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Rapid Isolation of Plasma Membranes in High Yield from Cultured Fibroblasts

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1. A method was developed which allows the rapid preparation of pure plasma membranes in high yield from cultured fibroblasts. 2. Cells are lysed in hypo-osmotic borate/EDTA and, after differential centrifugation, the membranes collected by centrifugation on a sucrose barrier. 3. Electron microscopy of the isolated material shows large membrane vesicles essentially free from contaminating organelles. 4. There is no detectable activity of the endoplasmic-reticulum enzyme marker, $NADH_2$–lipoamide oxidoreductase (EC 1.6.4.3), and that of succinate dehydrogenase (EC 1.3.99.1), a marker for mitochondria, is substantially decreased. Chemical compositions are in good agreement with previous observations. 5. This study confirms the usefulness of applied isotopic markers for isolating plasma membranes.

There is now overwhelming evidence that the surface of animal cells is a primary target for transduction of a wide variety of external stimuli controlling their behavioural properties. Isolation and characterization of the molecules involved in such processes, and of the assemblies of which they form part, requires a routine method for the isolation of plasma membranes. Ideally, this should be rapid, non-selective and capable of large-scale preparations.

The traditional use of enzyme activities as specific markers for membrane purification is beset with difficulties (Steck & Wallach, 1970; Warren & Glick, 1971; Steck, 1972). Later studies (Chang *et al.*, 1975; Rittenhouse-Simmons & Deykin, 1976) have illustrated the usefulness of an alternative approach for plasma membranes, namely labelled markers directed against the carbohydrate side chains of surface glycoproteins. By extending previously established methods for modifying sialoglycoproteins (Van Lenten & Ashwell, 1971; Liao *et al.*, 1973) we have now incorporated a radioactive marker into cultured fibroblasts which, under the mild conditions used, is almost exclusively limited to the outer cell surface.

Methods previously used for the cellular-disruption step include physical or mechanical devices and osmotic lysis when the plasma membrane is first stabilized by appropriate agents. McCollester (1970) used this latter approach to develop a rapid gentle method for the isolation of large membrane sacs from tumour cells by hypo-osmotic lysis in a solution of sodium borate/EDTA, although Warley & Cook (1973) found it necessary to incorporate additional purification steps. We have developed this method further by use of differential centrifugation and centrifugation at a sucrose barrier, to demonstrate that a high yield of large membrane vesicles, the purity of which is confirmed by several techniques, can be rapidly and reproducibly isolated from large-scale cell cultures.

Materials and Methods

All reagents were analytical grade unless otherwise specified.

Cell culture

An established line of rat dermal fibroblasts (16C Colworth strain) was maintained and grown in 8oz medicine bottles as previously described (Lloyd & Cook, 1974) in Dulbecco's modification of Eagle's medium (special formulation of Gibco–Biocult, Paisley, Renfrewshire, Scotland, U.K.) containing 10% (v/v) foetal calf serum, amphotericin B (fungizone; 2.5μg/ml), penicillin (50 units/ml) and streptomycin (50μg/ml) (all from Gibco–Biocult) in a gas phase of 10% (v/v) CO_2 in air. Large-scale cultures for membrane preparations were grown in 1.6-litre plastic spiral vessels (Sterilin, Richmond, Surrey, U.K.) as described by Maroudas (1974), by using an inoculum of 1×10^8 cells.

Membrane preparation

For the preparation of plasma membranes the following were freshly prepared from stock solutions. Harvesting solution: 0.05M-boric acid, 0.15M-NaCl, 1mM-$MgCl_2$, 1mM-$CaCl_2$, pH7.2. Extraction solution: 0.02M-boric acid, 0.2mM-EDTA, pH10.2,

Borate solution: 0.5M-boric acid, pH 10.2. Phosphate-buffered saline: 0.137M-NaCl, 2.7mM-KCl, 0.9mM-$CaCl_2$, 0.49mM-$MgCl_2$, 1.47mM-KH_2PO_4, 0.84mM-Na_2HPO_4, pH 7.4 or 7.8. All solutions were adjusted to the appropriate pH with 1M-NaOH. Cells grown in monolayer in 8oz bottles (substrate-attached cells) were washed with harvesting solution and scraped into the same solution with a 'rubber policeman' for glass bottles and by passing the Cellophane strip from roller bottles between fixed strips of rubber. They were collected by centrifugation at 450*g* for 5min (MSE bench centrifuge, 1300rev./min; swing-out head). The pellet was resuspended by pipetting in harvesting solution (2 pellet volumes) and the suspension added slowly with stirring to the extraction solution (100vol.) with the stirring speed pre-adjusted to aggregate the gelatinized cytoplasm in the vortex. After stirring for 10min, borate solution (8vol.) was added and the stirring continued for a further 5min. After filtration through two layers of nylon gauze (Henry Simon, Stockport, U.K.: average mesh size 900μm), the suspension was centrifuged at 450*g* for 10min at 2°C (MSE Major; 6×250ml rotor). The pellet contained any whole cells, nuclei and precipitate that were not removed by filtration; this was discarded and the supernatant was recentrifuged at 12000*g* for 30min at 2°C (MSE model HS 18; 8700rev./min; 6×250ml rotor). The plasma-membrane-rich pellet was gently suspended in phosphate-buffered saline and layered on top of 35% (w/w) sucrose solution in phosphate-buffered saline and centrifuged at 24000*g* for 1h at 2°C (MSE model SS50; 15000rev./min; 3×40ml swing-out rotor). The plasma-membrane fraction collected at the sucrose phosphate-buffered saline interface and was removed with a Pasteur pipette, resuspended in a convenient volume of phosphate-buffered saline and the membranes were collected by centrifugation at 100000*g* for 10min at 2°C (MSE model SS50; 40000rev./min; 10×10ml rotor). Membranes were stored frozen when necessary.

Electron microscopy

Samples were fixed in 3% (v/v) glutaraldehyde in 0.1M-sodium cacodylate, pH 7.4, for 4h at room temperature (21°C). After a overnight wash in 0.1M-sodium cacodylate/0.25M-sucrose they were post-fixed in 1% osmic acid in Michaelis buffer (0.143M-sodium barbitone/0.143M-sodium acetate) at pH 7.4 for 2h at 0°C and stained with 1% uranyl acetate for 2h at room temperature. After dehydration in a graded series of ethanol solutions, embedding in low-viscosity resin (Spurr, 1969) and sectioning with a Porter–Blum MT2 microtome [DuPont (U.K.) Ltd., Hitchin, Herts., U.K.] sections were examined with a JEM 7A electron microscope [JEOL (U.K.) Ltd., Collindale, U.K.].

Enzyme assays

$(Na^+ + K^+)$-ATPase* (EC 3.6.1.3) and $NADH_2$–lipoamide oxidoreductase (EC 1.6.4.3) were measured by the methods of Wallach & Kamat (1964). Succinate dehydrogenase (EC 1.3.99.1) and 5′-nucleotidase (5′-ribonucleotide phosphohydrolase, EC 3.1.3.5) were measured by the methods of Porteous & Clarke (1965) and Persijn *et al.* (1968) respectively. Cells were lysed in water and activities in this lysate were taken as original activities in the calculation of relative specific activities. Protein determinations were by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

Chemical analysis

Membrane pellets were extracted overnight in chloroform/methanol (2:1, v/v). Insoluble material was spun down (MSE Minor; 1500*g*; 10min) and the pellet washed twice in the chloroform/methanol mixture. The extract and washings were combined, shaken twice with 0.75% NaCl to remove salts and concentrated by rotary evaporation. Cholesterol was assayed by the method of Zlatkis *et al.* (1953) and phospholipid by the method of Rouser *et al.* (1966). Phospholipid was assumed to consist of 25μg of phospholipid/μg of inorganic P (Kopaczyk *et al.*, 1966). The pellet was dissolved by boiling in 0.1M-NaOH and the solution assayed for protein.

Radioactive labelling

Preliminary experiments with fetuin as a model compound showed that NaB^3H_4 was destroyed at pH 7.0 with little incorporation of ^3H into the pre-oxidized glycoprotein. At higher pH, incorporation was increased, presumably because NaB^3H_4 was hydrolysed more slowly. All reductions were at 0°C at a final pH of 8.5 as a compromise between maximizing incorporation and preventing elimination of carbohydrate side chains *O*-glycosidically linked to serine or threonine (Thomas & Winzler, 1969). Specific labelling of sialic acid residues has previously been observed (Liao *et al.*, 1973) at concentrations of sodium periodate less than 2mM, and our studies confirmed that short incubations with 1mM-periodate (30min) and reducing agent (10min) gave optimal incorporation into proteolytic-sensitive molecules while minimizing labelling in the absence of oxidizing agent.

All operations were with prechilled solutions in a tray of ice. The monolayer in each glass bottle (230ml)

* Abbreviation: $(Na^+ + K^+)$-ATPase, $(Na^+ + K^+)$-dependent adenosine triphosphatase measured by the increase in the rate of ATP hydrolysis that occurs in the presence of both Na^+ and K^+, over the rate found when K^+ is absent.

was washed twice with phosphate-buffered saline, pH7.4 (10ml), and then incubated in 1mM-sodium periodate in phosphate-buffered saline (15ml; pH7.4) for 30min. After this time any remaining periodate was inactivated by three washes of phosphate-buffered saline (10ml each) containing glucose (1g/litre). Phosphate-buffered saline, pH7.8 (15ml), was added, followed by NaB^3H_4 (The Radiochemical Centre, Amersham, Bucks., U.K.; low-specific-radioactivity material, code no. TRA45, was used for membrane-isolation studies, and high-specific-radioactivity material, code no, TRK45, for polyacrylamide-gel studies) in 0.01M-NaOH (1ml) at a nominal concentration of 0.5μmol/ml. The pH of the solution was 8.5. After incubation (10min) cells were washed three times with phosphate-buffered saline, scraped off the glass into phosphate-buffered saline with a 'rubber policeman' and collected by centrifugation at 450*g* for 5min (MSE bench centrifuge; 1300rev./min; swing-out head).

Metabolic labelling of cells

Cells in monolayer were washed once with phosphate-buffered saline and incubated for 1h at 37°C with the same (10ml) containing 250μCi of [1-^{3}H]-glucosamine hydrochloride (The Radiochemical Centre; 3000mCi/mmol). Complete medium (10ml) was then added, the cells were incubated for a further 6h, washed with phosphate-buffered saline, scraped off the glass with a 'rubber policeman' and collected by centrifugation at 450*g* for 10min (MSE bench centrifuge; 1300rev./min).

Polyacrylamide-gel electrophoresis

Whole cells were dissolved by boiling for 10min in electrophoresis buffer containing 2% (w/v) sodium dodecyl sulphate; the solution was made 1% (v/v) in mercaptoethanol and boiled for a further 5min. Gels were prepared and run as described by Fairbanks *et al.* (1971), except that pH7.1 was used. Loadings were based on radioactivity.

The gels were frozen and sliced with a Mickle slicer. Slices (1mm) were dissolved in 30% (v/v) H_2O_2 (0.5ml) containing 1 drop of ammonia at 50°C for 6h. Water (500μl), 4M-HCl (200μl) and 2% (w/v) butyl-PBD [5-(4-biphenylyl)-2-(4-t-butylphenyl-1-oxa-3,4-diazole] in toluene/Triton X-100 (2:1, v/v; 15ml) were added and the samples counted for radioactivity in a Philips liquid-scintillation analyser.

Results and Discussion

Periodate/[^{3}H]borohydride labelling

Under mild conditions, sequential treatment with $NaIO_4$ and reduction with NaB^3H_4 selectively modifies the *N*-acetylneuraminic acid residues of glycoproteins (Van Lenten & Ashwell, 1971). We have extended this approach, as have others (Novogrodsky & Katchalski, 1972; Liao *et al.*, 1973; Critchley *et al.*, 1976), to provide a specific cell-surface marker for rat fibroblasts. Because the labelling reagents are of low molecular weight, it cannot necessarily be assumed that the plasma membrane is impermeable to them and, in addition, significant incorporation into membrane lipids (30%) has also been observed (Liao *et al.*, 1973). However, by labelling growing fibroblasts in monolayer culture rather than membrane fractions and by using low-temperature incubations, we found that chloroform/methanol-extractable radioactivity can be substantially decreased (to $\leq$18%). As shown in Table 1, most of the radioactivity is then incorporated into proteolytic-sensitive surface glycoproteins. Thus short treatment with crystalline trypsin removes 72% of the chloroform/methanol-resistant radioactivity and a further 12% can be removed by subsequent mild treatment with Pronase. In agreement with previous studies (Liao *et al.*, 1973) exposure of control cell cultures to reducing agent without prior oxidation with $NaIO_4$ resulted in negligible (1.6%) incorporation of radioactivity. Electrokinetic (Cook *et al.*, 1960) and structural (Winzler *et al.*, 1967; Langley & Ambrose, 1964) studies have established that not all surface carbohydrate is released by proteolytic digestion and we likewise suggest that the remaining 16% of radioactivity is in residual sialoglycoproteins or gangliosides. After radiolabelling and trypsin treatment, replated cells attach and grow normally, thus

Table 1. *Susceptibility of the radioactive surface marker to proteolytic degradation*

Cells were labelled in monolayer cultures by using the periodate/[^{3}H]borohydride procedure and, after detachment with a 'rubber policeman', treated with the enzymes (100μg/ml) in suspension for 20min at room temperature. After lysis with 5% NaCl, cell protein was precipitated with cold 10% (w/v) trichloroacetic acid and washed 3 times with cold 5% (v/v) acetic acid. The precipitate was extracted with chloroform/methanol (2:1, v/v; three extensive washes) and finally dissolved in a small volume 0.5ml) of 0.5M-NaOH by boiling for 1h. Protein determinations and the measurement of radioactivity were as described in the Experimental section.

Treatment	Radioactivity (nCi/mg of protein)	(%)
Periodate/[^{3}H]borohydride	84.4	100
Periodate/[^{3}H]borohydride+trypsin	24.0	28.4
Periodate/[^{3}H]borohydride+trypsin followed by Pronase	13.5	16.0
[^{3}H]Borohydride (no periodate)	1.3	1.6

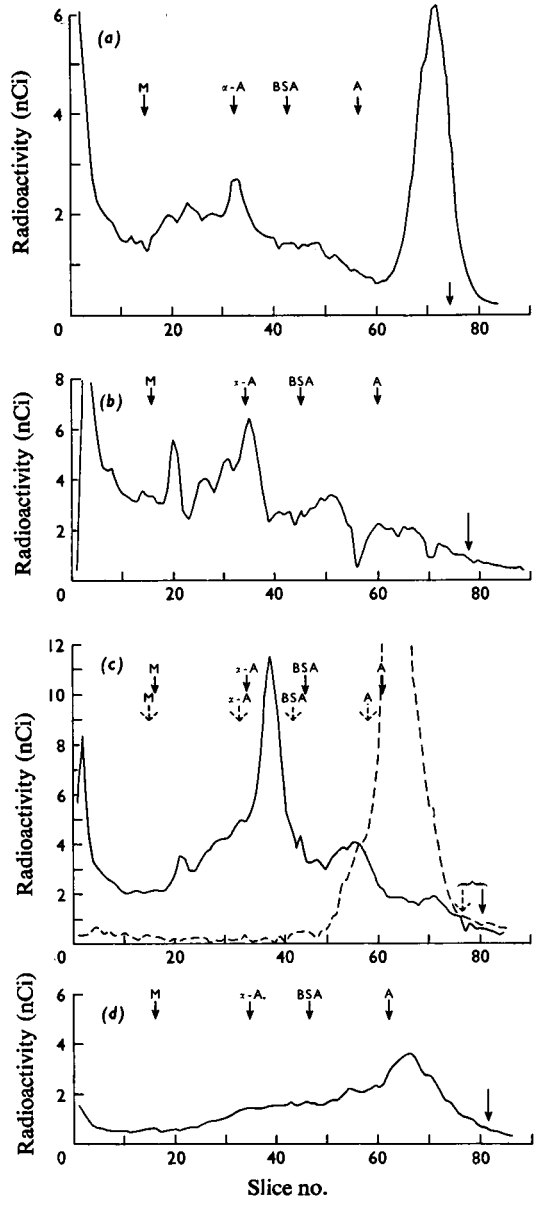


Fig. 1. *Radiolabelled preparations from 16C cultured fibroblasts*

Sodium dodecyl sulphate/polyacrylamide-gel (5%, w/v) electrophoresis of proteins of: (*a*) cells metabolically labelled with [^{3}H]glucosamine; (*b*) membrane preparations isolated by our method after periodate oxidation of attached cells followed by [^{3}H]borohydride reduction in suspension; (*c*) untreated (——) or mild-acid-hydrolysed (----) whole-cell preparations when both oxidation and reduction were on the attached cells; (*d*) whole-cell preparation labelled as in (*c*) and subsequently treated with trypsin (10 μg/ml; 4min; 22°C). All cultures were labelled when dense. The gels were loaded with equal radioactivity and they are lined up on the border between stacking and running gels (slice 0). Slice size was 1mm. Electrophoresis was from left (−) to right (+). Molecular-weight markers are: M, myosin (200000); α-A, α-actinin (90000); BSA, bovine serum albumin (68000); A, actin (43000). Arrow indicates position of marker dye (Bromophenol Blue).

confirming that our treatments do not significantly affect the viability of cultured fibroblasts.

Analysis of solubilized preparations by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis confirms the specificity of the present labelling procedure. Gel radioactivity profiles (Fig. 1) indicate that the efficiency of reduction of periodate-treated cells with NaB^3H_4 is markedly dependent on the conditions. Reduction of cells in suspension, after preoxidation in monolayer culture, gives a complex electrophoresis pattern where the radioactive distribution (but not the peak size) corresponds closely to that observed when cellular glycoproteins are metabolically labelled by using a short pulse of [^{3}H]-glucosamine. In contrast, when both oxidation and reduction steps are on the monolayer, the gel shows a significantly altered pattern, with a major peak of radioactivity at an apparent molecular weight of 70000–80000 for both whole cells and membranes derived from them (Figs. 1 and 2). Thus the accessibility of membrane macromolecules is strongly dependent on the cell state, and the major radioactive peak represents the most accessible glycoprotein(s) when cells are adhering and growing on an inert substratum. Trypsin treatment of cells prelabelled in monolayer (Fig. 1) confirms the susceptibility of incorporated radioactivity to proteolysis; the major peaks are almost completely removed by mild treatment with the enzyme (10μg/ml; 4min; 22°C). That the label is overwhelmingly incorporated into analogues of sialic acid can also be demonstrated electrophoretically (Fig. 1), since mild acid hydrolysis (0.1M; 80°C; 1h) of solubilized preparations almost completely removes the label from (glyco)protein acceptors under conditions specific for removal of sialic acid residues (Blix *et al.*, 1956). The residual peak running close to the marker dye is absent when the non-diffusible material is rerun after dialysis and can be attributed to the released analogues of sialic acid. Taken together, the evidence supports our use of this labelling procedure applied to monolayers of rat fibroblasts as a specific marker for plasma membranes.

Preparation of plasma membranes

Suspension of cultured cells in hypo-osmotic borate/EDTA can be readily seen by phase-contrast microscopy to cause controlled cell swelling and plasma-membrane lysis. Cytoplasmic contents are expelled and gelatinized and large empty membrane sacs are formed. Warley & Cook (1973) established that McCollester's (1970) partially purified preparations could be substantially improved by using a glass-bead column (Warren *et al.*, 1966). In our hands, however, lack of suitable glass beads and difficulties in the scaling-up and reproducibility of this procedure led to our development of alternative steps for isolating plasma-membrane-rich material. By using radiolabelling, optimal conditions were determined for further purification of McCollester's (1970) starting material. Cells are harvested and extracted at room temperature and subsequent steps are at 2°C. The distribution and yield of plasma membranes, monitored by using the radioisotopic marker to follow our final isolation procedure, is given in Table 2. Our investigation showed that a freshly prepared borate solution, pH10.2, was most reproducible in precipitating the cell contents. After the removal of gelatinous contaminants by filtration, a low-speed centrifugation (450*g*, 10min) is necessary to remove cell nuclei, with any remaining precipitate. About 40% of the label is precipitated with the gelatinized material and is removed by these combined operations. Microscopy indicated that this represented unlysed cells and entrapped membrane, and since preliminary experiments suggested that purification of this fraction to increase our overall yield would require extended centrifugation procedures, it was not characterized further. Subsequent centrifugation (12000*g*, 3min) of the supernatant isolates an additional 30% of the radioactivity, and this yield cannot be enhanced even by very-high-speed centrifugation (80000*g*, 30min). The remaining 30% of the surface marker is therefore in very light material, which also cannot be isolated by the simple preparations described here. The lightly packed membrane

Table 2. *Distribution and yield of plasma membranes during the isolation procedure, as monitored by the radioactive surface marker*

Samples were taken at each stage of the preparation. An equal volume of 1M-NaOH was added and the samples were dissolved by boiling at 100°C for 1h. Radioactivity was counted as described in the Experimental section and the total radioactivity in each fraction calculated. The results refer to the optimal isolation conditions described in the text.

Centrifugation step	Expt. 1 (nCi)	Expt. 2 (nCi)	Radioactivity (% of total)
Extraction mixture	651	1174	100
450*g* pellet and filtration	286	677	42–44
12000*g* pellet	195	333	28–30
Phosphate-buffered saline/35% sucrose interface	—	333	28

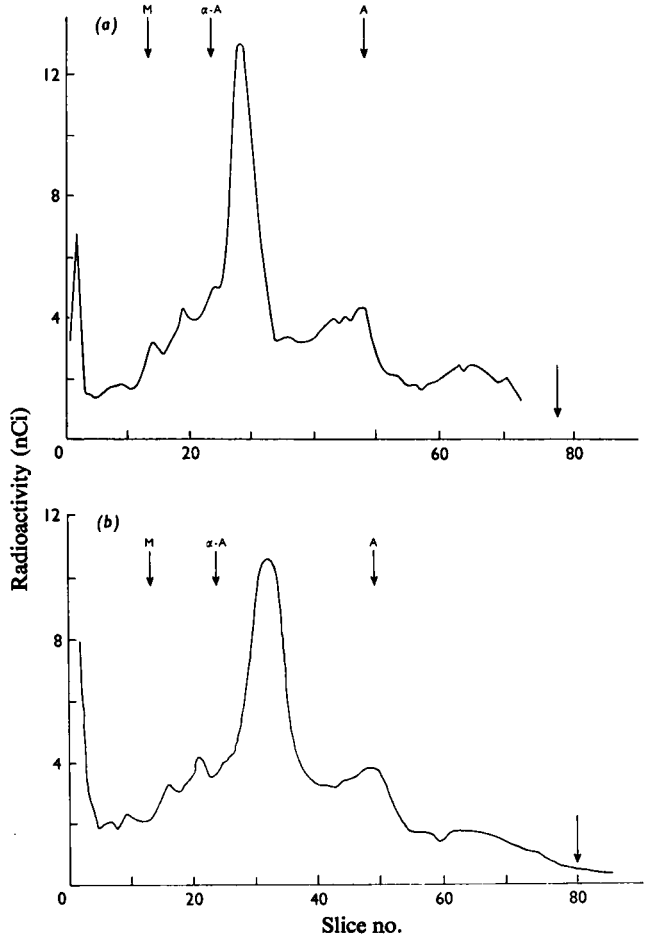
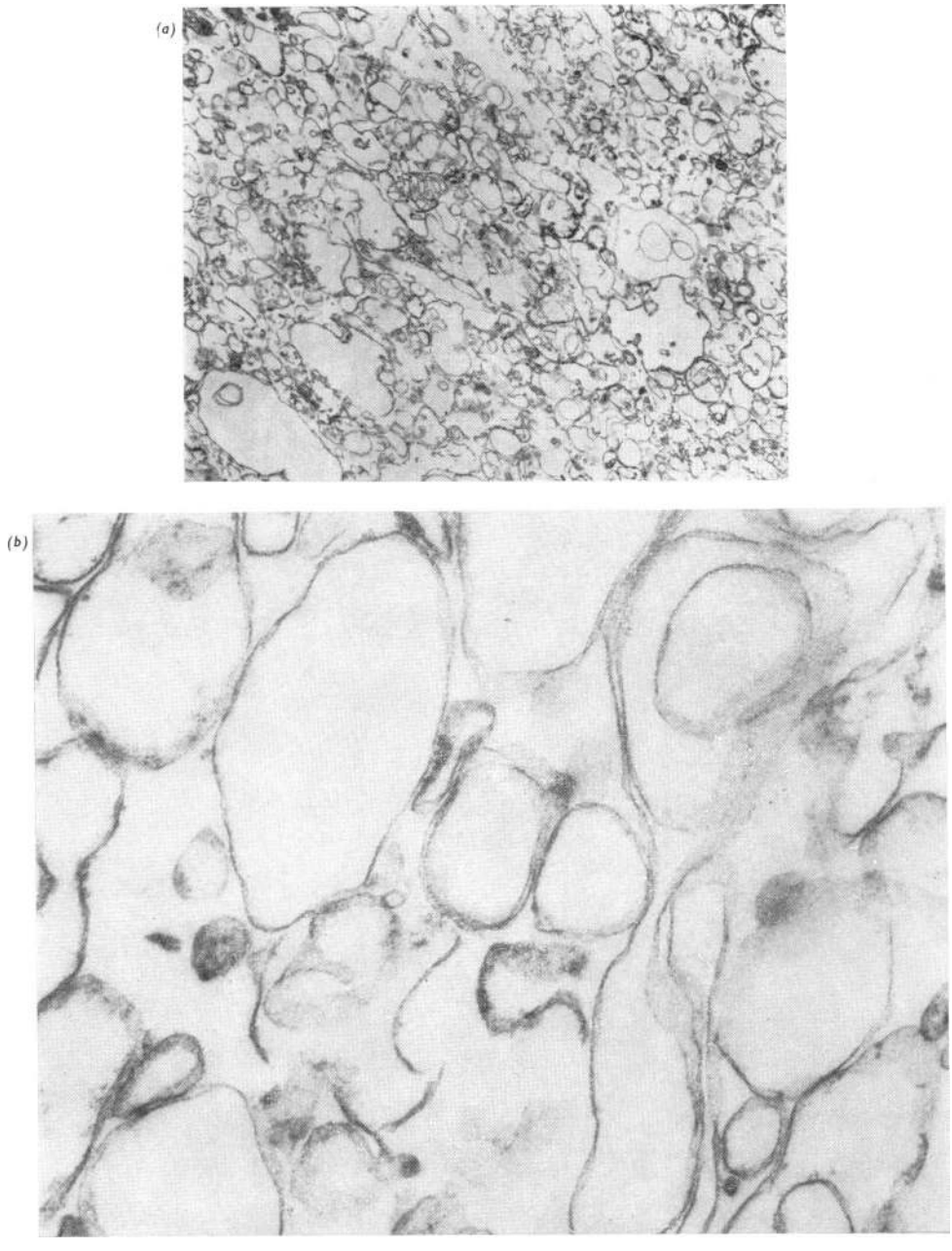


Fig. 2. *Representative nature of the isolated plasma membranes*
Comparison by sodium dodecyl sulphate/polyacrylamide-gel (5%, w/v) electrophoresis of (*a*) whole (16C) fibroblast preparations and (*b*) membrane fractions isolated from them by our preparative method. Minor variations can be attributed to slight differences in migration, as monitored by the marker dye (Bromophenol Blue), and in gel slicing (slice size 1 mm). Gels were loaded with equal radioactivity and are lined up on the border of stacking and running gels. Electrophoresis and molecular weight markers are as described in Fig. 1.

material is resuspended by gentle stirring in phosphate-buffered saline. After application to a discontinuous gradient of 55, 50, 45, 40, 35% (w/w) sucrose in phosphate-buffered saline, centrifugation (24000*g*, 1 h) gives quantitative recovery of fluffy white membrane-enriched material at the phosphate-buffered saline/sucrose interface, and the routine use of the gradient is unnecessary. Recovery of the surface label is quantitative in this step, giving an overall yield of about 30% of the total plasma-membrane radioactivity.

Electron micrographs of thin sections of the membrane pellets produced from cultured fibroblasts showed large membrane vesicles, as illustrated in Plate 1, with little sign of contamination by cytoplasmic organelles. Recent evidence (G. M. W. Cook, personal communication) indicates that the vesicles may be derived from large membrane sheets or 'ghosts' during their resuspension and application to the sucrose barrier. Analysis by gel electrophoresis confirms the representative nature of the isolated plasma membranes. As shown in Fig. 2, when the radioactive profiles of gels of intact cells are compared with those from membrane preparations derived from these cells, the two patterns can be completely superimposed.



EXPLANATION OF PLATE 1

Electron microscopy of isolated membranes

Typical fields of thin sections of the plasma-membrane fraction purified by using the final isolation procedure described in the text. The material consists of large membrane vesicles with no evidence of contaminating organelles: (*a*) low magnification (×6600); (*b*) high magnification (×53000).

Table 3. *Enzyme activities in membrane preparations and cell lysates*

The results from two preparations are shown. Specific activities are quoted as μmol/h per mg and total activities as μmol/h. Relative specific activity is $\frac{\text{membrane activity}}{\text{lysate activity}}$. % recovery is $\frac{\text{membrane total activity}}{\text{lysate total activity}} \times 100$. Preparation II is a duplicate of preparation I.

	Preparation I				Preparation II			
	Sp. activity	Total activity	Relative sp. activity	Recovery (%)	Sp. activity	Total activity	Relative sp. activity	Recovery (%)
$(Na^+ + K^+)$-ATPase								
Lysate	0.76	28.3	1.81	10.4	0.40	29.7	3.12	14.3
Membrane	1.37	2.94			1.26	4.26		
Succinate dehydrogenase								
Lysate	0.45	16.98	0.19	1.08	0.80	59.76	0.17	0.77
Membrane	0.086	0.18			0.14	0.48		
5′-Nucleotidase								
Lysate	204	7626	0.86	4.9	110	8250	1.36	6.1
Membrane	175	374			149	504		
NADH oxidoreductase								
Lysate	0.992	2.22	0	0	0.51	2.28	0	0
Membrane	0	0			0	0		

Enzymic and chemical analysis of membrane preparations

The recovery and specific activities of recognized enzymic markers and the chemical composition of the plasma-membrane-enriched material are illustrated in Tables 3 and 4 respectively. No $NADH_2$–lipoamide oxidoreductase activity, which is considered (Ferber *et al.*, 1972) to be a reliable marker for endoplasmic reticulum, could be demonstrated in the membrane preparations. This is particularly significant, since smooth endoplasmic reticulum is the most common contaminant (Brunette & Till, 1971; Warley & Cook, 1973) of most other plasma-membrane preparations. Our purification also removes most (99%) of the activity of the mitochondrial marker, succinate dehydrogenase, and gives a 5-fold decrease in its specific activity. The limited residual contamination may arise from co-sedimentation of mitochondria with the membrane fraction during differential centrifugation (Chang *et al.*, 1975), although electron microscopy shows no intact mitochondria in the final preparations. The activities of 5′-nucleotidase and $(Na^+ + K^+)$-ATPase were examined as potential markers for plasma membrane. With 5′-nucleotidase, our preparations show little concentration of the enzyme in the membrane fraction, thus confirming, as others have suggested (Gahmberg & Simons, 1970; Warley & Cook, 1973), that this enzyme is a poor marker for plasma membranes from tissue-culture cells. The 2–3-fold increase in the relative specific activity of the $(Na^+ + K^+)$-ATPase compared with the whole-cell lysate is consistent with, although marginally lower than, previous observations with fibroblast cell lines (Perdue & Sneider, 1970; Brunette & Till, 1971; Juliano & Gagalang, 1975). A further indication that these markers fail to represent the degree of purification is that the yield estimated by the radioactive marker is 30% whereas the average recoveries of 5′-nucleotidase and $(Na^+ + K^+)$-ATPase are approx. 5% and 12.5% respectively.

Table 4. *Chemical composition of the isolated plasma membranes*

Cholesterol/protein (μg/mg)	171
Phospholipid/protein (μg/mg)	976
Cholesterol/phospholipid (molar ratio)	0.55

The chemical composition of the isolated plasma membranes (Table 4) is in very good agreement with previous observations (Renkonen *et al.*, 1972) and is well within the range expected for plasma membranes.

Conclusions

Periodate/[^{3}H]borohydride labelling of membrane glycoproteins has been optimized for cultured

fibroblasts. Low incorporation into fractions extractable with a lipid solvent, predominant incorporation into sialic acid analogues of proteolytic-sensitive glycoproteins and the superimposability of whole-cell and isolated-membrane gel-electrophoresis patterns all suggest that application of this procedure to fibroblast monolayers provides a specific marker for plasma membranes.

Using this radioactive marker we have developed a membrane-isolation method applicable to large-scale fibroblast cultures. Plasma-membrane-enriched material can be isolated in under 3h, and morphological, biochemical and radioactive evidence confirms the high yield, purity and representative nature of the preparations.

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