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A COMPARISON OF THE PROPERTIES OF MEMBRANES ISOLATED FROM BOVINE SKIM MILK AND CREAM

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SUMMARY

Membrane material was isolated from skim milk and cream using the same samples of whole milk and similar purification techniques. The membranes from these two sources were characterised and compared by lipid, carbohydrate, enzymatic and electrophoretic analyses. The skim milk membranes contained higher levels of cholesterol, phospholipid and carbohydrate per mg of protein than the cream membranes. In general, the specific activities of the enzymes tested were also higher in the skim milk membranes, nucleotide pyrophosphatase, γ-glutamyltranspeptidase and sulphydryl oxidase being particularly active in these membranes. The major protein of the skim milk material had a molecular weight of approx. 85 000 and constituted 32 % of the total protein. This particular protein band was almost absent in the cream membranes (only 3 % of the total protein) where the major protein had a molecular weight of approx. 70 000 and constituted 34 % of the total protein. Glycoprotein bands were also located in both membrane preparations but the position of these bands did not correspond with the areas which were stained with the protein staining reagent Coomassie blue. The major glycoprotein in both skim milk and cream membranes had an apparent molecular weight of 115 000. The biochemical and compositional differences between these membranes in milk provide further evidence for the skim milk membranes being more closely related to secretory cell plasma membrane than to the cream membranes. The data also lend support to the hypothesis of Keenan et al. ((1970) J. Cell Biol. 44, 80) that the cream membrane undergoes morphological and structural changes while evolving from the plasma membrane of the secretory cell.

INTRODUCTION

The presence of membrane material in skim milk has been recognised for a number of years [1, 2], but only recently have attempts been made to purify and characterise this material [3, 4]. These skim milk membranes have been shown to be rich in membrane fragments, microvilli, and membrane bound vesicles [5]. Data obtained from lipid analysis, enzymic activity determination and density gradient experiments have supported the hypothesis that the membrane material is derived from the plasma

membranes or possibly the Golgi vesicles of the secretory cell [3]. Also, comparisons of the lipid composition of a number of membrane systems from milk and mammary gland show that skim milk membranes differ significantly from the milk fat globule membrane material, while showing considerable similarity to the plasma membrane of the secretory cell [4].

At present, it is difficult to make valid comparisons between the biochemical properties of skim milk and fat globule membranes because the data available in the literature have been obtained from the analysis of fractions isolated by a variety of techniques, and also from different samples of whole milk. In this present investigation, a number of biochemical properties of skim milk and cream membranes isolated by similar procedures from the same sample of whole milk have been studied. This has enabled a more direct comparison of their composition and properties and hence a better assessment can be made of their origins in the secretory cell of the mammary gland.

MATERIALS

p-Nitrophenyl phosphate (disodium salt), reduced glutathione, glucose 6-phosphate (disodium salt), ATP (disodium salt), and AMP were obtained from Calbiochem (Australia) Pty. Ltd. *N*-Acetylneuraminic acid (synthetic), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), L-γ-glutamyl-*p*-nitroanilide, and β-NADH were purchased from Sigma.

Protein standards for molecular weight determination on polyacrylamide gels were obtained as follows: bovine serum albumin (fatty acid poor), catalase (bovine liver, crystalline), ovalbumin (5×crystallized) and pepsin (porcine stomach mucosa, 3×crystallized) from Calbiochem, and trypsin (bovine pancreas, 2×crystallized) from Sigma. α-Lactalbumin (bovine) was a gift from Dr T. E. Barman, National Institute for Research in Dairying, Reading, U.K.

Lipid standards for thin-layer chromatography were purchased from Calbiochem (Australia) Pty. Ltd. All other materials were obtained from commercial sources and were used without further purification.

METHODS

Preparation of membrane material

Bulk raw uncooled milk was collected from a local dairy herd consisting mainly of animals of Friesian breed. Cream was separated by either centrifugation of the whole milk ($4000 \times g$, 10 min, 30–35 °C) followed by aspiration, or by separation in an Alfa-Laval separator. In both cases the skim milk obtained was centrifuged at $4000 \times g$ for 10 min at 5 °C in order to remove any remaining traces of fat. Cream was washed at least 4 times with 3 vol. of 10 mM Tris–HCl buffer (pH 7.5), containing 0.25 M sucrose and 0.15 M NaCl. The washed cream was suspended in distilled water to give a final fat concentration of about 35 %. After cooling to 10–12 °C, the cream was churned and the buttermilk recovered. The butter obtained was heated to 35 °C and the remaining membrane material was recovered by centrifugation and

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Abbreviation: DTNB, 5,5′-dithiobis(2-nitrobenzoic acid).

combined with the buttermilk. Free fat and unchurned fat globules were removed by centrifugation and aspiration. Membrane material was recovered by precipitation at 5 °C with $(NH_4)_2SO_4$ at 50 % satn. The precipitate was resuspended in 10 mM Tris–HCl buffer (pH 7.5) containing 90 mM KCl and dialysed exhaustively (100 vol.) against the same buffer. The dialysed 50 % $(NH_4)_2SO_4$ precipitate was centrifuged at 150 000 × *g* for 60 min at 5 °C, and the precipitate was resuspended in the above buffer with the aid of a Sorvall Omnimixer. This lipoprotein fraction was washed 3 times using the above buffer system and centrifugation conditions. The pellet obtained from the last washing was resuspended to give a protein concentration of approx. 10 mg/ml and this fraction was designated as the cream lipoproteins. In some instances, the $(NH_4)_2SO_4$ precipitation step was omitted, the lipoproteins being purified only by centrifugation and washing. Also, the lipoprotein could be purified by applying the dialysed 50 % $(NH_4)_2SO_4$ precipitate to a column (45 cm × 8 cm) of Sepharose 2B (Pharmacia) equilibrated in 10 mM Tris–HCl buffer (pH 7.5), containing 90 mM KCl. The lipoproteins were recovered from fractions which eluted at the void volume of the column and they were concentrated either by ultracentrifugation (150 000 × *g*, 60 min) or by ultrafiltration using a Diaflo XM-50 membrane (Amicon Corp., Lexington, Mass.).

Skim milk membrane material was prepared as follows. Rennet (0.1 ml per 100 ml of skim) was added to warm (30 °C) skim milk and after about 20 min whey could be removed by filtration of the cut curd through two layers of cheese cloth. The whey was clarified by centrifugation (4000 × *g*, 30 min) and the supernatant was precipitated at 5 °C with $(NH_4)_2SO_4$ at 50 % satn. The precipitate was recovered by centrifugation at 13 000 × *g* for 30 min at 5 °C, and resuspended with the aid of a Sorvall Omnimixer in 10 mM Tris–HCl buffer (pH 7.5) containing 90 mM KCl, and dialysed exhaustively (100 vol.) against the same buffer. Skim milk lipoproteins could be recovered from this dialysed $(NH_4)_2SO_4$ precipitate by either ultracentrifugation and washing or by Sepharose 2B chromatography as described for preparation of the cream lipoproteins. The membrane material was suspended in the above buffer at a protein concentration of approx. 10 mg/ml.

Enzyme assays

All assays were conducted under conditions which gave linear activity responses with respect to both protein concentration and time. Alkaline phosphatase was assayed at 30 °C and pH 10.2 using 5 mM *p*-nitrophenyl phosphate as substrate [6]. Acid phosphatase was determined at 37 °C and pH 5.0 using 1 mM *p*-nitrophenyl phosphate as substrate. The reaction was stopped with 2 M NaOH, and the *p*-nitrophenol released was estimated spectrophotometrically using an extinction coefficient of 18 300 at 400 nm [7]. For the above two enzymes, activity was expressed as μmoles of *p*-nitrophenol released/min per mg protein.

Sulphydryl oxidase activity was estimated by following the decrease in free sulphydryl groups in reduced glutathione (5 mM) in 100 mM Tris–HCl buffer (pH 7.5) containing 50 μM EDTA, using DTNB. The extinction coefficient of the thionitrobenzoate anion at 410 nm was taken to be 13 600 [8]. Activity was expressed as μmoles of reduced glutathione decomposed/min per mg of protein. γ-Glutamyl transpeptidase was assayed at 30 °C by the method of Orlowski and Meister [9] using 5 mM γ-glutamyl-*p*-nitroanilide as substrate in the presence of 10 mM $MgCl_2$.

The *p*-nitroaniline ($E = 8800$) released was monitored by recording the change in absorbance at 410 nm. Activity was expressed as μmoles of *p*-nitroaniline produced/min per mg of protein.

Assay of nucleotide pyrophosphatase was performed at 37 °C according to the procedure of Bachorik and Dietrich [10] using 1 mM NADH as substrate in the presence of 10 mM $MgCl_2$ in 100 mM 2-amino-2-methyl-1,3-propanediol–HCl buffer (pH 9). The production of NMNH from NADH was measured spectrophotometrically at 340 nm after oxidation of the remaining substrate using lactate dehydrogenase and sodium pyruvate. The number of μmoles of NADH decomposed/min per mg of protein was defined as 1 unit of activity. 5'-Nucleotidase activity at 37 °C using 5 mM AMP as substrate with 5 mM $MgCl_2$ in 50 mM Tris–HCl buffer pH 7.8 was determined essentially by the method of Huang and Keenan [11]. Mg^{2+}-ATPase was assayed at 37 °C using 2 mM ATP and 5 mM $MgCl_2$ in 100 mM Tris–HCl buffer (pH 7.5). The reaction was terminated with 5 % trichloroacetic acid. Inorganic pyrophosphatase activity was measured at 37 °C using 1 mM sodium pyrophosphate as substrate in the presence of 1 mM $MgCl_2$ and 20 mM Tris–HCl buffer (pH 8.0). The reaction was terminated with 10 % trichloroacetic acid. Glucose-6-phosphatase activity was determined at 37 °C using 5 mM glucose 6-phosphate as substrate in 50 mM Tris–HCl buffer (pH 7.5), and the reaction was terminated with 10 % trichloroacetic acid. In the assay procedures of the above enzymes which produce free phosphate the trichloroacetic acid filtrates were kept at approx. 5 °C before analysis for P_i by the method of Ames and Dubin [12].

Diaphorase (NADH–dichlorophenolindophenol oxidoreductase) was measured by the method of Edelock et al. [13] and activity was defined as the change in absorbance at 600 nm/min per mg of protein. The method for the determination of xanthine oxidase has been described in a previous publication [6].

Electrophoresis

Polyacrylamide-gel electrophoresis was performed according to the procedure of Weber and Osborn [14] using a 7.5 % gel with the normal amount of cross-linking reagent. Membrane fractions and standard proteins were treated with 1 % sodium dodecylsulphate and 1 % β-mercaptoethanol and heated for 2 min at 100 °C before electrophoresis. Protein bands were located by staining the gels for 3 h in 0.25 % Coomassie Blue in 40 % methanol–10 % acetic acid. Destaining was accomplished in 40 % acetone–10 % acetic acid for 1 h followed by washing overnight in 20 % acetone–10 % acetic acid and finally in 7 % acetic acid for about 2 days. Mobilities of protein bands were calculated relative to the marker dye bromophenol blue. Carbohydrate containing proteins were located using the periodate–Schiff method of Glossmann and Neville [15]. The molecular weights of the protein bands in the membranes were estimated from the relative mobilities of the following standards (molecular weight in parentheses): bovine serum albumin (68 000), catalase (60 000), ovalbumin (45 000), pepsin (35 000), trypsin (23 000), α-lactalbumin (14 600).

Extraction and analysis of lipids

Lipids were extracted from membrane fractions containing known amount of protein using the procedure of Folch et al. [16]. The lipid extracts were evaporated to dryness (under O_2-free nitrogen) in preweighed vials. The amount of material

recovered was used to determine the ratio of lipid to protein in the membrane. Digestion and total phosphorus content of the lipid extracts was determined by the method of Ames and Dubin [12]. Phospholipid content was calculated by multiplying the value for phosphorus by 25. In some instances phospholipid content was determined by ashing whole membrane suspensions using the above procedure [12]. Similar results were obtained with either method.

Neutral lipids were separated on Merck precoated silica gel plates (0.25 mm thickness) without prior activation using a solvent system of hexane–diethyl ether–acetic acid (40:60:1, by vol.). The spots were detected by chromic acid charring [13], and individual spots were identified by comparison with R_F values of standards. Cholesterol was quantitized by densitometry by comparing the peak area with the peak areas of known amounts of cholesterol run on the same plate and detected under the same conditions. Separation of polar lipids was carried out using a solvent system of chloroform–methanol–water (70:25:3, by vol.). Overall polar and lipid distribution was visualised using chromic acid charring, and individual spots were identified by comparison with R_F values of standards and by the use of specific spray reagents [13] (viz. ninhydrin spray for phosphatidylserine and ethanolamine; orcinol–H_2SO_4 spray for glycolipids).

Other analytical methods

Neutral sugars were determined in the membrane fractions using the anthrone reagent as described by Spiro [18]. Galactose was used as a standard. Sialic acid content of the membranes was determined after hydrolysis in 0.05 M H_2SO_4 for 60 min at 80 °C using the method of Warren [19] with *N*-acetylneuraminic acid as a standard.

Membranes, extracted with 20 vol. of chloroform–methanol (2:1, by vol.), were recovered by centrifugation and the residues were lyophilized. Protein was hydrolysed by the method of Roach and Gehrke [20] and the amino acids analysed by the gas–liquid chromatography procedure of Roach and Gehrke [21].

Protein was determined according to the procedure of Lowry et al. [22] using bovine serum albumin as a standard.

RESULTS AND DISCUSSION

The overall composition of membranes isolated from skim milk and cream is summarised in Table I. There was no significant difference in the values obtained for the various constituents whether the membrane material was recovered by ultracentrifugation and washing or by chromatography on Sepharose 2B. Consequently, results from both of these procedures were included to determine the mean values and standard deviations as shown in Table I. Skim milk and cream membranes had similar lipid–protein ratios; however, there was a distinct difference in the lipid class distribution between these membranes. Both phospholipid and cholesterol levels were higher in skim milk membranes by factors of 30 and 36 %, respectively. Total hexoses and sialic acid levels were also higher in the skim milk material. The results obtained for the levels of some of the constituents of the cream membranes listed in Table I (viz. total lipid and sialic acid) compare favourably with those obtained by other workers [22, 23]. However, phospholipid, cholesterol and neutral hexose levels showed considerable deviation from previously published data [25]. The reason for

TABLE I

COMPOSITION OF MEMBRANE MATERIAL FROM SKIM MILK AND CREAM

In all cases at least 4 different preparations were analysed and the data are expressed as the mean ± S.E. Values are expressed as mg/mg protein.

Constituent	Skim milk	Cream
Total lipid	1.01 ± 0.07	1.06 ± 0.11
Phospholipid	0.43 ± 0.03	0.33 ± 0.03
Cholesterol	0.110 ± 0.021	0.035 ± 0.012
Neutral hexose	0.154 ± 0.005	0.111 ± 0.025
Sialic acid	0.052 ± 0.014	0.019 ± 0.005

these differences is hard to ascertain but it is most likely related to such factors as animal breed used in the experiments, stage of lactation, health and nutrition of the animals, as well as to the membrane preparation procedures. Kobylka and Carraway [24] also noted that their milk fat globule membrane preparation differed in lipid composition from those reported by other workers. Very little information is available on the absolute composition of skim milk membranes; however, Plantz and Patton [3] have quoted cholesterol–phospholipid molar ratios in the range 0.3–0.6 for skim milk membranes isolated by density gradient centrifugation. The value obtained in this present investigation of 0.5 falls within this range.

The enzyme complement of the purified milk membranes is shown in Table II. The enzyme activities from the different preparations varied over a fairly narrow range

TABLE II

ENZYME LEVELS IN PURIFIED MILK MEMBRANES

Activities are quoted as units/mg as defined in the text. The values listed represent an average activity from 5 different preparations. The range in activities obtained in these different preparations is shown in brackets.

Enzyme	Skim milk membranes	Cream membranes	Ratio skim/cream
Alkaline phosphatase	0.56 (0.50–0.64)	0.32 (0.30–0.40)	1.6
Acid phosphatase	0.0058 (0.005–0.007)	0.0125 (0.01–0.014)	0.5
5′-Nucleotidase*	0.59 (0.56–0.68)	0.48 (0.38–0.54)	1.2
Mg^{2+}-ATPase*	0.023 (0.014–0.025)	0.007 (0.006–0.009)	3.3
Nucleotide pyrophosphatase	1.85 (1.7–1.96)	0.39 (0.32–0.44)	4.7
Inorganic pyrophosphatase	0.047 (0.042–0.053)	0.024 (0.0225–0.026)	2.0
Glucose-6-phosphatase*	0.008 (0.0071–0.0098)	0.003 (0.0025–0.0036)	2.7
Sulphydryl oxidase	0.70 (0.65–1.0)	0.27 (0.24–0.34)	2.7
γ-Glutamyl transpeptidase	3.8 (2.65–5.1)	0.65 (0.59–0.77)	5.8
Xanthine oxidase**,***	0.075	0.24	0.3
Diaphorase***	0.11	0.39	0.3

* Activity in μmoles of P_i liberated/min per mg of protein.

** Activity in μmoles of O_2 consumed/min per mg of protein.

*** No ranges are given because, for these enzymes, only two different membrane preparations were analysed.

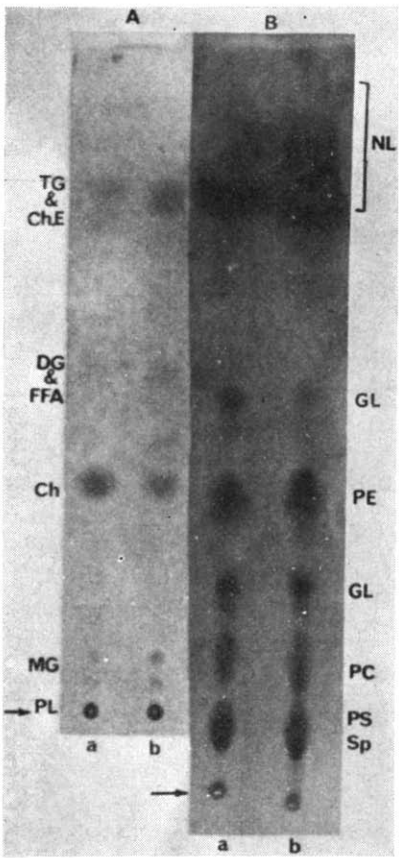
and, in general, activities of most enzymes were higher in the skim milk membranes. For example, skim milk membranes had considerably more nucleotide pyrophosphatase (4.7 times) and γ-glutamyl transpeptidase (5.8 times) activity than the cream membranes. However, diaphorase, acid phosphatase and xanthine oxidase were 2–4 times more highly concentrated in the cream membranes. The specific activities obtained for alkaline phosphatase, 5′-nucleotidase, and nucleotide pyrophosphatase were higher than those quoted by Plantz and Patton [3] for a skim milk membrane material prepared by ultracentrifugation. The preparation used by these authors though was most likely still contaminated with casein and whey proteins, so specific activities would be expected to be considerably less than a completely pure membrane preparation. Similar reasoning would apply to explain the results of Morton [2] who obtained similar results to Plantz and Patton [3] for alkaline phosphatase activities.

The levels found for 5′-nucleotidase and Mg^{2+}-ATPase in the cream membrane fraction compared favourably with the data of Huang and Keenan [11, 25] as does the glucose-6-phosphatase level with the value quoted by Dowben et al. [26]. The skim membrane preparation exhibited higher Mg^{2+}-ATPase activities than the membranes isolated from cream, a result not in accord with the finding of Plantz and Patton [3] who were unable to demonstrate ATPase activity in their preparation. The low activities found in these membranes towards some of the phosphate esters (viz. ATP, glucose 6-phosphate and pyrophosphate) may be a result of the action of other phosphatases of wide specificity. Inhibitor studies and specificity studies of purified enzymes isolated from these membranes would be necessary to determine the extent of this proposal. Some work along these lines has been already carried out by Huang and Keenan [11, 25].

Both membrane fractions contain significant levels of γ-glutamyl transpeptidase, an enzyme important in amino acid transport through some membranes [27]. Thus, the presence of this enzyme, together with other typical plasma membrane markers (5′-nucleotidase and alkaline phosphatase), helps to support the cellular membrane origins of the material being studied.

Fig. 1 shows photographs of thin-layer plates used to demonstrate qualitative differences in the neutral and polar lipids of the milk membranes. Skim milk lipids had observably more cholesterol than cream membrane lipids and this result was confirmed by quantitative densitometry (see Methods). The level of polar lipid classes did not show any significant differences between the two membrane samples. Plantz and Patton [3] have quantitated the phospholipid classes of skim milk membrane material and have shown its phospholipid composition is similar to that of milk fat globule membrane and mammary cell plasma membrane [28].

The sodium dodecylsulphate polyacrylamide-gel electrophoresis patterns obtained for the skim milk and cream membranes and their respective densitometer tracings are shown in Figs 2 and 3. The molecular weight of each major protein fraction and the relative abundance of each band is summarised in Table III. The patterns obtained for the cream membrane together with the estimated molecular weights are in good agreement with the data of other workers [23, 24, 29] who have studied the protein components of purified milk fat globule membranes. It may be noted from these results that neither the skim nor cream membranes were contaminated by any major milk proteins, since the relative mobilities of these milk proteins were found to be considerably higher than any of the bands found in the purified membrane samples.



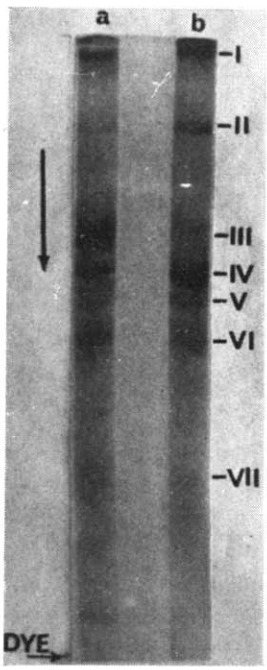


Fig. 1. Thin-layer chromatograms of lipid extracts of milk membranes. (A) Neutral lipids, 360 μg of lipid applied at origin (arrow). Ph, phospholipid; MG, monoglycerides; Ch, cholesterol; DG, diglycerides; FFA, free fatty acids; TG, triglycerides; ChE, cholesterol esters. (B) Polar lipids, 240 μg of lipid applied at origin (arrow). Sp, sphingomyelin; PS, phosphatidylserine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; GL, glycolipid; NL, neutral lipid. a, lipids from skim milk membrane; b, lipids from cream membranes.

Fig. 2. Polyacrylamide-gel electrophoresis patterns of (a) skim milk and (b) cream membrane material. Approx. 50 μg of protein was applied to each gel. Gels were stained and destained as described in Methods. Numerals to the right of the gels represent the major protein components of the membrane.

The results obtained show that the overall protein composition of each membrane was very similar, but certain quantitative differences in the concentration of individual components were apparent. Component III (mol. wt = 85 000) was the major band for the skim milk membranes (32 % of total) while the same band in the cream membrane represented only 3 % of the total protein. The major band of the cream membrane (Component IV, mol. wt = 70 000) was present in the skim milk preparation but it constituted only about 20 % of the total protein. This quantitative difference between the protein components of the two membranes was reproducibly observed to nearly the same extent with six different preparations.

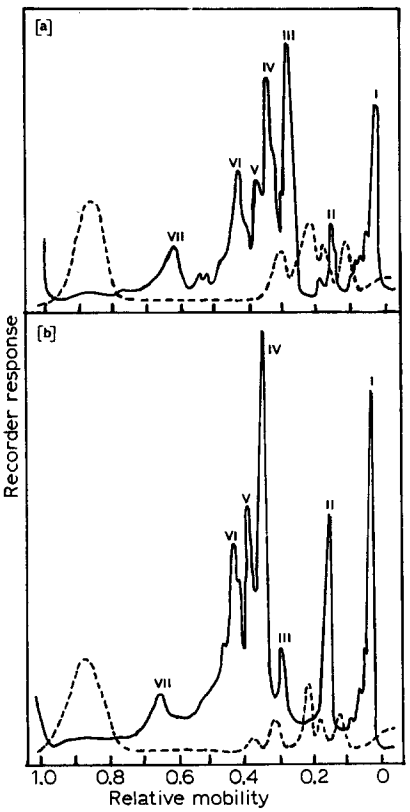


Fig. 3. Densitometer tracing of Coomassie Blue stained gels (—) and periodate–Schiff stained gels (- - -). Numbering of bands is the same as in Fig. 2.

TABLE III

MOLECULAR WEIGHTS ON MAJOR PROTEIN COMPONENTS IN PURIFIED MEMBRANES

Band No.	Mol. wt	Relative abundance* (%)	
		Skim-milk	Cream
I	200 000	10.7	16
II	132 000	3.3	11.7
III	85 000	32.5	3.1
IV	70 000	23.5	34
V	63 500	6.4	14.4
VI	52 500	11.8	11.8
VII	27 500	10	8.5

* This was determined by measuring the total area under the protein peaks from the densitometer tracing using a digital integrator and taking the area of each peak as a percentage of the total area.

The glycoprotein components in each membrane are shown as a densitometer tracing (dashed line) in Fig. 2. Essentially similar patterns were found in each case. However, the bands in the skim milk preparations gave higher colour yields than those from the cream membrane. This result was consistent with the chemical analysis (see Table I) which showed that there was more neutral hexose and sialic acid in the skim milk membranes. The position of most of the glycoprotein bands did not correspond exactly with any of the major protein components; this was confirmed by treating the periodate–Schiff-stained gels with 8-anilinonapthalene sulphonic acid and visualising under ultraviolet light (see [15]). The major glycoprotein band in each case had an estimated molecular weight of 115 000 under the conditions used. As glycoproteins behave anomalously on dodecylsulphate gels [24, 30], this molecular weight may not be its true value. Jackson et al. [31] have purified a glycoprotein from cream membranes and its molecular weight determined by ultracentrifugation was 123 000, a value in reasonable agreement with the above figure.

TABLE IV

AMINO ACID COMPOSITION OF SKIM MILK AND CREAM MEMBRANES

Values are expressed as mole% of total amino acids. Tryptophan and cysteine were not determined. The results quoted are an average of duplicate determinations.

Amino acid	Mole%	
	Skim membrane	Cream membrane
Ala	5.4	5.4
Val	6.5	6.3
Gly	4.8	4.8
Ile	4.7	4.9
Leu	9.7	9.8
Pro	6.1	5.7
Thr	6.5	6.5
Ser	8.1	8.2
Met	—	—
Phe	6.1	6.1
Asp	10.4	10.2
Glu	13.5	13.6
Tyr	3.4	3.0
Lys	6.7	6.8
His	1.8	1.4
Arg	6.0	6.1
Cys	0.7	0.1

The amino acid composition of the purified membranes is shown in Table IV. There was practically no difference in the overall amino acid composition of the membranes from the two sources studied. Both membrane preparations were characterised by high levels of leucine, aspartic acid and glutamic acid, and the values obtained for cream membranes were in good agreement with previously published data on purified fat globule membranes [24].

Overall, this investigation may be said to have established that purified skim milk membranes do differ compositionally with respect to the lipid, protein, and carbohydrate components, from their counterpart membranes in cream. Variation in analytical results has been minimised in the present studies by purifying these membranes from the same whole milk sample and using the same isolation procedure. Also, skim milk and cream membranes possessed different levels of a variety of enzymes. Solyon and Trams [32] have pointed out that no enzyme activity has been proven to be an exclusive marker of plasma membranes. Thus, identity of the milk membranes with mammary gland plasma membranes based on enzymic composition must be viewed with reservation until detailed enzymic analysis of purified mammary gland plasma membranes is carried out. However, the higher specific activities of certain more common plasma membrane enzymes in the material from skim milk, as compared to the material from cream, would indicate, either that the skim membranes had closer evolutionary links to the cellular plasma membrane than did the cream membranes, or that changes in enzyme activities in the cream membranes had occurred, possibly due to the environment from which they were isolated. As morphological and radioactive tracer data do not support the concept that the skim membranes arise by disintegration of the milk fat globule membrane material [33], it seems likely that the skim milk membrane represents material which is the precursor of the milk fat globule membrane and thus it represents the membrane material (true plasma membrane plus fused Golgi membrane) which accumulates at the apex of the secretory cell [34, 36]. This material is structurally rearranged [23, 28] while enveloping the emerging fat globule, so that distinct differences may occur in the composition and properties of this material and the isolated milk fat globule membrane. Also, at the same time, any excess cell membrane material is sloughed off, appearing apparently unaltered in the skim milk. A summary of the above concepts is shown in Fig. 4. It is possible, however, that even this skim milk membrane material has undergone small compositional changes compared to the material present in the secretory cell. If the secretory cell material represents a mixture of true plasma membrane and Golgi apparatus membrane, then it would be expected that the material in skim milk should contain typical Golgi enzyme markers such as galactosyl transferase. This enzyme occurs in a soluble form in milk [36] and its absence in skim milk membranes has been confirmed by Plantz et al. [3]. Galactosyl transferase is strongly bound to the

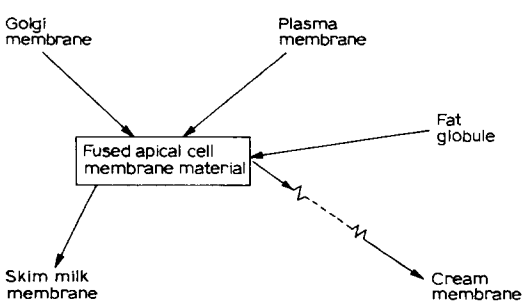


Fig. 4. Possible interrelationships of mammary gland and milk membranes. The break in the arrow between the fused membrane material and cream membrane represents the postulated morphological and structural changes which occur.

Golgi vesicles in the mammary gland [37–39], and as fairly extreme conditions will not release it from these membranes in vitro [40], it appears that some, as yet, unknown factors operate during milk secretion to release this enzyme in a soluble form in milk. The loss of this one integral protein from the cell membrane material during milk secretion would mean that the material finally appearing in skim milk would have a slightly different composition, as suggested above.

The relatively easy preparation of large amounts of this plasma membrane-like material in skim milk makes it an excellent source for preparation of a number of membrane bound enzymes and structural proteins, and the study of their characteristics and interactions. Work along these lines is presently being undertaken.

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