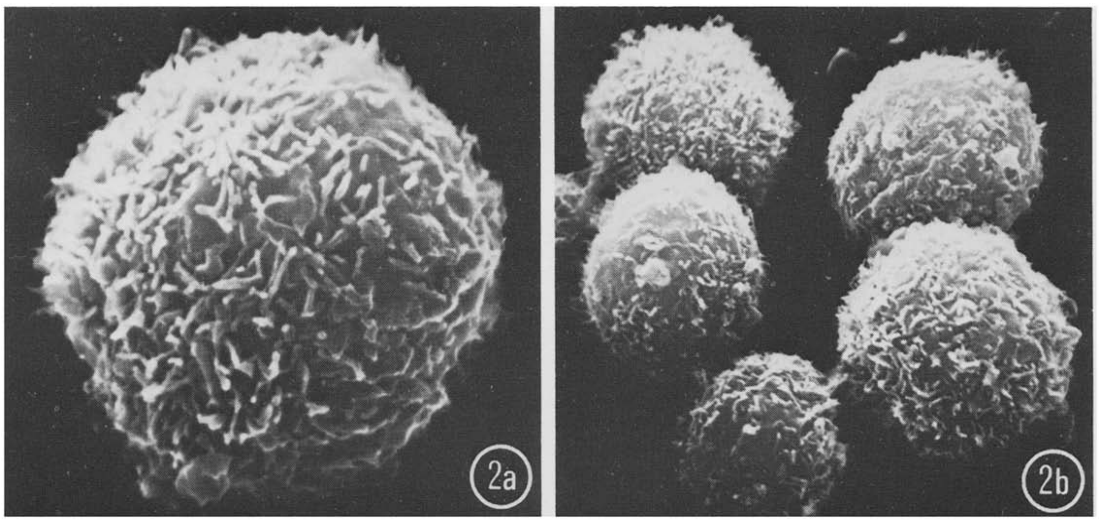


Fig.1. Effect of sonication on glutaraldehyde-treated Lettree cells. Cells were treated as described in section 2, allowed to settle on to gelatin-coated cover-slips and fixed with 3% glutaraldehyde in phosphate-buffered saline for 1 h. After washing in buffer, the cover-slips were taken through a graded series of acetone washes, critical point dried, coated with gold, and viewed in a type 2A stereoscan. 1a, 1b: cells before sonication; 2a, 2b: cells after sonication. Magnification: 1a, 2a × 6490; 1b, 2b × 3180. Cells after sonication can be seen to have fewer, and shorter, microvilli.

rupture. The protein content of the supernatant fraction is also higher than would be expected if it were pure plasma membrane, which is not suprising in view of the fact that microvilli are known to contain bundles of microfilaments [16]. A method for the purification of microvillar membranes is being developed.

In summary, this communication outlines a simple approach for the isolation of microvilli from cultured cells. It should prove useful for elucidating the extent to which the composition of microvillar membrane differs from that of adjacent plasma membrane, as well as for studies on the structure and turnover of the microvillus–microfilament system.

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