Membranes of Mammary Gland. VI. Lipid and Protein Composition of Golgi Apparatus and Rough Endoplasmic Reticulum from Bovine Mammary Gland

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Abstract

The lipid and protein composition of Golgi apparatus and rough endoplasmic reticulum fractions from bovine mammary gland was determined and compared with plasma membrane and milk fat globule membrane fractions. Golgi apparatus was intermediate between endoplasmic reticulum and the plasma membrane-globule membrane with respect to content of lipid phosphorus, cholesterol, protein bound sialic acid, cerebrosides, phosphatidyl choline and sphingomyelin. Fatty acid composition of total polar lipids and individual phospholipids were similar if not identical among all membrane fractions. Membrane fractions washed by procedures which remove adsorbed and intraluminal (secretory) proteins had similar polyacrylamide gel electrophoretic patterns. Endoplasmic reticulum, Golgi apparatus, and milk fat globule membrane had eight electrophoretically identical protein bands. Molecular weights of membrane proteins ranged from 11,500 to 102,000. Washed endoplasmic reticulum, Golgi apparatus and milk fat globule membrane fractions had similar amino acid compositions. These results are consistent with the hypothesis of Golgi apparatus-mediated endomembrane differentiation. The interrelation between lipid and protein and lactose secretion is discussed.

Introduction

In a preceding paper we detailed methods for isolation of purified Golgi apparatus and rough endoplasmic reticulum (RER) fractions from bovine mammary gland (10). The present communication contains results from studies of the lipid and protein composition of these fractions in comparison with milk fat globule membranes and plasma membranes.

These comparisons are of importance for an eventual understanding of the structure and function of membranes of the mammary gland. In particular, they serve as a basis for relating morphological differences among cell components to differences in membrane constituents and also for clarification of the proposed functional role of Golgi apparatus as a site of endomembrane differentiation (18,24,25,26). Membrane differentiation appears to be a crucial feature of milk secretion since the available evidence indicates exocytosis of milk constituents involves discharge of membrane material from the secretory cell (14,18,19,27).

Materials and Methods

Golgi apparatus- and RER-rich fractions were isolated from mammary tissue obtained from lactating Holstein cows at slaughter by methods described in a preceding paper (10). In an attempt to overcome variations among animals, tissue from three or four animals was combined for each isolation. Data were collected with either four preparations individually or from four combined preparations. For comparative purposes milk fat globule membranes, which are derived directly from the secretory cell plasma membrane (see e.g. 15), were also analyzed. These were prepared from Holstein milk samples obtained at the time of normal milking as described previously (7). Combined plasma membrane fractions from five preparations were subjected to analyses. These were prepared as described (15) by density gradient centrifugation using as starting material smooth membrane fractions obtained during isolation of RER (10). This represents a modification of the procedure of Touster et al. (38). Due to low yields, plasma membrane fractions were not assayed enzymologically; however, as evaluated by electron microscopy, these fractions were equally as pure as those studied previously (15).

Membrane fractions were analyzed as obtained or after washing by a method based on procedures which remove most of the adsorbed and intraluminal materials while preserving the morphology and most of the constitutive en-
zymes and phospholipids of membranes (28, 30). This procedure involved washing fractions twice with .14 M NaCl, twice with 1.0 M NaCl and once with .1 M Na$_2$CO$_3$ — .1 M NaHCO$_3$. Membrane fractions were suspended in these solutions (1 ml/mg original broside content of fractions, lipid extracts were also analyzed for total cholesterol (35)). Supernatants and floating lipids were removed by aspiration and discarded. These fractions are referred to as washed membranes hereafter.

Methods for lipid extraction, chromatographic separation and analysis were identical to those used previously (8, 11). Lipid extracts were also analyzed for total cholesterol (35) and ester (34) content. To estimate the cerebroside content of fractions, lipid extracts were fractionated on silica columns with acetone-methanol (9:1 v/v) to elute a crude glycolipid fraction (39). The cerebroside content of these fractions was determined by colorimetric assay for sphingosine (32) with sphingosine, which would interfere with the assay, from these glycolipid fractions.

Sialic acid was determined in membrane residues recovered after lipid extraction by the thio-barbituric acid method (41) with N-acetylneuraminic acid as standard. Protein was determined with the Folin phenol reagent as standard. Thin-layer chromatographic analysis revealed the absence of sphingomyelin, which would interfere with the assay, from these glycolipid fractions.

For characterization of membrane proteins, lipids were extracted from washed fractions and the insoluble residues were recovered. Portions of these residues were dispersed into .01 M sodium phosphate buffer, pH 7.0, containing 1% sodium dodecyl sulfate (SDS) and 1% 2-mercaptoethanol with stirring for 4 hr at 37 C. Insoluble material was removed by centrifugation and the solubilized protein was subjected to electrophoresis in SDS-containing polyacrylamide gels according to Weber and Osborn (42). Bromophenol blue dye was added to samples and served as a reference standard for calculation of relative mobilities. Gels were stained with commassie blue, de-stained with aqueous methanol-acetic acid (42), and analyzed by densitometry in a Gilford spectrophotometer equipped with a gel scanning attachment. Since proteins are separated according to molecular weight in SDS gels (42), it was possible to estimate molecular weights for the protein bands separated. For this purpose the following protein standards were used for preparation of standard curves (molecular weights in parentheses): Ribonuclease (13,700), trypsin (23,300), pepsin (35,000), fumarase (49,000), catalase (60,000) and bovine albumin (68,000). Other portions of residues recovered after lipid extraction were suspended in 6 N HCl and hydrolyzed in evacuated, sealed tubes for 12 hr at 121 C in an autoclave. After cooling, solutions were lyophilized, washed with water and analyzed for amino acid composition in a Technicon amino acid analyzer by standard procedures (23). Amino acid compositions were calculated as mole percentage of the total recovered and were not corrected for losses of acid-labile amino acids.

Authentic phospholipids, methyl ester standards and gas chromatographic columns were from Supelco, Inc., Bellefonte, Pennsylvania. N-acetyleneuraminic acid, sphingosine sulfate and reference proteins were purchased from Sigma Chemical Co., St. Louis, Missouri. A quantitative neutral lipid reference mixture, used in densitometric analysis, was from the Hormel Institute, Austin, Minnesota.

Results

Golgi apparatus fractions contained large amounts of lipid, 1.24 mg/mg protein (Table 1). Plasma membrane and washed milk fat globule membrane (MFGM) fractions also contained in excess of 1 mg of lipid per milligram protein. On this basis, RER fractions contained only about one-third the amount of lipid found in Golgi apparatus fractions. Washing of RER and Golgi apparatus increased their lipid content on a protein basis. The amount of lipid phosphorus in total lipid fractions was highest in RER and MFGM and lowest in Golgi apparatus; similar results were obtained for percent total polar lipids. However, this comparison is misleading due to the much lower lipid in RER. When compared on a protein basis, lipid phosphorus increased in the order RER, Golgi apparatus, plasma membrane and MFGM. Similarly total cholesterol, as micrograms per milligram lipid, and protein bound sialic acid increased in this same order (Table 1). Of the fractions analyzed, Golgi apparatus contained the largest amount of neutral lipids.

The ester content of Golgi apparatus total lipids was also higher than that encountered in RER, plasma membrane and MFGM.

Only trace amounts of cerebrosides were in RER lipids. However, cerebrosides were in the other fractions and increased in the order Golgi apparatus, plasma membrane and MFGM.
When analyzed by thin-layer chromatography, plasma membrane and MFGM cerebroside fractions were composed of nearly equal amounts of monohexose and dihexose cerebrosides. In contrast, this fraction from Golgi apparatus was composed nearly entirely of monohexosyl cerebroside, the precursor of dihexose cerebrosides (6). This result is in contrast to our failure to detect cerebrosides in Golgi apparatus fractions from other tissues (11,12). In retrospect, it appears that these previous failures may have been due to sole reliance on bi-dimensional chromatography of total lipid extracts. The large amounts of neutral lipids in Golgi apparatus may have obscured the chromatographic spot for cerebroside monohexoside. The method in this study, which involved column chromatographic purification of glycolipids prior to analysis, removes this ambiguity.

The same five major phospholipids, sphingomyelin and the choline, ethanolamine, inositol and serine phosphatides, were in all fractions (Table 2). Lesser amounts of the choline and ethanolamine lysophosphatides were also in all fractions, but total lysophosphatide content was below 4.5% in all cases. Only low or negligible amounts of cardiolipin, a nearly exclusively mitochondrial phospholipid (8) were encountered in the fractions analyzed, confirming the relative freedom from mitochondrial contamination established by morphological and enzymatic analysis (10). The percentage of sphingomyelin increased going from RER to Golgi apparatus to plasma membrane-MFGM. This increase in sphingomyelin was paralleled by a decrease in phosphatidyl choline from RER to Golgi apparatus to plasma membrane-MFGM. The net result of this disproportionation was that all membranes had a nearly constant amount of total choline containing phospholipids, the range being from approximately 60 to 64% of the total lipid phosphorus. When these values were calculated on a protein basis, there was a progressive increase in sphingomyelin and in total choline containing phospholipids in going from RER to Golgi apparatus to plasma membrane-MFGM. In contrast, on this basis phosphatidyl choline was relatively constant in RER, Golgi apparatus, and plasma membrane. Although higher phosphatidyl choline was encountered in MFGM, this can be attributed to the fact that the phospholipids, which are primarily choline phosphatide, of forming fat globules are added to those which preexist in the plasma membrane-MFGM (15,28,29). No large or consistent differences were in the distribution of ethanolamine, serine, and inositol phosphatides in any of the fractions analyzed.

**Table 1. Composition of membrane fractions from bovine mammary gland.**

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Endoplasmic reticulum</th>
<th>Golgi apparatus</th>
<th>Plasma membrane</th>
<th>Globule membrane</th>
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<tr>
<td>Total lipid (mg/mg protein)</td>
<td>.43± .01 (.92)</td>
<td>1.24± .01 (1.36)</td>
<td>1.03 (1.14)</td>
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<td>Lipid phosphorus (µg/µg lipid)</td>
<td>29.46±1.27 (33.06)</td>
<td>14.27± .08 (32.2)</td>
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<td>Lipid phosphorus (µg/mg protein)</td>
<td>12.67±1.20</td>
<td>17.69 ±.13</td>
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<td>Polar lipids (% of total lipids)</td>
<td>72.4</td>
<td>33.1</td>
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<td>Cerebrosides (µmole/µg lipid P)</td>
<td>8.2</td>
<td>16.0</td>
<td>(20.0)</td>
<td></td>
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<tr>
<td>Cholesterol (µg/µg lipid)</td>
<td>36.7 ±2.52 (54.0)</td>
<td>54.9 ±9.51 (62.0)</td>
<td>76.0 (48.0)b</td>
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</tr>
<tr>
<td>Ester (µeq/µg lipid)</td>
<td>2.34± .80 (2.42)</td>
<td>3.54± .21 (2.12)</td>
<td>2.20 (2.58)</td>
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</tr>
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<td>Sialic acid (µmole/mg protein)</td>
<td>8.40</td>
<td>21.2 ±1.03</td>
<td>51.0</td>
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</table>

*a Data expressed as mean ± standard deviation of four preparations or the value obtained with four combined preparations. Figures in parentheses are values obtained with washed membrane fractions.

*b This cholesterol content is low due to extraction of large amounts of cholesterol during the washing procedure.
MEMBRANES OF MAMMARY GLAND VI

Table 2. Distribution of phospholipids in membranes from bovine mammary gland.*

<table>
<thead>
<tr>
<th>Phospholipid</th>
<th>Percent of total lipid phosphorus</th>
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</thead>
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<tr>
<td></td>
<td>Endoplasmic reticulum</td>
</tr>
<tr>
<td>Sphingomyelin</td>
<td>5.7±0.26 (5.2)</td>
</tr>
<tr>
<td>Phosphatidyl choline</td>
<td>57.1±3.99 (55.1)</td>
</tr>
<tr>
<td>Phosphatidyl serine</td>
<td>4.1±0.98 (3.9)</td>
</tr>
<tr>
<td>Phosphatidyl inositol</td>
<td>5.7±1.39 (6.6)</td>
</tr>
<tr>
<td>Phosphatidyl ethanolamine</td>
<td>23.8±1.59 (27.2)</td>
</tr>
<tr>
<td>Lysophosphatidyl choline</td>
<td>1.6±0.39 (.8)</td>
</tr>
<tr>
<td>Lysophosphatidyl ethanolamine</td>
<td>2.1±1.50 (1.3)</td>
</tr>
<tr>
<td>Cardiolipin</td>
<td>.2±.40</td>
</tr>
</tbody>
</table>

*Results expressed as mean ± standard deviation of four separate preparations or as the value obtained with four combined preparations. Figures in parentheses are values obtained with washed membrane fractions.

In the aggregate, these results suggest that Golgi apparatus-mediated cytomembrane differentiation from endoplasmic reticulum to plasma membrane involves a net increase, on a protein basis, in the content of all phospholipids except phosphatidyl choline. The high degree of similarity in the phospholipid distribution patterns of plasma membrane and MFGM confirms our previous observations (15,17). No large differences were observed between phospholipid distribution patterns of washed membranes and the respective unwashed controls (Table 2). This suggests that the washing procedure removed little membrane structural material and, thus, confirms the observation of Phillips et al. (30).

Neutral lipid distribution values for these membranes are in Table 3. Cholesterol, triglycerides, free fatty acids and cholesterol esters were the major neutral lipids encountered in all fractions. Diglycerides were present in all fractions, and a component migrating with methyl esters on thin-layer chromatography was in RER and plasma membrane fractions. The latter may be artefactual in that they may have been formed through the action of an acylase during the initial stages of lipid extraction. The most striking differences in the neutral lipid distribution of these membranes was the much higher triglycerides and the correspondingly lower amounts of other major constituents in Golgi apparatus. With one exception, washing of membranes did not appreciably alter the neutral lipid distribution patterns. The exception was that the triglyceride content of the Golgi apparatus fraction was decreased by nearly one-half. Washing did, however, decrease the total neutral lipid contents of both RER and Golgi apparatus (c.f. Table 1). While these data do not allow conclusions as to whether neutral lipids are true membrane constituents, the decreased neutral lipid content in washed membranes suggests that a portion of the neutral lipid may be artefactually adsorbed or loosely bound to the membrane surfaces.

All membrane fractions displayed similar total polar lipid fatty acid compositional patterns with respect to content of major fatty acids and in total percent unsaturation (Table 4). Deviations in fatty acid distribution were slight among RER, Golgi apparatus and MFGM; more variation was observed with the plasma membrane fraction. No significant differences in fatty acid composition were found when washed membrane fractions were compared to unwashed controls. The major saturated acids encountered were 16:0 and 18:0 and the major unsaturated acids were 18:1 and 18:2. Only low levels of long chain unsaturated acids were encountered in these membrane fractions.

Table 3. Neutral lipid composition of membranes from bovine mammary gland.*

<table>
<thead>
<tr>
<th>Neutral lipid</th>
<th>Percent of total neutral lipid</th>
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<td></td>
<td>Endoplasmic reticulum</td>
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<tr>
<td>Diglycerides</td>
<td>2.5</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>45.8</td>
</tr>
<tr>
<td>Fatty Acids</td>
<td>20.4</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>13.4</td>
</tr>
<tr>
<td>Methyl esters</td>
<td>5.8</td>
</tr>
<tr>
<td>Cholesterol esters</td>
<td>12.0</td>
</tr>
</tbody>
</table>

*Values were obtained with four combined preparations.
TABLE 4. Fatty acid composition of total polar lipids from bovine mammary gland membranes. *

<table>
<thead>
<tr>
<th>Acid ( b )</th>
<th>Endoplasmic reticulum</th>
<th>Golgi apparatus</th>
<th>Plasma membrane</th>
<th>Globule membrane</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>1.8 (2.6)</td>
<td>1.4 (2.3)</td>
<td>2.1</td>
<td>3.3 (4.3)</td>
</tr>
<tr>
<td>16:0</td>
<td>18.4 (17.3)</td>
<td>15.7 (19.6)</td>
<td>21.5</td>
<td>17.1 (16.3)</td>
</tr>
<tr>
<td>16:1</td>
<td>2.0 (2.3)</td>
<td>2.2 (1.7)</td>
<td>1.6</td>
<td>1.4 (1.7)</td>
</tr>
<tr>
<td>18:0</td>
<td>15.4 (16.1)</td>
<td>14.8 (17.0)</td>
<td>17.4</td>
<td>15.9 (15.2)</td>
</tr>
<tr>
<td>18:1</td>
<td>38.7 (41.1)</td>
<td>39.7 (33.1)</td>
<td>35.2</td>
<td>42.8 (40.7)</td>
</tr>
<tr>
<td>18:2</td>
<td>14.4 (12.1)</td>
<td>12.7 (15.0)</td>
<td>10.4</td>
<td>14.1 (14.2)</td>
</tr>
<tr>
<td>18:3</td>
<td>1.8 (1.8)</td>
<td>1.5 (1.8)</td>
<td>1.7</td>
<td>1.6 (1.7)</td>
</tr>
<tr>
<td>20:3</td>
<td>1.5 (1.5)</td>
<td>2.6 (2.0)</td>
<td>1.9</td>
<td>0.9 (1.9)</td>
</tr>
<tr>
<td>20:4</td>
<td>3.8 (3.3)</td>
<td>6.4 (4.8)</td>
<td>8.8</td>
<td>1.2 (2.5)</td>
</tr>
<tr>
<td>20:5</td>
<td>6 (4.4)</td>
<td>8 (.6)</td>
<td>8 (.8)</td>
<td>0.3 (.3)</td>
</tr>
<tr>
<td>22:4</td>
<td>1 (4.4)</td>
<td>1.5 (1.6)</td>
<td>1.5</td>
<td>0.6 (.5)</td>
</tr>
<tr>
<td>22:5</td>
<td>1.4 (1.2)</td>
<td>1.4 (1.5)</td>
<td>1.5</td>
<td>0.6 (.7)</td>
</tr>
<tr>
<td>% Unsaturated</td>
<td>64.5 (64.1)</td>
<td>68.1 (61.1)</td>
<td>59.0</td>
<td>63.5 (64.2)</td>
</tr>
</tbody>
</table>

* Data obtained with four combined preparations. Figures in parentheses were obtained with washed membrane fractions. 

b Fatty acids expressed as number of carbons: number of double bonds.

The most striking result of this comparison was the relatively constant amount of total unsaturated acids in all fractions. This is suggestive of a need for a controlled unsaturation for the maintenance of function in these cytologically distinct components of the endomembrane system.

More variation in fatty acid composition was evident on comparison of individual major phospholipids from each fraction (Table 5). However, with few exceptions the same fatty acids were in each phospholipid from all membrane fractions. Sphingomyelin was the only phospholipid with appreciable amounts of long-chain, saturated acids. These acids are characteristic of sphingolipids from a great

TABLE 5. Fatty acid composition of major phospholipids from bovine mammary gland membranes. *

<table>
<thead>
<tr>
<th>Acid ( b )</th>
<th>Sphingomyelin</th>
<th>Phosphatidylcholine</th>
<th>Phosphatidylethanolamine</th>
<th>Phosphatidylinositol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid ( b )</td>
<td>RER</td>
<td>GA</td>
<td>PM</td>
<td>RER</td>
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<tr>
<td>14:0</td>
<td>3.5</td>
<td>2.3</td>
<td>2.2</td>
<td>1.6</td>
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<tr>
<td>16:0</td>
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<td>18:0</td>
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<td>24:0</td>
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<tr>
<td>% Unsaturated</td>
<td>7.9</td>
<td>2.5</td>
<td>3.4</td>
<td>57.9</td>
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</table>

* Values obtained with four combined preparations.

b Fatty acids expressed as number of carbons: number of double bonds. Abbreviations: RER, rough endoplasmic reticulum; GA, Golgi apparatus; PM, plasma membrane.
number of mammalian tissues. There was a tendency toward higher unsaturation and lower long-chain acids in RER in comparison with Golgi apparatus and plasma membrane sphingomyelins. In contrast, phosphatidyl cholines of RER and Golgi apparatus were virtually

Table 6. Summary of electrophoretic comparison of major protein components of washed membranes from bovine mammary gland.

<table>
<thead>
<tr>
<th>Band number *</th>
<th>Endoplasmic reticulum</th>
<th>Golgi apparatus</th>
<th>Globule membrane</th>
<th>Molecular weight ( \times 10^3 )</th>
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<td>24</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>12.1</td>
</tr>
<tr>
<td>25</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>11.5</td>
</tr>
</tbody>
</table>

* Numbers refer to bands as numbered in Figure 1. + Denotes presence of protein band in the respective fraction.
identical while this lipid from plasma membrane differed in that it contained more of 18:1 and total unsaturated acids and less of 16:0. Phosphatidyl ethanolamines from all three membrane fractions displayed a high degree of similarity, while phosphatidyl inositol from Golgi apparatus and plasma membrane were related and differed, especially in degree of unsaturation, from RER phosphatidyl inositol.

Electrophoretic analysis revealed complex protein patterns for washed RER, Golgi apparatus and MFGM preparations (Fig. 1). Twenty-five bands, differing with respect to mobility relative to the tracking dye, were detected. There was a tendency toward decreasing complexity from RER, which had 17 distinct bands, to Golgi apparatus (15 bands) to MFGM (14 bands). The molecular weights of these proteins, estimated from their mobilities relative to those of reference proteins of known molecular weight, ranged from a high of 102,000 to a low of 11,500 (Table 6). The more provocative finding of this comparison was the existence of 8 protein components which were common to all fractions (bands 7, 9, 10, 12, 13, 17, 18 and 23 in Fig. 1 and Table 6). Several of these common proteins were major components in all membrane fractions. In previous work, with acidic polyacrylamide gels, MFGM and plasma membrane had identical electrophoretic profiles with one major protein component (15). In agreement with these observations, one major protein component (band 7) of molecular weight 68,000 was in MFGM when separations were made in sodium dodecylsulfate polyacrylamide gels (Fig. 1). Band 7 was also a prominent component of the RER and Golgi apparatus patterns. Since prosthetic groups (such as carbohydrates; c.f. Table 1 and ref. 31, 40) can be added to membrane proteins, it was possible that certain of the proteins in RER membranes were precursors of Golgi apparatus and MFGM proteins. Examination of data in Table 6 reveals four such possible band patterns which differ, within reasonable limits, in molecular weight. The band pairs 2-3-4, 4-5-6, 15-16 and 24-25 would fulfill this requirement. Although structural studies of purified proteins will be required to prove such a relationship, it does represent an attractive hypothesis.

Because of difficulties of differential solubilization of different membranes and the inscrutability of a certain portion of the solubilized protein fractions into gels, it was possible that certain of the electrophoretic similarities were artefactual. If all of the membrane fractions had similar proteins, it would be expected that they would have similar amino acid compositions. In fact, acid hydrolysates of washed membrane fractions were similar in amino acid composition (Table 7). That some contamination of RER and Golgi apparatus with non-endomembrane components (mitochondria and collagen, for example) was inevitable while MFGM represents highly purified apical plasma membrane, then the similarities observed are indeed striking. Glutamic acid and leucine were the major residues encountered and together accounted for 22.5 to 24.5 mole percent of the total residues recovered. Other major amino acids included aspartic acid, threonine, glycine, alanine, lysine and arginine.

Discussion

Extensive morphological and biochemical evidence have been presented to document the hypothesis that the milk fat globule membrane is derived from the apical plasma membrane of mammary secretory cells (see, for example, 2, 14, 19, 27). Thus, to maintain cellular integrity an efficient mechanism must exist to replenish that portion of the plasma membrane given up to fat globules. This replacement mechanism follows as a natural consequence of the proposed functional role of the Golgi apparatus in membrane transformation and product compartmentalization and integrates the processes of lipid secretion with protein and lactose secretion. In this concept Golgi apparatus is
postulated to derive its membranes from endo-
plasmic reticulum and to transform this mem-
baine into secretory vesicle membranes which 
are identical to and fuse with the apical plasma 
membrane (18,24,25,26). Secretory vesicles 
are a major vehicle for transport of milk pro-
teins to the cell surface for secretion (7,43). 
That lactose is also contained within secretory 
vesicles has not been directly demonstrated, 
but the available evidence strongly suggests 
this as the most likely mode of extrusion of lac-
tose from the cell. This evidence includes the 
observations that lactose synthesis is localized 
specifically in the Golgi apparatus of rat (13) 
and bovine (10) mammary glands and the 
demonstrated impermeability of the apical cell 
surface to lactose (20). Furthermore, there is 
a close correlation between the rates of protein 
and lactose secretion from the cell (33). Mor-
phologically, the transitional nature of Golgi 
apparatus membranes is seen as a gradual 
thickening of membranes from those which are 
dioplasmic reticulum-like at the proximal 
face of the dictyosome to those which are plas-
ma membrane-like at the distal face and in 
forming secretory vesicles (5,7,25). The com-
positional intermediacy of Golgi apparatus 
between RER and plasma membranes has been 
extensively documented with rat liver (12,24, 
25) and, to a lesser extent, with rat mammary 
gland (11).

Results presented herein are fully consonant 
with the hypothesis of Golgi apparatus func-
tion in membrane transformation in the lactat-
ing bovine mammary gland. Golgi apparatus 
membranes are truly intermediate between 
RER at one extreme and plasma membranes 
and MFGM at the other in content of phos-
holipids, cerebrosides, cholesterol, and sialic 
acid. Sphingomyelin increased as both percent-
age of total lipid phosphorus and on a protein 
basis from RER to Golgi apparatus to plasma 
membrane. Phosphatidyl choline decreased as 
percent of total lipid phosphorus but the net 
content, on a protein basis, remained constant. 
These results suggest that transformation of 
endomembranes from RER-like to plasma 
membrane-like involves the net addition of 
polar lipids and cholesterol. The fatty acid 
compositional patterns of both total polar lip-
ids and the individual phospholipids also agree 
with the hypothesis of Golgi apparatus func-
tion. The low glycolipids and protein-bound 
sialic acid in RER and their increasing content 
in Golgi apparatus, plasma membranes and 
MFGM is consistent with the known localiza-
tion of glycosyl transferases in Golgi apparatus 
(13,31,40). These results further imply that 
membrane flow is unidirectional toward the 
cell surface. Moreover, the membrane protein 
profiles and the amino acid composition is also 
consistent with this hypothesis. Our results 
with proteins suggest that these membrane 
transformations involve concentration of cer-
tain proteins and/or addition of prosthetic 
groups to polypeptide chains contained in 
RER membranes. The intermediate position 
of Golgi apparatus between RER and plasma 
membrane-MFGM with respect to enzyme 
specific activities is also consistent with this 
hypothesis (10).

One deviation from this hypothesis is evi-
dent in that Golgi apparatus fractions contain 
more neutral lipid, on a protein basis, than do 
the other fractions. However, it is possible that 
portions of this neutral lipid are adsorbed 
by the membrane and, thus, do not represent true 
membrane constituents. High lipid to protein 
ratios are also characteristic of Golgi apparatus 
frations from liver (3), rat mammary gland 
(11), and rat testis (16).

With respect to distribution of the serine 
and inositol phosphatides and the fatty acid 
composition of certain of the phospholipids, 
plasma membranes deviate from the trends 
evident among RER, Golgi apparatus, and 
MFGM. While it is natural to assume that 
these deviations are due to contamination of 
the plasma membrane fraction, correcting data 
for the major contaminants of this fraction, 
RER and mitochondria, does not yield the 
observed results. One possible explanation 
for fatty acid differences is the fact that 
plasma membranes are known to contain 
enzymes (36,37) which function in transacyla-
tion of phospholipids. However, this does not 
explain the deviations with respect to the inosi-
tol and serine phosphatide contents. A more 
likely explanation for these differences emerges 
from the knowledge that plasma membrane 
frations are enriched in membrane fragments 
obtained from the lateral surfaces of the cells 
where the membrane is stabilized by junctional 
complexes (15). The MFGM is derived from 
the apical plasma membrane and thus the com-
positional differences observed may reflect 
slight differences in composition between the 
apical and lateral plasma membrane.

All of our results are consistent with the hy-
pothesis that Golgi apparatus of bovine mam-
mary gland functions in membrane transfor-
mation as well as in product compartmentaliza-
tion. Thus, secretion of the three major com-
ponents of milk are phenomena interrelated

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Fig. 2. Diagram summarizing the interrelationships among Golgi apparatus, endoplasmic reticulum, secretory vesicles and plasma membrane in the transformation of membrane and the secretion of the lipid and proteins of milk. The concept of Golgi apparatus function diagrammed involves input of new membrane material from endoplasmic reticulum at the proximal or forming face of the dictyosome and its transformation and utilization in the elaboration of secretory vesicles at the distal or maturing face of the dictyosome. Secretory proteins are contained in these vesicles and the caseins appear to undergo micelle formation within the vesicle. Lactose is most probably also contained in the secretory vesicles. Fusion of the secretory vesicles with plasma membrane provides new plasma membrane to replenish that lost from the envelopment of lipid globules during milk fat globule formation. Abbreviations: LG, lipid globule; R, ribosome; D1, D2 and D3, dictyosomes; MFG, milk fat globule.

through membrane flow and differentiation. These concepts of integration of membrane and product flow are summarized diagrammatically in Figure 2.

This hypothesis predicts concomitant synthesis of both secretory and membrane protein. Although this has yet to be studied with mammary gland, concomitant synthesis of membrane and secretory protein has been demonstrated in rat liver (4) and in rat parotid gland (1). The availability of methods for isolation of endomembrane components from mammary gland (10) coupled with the compositional analyses reported herein make kinetic studies of the turnover of membrane constituents possible.

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References


