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ISOLATION AND CHARACTERIZATION OF THE PLASMA MEMBRANES OF CULTURED LYMPHOBLASTS FROM PATIENTS WITH CYSTIC FIBROSIS AND NORMAL INDIVIDUALS

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Summary

Plasma membranes were isolated from two cystic fibrosis lymphoblastoid cell lines and two age and sex-matched normal cell lines after controlled cell disruption. The preparations were enriched approx. 20-fold in plasma membrane markers and were essentially free of markers for other organelles except the Golgi-localized enzyme, galactosyltransferase. Sodium dodecyl sulphate polyacrylamide gel electrophoresis showed virtually identical polypeptide profiles after staining with Coomassie blue. Membranes from normal cells contained 0.49—0.67 μmol of total phospholipid/mg membrane protein and those from cystic fibrosis cells, 0.53—0.67 μmol. The cholesterol content was 0.35—0.41 μmol/mg protein for normal, and 0.43—0.49 μmol for cystic fibrosis cell membranes. The proportions of individual phospholipid classes and their fatty acid compositions as well as that of the total membrane lipid also did not differ significantly in the two cell types. The bulk lipid fluidity as reflected by the fluorescence polarization of β-parinaric acid and diphenylhexatriene was also not changed. The carbohydrate composition of cell surface glycopeptides released with papain was not consistently different with the normal and cystic fibrosis cells. The quantity of fucose in intact plasma membranes was also quantitated enzymatically and found to be very similar in both cell types.

Supplementary data to this article are deposited with, and can be obtained from, Elsevier/North-Holland Biomedical Press B.V., BBA Data Deposition, P.O. Box 1345, 1000 BH Amsterdam, The Netherlands. Reference should be made to No. BBA/DD/136/78725/598 (1980) 1—15. The supplementary information includes: The fatty acid composition (%) of phospholipid classes.

Abbreviations: GlcNAc, *N*-acetylglucosamine; GalNAc, *N*-acetylgalactosamine; SDS, sodium dodecyl sulphate.

Introduction

Primarily because of the elevation in Na^+ and Cl^- concentrations which is diagnostic of the disease [1,2] and changes in serous and mucus secretions of other exocrine glands [3,4], a defect in cell membrane function has long been suspected in cystic fibrosis [5]. There have also been reports of changes in cells from patients with the disease in other specific membrane functions including certain enzyme activities [6—8] and permeability to ions [9—12] and other solutes [5]. In order to attempt to determine what molecular changes in the cell surface membrane of cystic fibrosis cells might be responsible for these observations, we have purified and characterized plasma membranes of cultured cells. Some of the findings with fibroblast membranes have already been described [13,14]. The other type of cultured cell available for such studies is the lymphoblast. It has the major advantages that it does not senesce and that it can be grown to large quantities in suspension culture. There is evidence that it expresses a cystic fibrosis phenotype in that it secretes the ciliary dyskinesia factor into its growth medium [15]. This report describes the isolation of and an analysis of the major molecular constituents of plasma membranes from cultured lymphoblasts from patients with cystic fibrosis and age and sex-matched normal individuals. Comparative examinations of both the chemical composition [16,17] and fluidity [18] of membrane lipid and of the sugar composition of membrane glycoproteins [19] were stimulated by previous reports of changes in patient tissues or cultured fibroblasts. An abstract describing part of this work has already been published [20].

Materials

Ficol-Pague was obtained from Pharmacia Fine Chemicals. Fetal calf serum was purchased from microbiological associates. [^{14}C]AMP, [^{14}C]-UDPgalactose and ^{125}I were obtained from Amersham, U.K. 5′-AMP, 4-methylumbelliferyl phosphate, 4-methylumbelliferyl-2-acetamido-2-deoxy-β-D-glucopyranoside, *p*-iodonitrotetrazolium violet, wheat germ agglutinin and papain were purchased from Sigma. UDPgalactose was obtained from Calbiochem and fluorescamine from Roche Diagnostics. Diphenylhexatriene was obtained from Eastman and β-parinaric acid from Molecular Probes. Phosphate-buffered saline consisted of 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.3 mM KH_2PO_4, 0.9 mM $CaCl_2$ and 0.3 mM $MgCl_2$. Desialyldegalactosyl orosomucoid and fucose dehydrogenase were kindly provided by Dr. H. Schachter. Pig muscle actin was kindly provided by Dr. Brian Barber.

Methods

Cell culture. Permanent lymphoblast cell lines were established according to the method of Glade and Broder [21]. Peripheral blood lymphocytes were isolated on a Ficol gradient and transformed with a cell lysate from previously transformed cell lines. The cell lysate containing Epstein-Barr virus was prepared by freezing and thawing ten times. Once established, all cell lines were maintained on a minimal essential (Eagle) modified medium supplemented

with 15% fetal calf serum according to Stanners et al. [22]. The cells to be transformed were treated with a lysate from cells of the opposite sex so that karyotyping could be used to test for effectiveness of the transformation. The cells used for isolation of membranes were grown from frozen stocks and subcultured every 2 days by 1 : 1 dilution with fresh growth medium. The stock cultures were maintained without antibiotics, but large quantities of cells necessary for membrane isolation were grown in the presence of penicillin and streptomycin. All cell lines were routinely screened for mycoplasma contamination. The normal cell lines were numbers 93 and 50. The age and sex-matched cystic fibrosis lines were 115 and 135, respectively.

Cell disruption. Cells were pelleted at $400 \times g$ and washed five times by repeated resuspension in phosphate-buffered saline and centrifugation at $400 \times g$. The cell pellet was resuspended in phosphate-buffered saline to a concentration of $5 \cdot 10^7$ cells/ml and disrupted using a Stansted cell-disrupting pump, Model A0612 with No. 716 disrupting valve, Stansted Fluid Power Ltd., Essex, U.K. Various disrupting pressures were tested and a value of 100 lb/inch^2 arrived at, which released only about 10% of the DNA from the nuclei. Under these conditions, 40—60% of the cells remained undisrupted and approx. 5% were still viable.

Plasma membrane isolation. Plasma membranes were isolated essentially according to Crumpton and Snary [23]. The homogenate was centrifuged at $400 \times g$ for 10 min; the nuclear supernatant was centrifuged at $4000 \times g$ for 15 min; the resulting mitochondrial supernatant was centrifuged at $20\,000 \times g$ for 30 min and the microsomal pellet was suspended in 36% sucrose/10 mM Tris-HCl buffer (pH 7.5); overlaid with 25% sucrose/Tris-HCl buffer and centrifuged at 25 000 rev./min in a SW 25.1 rotor for 15 h. The interfacial band was collected, diluted 4-fold with 10 mM Tris-HCl and pelleted at 25 000 rev./min in a SW 25.2 rotor for 2 h. The pellet containing the plasma membrane was homogenized in 1 mM $NaHCO_3$ (pH 7.5) and pelleted. This washing step was repeated once more. Samples for electron microscopy were taken before and after this hypotonic wash.

Electron microscopy. Small portions of freshly prepared membrane pellets were fixed in 2.7% glutaraldehyde in 0.07 M NaH_2PO_4 (pH 7.4). After rinsing in the same buffer the fractions were postfixed with 1% uranyl acetate in 25% ethanol, dehydrated, embedded and sectioned. The sections were viewed in a Phillips EM300.

Enzyme assays. 5'-Nucleotidase was assayed at 37°C in a solution containing 0.05 M Tris-HCl (pH 7.5), 10 mM $MgCl_2$ and 5 mM 5'-AMP. Tracer amounts of [8-^{14}C]AMP permitted the conversion of AMP to be followed radiochemically according to Suran [24].

Alkaline phosphatase was assayed according to Wijcik et al. [14] with 4-methylumbelliferyl phosphate as a substrate. Fluorescence intensity was measured at 449 nm with excitation at 368 nm.

Succinate dehydrogenase was determined as described by Pennington [25] using *p*-iodonitrotetrazolium violet as substrate.

Hexosaminidase was assayed according to Robinson and Stirling [26] using 4-methylumbelliferyl-2-acetamido-2-deoxy-β-D-glucopyranoside as substrate. Fluorescence of the methylumbelliferone was measured as described above for alkaline phosphatase.

Galactosyltransferase was assayed according to Treloar et al. [27] using desialyldegalactosyl orosomucoid as acceptor. Radioactive glycoprotein was precipitated with 1% phosphotungstic acid in 0.5 N HCl according to Grimes [28], washed and counted.

Protein assay. Protein was measured by the fluorometric (fluorescamine) method of Bohlen et al. [29] or by the method of Lowry et al. [30] using bovine serum albumin as standard.

^{125}I labelling of wheat germ agglutinin. 100 μg of wheat germ agglutinin in 20 μl of water was mixed with 50 μl of phosphate-buffered saline containing 0.8 mCi of $Na^{125}I$. Iodination was achieved by the addition of two 10-μl aliquots of 2.5 mg/ml chloramine T at 1 min intervals. The reaction was terminated by the addition of 50 μl of 2.5 mg/ml sodium metabisulphate. The reaction mixture was then applied to a column (28 × 1.3 cm) of Sephadex G-25 and the ^{125}I-labelled wheat germ agglutinin obtained from the void volume fractions. The specific radioactivity of ^{125}I-labelled wheat germ agglutinin was approx. 1 Ci/g.

Extensively washed cells were labelled with ^{125}I-labelled wheat germ agglutinin prior to cell disruption. In a typical experiment, $1.6 \cdot 10^7$ dpm of ^{125}I-labelled wheat germ agglutinin was incubated with $5 \cdot 10^6$ cells in a total volume of 4 ml of phosphate-buffered saline and 0.35% bovine serum albumin. After 15 min at 4°C, cells were pelleted, washed three times to remove unbound ^{125}I-labelled wheat germ agglutinin and mixed with the bulk of cells to be used for membrane isolation. The cells bound 20—25% of the total radioactivity.

SDS-polyacrylamide gel electrophoresis. The polypeptide composition of plasma membranes was analysed on 12% slab gels according to Laemmli [31]. The gels were stained with Coomassie blue.

Lipid analysis. Lipids were extracted from isolated plasma membranes essentially according to Folch et al. [32] with 7 vols. of $CHCl_3/CH_3OH$ (2 : 1). Total phosphorus was determined in a portion of this extract by the method of Bartlett [33]. A second portion was used for separation of phospholipid classes by two-dimensional thin-layer chromatography according to Rouser et al. [34]. The individual phospholipid spots were scraped from the plate and the fatty acids as well as the total membrane fatty acids were determined by gas-liquid chromatography on 10% DEGS (stabilized) on W_1AE (100/200 mesh) at 185°C as described by Riordan et al. [35]. In addition to the total fatty acids, a third portion of the total lipid extract was used for analysis of cholesterol by gas-liquid chromatography on 3% QF-1 on W_1AW-DHCS (100/200 mes) according to van Hoeven and Emmelot [36] with cholestane as internal standard.

Surface carbohydrate analysis. Surface glycopeptides were cleaved from intact extensively washed viable cells with papain essentially according to Droege et al. [37]. $2 \cdot 10^9$ cells were resuspended in 5 ml phosphate-buffered saline and mixed with 0.5 ml papain solution containing 7.8 mg papain (11 units/mg), 0.1 ml of 0.5 M cysteine-HCl and 0.4 ml phosphate-buffered saline. Digestion was carried out at 37°C for 1 h with occasional shaking. The suspension was then centrifuged at 800 × *g* for 10 min, 0.35 ml of 12.5% trichloroacetic acid was added and kept on ice for 1 h. The trichloroacetic

acid precipitate formed was centrifuged for 10 min at 800 × *g*, the supernatant taken to pH 6.9 with NaOH and exhaustively dialysed using tubing which retained molecules of molecular weight greater than 3500. After dialyses, an aliquot of the supernatant was taken for protein assay and the rest was lyophilised and prepared for analysis by gas-liquid chromatography. Monosaccharides were analysed according to Zawetta et al. [38] as trifluoroacetate derivatives. This procedure entailed methanolysis with 0.5 N methanolic HCl (20 h at 80°C) and derivatisation of the methanolysed residue with trifluoroacetic acid anhydride (30 min at 80°C). The derivatives were analyzed on 5% (w/w) OV-210 on Varaport 30. Inositol was used as internal standard.

The fucose content of isolated plasma membranes was measured by the method of Finch et al. [39] using fucose dehydrogenase. The formation of NADH was monitored from the increase in absorbance at 340 nm. The enzyme reaction was carried out at 37°C for 90 min in a total volume of 0.5 ml of a solution containing 2 mM NAD^+; 100 mM Tris-HCl (pH 8.0); 0.1 mg L-fucose dehydrogenase; 0.20 nmol α-L-fucose or 250 mg of plasma membrane protein previously hydrolyzed with 0.1 N HCl at 100°C for 1 h and evaporated to dryness to remove HCl.

The details and applicability of this method to the analysis of small amounts of membrane-bound fucose will be presented elsewhere.

Fluorescence polarization Diphenylhexatriene was allowed to partition into the lipid phase of the plasma membranes according to Shinitzky and Inbar [40] and polarization of its fluorescence measured in an SLM System 4000 Scanning Polarization Spectrofluorometer with excitation at 357 nm and emission greater than 399 nm. β-Parinaric acid [41] was incorporated in the same manner in an argon atmosphere and fluorescence polarization determined with excitation at 321 nm and emission greater than 386 nm.

Results

Plasma membrane purification

Table I shows the distribution of markers of the plasma membrane and intracellular organelles after fractionation of normal lymphoblasts. The recovery of 2% of the total cell protein in the plasma membrane fraction was typical of a large number of preparations from cell lines originally derived from either normal individuals or patients with cystic fibrosis. The plasma membrane marker enzyme 5′-nucleotidase was enriched 21.7-fold in the plasma membrane fraction relative to the homogenate and 6.7% of the total activity was recovered. ^{125}I-labelled wheat germ agglutinin bound to the cell surface before disruption exhibited a 12-fold enhancement in specific binding activity in the plasma membrane compared to the homogenate. The enrichment of alkaline phosphatase was intermediate between the value for 5′-nucleotidase and ^{125}I-labelled wheat germ agglutinin at 15-fold.

The specific activity of succinate dehydrogenase which was utilized to detect mitochondrial contamination was reduced in the plasma membrane fraction by a factor of three relative to the homogenate. Only 0.1% of the total cellular amount of this enzyme was recovered with the plasma membrane. Hexosaminidase specific activity was less in the plasma membrane than in the

TABLE I

MARKERS OF SUBCELLULAR FRACTIONS

Assays were described in Methods. Protein values are in mg. Specific activities of 5'-nucleotidase, succinic dehydrogenase and NADH oxidoreductase are in units of μmol of substrate converted/mg protein per h, those of alkaline phosphatase and hexosaminidase in units of nmol 4-methylumbelliferone liberated/mg protein per h, and galactosyltransferase in units of dpm of UDP[^{14}C] Gal transferred/mg protein per h. Total activities of all enzymes are in their respective units transferred per h. ^{125}I-labelled wheat germ

	5'-Nucleotidase			^{125}I-WGA		Alkaline phosphatase	
	Protein	Total	Spec. act.	Total	Spec. act.	Total	Spec. act.
Homogenate	647	58.9	0.09	3 423 520	5 267	10 427	16.1
Nuclear pellet	114	10.5	0.09	1 105 236	10 532	5 376	47.3
Nuclear supernatant	432	47.2	0.11	1 979 736	4 897	10 647	24.3
Mitochondrial pellet	54	6.8	0.13	622 450	13 372	1 472	27.4
Mitochondrial supernatant	418	28.4	0.07	1 170 382	3 229	5 047	12.1
Microsomal pellet	58	20.0	0.35	878 442	15 362	1 558	27.1
Microsomal supernatant	284	6.9	0.04	400 796	2 147	3 896	20.9
Gradient pellet	14	4.1	0.30	134 438	9 897	340	25.1
Sucrose pellet	1	1.4	1.11	35 429	42 463	194	155.0
Plasma membrane	2	3.9	1.78	128 865	58 838	515	235.0

homogenate with only 0.3% of the total in the membrane fraction. Almost identical relative specific activity and recovery of the microsomal marker NADH oxidoreductase were determined. Galactosyltransferase which is localized at least primarily in the Golgi apparatus was in fact enriched 4.5-fold and 1.5% of the total activity was recovered in the plasma membrane fraction.

The purification of plasma membranes from normal and cystic fibrosis cells was compared utilizing 5'-nucleotidase and bound ^{125}I-labelled wheat germ agglutinin as markers and the results presented in Table II. The enrichments and recoveries were nearly the same for both cell types.

The morphological appearance of the plasma membrane fractions from normal and cystic fibrosis cells are shown in Fig. 1. When first obtained from the sucrose gradient and pelleted, the preparations consisted almost entirely of closed vesicles ranging from less than 0.1 μm to approx. 0.7 μm in diameter.

TABLE II

PURIFICATION AND RECOVERY OF PLASMA MEMBRANE MARKERS

5'-Nucleotidase was assayed and ^{125}I-labelled wheat germ agglutinin (^{125}I-WGA) measured as described in Methods. The specific activities of 5'-nucleotidase are in units of μmol P_i released/mg protein per h and total activities in μmol/h. ^{125}I-WGA specific activities are in dpm/mg protein and total activities in dpm. CF, cystic fibrosis cells.

	5'-Nucleotidase activity				^{125}I-WGA binding			
	Total	Spec. act.	% enrichment		Total	Spec. act.	% enrichment	
Normal homogenate	59	0.09	100	1.0	3 423 520	5 291	100	1.0
Plasma membrane	4	1.95	7	21.7	128 865	64 432	4	12.0
CF homogenate	67	0.05	100	1.0	441 830	305	100	1.0
Plasma membrane	4	0.90	6	18.0	16 535	3 307	4	11.0

agglutinin specific radioactivity is in cpm/mg protein and total radioactivity in cpm. The 'sucrose pellet' was obtained by further centrifuging the supernatant obtained from the 2 h spin of the gradient interface. This centrifugation was done at 25 000 rev./min for 6 h in a SW 25.1 rotor. The 'gradient pellet' is the material pelleted during the sucrose density centrifugation. ^{125}I-WGA, wheat germ agglutinin.

Succinate dehydrogenase		Hexosaminidase		NADH oxidoreductase		Galactosyltransferase	
Total	Spec. act.	Total	Spec. act.	Total	Spec. act.	Total	Spec. act.
3644	5.6	23 882	36.9	51.4	0.08	46.6	7 205
612	5.4	4 291	37.7	19.3	0.17	5.3	4 657
835	1.9	19 823	45.3	30.4	0.07	34.1	7 809
887	16.5	8 493	158.4	18.7	0.35	25.2	47 109
1362	3.3	12 868	30.8	13.1	0.03	24.9	5 962
131	2.3	4 113	71.5	7.0	0.12	9.1	15 764
1056	3.7	7 483	40.1	4.3	0.02	16.6	3 839
—	—	786	57.9	2.1	0.15	1.3	9 614
6	5.0	22	17.8	0.2	0.14	0.4	27 948
4	2.0	67	30.6	0.2	0.08	0.7	33 054

There is no obvious contamination with other identifiable organelles. Most vesicles contain poorly defined electron-opaque material. Much of this is lost after hypotonic washing. This treatment also results in the appearance of many flattened sacs, presumably due to collapse of vesicles. The product from cystic fibrosis cells did not appear different from that from normal cells.

SDS-polyacrylamide gel electrophoresis of plasma membrane proteins

Approx. 50 different protein-staining bands were resolved in membranes from two different matched pairs of cells (Fig. 2). Of these there are six areas of more intensely staining bands in the molecular weight ranges 102—112 000; 82—92 000; 58—66 000; 40—45 000; 29—31 000 and 25—27 000 (Fig. 2). Many other more faintly staining bands span the entire molecular weight range (approx. 15 000—200 000). None of these proteins are readily identified with the exception of the major component of group IV which comigrates with actin isolated from pig muscle. No consistent differences between members of the two pairs of normals and cystic fibrosis membranes could be discerned.

Membrane lipids

The total phospholipid and cholesterol contents of plasma membranes from normal and cystic fibrosis lymphoblasts are indicated in Table III. The amounts of phospholipid relative to membrane protein were approximately the same for both membranes whereas the cholesterol was approx. 25% higher in the cystic fibrosis membranes of one pair; it was nearly identical in the second pair of membranes. The proportions of each of the different phospholipid classes are expressed in Table IV. In both membranes the major phospholipid is phosphatidylcholine representing approx. 40% of the total. Phosphatidylethanolamine made up about 25% with the other classes contributing lesser amounts.

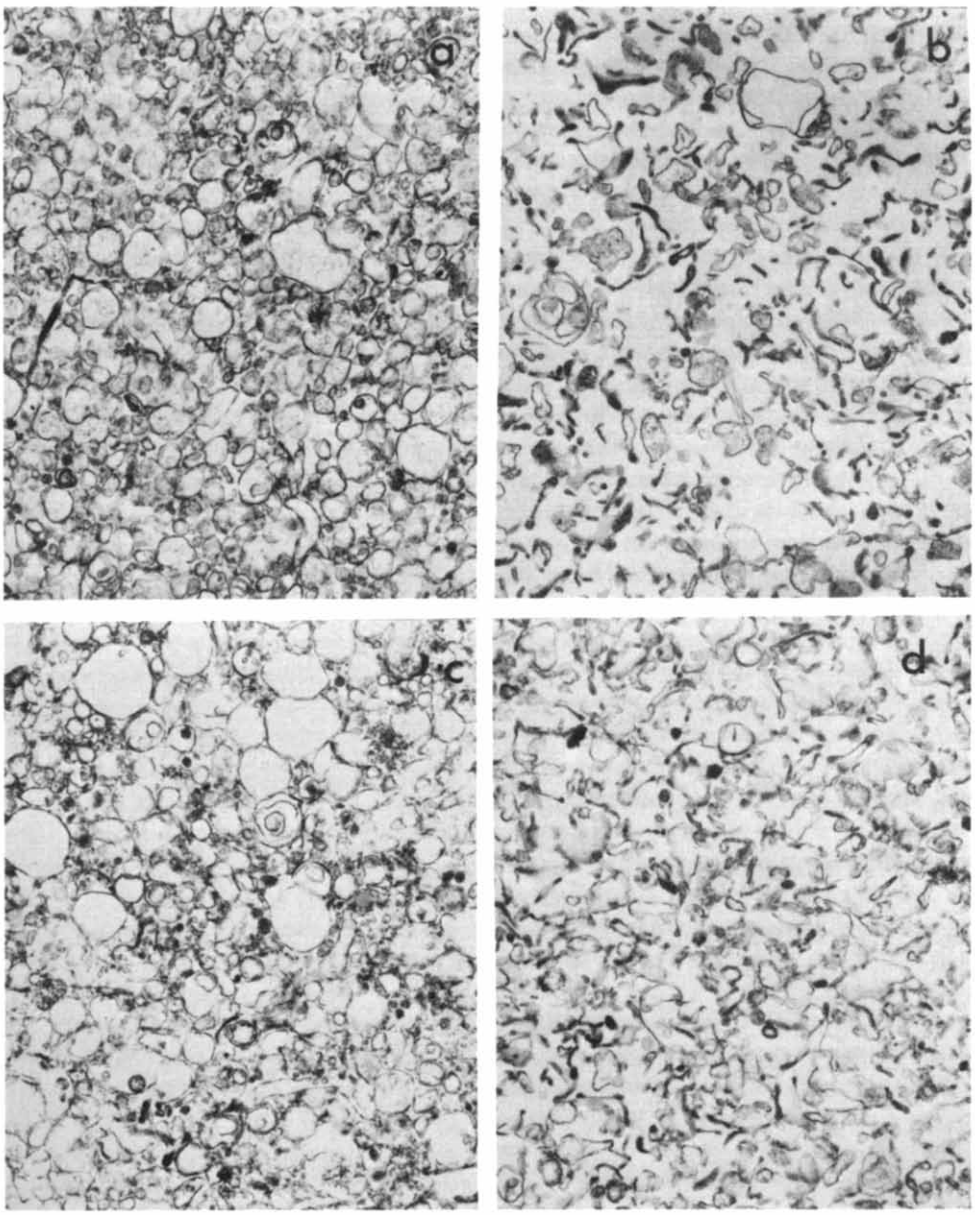

Fig. 1. Electron micrographs of thin sections through plasma membrane pellets before and after hypotonic washing. (a) Normal, before (magnification 29 040); (b) normal, After (magnification 29 040); (c) cystic fibrosis, before (magnification 29 040); (d) cystic fibrosis, after (magnification 32 670).

The fatty acid compositions of the total membrane lipid fraction as shown in Table V revealed absolutely no difference between the normals and cystic fibrosis cells. Oleate, stearate and palmitate comprise 70% of the total fatty acids. The predominant unsaturated acid is oleate with considerable amounts of arachidonic and nervonic acids as well.

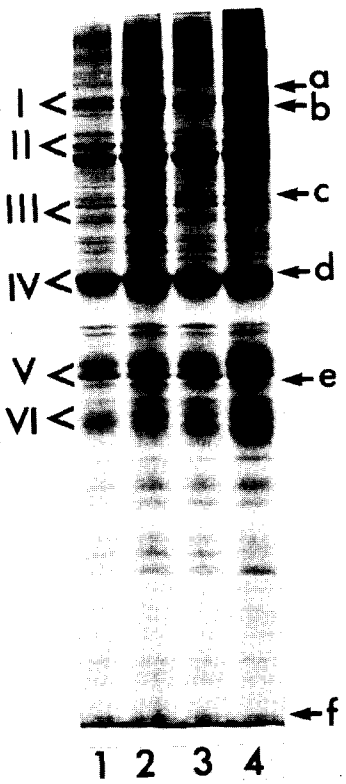

Fig. 2. SDS-polyacrylamide gel electrophoresis (Ref. 31; 12.5% acrylamide) of two normal and two cystic fibrosis lymphoblast lines. Gel 1, 88 μg CF line No. 135; gel 2, 141 μg normal line No. 50; gel 3, 105 μg CF line No. 115; gel 4, 175 μg normal line No. 93. Positions of the following standard proteins are indicated by arrows: a, β-galactosidase, 130 000; b, phosphorylase *a*, 94 000; c, bovine serum albumin, 68 000; d, pig muscle actin, 43 000; e, DNAase I, 31 000; f, lysosyme, 14 000. The six more intensely staining groups of bands have molecular weight ranges: I, 102—112 000; II, 82—92 000; III, 58—66 000; IV, 40—45 000; V, 29—31 000; VI, 25—27 000.

The fatty acid contents of each individual phospholipid class were determined and were tabulated (data bank). The normal and cystic fibrosis lines used in this experiment were 93 and 115, respectively. The most abundant phospholipid, phosphatidylcholine contains about 40% palmitate, 25% stearate and 25% oleate. It contains less than 5% arachidonate, myristate and palmitoleate and 1% of myristoleate, linoleate and 11-eicosenoic acid. Phosphatidylethanolamine differs markedly from phosphatidylcholine by its lower palmitate content (10%), increased stearate, increased arachidonate and the presence of higher carbon fatty acids, namely lignoceric acid and nervonic acid. Phosphatidylserine and sphingomyelin differ from each other in stearate content and in arachidonate content. There is a total absence of arachidonate in phosphatidylserine. Their high stearate content distinguishes these two phospholipids from the others. Myristate and myristoleate are increased in phos-

TABLE III

PHOSPHOLIPID AND CHOLESTEROL CONTENTS

Total phospholipids were quantitated on a portion of the lipid extract of the plasma membranes as inorganic phosphate. Cholesterol was analysed using gas-liquid chromatography. The means and S.D. of three determinations are presented for plasma membranes isolated from four cell lines.

	Normal		CF	
	Line 93	Line 50	Line 115	Line 135
Total phospholipid (μM/mg protein)	0.49 ± 0.01	0.67 ± 0.07	0.53 ± 0.03	0.67 ± 0.06
Cholesterol (μM/mg protein)	0.35 ± 0.04	0.41 ± 0.11	0.49 ± 0.02	0.43 ± 0.08
Cholesterol/phospholipid	0.71 ± 0.05	0.61 ± 0.18	0.92 ± 0.05	0.64 ± 0.14

TABLE IV

PHOSPHOLIPID COMPOSITION OF PLASMA MEMBRANES

Phospholipids in the total lipid extract were separated by two-dimensional TLC and quantitated by summation of the amounts of the different fatty acids which they contain. The data for cell line Nos. 50 and 135 are means of duplicate determinations with S.D.; data for line Nos. 93 and 115 are from single experiments. Data are in percent.

	Normal		CF	
	Line 50	Line 93	Line 135	Line 115
Phosphatidylcholine	48 ± 7	38	47 ± 4	40
Phosphatidylethanolamine	20 ± 3	26	20 ± 5	22
Phosphatidylserine	7 ± 1	7	8 ± 1	9
Sphingomyelin	4 ± 1	10	5 ± 1	10
Phosphatidylinositol	4 ± 1	4	4 ± 1	4
Lysophosphatidylcholine	5 ± 1	7	2 ± 1	6
Phosphatidic acid	4 ± 1	5	4 ± 0	5

TABLE V

FATTY ACID COMPOSITION OF TOTAL MEMBRANE LIPID

Quantitation was by GLC of methyl esters and the data for all four cell lines were derived from the same experiment. —, not detectable. Data are in percent.

Fatty acid	Normal		CF	
	Line 93	Line 50	Line 115	Line 135
14 : 0	1.0	1.0	1.0	1.0
14 : 1	0.4	0.4	0.5	0.4
16 : 0	17.0	16.0	18.0	18.0
16 : 1	2.5	3.0	2.2	2.0
18 : 0	19.0	24.0	20.0	21.0
18 : 1	32.0	30.0	29.0	32.0
18 : 2	1.2	1.0	1.1	1.0
20 : 1	0.7	0.8	0.3	0.3
20 : 2	0.3	0.8	0.3	—
20 : 4	9.3	8.4	8.4	8.2
24 : 0	4.4	3.4	4.5	3.8
24 : 1	7.6	6.5	8.4	7.9

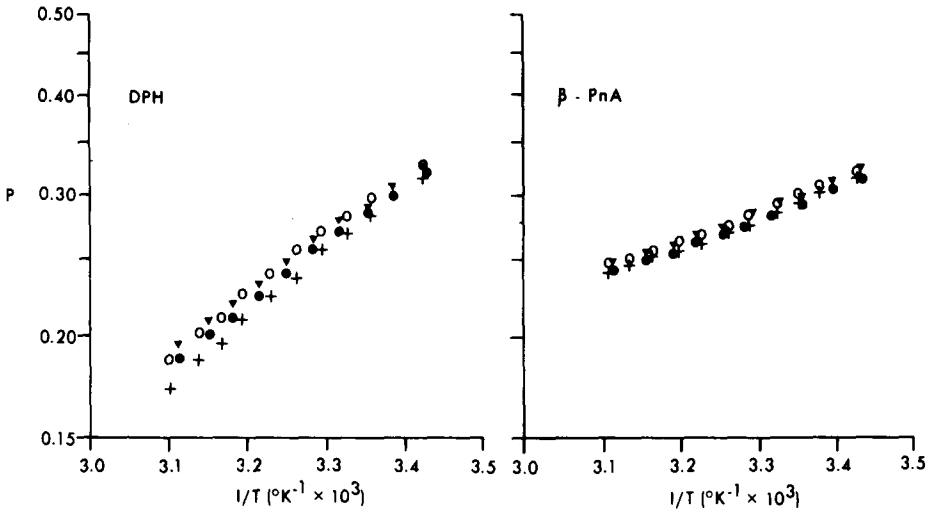

Fig. 3. Arrhenius plots of degree of fluorescence polarization (*P*) of diphenylhexatriene (DPH) and β-parinaric acid (β-PnA) in plasma membranes from normal (+, line No. 50; ▼, line No. 93) and cystic fibrosis (○, line No. 135; ●, line No. 115) cells measured at temperatures between 19°C and 49°C.

phatidylinositol, phosphatidic acid and lysophosphatidylcholine relative to the other phospholipids. The fatty acid composition of the latter three phospholipids is very similar with the exception of higher content of palmitate in phosphatidylinositol and the presence of 11-eicosenoic acid in lysophosphatidylcholine. This phospholipid differs from phosphatidylcholine by its higher palmitoleate and lower stearate content. In no case have consistent differences in fatty acid composition of phospholipids from plasma membranes of cystic fibrosis and normal control cells been found. Similar data were obtained with membranes from cystic fibrosis line No. 135 and normal control line No. 50.

As one index of the fluidity of the membrane lipid phase, the degree of fluorescence polarization of diphenylhexatriene and β-parinaric acid were determined between 19 and 49°C. The Arrhenius plots of the data from the four cell lines are shown in Fig. 3. With both probes nearly linear curves inversely relating polarization and temperature were obtained, the slopes being greater in the case of diphenylhexatriene. With membranes from one pair of cell lines (CF135 and N 50) the normals caused less polarization of the fluorescence of each of the probes. However, with the other pair (CF115 and N 93), the difference was much less and opposite in direction.

Cell surface carbohydrate

The monosaccharide compositions of glycopeptides cleaved from the cell surface membrane with papain are listed in Table VI. Those sugars commonly found in mammalian cell glycoproteins were present. The neutral sugars were fucose, galactose, mannose and glucose in the approximate ratios of 1 : 1 : 1 : 0.5. The amino sugars were *N*-acetylglucosamine and *N*-acetylgalactosamine in the proportions of about 2 : 1. Sialic acid is present in amounts approx. 30%

TABLE VI

CARBOHYDRATE COMPOSITION OF CELL SURFACE GLYCOPEPTIDES

Sugars were analysed by gas-liquid chromatography as trifluoroacetate derivatives following digestion of the cell surface with papain. Results represent means and S.D. from two or three determinations on each of the four cell lines. Results are expressed as nmol of sugar/mg of protein. The fucose values in parentheses were determined enzymatically using whole plasma membranes. 250 mg of plasma membrane protein was hydrolyzed with 0.1 N HCl at 100°C for 1 h, evaporated to dryness, the residue resuspended in 0.2 ml water, centrifuged at 12 000 × *g* and the supernatant assayed for fucose with fucose dehydrogenase. Results represent means and S.D. of two determinations. n.d., not determined.

Monosaccharide	Normal		CF	
	Line 93	Line 50	Line 115	Line 135
Fucose	19.1 ± 1.3	12.3 ± 1.0	15.8 ± 0.7	17.8 ± 1.4
	(21.5 ± 2.5)	(20.8 ± 2.5)	(17.1 ± 0.1)	(22.1 ± 1.9)
Gal	19.6 ± 0.5	16.3 ± 1.0	19.1 ± 1.2	18.0 ± 0.3
Man	13.6 ± 0.3	14.2 ± 1.2	16.0 ± 1.0	16.8 ± 1.3
GlcNAc	16.3 ± 1.1	17.8 ± 0.9	16.6 ± 0.4	17.3 ± 0.9
GalNAc	10.6 ± 2.0	6.5 ± 1.2	10.6 ± 1.8	8.0 ± 0.4
Sialic acid	25.2 ± 0.2	19.4 ± 1.4	27.9 ± 1.2	24.3 ± 0.6
Glc	n.d.	7.5 ± 1.8	8.6 ± 0.7	7.6 ± 0.7

greater than those of fucose, galactose or mannose. With the possible exception of sialic acid, the relative amounts of the individual sugars as well as the total carbohydrate/mg membrane protein exhibited no significant differences between the two pairs of normal and cystic fibrosis cells. There was slightly more sialic acid on each of the normal cell lines than on their cystic fibrosis counterparts.

Because some difficulties were encountered in obtaining reproducible fucose values due to interference by amino acids also eluting near the solvent front and because of other reports of altered fucose content in cystic fibrosis [50, 51,54,56], this sugar was determined by an independent assay. Table VI also lists the fucose contents of isolated plasma membranes measured using fucose dehydrogenase and shows that two normal and two cystic fibrosis cell lines contents were similar. In addition, the papain-released glycopeptides described above did not reveal any differences between normal and cystic fibrosis cells when assayed in this way.

Discussion

Because of the contention that compromised membrane function contributes to the pathology of cystic fibrosis [5,42], we have isolated and analysed plasma membranes from one type of cultured cell which does apparently express the cystic fibrosis phenotype [15]. Lymphoblast lines established by transformation with Epstein-Barr virus of peripheral blood lymphocytes from patients with cystic fibrosis and normal individuals of the same age and sex were grown in large quantities (approx. $2 \cdot 10^{10}$ cells per batch). Plasma membranes were isolated after controlled rupture of these cells according to Crumpton and Snary [23] using a Stansted Cell Disruptor. The preparation was

enriched approx. 20-fold in several plasma membrane markers, was relatively free of markers for other organelles except Golgi and appeared primarily vesicular by transmission electron microscopy. The osmotic properties of these vesicles have not yet been carefully tested but they are capable of ATP-dependent Ca^{2+} transport (Katz, S., unpublished data). No gross differences between membranes from cystic fibrosis and normal cells could be discerned on the basis of marker enzyme activities or morphology.

Resolution of the proteins contained in these membranes on the basis of their size using SDS-polyacrylamide gel electrophoresis showed essentially identical profiles for the matched pairs of cystic fibrosis and normal cells. This is consistent with the findings from similar analyses of the proteins of pairs of fibroblast membranes [43,44]. Of course, putative alterations in specific functional membrane proteins such as Ca^{2+}-ATPase, for example, would presumably not be major enough to be detected in this manner. However, if there are changes in membrane proteins in cystic fibrosis, it seems that they must be restricted to such functional molecules and are probably minor, possibly single amino acid substitutions. Alternatively, these changes may even be in the regulation of the activities of these proteins rather than in the molecules themselves.

Changes in membrane lipids have been reported in erythrocytes from patients with cystic fibrosis [17], reflecting primarily the decrease in linoleic acid also observed in serum [5], adipose tissue [6] and mucus-associated phosphatidylcholine [7]. Some investigators have attributed these observations to the general malabsorption by the gut [8], whereas, others have suggested a possible defect in fatty acid metabolism [49]. Therefore, it was of particular interest to examine the fatty acid composition of membrane lipids from cells containing the cystic fibrosis genotype but not subject to the secondary complications of the disease. However, in terms of both total membrane lipid fatty acids and those of individual phospholipid classes, no differences were found. The quantities of these phospholipids as well as of cholesterol also did not differ. These findings are in agreement with those which we have recently reported for membranes of normal and cystic fibrosis fibroblasts [35]. Despite this apparent lack of difference in the chemical composition of membrane lipids, a report of increased lipid fluidity in cystic fibrosis fibroblast membranes [18] prompted us to also assess this parameter of the lymphoblast membranes. Fluorescence polarization measurements using two lipid-soluble probes failed to reveal differences in bulk lipid fluidity. We have also found this to be the case with carefully purified fibroblast plasma membranes [35].

Changes in the carbohydrate composition of glycoproteins in cystic fibrosis have been reported since the analyses of mucoid fractions of duodenal secretions by Dische and coworkers [50,51]. This initial claim of an elevated ratio of L-fucose to sialic acid in some of these fractions in cystic fibrosis has not been consistently reproduced by other investigators [52,53]. However, more recent studies have continued to implicate alterations in the metabolism of complex carbohydrates in cystic fibrosis with recurrent reports of changes involving L-fucose. Two laboratories have presented some evidence of increased fucose in extracellular glycoproteins of serum [4] and fibroblast culture medium [5] in cystic fibrosis. Scanlin and Glick [19] have reported alterations

in the monosaccharide composition of glycopeptides from the fibroblast membrane surface in cystic fibrosis. Incorporation of L-[^{3}H]fucose and D-[^{14}C]-glucosamine into the plasma membrane of cultured fibroblasts [43] did not reveal any differences between normal and cystic fibrosis cell strains. We analysed the monosaccharide composition of glycopeptides cleaved from the surface of lymphoblasts with papain and did not detect consistent and reproducible differences between the normal and cystic fibrosis cells. Fucose was also determined enzymatically in whole membranes and the values obtained from normal and cystic fibrosis cells were similar.

In conclusion, it appears that in this study which in contrast to others integrates the analysis of all the main constituents of a cell membrane, there are no gross structural or compositional differences between those of normal and cystic fibrosis cells. Studies of the functional properties of these membranes are in progress.

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