ENZYMIC CHARACTERISTICS OF FAT GLOBULE MEMBRANES FROM BOVINE COLOSTRUM AND BOVINE MILK

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ABSTRACT
Fat globule membranes have been isolated from bovine colostrum and bovine milk by the dispersion of the fat in sucrose solutions at 4°C and fractionation by centrifugation through discontinuous sucrose gradients. The morphology and enzymic characteristics of the separated fractions were examined.

Fractions comprising a large proportion of the total extracted membrane were thus obtained having high levels of the Golgi marker enzymes UDP-galactose N-acetylglucosamine β-4-galactosyltransferase and thiamine pyrophosphatase. A membrane-derived form of the galactosyltransferase has been solubilized from fat and purified to homogeneity. This enzyme is larger in molecular weight than previously studied soluble galactosyltransferases, but resembles in size the galactosyltransferase of lactating mammary Golgi membranes.

In contrast, when fat globule membranes were prepared by traditional procedures, which involved washing the fat at higher temperatures, before extraction, galactosyltransferase was not present in the membranes, having been released into supernatant fractions. When the enzyme released by this procedure was partially purified and examined by gel filtration, it was found to be of a degraded form resembling in size the soluble galactosyltransferase of milk. The release is therefore attributed to the action of proteolytic enzymes.

Our observations contrast with previous biochemical studies which suggested that Golgi membranes do not contribute to the milk fat globule membrane. They are, however, consistent with electron microscope studies of the fat secretion process, which indicate that secretory vesicle membranes, derived from the Golgi apparatus, may provide a large proportion of the fat globule membrane.

While the composition and origin of the membranes surrounding the fat globules of milk have been the subject of many studies (1, 8, 16, 23, 24), there are serious differences between the conclusions reached on the basis of electron microscope and biochemical analyses.

The elegant microscope studies of Wooding (23, 24) suggest that, within the lactating mammary epithelial cell, fat droplets originate near the basal portion with no surrounding membrane, but that during passage to the apex of the cell before secretion they become associated with membrane-bounded vesicles derived from the Golgi apparatus. Under some conditions, fat droplets are observed within the cell included in vesicles which also contain aqueous constituents and casein micelles (25). Fat globule membranes can therefore originate without any contribution from the plasmalemma. Normally the acquisition of the membrane occurs at the plasmalemma during secre-
tion, but the studies of Wooding suggest that the membrane of vesicles associated with the fat droplet makes the major contribution to the membrane surrounding the secreted droplet.

An autoradiographic study of the biosynthesis of milk fat also supports the association of fat droplets with intracellular membranes, including those of the Golgi apparatus (22).

Some secreted fat droplets have been shown to contain cytoplasmic crescents (25, 26), and other studies of Wooding (24, 25) indicate that, after secretion, some of this surrounding membrane may be lost from fat droplets by "blebbing". Membrane fractions that may have originated in this way have been isolated from milk by Morton (15) and successive workers.

Analyses of the enzymic, phospholipid, and protein composition of membranes isolated from cream and from skim milk have been interpreted to suggest that these membranes are a nearly homogeneous form of the plasma membrane which the fat is seen as acquiring during its passage across the apical plasmalemma of the mammary epithelial cell. This point of view has been expressed forcibly in a recent review by Patton and Keenan (16), who particularly emphasize the complete absence from bovine milk fat globule membranes of enzymes generally accepted as markers for Golgi membranes. Patton and Keenan argue that similarities in the protein and phospholipid composition of milk fat globule membranes and plasma membrane support this hypothesis, and conclude that intracellular membranes do not participate in any way in the secretion of milk fat. The membranes used in these biochemical studies were isolated from washed cream by a process involving repeated washing at 38°-40°C to promote coalescence of the lipid droplets and to remove aqueous phase contaminants.

In contrast to these studies, we have found that the fat of both colostrum and milk is an excellent source of a membrane-bound form of UDP-galactose N-acetylglucosamine β-4-galactosyltransferase, a marker enzyme for Golgi membranes (2, 4, 19), and the catalytic component of lactose synthase (2). We describe the isolation, from the total fat globule membrane, prepared at 4°C, of major membrane fractions enriched with this galactosyltransferase. These and other observations lend support to the proposals made by Wooding (23, 24) that the fat globule membrane is derived in some part from membranes of Golgi origin, although it cannot be excluded that a portion of the apical plasma membrane may also be acquired by the fat droplets during exocytosis.

MATERIALS AND METHODS

Bovine colostrum was obtained from the Department of Agriculture, University of Florida (Gainesville, Fla.) 1 day after secretion. The fat layer was immediately separated by flotation (4,000 g, 15 min); some was used the same day and the remainder stored in batches at -15°C. Cream (40% fat) was obtained from Borden Inc., Dairy Services Division (Miami, Fla.) on the day of milking and used immediately. Other materials were obtained as reported previously (18, 21).

A packed fat layer was separated from the aqueous phase of both cream and colostrum by flotation (4,000 g, 15 min) at 4°C. The fat (100 g) was dispersed in 0.5 M sucrose (300 ml) containing 1% dextran, 10 mM MgCl₂, 1 mM β-mercaptoethanol and 0.1 M cacodylate buffer, pH 7.0. (0.1 M sodium cacodylate adjusted to pH 7.0 with HCl) (medium A), at 4°-10°C, using an overhead drive stirrer with stainless steel blades (5 cm diameter) at 1,725 rpm for 5 min. Free fat was removed by centrifugation for 10 min at 4,000 g, and the supernate (S₁) used to prepare membrane fractions.

S₁ was centrifuged (10,000 g, 1 h, 4°C) to yield a precipitate P₂ and a supernate S₂. P₂ was suspended in 18 ml of S₂ and applied to a discontinuous sucrose gradient (I) and centrifuged at 100,000 g for 1 h in an SW 27 rotor (Beckman Instruments, Inc., Palo Alto, Calif.) at 4°C. Discontinuous gradient I comprised P₂ suspended in S₂ (6.5 ml, 60 mg protein/ml) layered over 1.8 M sucrose (3 ml), 1.5 M sucrose (13 ml), and 1.25 M sucrose (14 ml); all sucrose solutions contained 10 mM cacodylate buffer at pH 7.0. When applied to milk fat, this gradient yielded a supernate (S₃), a membranous layer at the 0.5/1.25 M sucrose interface (M₃), and a smaller membranous layer at the 1.25/1.5 M interface (N₃), and a precipitate (P₃). For preparations from colostrum a simpler discontinuous gradient (II), comprised only of P₂ suspended in S₂ (6.5 ml, 60 mg protein/ml) layered over 1.25 M sucrose, was used to give M₃ and P₃; when discontinuous gradient I was used, no membranes were collected at the 1.25/1.5 M sucrose interface. The fractions M₃, N₃, and P₃ were suspended in 0.25 M sucrose, containing 10 mM cacodylate buffer pH 7.0. Pellets for electron microscopy (M₃', N₃', and P₃') were obtained by sedimentation at 10,000 g for 20 min in an L50 rotor (Beckman Instruments, Inc., Spinco Div.), and were fixed with 2.5% glutaraldehyde in 50 mM cacodylate buffer, pH 7.4, containing 2% sucrose, postfixed with 1% OsO₄ and embedded in Araldite. Thin sections were cut on an LKB Ultrotome III (LKB Instruments, Inc., Rockville, Md.), stained with saturated ethanolic uranylacetate and lead citrate, and examined in a Philips EM 300.
MIXTURE
(100 g fat in 300 ml medium A)
centrifuge: 4,000 g, 10 min

fat (discarded)

S1 (250 ml)
centrifuge: 100,000 g, 1 h

precipitate (discarded)

S2

P2 (precipitate, resuspended in 18 ml S2)
centrifuge: 100,000 g, 1 h
discontinuous gradient*

0.5 M sucrose

1.25 M sucrose

1.25 M sucrose

1.5 M sucrose

S3

M3

N3

P3

M3'

N3'

centrifuge: 10,000 g, 20 min

Scheme 1 Preparation of fat globule membranes from milk and colostrum.
* The discontinuous gradient used for colostrum comprised 1.25 M sucrose only, there being no 1.25/1.5 M interface.

Protein concentrations were obtained by the method of Lowry et al. (9), 5'-nucleotidase, thiamine pyrophosphatase, glucose-6-phosphatase, and succinic dehydrogenase (14), xanthine oxidase (12), and galactosyltransferase (18, 21) as described in the literature. UDPase assays were performed as for thiamine pyrophosphatase, using 2 mM UDP.

Membranes were also prepared following a procedure described by Patton and Trams (17) in which the cream was washed with 0.25 M sucrose at 38°-40°C (2 × 3 vol), extracted by churning at 12°C, and the membranes were collected by centrifugation at 100,000 g for 1 h.

The preparation of galactosyltransferase from fat globule membranes was performed as described in detail elsewhere. The enzyme was characterized by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis and by chromatography on a column (1.5 × 95 cm) of Bio-Gel P150 (Bio-Rad Laboratories, Richmond, Calif.) (200-400 mesh), equilibrated with 0.5 M NaCl containing 50 mM cacodylate buffer, pH 7.4, and 1 mM β-mercaptoethanol.

RESULTS
Fat globule membranes were prepared from bo-

vain cream and colostrum, using sucrose density gradient fractionation as described above. The yields of protein and galactosyltransferase in the various fractions from cream and colostrum are summarized in Table I. A very similar pattern can be seen for both sources, where the initial extract from 100 g of material concentrated by flotation, after removal of free fat and material sedimentable at 4,000 g for 10 min, contained slightly more than 3 g of protein, which yielded 1.1-1.2 g of membrane material (P2). Fraction P2 from colostrum divided equally on a sucrose gradient, described for the isolation of Golgi membranes (14), between fractions M3 and P3, where M3 contained twice as much galactosyltransferase at twice the specific activity of P3, a threefold enrichment over the initial extract, S1. The protein content and specific activity of galactosyltransferase in each fraction are shown in Table I. Electron microscope examination showed that the fractions M3' and P3 are closely similar in their morphological appearances, consisting of fragments of smooth membrane and some small vesicles (Fig. 1). Ribosomes are absent and little or no casein can be seen within the vesicles.

The total membrane fraction P2 of cream fractionated differently on the single-step sucrose gradient (gradient II); although the membrane fraction (equivalent to M3) showed a specific activity threefold higher than that of the pellet, the total galactosyltransferase activity of the pellet was greater than that of the membrane fraction. Consequently, a different discontinuous gradient (gradient I) was used. This yielded M3 at the 0.5/1.25 M sucrose interface, another smaller fraction N3 at the 1.25/1.5 M sucrose interface, and a pellet P3. Both M3 and N3 had high specific activities for galactosyltransferase, while P3 contained a negligible amount of galactosyltransferase activity (see Table I). The levels of other marker enzymes are given in Table II. The membranes, enriched in galactosyltransferase, from cream may have a slightly higher density than those from colostrum fat, as a result of the different characteristics of the two secretions. The electron micrographs of the fractions from cream (M3, N3) (Fig. 1b) were closely similar to the micrographs of the material from colostrum fat (M3) (Fig. 1a). Both were homogeneous through the pellet. In contrast, micrographs of fractions P3 from cream showed it to be rich in casein.

While a reasonably quantitative recovery of ga-

### Table I

#### Progress of Fractionation by Scheme I

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total protein (mg)</th>
<th>Total galactosyltransferase activity (mU)</th>
<th>Sp act per mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colostrum Fat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S1</td>
<td>3,150</td>
<td>$17.6 \times 10^3$</td>
<td>5.6</td>
</tr>
<tr>
<td>P2</td>
<td>1,200</td>
<td>$17.0 \times 10^3$</td>
<td>14.2</td>
</tr>
<tr>
<td>S2</td>
<td>1,950</td>
<td>$5.46 \times 10^3$</td>
<td>2.8</td>
</tr>
<tr>
<td>M3</td>
<td>500</td>
<td>$9.4 \times 10^3$</td>
<td>18.8</td>
</tr>
<tr>
<td>P3</td>
<td>600</td>
<td>$5.28 \times 10^3$</td>
<td>8.8</td>
</tr>
<tr>
<td>S3</td>
<td>20</td>
<td>$0.01 \times 10^3$</td>
<td>0.7</td>
</tr>
<tr>
<td>M3'</td>
<td>85</td>
<td>$1.57 \times 10^3$</td>
<td>18.5</td>
</tr>
<tr>
<td>Cream</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S1</td>
<td>3,500</td>
<td>$6.65 \times 10^3$</td>
<td>1.9</td>
</tr>
<tr>
<td>P2</td>
<td>1,120</td>
<td>$2.58 \times 10^3$</td>
<td>2.3</td>
</tr>
<tr>
<td>S2</td>
<td>2,125</td>
<td>$1.06 \times 10^3$</td>
<td>0.5</td>
</tr>
<tr>
<td>S3</td>
<td>30</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>M3</td>
<td>368</td>
<td>$6.0 \times 10^3$</td>
<td>16.5</td>
</tr>
<tr>
<td>N3</td>
<td>180</td>
<td>$2.8 \times 10^3$</td>
<td>15.8</td>
</tr>
<tr>
<td>P3</td>
<td>315</td>
<td>$0.28 \times 10^3$</td>
<td>0.9</td>
</tr>
<tr>
<td>'M3'</td>
<td>125</td>
<td>$2.0 \times 10^3$</td>
<td>16.0</td>
</tr>
<tr>
<td>N3'</td>
<td>84</td>
<td>$2.0 \times 10^3$</td>
<td>16.3</td>
</tr>
<tr>
<td>M3 + N3</td>
<td>548</td>
<td>$1.37 \times 10^3$</td>
<td>16.1</td>
</tr>
</tbody>
</table>

Galactosyltransferase is expressed as nanomoles [14C]galactose transferred per minute.

* Total (corrected from Table III).
lactosyltransferase was obtained in the fractions from colostrum, in the case of the cream preparation it appeared that more enzyme was recovered in the various fractions than in the starting material. This could possibly be a result of the presence of α-lactalbumin, an inhibitor of the transfer of
Galactosyltransferase is expressed as nanomoles [\(^{14}\)C]galactose transferred per minute. Phosphatase activities are expressed as nanomoles inorganic phosphate released per minute.

galactose to \(N\)-acetylglucosamine, or to glycoproteins in the initial extract. Glycoproteins may inhibit by acting as alternative acceptors of galactose. This same phenomenon has also been observed by other workers in the preparation of Golgi membranes from rat liver (13).

To test for the presence of such inhibitors, the activity of exogenous bovine colostrum galactosyltransferase was examined in the presence of the various fractions (S1, S2, P2, S3, M3, N3, and P3). The levels of inhibition indicated in Table III were found. The inhibition in S1 and P2 resulted, at least in part, from the presence of \(\alpha\)-lactalbumin, since both these fractions showed lactose synthase activity when assayed in the presence of 10 mM glucose (Table III). Significant lactose synthesis by galactosyltransferase at this concentration of glucose occurs only in the presence of \(\alpha\)-lactalbumin (2). The inhibition in S2 and S3 must result from the presence of glycoproteins or other inhibitors. From the levels of inhibition of exogenous galactosyltransferase, we can approximate the true level of enzyme activity during membrane preparation. The corrected levels of total galactosyltransferase activity are given in Table I. The small apparent increase observed upon fractionation of P2 probably results from the inherent inaccuracies of this type of calculation.

The galactosyltransferase purified from fat globule membranes is larger, by about 12,000 daltons, than colostral galactosyltransferase (mol wt 51,000, see reference 18), as shown by SDS polyacrylamide gel electrophoresis, gel filtration, and analytical ultracentrifugation. The gel filtration evidence is shown in Fig. 2a. The elution profiles of galactosyltransferase purified from fat globule membranes and bovine colostrum galactosyltransferase on a column of Bio-Gel P150 are compared; the elution positions of marker proteins used to calibrate the column are also known.

When the fat globule membranes were prepared by the procedure of Patton and Trams (17),
the 38°-40°C 0.25 M sucrose washes and the final extract obtained after churning the cream at 12°C were centrifuged at 100,000 g for 1 h. Only trivial galactosyltransferase activity was detected in the pellets, but both wash supernates exhibited galactosyltransferase activity (Table IV), with specific activities much higher than that of milk (0.2 mU/mg). This galactosyltransferase was partially purified. The supernate from a 30% ammonium sulphate cut was loaded onto a column of leucine-Sepharose (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) (5 ml), washed with 30% ammonium sulphate (5 ml), then 25% ammonium sulphate (5 ml), and the enzyme eluted with 0.5 M NaCl. A further purification step of affinity chromatography with a column of α-lactalbumin-Sepharose (2 ml) was performed (18). Membrane and soluble galactosyltransferase copurify by these procedures (18, 19). The partially purified galactosyltransferase was then examined by gel filtration on Bio-Gel P150; the elution profile is shown in Fig. 2b. The main part of the activity peak is of similar size to the soluble colostrum enzyme, but the shoulders on the trailing edge suggest the presence of further proteolytically degraded forms as found in the galactosyltransferase from bovine milk (18).

DISCUSSION
Fat globule membranes with a high specific activity for galactosyltransferase, a Golgi membrane marker enzyme, were prepared from bovine colostrum and bovine milk fat by fractionation on sucrose gradients. The morphology of the membranes isolated in this study is closely similar to that reported by previous workers (10, 16, 25). Although galactosyltransferase has not been found in bovine milk fat globule membranes prepared by traditional procedures (16, 17), it has been reported to be present in human milk fat globule membranes (10, 11).

The specific enzyme activities of our membrane fractions (M3 and N3), comprising 40–50% of the total protein sedimenting at 10,000 g for 1 h, are compared with those of bovine mammary gland Golgi and endoplasmic reticulum membranes (7) and sheep mammary gland Golgi membranes (20) in Table V. Both the enzymic properties and the densities of our membrane fractions are similar to those of Golgi membranes, having high levels of galactosyltransferase and thiamine pyrophosphatase, moderate amounts of 5'–nucleotidase, and no glucose-6-phosphatase. (Although 5'–nucleotidase has been widely considered as a marker for plasma membrane, recent studies with rat liver have called this specificity into question [5]). The precipitate fractions (P3) we obtain have moderate levels of 5'–nucleotidase and some glucose-6-phosphatase activity, and are possibly of mixed origin (endoplasmic reticulum and plasma membrane); electron microscope examination indicates that the precipitate from milk fat, in contrast to that of colostrum, also contains casein.

The specific activities of galactosyltransferase in M3 and N3 are higher than those reported by others for bovine mammary gland Golgi membranes (7) but much lower than that for sheep mammary gland Golgi membranes. The low level reported for the bovine mammary gland Golgi membranes (7) is perhaps a result of the suboptimal assay conditions used in the previous study, with respect to the concentration of the acceptor substrate N-acetylglucosamine (2.5 mM, compared with a $K_m$ of about 10 mM, see reference 21). The lower specific activity of galactosyltransferase in fat globule membranes prepared by our procedure in comparison with that of sheep mammary gland Golgi membranes may reflect a mixed origin for the membranes (partly Golgi and partly plasmalemma) or could be a result of enzyme release by proteolysis during storage in the mammary gland. The hypothesis of proteolytic release would be supported by our evidence of the proteolytic release and degradation of the membrane galactosyltransferase at 38°-40°C (vide infra).

The differences between our observations and those of Keenan and Huang (6), Patton and Keenan (16), and Patton and Trams (17) can be attributed simply to the methods used previously for obtaining fat globule membranes from bovine milk. Our studies strongly suggest that procedures involving heating to 38°-40°C lead to the release of galactosyltransferase activity into supernatant fractions, no activity being detected in "microsomal" or fat globule membrane pellets. Since the specific activity of this released galactosyltransferase is much greater than that of milk (Table IV), the possibility that it derives principally from aqueous phase components associated with the fat can be eliminated. The evidence that the galactosyltransferase is released by proteolysis is strengthened by the fact that the elution profile on gel filtration of the released enzyme, after partial purification, resembles closely that of the proteolytically degraded enzyme forms found in bovine milk (18), with the initial activity peak corre-
**TABLE IV**

Galactosyltransferase Activity during Preparation of Fat Globule Membranes by Washing Cream at 38-40°C

<table>
<thead>
<tr>
<th>Specific activity, mU/mg protein</th>
<th>1st Sucrose wash 100,000 g</th>
<th>2nd Sucrose wash 100,000 g</th>
<th>After churning 100,000 g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Supematate</td>
<td>Precipitate</td>
<td>Supematate</td>
</tr>
<tr>
<td>Galactosyltransferase</td>
<td>2.3</td>
<td>0.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Lactose synthase</td>
<td>2.5</td>
<td>0.4</td>
<td>0.8</td>
</tr>
<tr>
<td>Lactose synthase + 200 μg/ml α-lactalbumin</td>
<td>5.1</td>
<td>0.6</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Lactose synthase activity was determined in the presence of 10 mM glucose. The specific activity of galactosyltransferase in milk is 0.2 mU/mg.

**TABLE V**

A Comparison of Fat Globule Membrane Enzymes with Those in Golgi and Endoplasmic Reticulum Membranes of Bovine Mammary Gland

<table>
<thead>
<tr>
<th>Specific activity nmol/min/mg protein</th>
<th>Galactosyltransferase (GlcNAc-1)</th>
<th>5'-Nucleotidase</th>
<th>TPase</th>
<th>Glucose-6-Pase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine mammary Golgi*</td>
<td>6.0</td>
<td>37.3</td>
<td>46.7</td>
<td>6.7$</td>
</tr>
<tr>
<td>Bovine mammary gland* endoplasmic reticulum</td>
<td>0.47</td>
<td>12.7</td>
<td>ND</td>
<td>10.7$</td>
</tr>
<tr>
<td>Sheep mammary gland Golgi‡</td>
<td>176</td>
<td>27</td>
<td>420</td>
<td>6</td>
</tr>
<tr>
<td>M3 colostrum</td>
<td>18.8</td>
<td>46</td>
<td>77</td>
<td>0</td>
</tr>
<tr>
<td>P3 colostrum</td>
<td>8.8</td>
<td>164</td>
<td>75</td>
<td>18</td>
</tr>
<tr>
<td>M3 + N3 cream</td>
<td>16.1</td>
<td>73.2</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>P3 cream</td>
<td>0.9</td>
<td>67</td>
<td>ND</td>
<td>28</td>
</tr>
</tbody>
</table>

* Calculated from data in Keenan, et al. 1972 (7).
‡ Taken from data in Smith et al., 1976 (20).
§ The activity units in this case are not defined, but comparable values for rat liver Golgi and endoplasmic reticulum are 28 and 200, respectively (14).

In contrast, when galactosyltransferase is solubilized and purified from fat globule membranes it is higher in molecular weight and different in properties$^1$ than the soluble enzymes of milk and colostrum. However, it resembles in size the galactosyltransferase purified from sheep mammary gland Golgi membranes (20). This evidence together with (a) the presence in our preparation of only low levels of vesicular material and few milk aqueous phase components such as casein (Fig. 1) and (b) the higher specific activity of galactosyltransferase in the membranes in comparison with milk or colostrum (0.2 and 2 mU/mg, respectively) suggests that the high level of galactosyltransferase in M3 and N3 does not originate from.

**FIGURE 2 (a)** Gel filtration profile of galactosyltransferase purified from colostrum fat globule membrane, compared with that of the enzyme purified from the aqueous phase of colostrum. Fat globule membrane enzyme, full line; colostrum enzyme, broken line. The column of Bio-Gel P150 (200-400 mesh) (1.5 × 95 cm) was equilibrated and eluted with 0.5 M NaCl containing 50 mM cacodylate, pH 7.4, and 1 mM β-mercaptoethanol at 4°C. The elution positions of marker proteins are indicated by arrows. Catalase was used as an internal marker protein in all runs, but did not affect the elution position of any protein. (b) Gel filtration profile of partially purified galactosyltransferase, obtained from washing cream at 38-40°C. The elution profile is compared with those of colostrum galactosyltransferase and fat globule membrane galactosyltransferase. The conditions were as for Fig. 2a. Fat globule membrane enzyme, dotted line; colostrum enzyme, broken line; partially purified enzyme from 38-40°C washes, full line.
the inclusion into vesicles of the aqueous phase of milk.

Our results provide enzymic evidence for the cellular origin of the fat globule membrane. Arguments previously put forward supporting a plasmalemma origin for bovine milk fat globule membranes have been less compelling than enzymic evidence. For example, it has been remarked that the phospholipid composition reported for the bovine fat globule membrane resembles that of the plasma membrane rather than intracellular membranes. However, the composition is equally consistent with a membrane intermediate between the plasmalemma and the Golgi membranes, both of which have a high content of sphingomyelin in comparison with all other membranes. It is also possible that the phospholipid composition may be changed during purification. A recent report of the phospholipid composition of the fat globule membrane from mouse milk gives values similar to those for the composition of Golgi rather than plasma membranes (3). The SDS acrylamide gel patterns of total proteins from the different membranes have been used to support the case for the plasmalemma origin for the fat globule membrane. Gel patterns obtained with Golgi membranes may be dominated by heavily staining intravesicular proteins which are absent from plasma membrane and fat globule membrane preparations. Recent studies suggest that the SDS gel patterns of proteins from Golgi and plasma membranes of rat liver are almost indistinguishable (5). Gel electrophoresis patterns are obviously not a sensitive criterion for determining the origin of membranes.

Analyses of the apical plasmalemma of the lactating mammary cell are not accessible, and it is not possible therefore to exclude this membrane as the immediate origin of the fat globule membrane. It is possible, but unlikely, that in its enzymic composition the apical membrane may partially resemble the Golgi membranes, owing to the continuous flux of membranes from the Golgi region during secretion. However, our results, when taken in conjunction with electron microscope studies by Wooding and others, do suggest that the milk fat globule membrane is derived in large part from secretory vacuole membranes of Golgi origin, but that some plasma membrane is acquired during extrusion from the cell. The involvement of Golgi apparatus in the secretion of all major components of milk, i.e. lactose, salts, protein, and fat, suggests that this organelle may mediate the regulation of milk secretion. The cream and colostrum fat globule membranes are excellent sources of Golgi-specific enzymes, and studies of the structure and properties of the membrane-derived galactosyltransferase are in progress.

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