FREEZE-FRACTURE OBSERVATIONS OF THE LACTATING RAT MAMMARY GLAND

Membrane Events during Milk Fat Secretion

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ABSTRACT

Membrane events during milk fat secretion were analyzed by freeze-fracture of the rat mammary gland. Two modes of milk fat secretion were observed: extrusion of fat droplets surrounded by a portion of the apical plasma membrane of the alveolar epithelial cells and, less frequently, release into the alveolar lumen of fat droplets contained in intracytoplasmic vacuoles. The extrusion process consists of two asynchronous events: clearing of membrane particles (probably including integral membrane proteins) and bulging of the apical plasma membrane. Most fat droplets are extruded with a bilayer membrane envelope (milk fat globule membrane) partially devoid of particles. The segregation of membrane particles may represent the onset of a process of structural degradation of the milk fat globule membrane.

KEY WORDS membrane structure · secretion · lactation · milk fat globule membrane · freeze-fracture

Under the complex hormonal environment and other stimuli of pregnancy and onset of lactation, the resting mammary gland is transformed into an actively secreting organ (8, 40). This process of growth and differentiation leads to the extensive development in the alveolar epithelial cells of rough endoplasmic reticulum, Golgi apparatus, and other endomembranes which are involved with the plasma membrane in the synthesis and secretion of milk (2–4, 9, 15, 16, 20, 28, 35, 37, 42).

The ultrastructural, physiological, and biochemical aspects of lactation have been investigated (4, 9, 16, 23, 35, 42). Early ultrastructural observations demonstrated that, during milk fat secretion, most lipid droplets are extruded into the alveolar lumen with a bilayer membrane envelope derived from the cell plasma membrane (2, 3). This secretion mechanism, as well as biochemical (10, 21, 28, 30) and morphological (10) evidences, led to the deduction that in freshly drawn milk the fat globule is still surrounded by a membrane (milk fat globule membrane, MFGM; for review see references 1, 29). Other studies provided additional aspects of milk fat secretion, namely on the origin, composition, and mechanism of formation and stabilization of the MFGM: (*a*) Golgi vesicles have been reported to contribute directly to the formation of the MFGM (45); (*b*) lipid droplets included in intracellular secretory vacuoles can be secreted without participation of the plasmalemma (47); (*c*) after secretion, the membrane of the milk fat globule may undergo a process of vesiculation and fragmentation and is gradually lost (14, 38, 44, 46); (*d*) some fat droplets are extruded with a crescent of cytoplasm

(12, 13, 22, 36, 37, 39, 48); and (*e*) enzymic and chemical analysis of the MFGM detected "marker enzymes" of Golgi apparatus (24, 25, 34) and endoplasmic reticulum (17, 25).

We have studied the freeze-fracture morphology of the apical membrane of the rat mammary gland during lactation. Our results show that the process of extrusion of fat droplets generally coexists with regional segregation of integral components of the apical plasmalemma.

MATERIALS AND METHODS

The inguinal mammary glands of Sprague-Dawley rats, aged 8–10 wk during the first pregnancy and 20–22 wk during the second pregnancy, were used 2 days before and 1, 4, 6, and 14 days after parturition. The animals were anesthetized with 0.2 g of sodium pentobarbital, intraperitoneally, and the tissue samples were fixed by immersion in 3% glutaraldehyde in 0.1 M sodium cacodylate, pH 7.4, for 2 h at 4°C. After fixation the specimens were washed in buffer, gradually impregnated in 28% glycerol in 0.1 M sodium cacodylate, pH 7.4, placed on gold specimen holders, and rapidly frozen in partially solidified Freon 22 (26). The specimens were freeze-fractured at −110°C in a Balzers 300 apparatus (Balzers AG, Balzers, Lichtenstein) equipped with a turbomolecular pump, shadowed at 45° with a platinum-carbon electron gun, and reinforced by carbon at 90°. The replicas were cleaned with a 5% sodium hypochlorite solution containing 2.5% sodium deoxycholate and 2.5% Triton X-100 for 24 h, followed by a 2:1 chloroform:methanol mixture, rinsed in distilled water, mounted on uncoated grids, and observed with a Jeol 100C electron microscope at 80 kV. The micrographs are mounted with shadow direction from bottom to top; shadows are white. Interpretation and nomenclature of fracture faces are given elsewhere (32).

RESULTS

The lactating rat mammary alveoli are formed by actively secreting cuboidal epithelial cells, arranged in a monolayer around a lumen where casein micelles, fat droplets, and other secretory products are collected (Fig. 1). The apical plasma membranes of these cells delimitate the lumen and are directly involved in the secretory process. The lateral membranes between neighboring cells present junctional complexes for intercellular contact and sealing of the alveolar lumen (Fig. 1; see reference 33 for illustrations). The basal membranes, in close relation with myoepithelial cells, capillaries, and extracellular connective structures that surround the alveoli, are involved in the uptake of blood-borne substances essential for milk biosynthesis.

Before the onset of fat secretion, a large amount of fat droplets is accumulated in the apex of the mammary epithelial cells. The lumina of the alveoli are already filled with numerous milk protein components. The casein granules are easily identified by their typical profiles consisting of flat, circular clusters of rugosities without enveloping membrane. The fracture-face A of the apical plasma membrane presents an apparently random distribution of numerous membrane particles, and views of microvilli. The particles are also present in the fracture faces of the microvilli.

The observation of the apical plasmalemma during lactation revealed structural alterations related to milk fat secretion (Figs. 2–11, and 13). A mild or pronounced bulging of microvilli-free regions of the membrane was observed. These areas were either smooth, i.e. devoid of particles (Fig. 2) or, less frequently, showed no alteration of particle distribution in relation to the surrounding apical plasmalemma. Fractures which allowed for simultaneous view of the plasma membrane and cytoplasm (Figs. 3, 5, and 7–9) showed that the bulges were occupied by fat droplets, sometimes associated with small portions of intracellular components. The events of fat droplet extrusion could be reconstructed from views of A and B faces of the apical plasma membrane and cross-fracture profiles. These included the gradual enveloping of the fat droplets by the apical plasmalemma. Frequently, the fracture-face A of the fat droplet membrane envelope displayed a smooth, particle-free surface in continuity with apical plasma membrane regions which presented an unaltered pattern of particle distribution (Figs. 3–6). Equivalent aspects of membrane particle clearing on the fracture-face B were also observed (Fig. 8). Less frequently, the fat droplets were enveloped by a membrane presenting a fracture face with particles (Fig. 9). Intermediate aspects consisting of emerging droplets surrounded by a membrane with some particle-free areas and the consecutive extrusion of more than one fat droplet were also observed. Other observations suggested that the secretion of larger fat droplets could also occur by extrusion of its components enveloped by a particle-free membrane as if the fat was being squeezed through a "locus minoris resistentiae" of the cell membrane (Figs. 10 and 11).

In the cytoplasm of the lactating cells most fat droplets showed smooth fracture faces. By cross fracture they often displayed a lamellar or paralamellar organization. Intracellular vacuoles con-

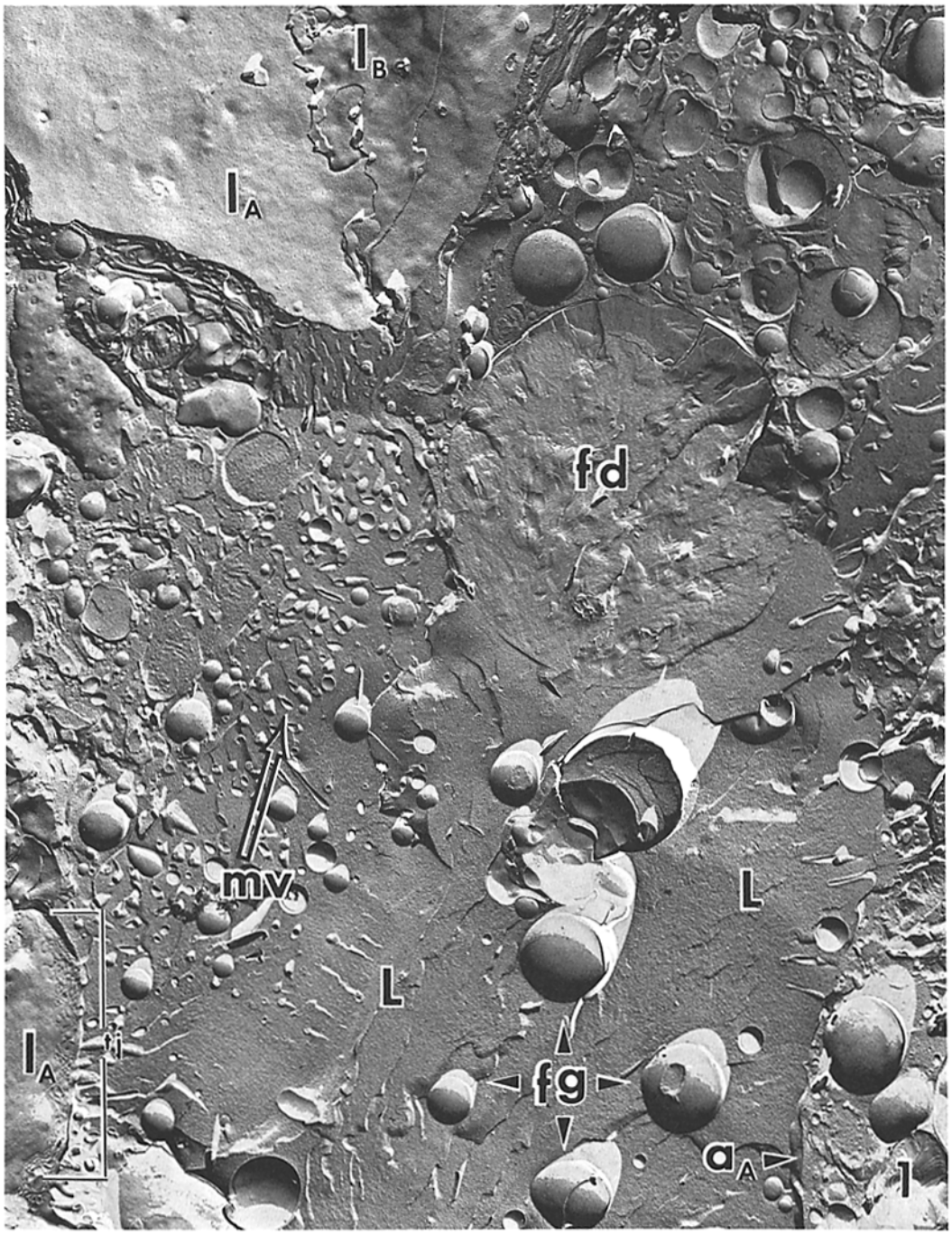
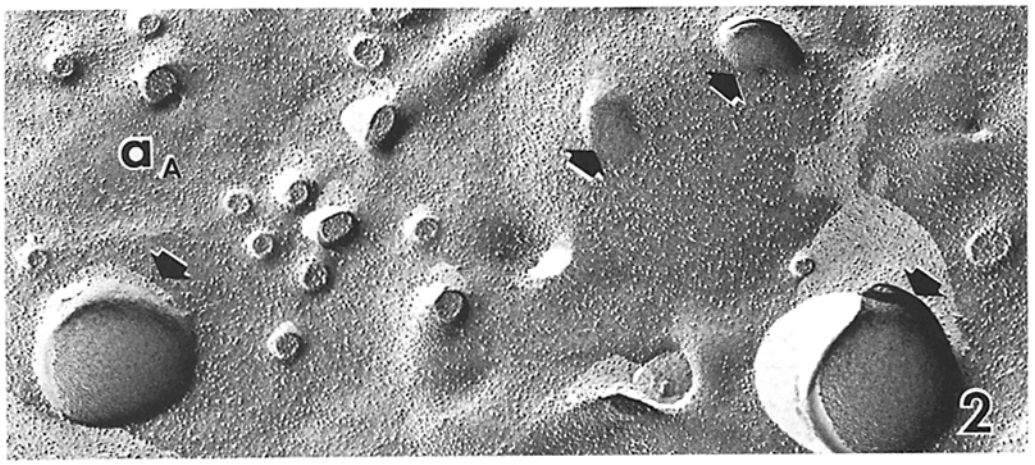


FIGURE 1 The freeze-fracture survey of a lactating alveolus of rat mammary gland shows the apex of several cells, a segment of apical plasma membrane (a_A), microvilli on longitudinal and cross views (*mv*), and lateral plasma membranes (l_A, l_B) where the tight junction band (*tj*) can be seen. In the alveolar lumen (*L*), fat flobules (*fg*) show a smooth fracture face. A cross fracture through a large fat droplet (*fd*) during extrusion is also observed. × 10,000.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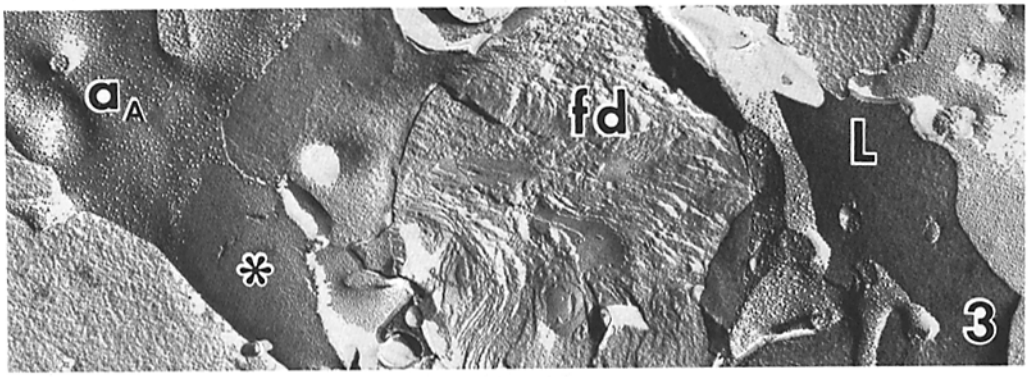
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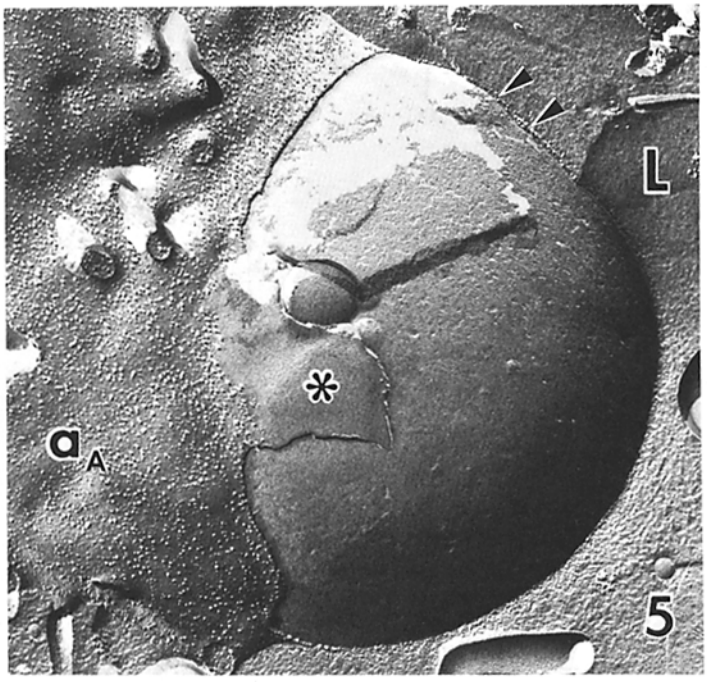
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taining lipid droplets surrounded by numerous, small (fat?) globules with a smooth fracture face were occasionally observed (Fig. 12). The following observation suggested that the secretion of these fat droplets followed a mechanism different from that of fat droplets which have no membrane: intracytoplasmic vacuoles were observed in close contact with the apical plasma membrane, and the region of the alveolar lumen directly opposite to these vacuoles presented emerging particle-free globules suggesting that fat was being released through a small opening in the plasmalemma (Fig. 13).

In the alveolar lumen, milk fat appeared as globules, heterogeneous in size and structure (Figs. 1, 14, and 15). Fracture usually followed a plane close to the surface; most fracture faces were smooth (Fig. 1).[1] Less frequently, a membrane with particles was seen around one or more globules (Fig. 14). In this case, the pattern of distribution of particles on the membranes enveloping fat globules ranged from uniform to segregated (Fig. 14) or reticulated. Cross fracture of the fat in the lumen revealed aspects similar to those observed in the cytoplasm (Figs. 14 and 15). Fat globules surrounded by cytoplasmic crescents were also observed. Other observations suggested the incorporation of small lipid droplets in larger globules, as well as the blebbing off of segments of the membrane envelope of the fat globules (Fig. 15).

[1] The smooth surface of most fat globules observed in the alveolar lumen probably corresponds to the fracture face of the fat globule membrane in view of the clearing of membrane particles during extrusion, and the concept of membrane splitting in freeze-etching (31). In some instances it might also correspond to the fracture through an outer lipid lamella of the fat globule. In this case, the cross-fracture profile of the fat globule membrane should often be evident (see Figs. 5 and 14).

DISCUSSION

Analysis of freeze-fracture results indicates two possible modes of milk fat secretion: (*a*) extrusion of the fat globule surrounded by a portion of the apical plasma membrane, the prevalent mechanism; and (*b*) release into the alveolar lumen of free fat globules, i.e. without enveloping membrane, a process observed much less frequently and restricted to the secretion of small fat droplets contained within intracellular vacuoles.

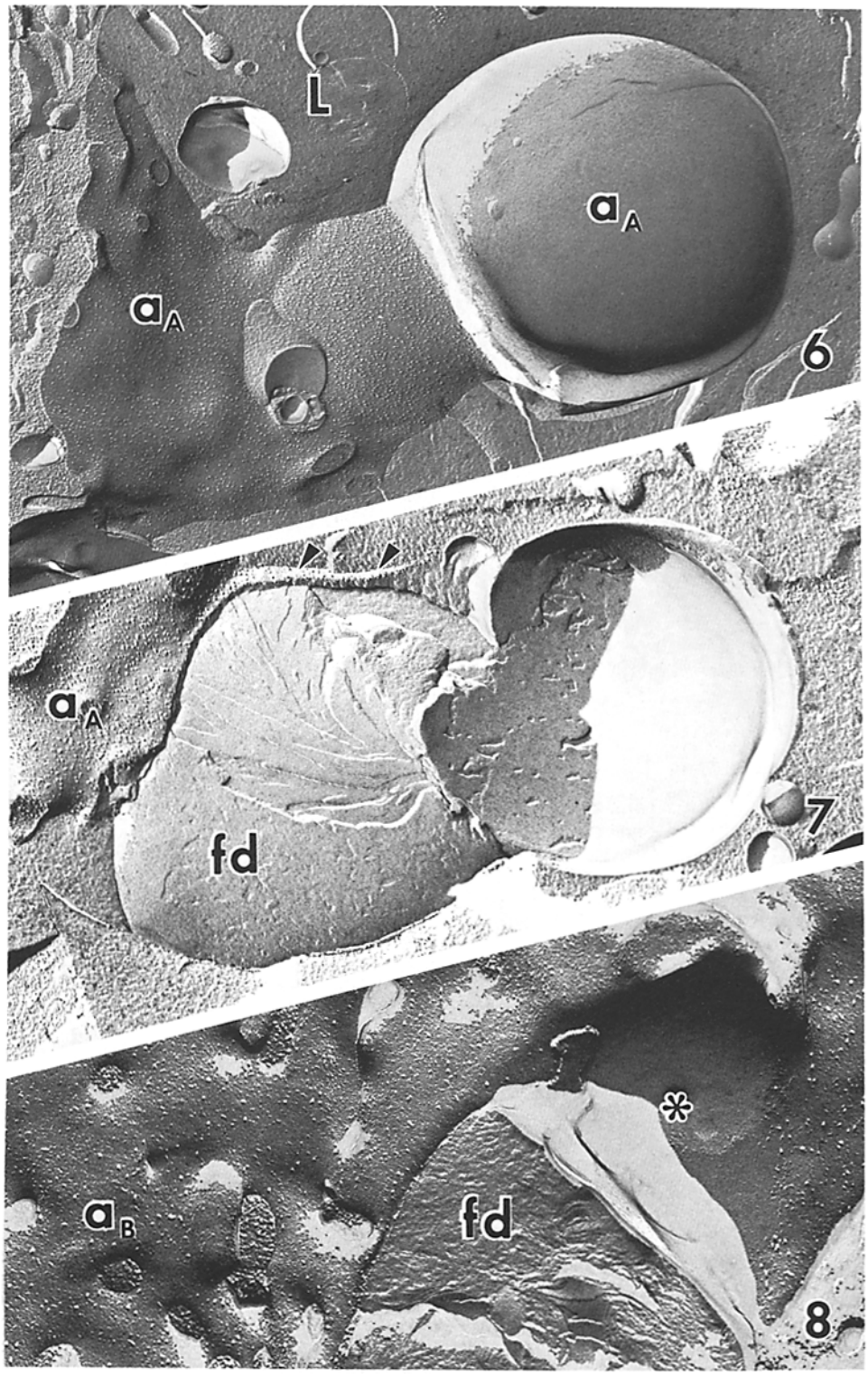
The fracture faces of the apical plasmalemma during the extrusion of fat droplets illustrate two membrane events: clearing of membrane particles and bulging of the plasma membrane. In some instances, as the droplets are pushed out of the cell there is a progressive clearing of particles in the areas of membrane bulging so that, at the moment of separation from the cell, the globule is surrounded by a bilayer membrane without particles. In others, the extrusion process is not preceded nor accompanied by clearing of membrane particles, and so, the extruded fat globules are enveloped by a membrane with particles. Intermediate aspects, i.e. fat droplets extruded with a variable degree of membrane particle clearing, are also found. In consequence, our results demonstrate an asynchronism between bulging of the plasma membrane and redistribution of integral membrane components, as revealed by the progressive clearing of particles. The extrusion of fat droplets with a bilayer membrane envelope supplied by the apical plasmalemma of mammary epithelial cells has been described in thin-section studies in several species (2–4, 11–13, 16, 22, 35, 36, 39, 41–43). Our freeze-fracture results

FIGURE 2 Initial events of fat droplet extrusion. The fracture-face A (a_A) of the apical plasma membrane shows bulging of particle-free areas (arrows). Microvilli are observed on cross fracture. × 40,000.

FIGURE 3 Fracture-face A (a_A) of an area of bulging of the apical plasma membrane, allowing a simultaneous view, on cross fracture, of a fat droplet (*fd*) underneath. The smooth area of the apical plasmalemma (*) is clearly demarcated from the area with particles. *L*, alveolar lumen. × 30,000.

FIGURE 4 High magnification of the transition from the area with particles to the smooth area of the apical plasmalemma of Fig. 3 shows the continuity of these membrane segments. × 120,000.

FIGURE 5 Fracture-face A (a_A) of an area of the apical plasmalemma with particle clearing (*). The outer lipid lamella of a fat droplet is exposed, and the cross-fracture profile of the surrounding apical membrane is evident (arrowheads).[1] *L*, alveolar lumen. × 40,000.


L
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show that this process is often accompanied by segregation of membrane integral components.[2] It is also possible that, during extrusion, peripheral membrane components at the inner surface are segregated. This could facilitate the extensive bulging of the apical cell membrane during the extrusion process and account for the absence of actin and spectrin in MFGM preparations (19).[3]

Less frequently, fat droplets seemed also to be released from intracytoplasmic vacuoles into the alveolar lumen. Our findings agree with previous thin-section observations (47). This exocytotic mechanism presumably involves the fusion of the secretory vacuoles, containing lipid droplets and other secretory products (47), with the plasmalemma, and results in the release of fat globules without an enveloping membrane.

Morphological and biochemical studies of secretion in different cells, including mammary epithelial cells during lactation, provide evidence for a continuous turnover between endomembranes and the plasma membrane segments related to the secretory processes (20). These events could explain the presence of Golgi (24, 25, 34) and endoplasmic reticulum enzymes (17, 25) in MFGM preparations and their phospholipid composition intermediate between that of the Golgi and the plasma membranes (34), even if contaminations from cytoplasmic components extruded with some fat droplets could be avoided (29) or if the Golgi vesicles did not contribute directly to the formation of the MFGM (45). In consequence, the enzymic or chemical analysis alone cannot provide definitive evidence for the origin of the membrane of the fat globule, but must be seen in perspective with cell membrane dynamics. After fat secretion, electron microscope observations suggest that, in some globules, the membrane is gradually lost by a process of fragmentation and vesiculation (5, 14, 38, 44, 46). It is possible that during and after structural degradation of the membrane envelope of the fat globules, lipid and protein components of the initial (44) fat globule membrane may be adsorbed to the surface of the fat globules (14). Thus, the unit membrane profiles observed on thin sections of MFGM preparations, obtained from cream by freezing, sonication, and other procedures (20, 21), may represent not only initial MFGM but also reflect bilayered structures reconstituted before and during the isolation procedures. The structural rearrangement of the MFGM during or after secretion has been postulated to account for some biochemical and ultrastructural results (14, 21, 27). However, it has been recently assumed that the overall composition of the MFGM relative to the plasma membrane of the alveolar epithelial cells is not affected (19). Unfortunately, analysis of the apical plasmalemma of the lactating mammary cell is not accessible (34).

Our freeze-fracture observations suggest that the events which occur within the apical membrane during milk fat secretion may account for some compositional (19), physicochemical (7), and ultrastructural (14, 21) differences between MFGM and plasma membrane preparations. The mechanism of segregation of membrane particles is, at present, unclear. It is possible that the

[2] Although clearing of membrane particles was more frequently observed, it may eventually not correspond to the prevalent mechanism if the extrusion without particle segregation is accomplished faster.

[3] The segregation of actin during extrusion is, in view of the particle rearrangements which we observed, a preferable alternative to account for the absence of actin in MFGM preparations. Mammary epithelial cell surface antigens (6) and concanavalin A binding sites (18) have been detected in MFGM. It is not established, however, whether these sites correspond to integral or peripheral membrane proteins.

FIGURE 6 Final events of fat droplet extrusion. Membrane particles are absent on fracture-face A (a_A) of the apical plasma membrane which envelops the fat droplet. *L*, alveolar lumen. × 27,000.

FIGURE 7 Stage equivalent to Fig. 6. The fracture process exposed the lamellar appearance of the fat droplet (*fd*). Membrane particles are observed on the area of the plasma membrane which surrounds but is not in direct contact with the fat droplet (arrowheads). a_A, fracture-face A of the apical plasma membrane. × 40,000.

FIGURE 8 Fat droplet extrusion as observed from the inside of a cell. A smooth area of membrane bulging (*) under the fat droplet (*fd*) is demarcated from the surrounding fracture-face B (a_B) of the apical membrane. × 40,000.

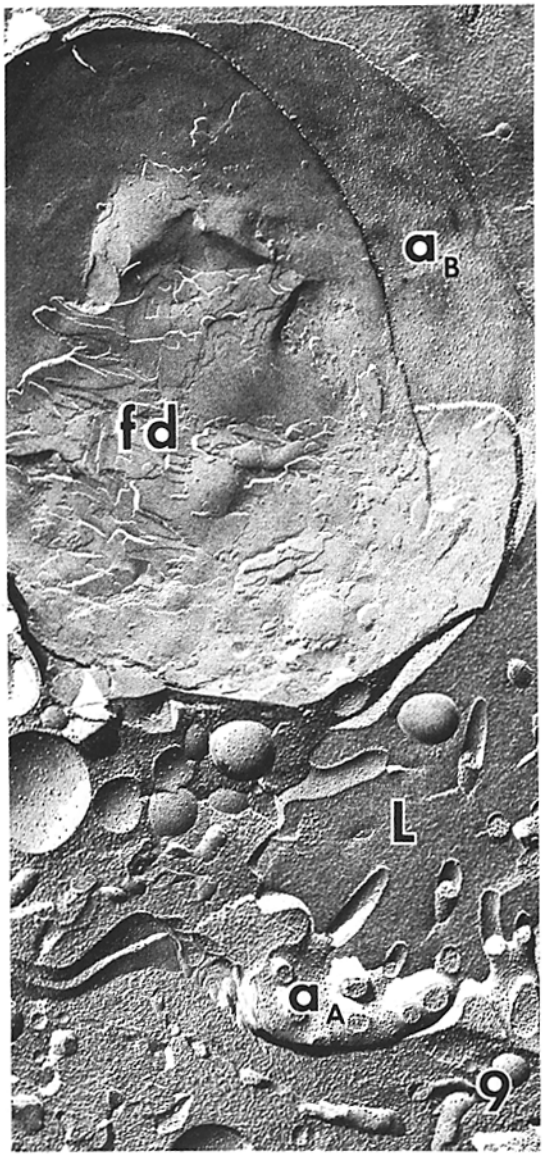


FIGURE 9 Emergence of a fat droplet (*fd*) surrounded by a membrane with particles (a_B). a_A, fracture-face A of the apical plasma membrane; *L*, alveolar lumen. × 30,000.

reorganization of the membrane integral components results from the interaction between the fat droplet core and the apical plasma membrane, during extrusion. It might also involve the concomitant displacement of peripheral membrane proteins at the inner surface.[3]

In summary, the process of segregation of integral components from areas of plasmalemma which envelop the fat droplets may differentially restrict the membrane components which form the milk fat globule membrane. This could correspond to an initial event of membrane structural degradation, which progresses after extrusion. Although most of the components found in purified preparations of MFGM were at one time associated with the apical plasmalemma, the fat globule membrane cannot be considered as structurally identical to the apical membrane of the mammary epithelial cells.

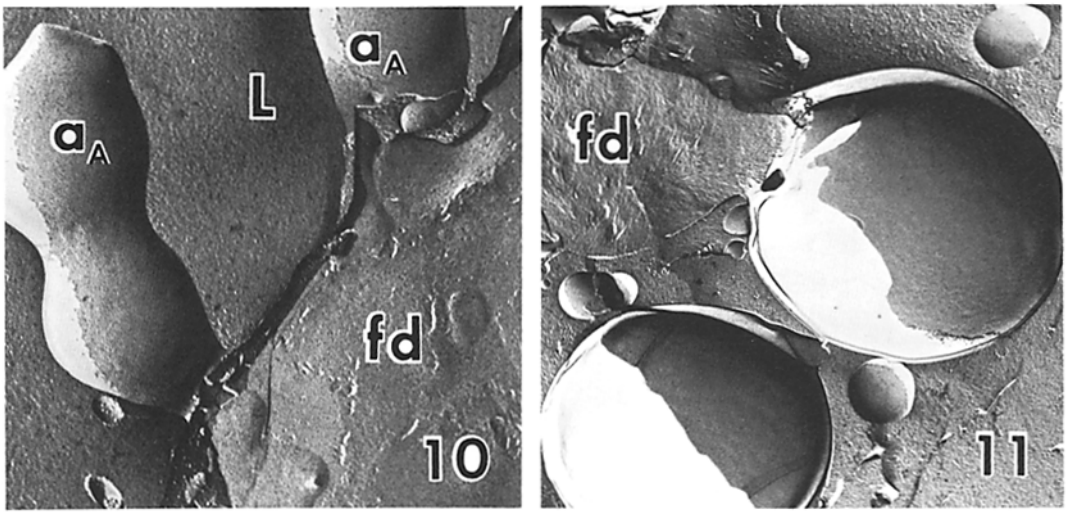
We wish to thank Dr. Pietro M. Gullino for helpful discussions, and Messrs. Clifford Parkison and Bela Berghoffer for technical assistance.

A. Peixoto de Menezes was supported in part by Instituto Nacional de Investigação Científica, Portugal.

Received for publication 13 July 1977, and in revised form 24 October 1977.

REFERENCES

1. ANDERSON, M., and T. E. CAWSTON. 1975. Reviews of the progress of dairy science. The milk-fat globule membrane. *J. Dairy Res.* **42**:459–483.
2. BARGMANN, W., K. FLEISCHHAUER, and A. KNOOP. 1961. Über die Morphologie der Milchsekretion. II. Zugleich eine Kritik am Schema der Sekretions-morphologie. *Z. Zellforsch. Mikrosk. Anat.* **53**:545–568.
3. BARGMANN, W., and A. KNOOP. 1959. Über die Morphologie der Milchsekretion. Licht- und elektronenmikroskopische Studien an der Milchdrüse der Ratte. *Z. Zellforsch. Mikrosk. Anat.* **49**:344–388.
4. BARGMANN, W., and U. WELSCH. 1969. On the ultrastructure of the mammary gland. *In* Lactogenesis: The Initiation of Milk Secretion at Parturition. M. Reynolds and S. J. Folley, editors. University of Pennsylvania Press, Philadelphia, Pa. 43–52.
5. BAUER, H. 1972. Ultrastructural observations on the milk fat globule envelope of cow's milk. *J. Dairy Sci.* **55**:1375–1387.
6. CERIANI, R. L., K. THOMPSON, J. A. PETERSON, and S. ABRAHAM. 1977. Surface differentiation antigens of human mammary epithelial cells carried on the human milk fat globule. *Proc. Natl. Acad. Sci. U. S. A.* **74**:582–586.
7. CHANDAN, R. C., J. CULLEN, and D. CHAPMAN. 1972. Physicochemical analysis of the bovine milk fat globule membrane. III. Proton magnetic resonance spectroscopy. *J. Dairy Sci.* **55**:1232–1236.
8. COWIE, A. T. 1961. The hormonal control of milk secretion. *In* Milk: The Mammary Gland and Its



FIGURES 10 and 11. Extrusion of fat droplets (*fd*) suggesting the sqeezing through a "locus minoris resistentiae" of the apical plasma membrane. Fig. 10, the fat material is surrounded by the apical plasma membrane, without particles (a_A). *L*, alveolar lumen. × 35,000. Fig. 11, dumbbell profile as a result of a constriction at the plasma membrane level. × 17,000.

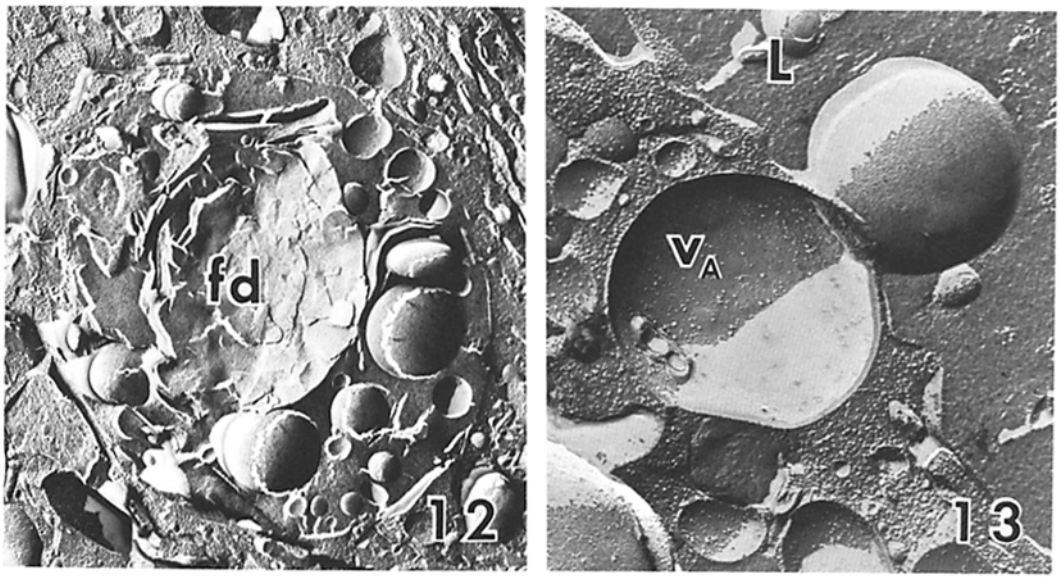
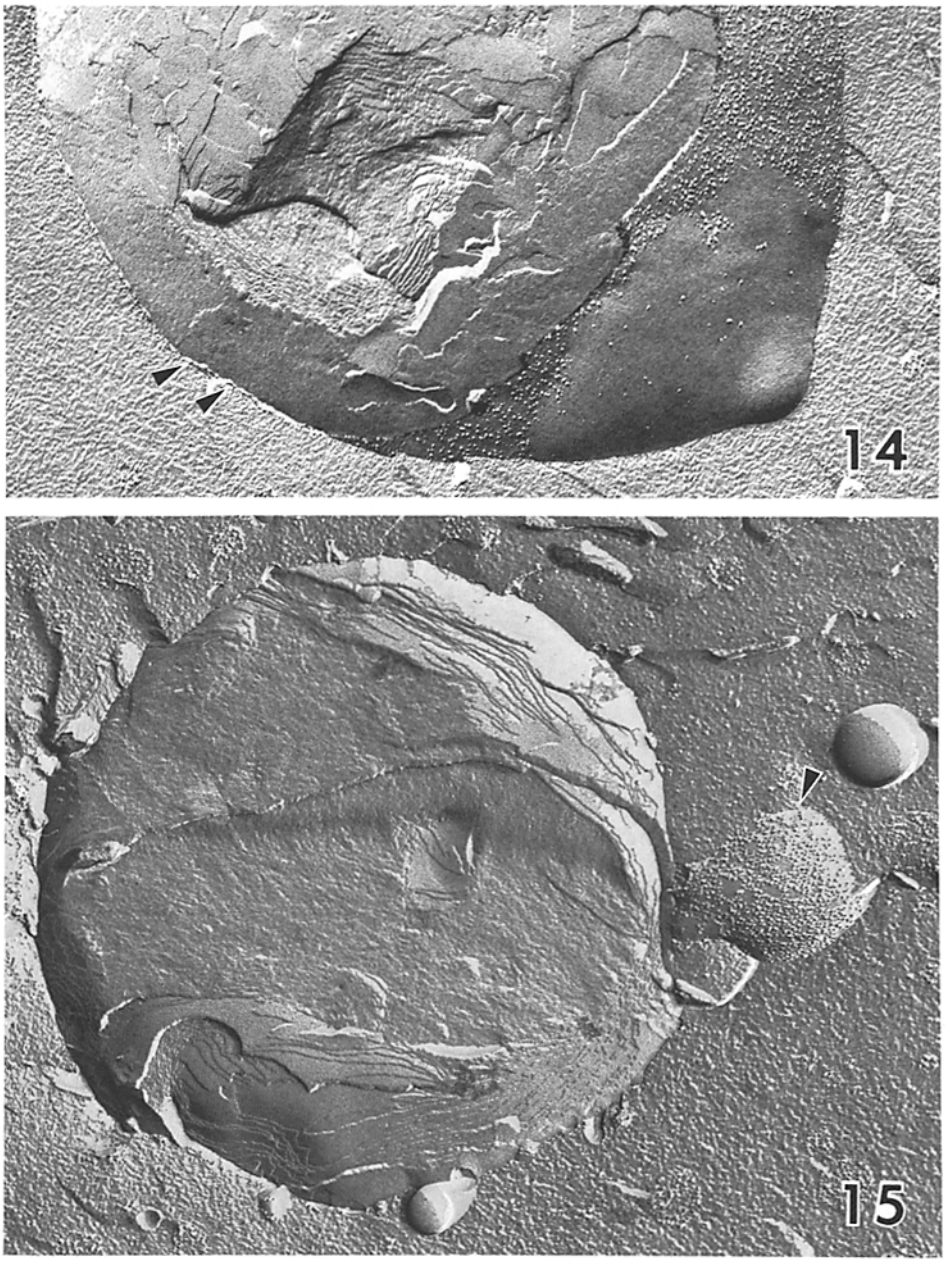


FIGURE 12. Intracytoplasmic vacuole containing a fat droplet (*fd*). × 17,000.

FIGURE 13 Intracellular vacuole with a membrane with particles (v_A) in close contact with the apical plasmalemma. From the opposite outer side of the cell membrane a globule with a smooth fracture face is emerging. *L*, alveolar lumen. × 35,000.



FIGURES 14 and 15. Aspects of globules in the alveolar lumen. Fig. 14, fracture-face A of a fat globule membrane with a smooth particle-free area. The arrowheads point to the cross-fracture profile of the fat globule membrane.[1] × 38,000. Fig. 15, vesiculation process of the fat globule membrane. The segment of blebbing (arrowhead) presents a fracture face with an irregular distribution of particles. × 38,000.

Secretion. S. K. Kon and A. T. Cowie, editors. Academic Press, Inc., New York. **1**:163–203.

9. Davis, C. L., and D. E. Bauman. 1974. General metabolism associated with the synthesis of milk. *In* Lactation: A Comprehensive Treatise. B. L. Larson and V. R. Smith, editors. Academic Press, Inc., New York. **2**:3–30.
10. Dowben, R. M., J. R. Brunner, and D. E. Philpott. 1967. Studies on milk fat globule membranes. *Biochim. Biophys. Acta.* **135**:1–10.
11. Feldman, J. D. 1961. Fine structure of the cow's udder during gestation and lactation. *Lab. Invest.* **10**:238–255.
12. Girardie, J. 1968. Histo-cytomorphologie de la glande mammaire de la souris C3H et de trois autres rongeurs. *Z. Zellforsch. Mikrosk. Anat.* **87**:478–503.
13. Helminen, H. J., and J. L. E. Ericsson. 1968. Studies on mammary gland involution. I. On the ultrastructure of the lactating mammary gland. *J. Ultrastruct. Res.* **25**:193–213.
14. Henson, A. F., G. Holdsworth, and R. C. Chandan. 1971. Physicochemical analyses of the bovine milk fat globule membrane. II. Electron microscope. *J. Dairy Sci.* **54**:1752–1763.
15. Hollmann, K. H. 1969. Quantitative electron microscopy of sub-cellular organization in mammary gland cells before and after parturition. *In* Lactogenesis: The Initiation of Milk Secretion at Parturition. M. Reynolds and S. J. Folley, editors. University of Pennsylvania Press, Philadelphia, Pa. 27–41.
16. Hollmann, K. H. 1974. Cytology and fine structure of the mammary gland. *In* Lactation: A Comprehensive Treatise. B. L. Larson and V. R. Smith, editors. Academic Press, Inc., New York. **1**:3–95.
17. Jarasch, E. D., G. Bruder, T. W. Keenan, and W. W. Franke. 1977. Redox constituents in milk fat globule membranes and rough endoplasmic reticulum from lactating mammary gland. *J. Cell Biol.* **73**:223–241.
18. Keenan, T. W., W. W. Franke, and J. Kartenbeck. 1974. Concanavalin A binding by isolated plasma membranes and endomembranes from liver and mammary gland. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **44**:274–278.
19. Keenan, T. W., C. Freudenstein, and W. W. Franke. 1977. Membranes of mammary gland. XIII. A lipoprotein complex derived from bovine milk fat globule membrane with some preparative characteristics resembling those of actin. *Cytobiologie.* **14**:259–278.
20. Keenan, T. W., D. J. Morré, and C. M. Huang. 1974. Membranes of the mammary gland. *In* Lactation: A Comprehensive Treatise. B. L. Larson and V. R. Smith, editors. Academic Press, Inc., New York. **2**:191–233.
21. Keenan, T. W., D. J. Morré, D. E. Olson, W. N. Yunghans, and S. Patton. 1970. Biochemical and morphological comparison of plasma membrane and milk fat globule membrane from bovine mammary gland. *J. Cell Biol.* **44**:80–93.
22. Kurosumi, K., Y. Kobayashi, and N. Baba. 1968. The fine structure of mammary glands of lactating rats, with special reference to the apocrine secretion. *Exp. Cell Res.* **50**:177–192.
23. Linzell, J. L., and M. Peaker. 1971. Mechanism of milk secretion. *Physiol. Rev.* **51**:564–597.
24. Martel, M. B., and R. Got. 1976. Transfert du galactose dans les membranes des globules lipidiques du lait humain. *Biochim. Biophys. Acta.* **436**:789–799.
25. Martel-Pradal, M. B., and R. Got. 1972. Presence d'enzymes marqueurs des membranes plasmiques, de l'appareil de Golgi et du reticulum endoplasmique dans les membranes des globules lipidiques de lait maternel. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **21**:220–222.
26. Martinez-Palomo, A., P. Pinto da Silva, and B. Chavez. 1976. Membrane structure of *Entamoeba histolytica*: fine structure of freeze-fractured membranes. *J. Ultrastruct. Res.* **54**:148–158.
27. Mather, I. H., and T. W. Keenan. 1975. Studies on the structure of milk fat globule membrane. *J. Membr. Biol.* **21**:65–85.
28. Patton, S., and F. M. Fowkes. 1967. The role of the plasma membrane in the secretion of milk fat. *J. Theor. Biol.* **15**:274–281.
29. Patton, S., and T. W. Keenan. 1975. The milk fat globule membrane. *Biochim. Biophys. Acta.* **415**:273–309.
30. Patton, S., and E. G. Trams. 1971. The presence of plasma membrane enzymes on the surface of bovine milk fat globules. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **14**:230–232.
31. Pinto da Silva, P., and D. Branton. 1970. Membrane splitting in freeze-etching. Covalent bound ferritin as a membrane marker. *J. Cell Biol.* **45**:598–605.
32. Pinto da Silva, P., and M. L. Nogueira. 1977. Membrane fusion during secretion. A hypothesis based on electron microscope observations of *Phytophthora palmivora* zoospores during encystment. *J. Cell Biol.* **73**:161–181.
33. Pitelka, D. R., S. T. Hamamoto, J. G. Duafala, and M. K. Nemanic. 1973. Cell contacts in the mouse mammary gland. I. Normal gland in postnatal development and the secretory cycle. *J. Cell Biol.* **56**:797–818.
34. Powell, J. T., U. Järlfors, and K. Brew. 1977. Enzymic characteristics of fat globule membranes from bovine colostrum and bovine milk. *J. Cell Biol.* **72**:617–627.
35. Saacke, R. G., and C. W. Heald. 1974. Cytological aspects of milk formation and secretion. *In* Lactation: A Comprehensive Treatise. B. L. Larson

and V. R. Smith, editors. Academic Press, Inc., New York. **2:**147–189.

36. SEKHRI, K. K., D. R. PITELKA, and K. B. DEOME. 1967. Studies of mouse mammary glands. I. Cytomorphology of the normal mammary gland. *J. Natl. Cancer Inst.* **39:**459–490.
37. STEIN, O., and Y. STEIN. 1967. Lipid synthesis, intracellular transport, and secretion. II. Electron microscopic radioautographic study of the mouse lactating mammary gland. *J. Cell Biol.* **34:**251–263.
38. STEWART, P. S., and D. M. IRVINE. 1969. Electron microscopic observations of the fat globule membrane. *J. Dairy Sci.* **52:**917–918 *a* (Abstr.).
39. STOCKINGER, L., and J. ZARZICKI. 1962. Elektronenmikroskopische Untersuchungen der Milchdruse des laktierenden Meerschweinchens mit Berucksichtigung des Saugaktes. *Z. Zellforsch. Mikrosk. Anat.* **57:**106–123.
40. TUCKER, H. A. 1974. General endocrinological control of lactation. *In* Lactation: A Comprehensive Treatise. B. L. Larson and V. R. Smith, editors. Academic Press, Inc., New York. **2:**277–326.
41. WAUGH, D., and E. VAN DER HOEVEN. 1962. Fine structure of the human adult female breast. *Lab. Invest.* **11:**220–228.
42. WELLINGS, S. R. 1969. Ultrastructural basis of lactogenesis. *In* Lactogenesis: The Initiation of Milk Secretion at Parturition. M. Reynolds and S. J. Folley, editors. University of Pennsylvania Press, Philadelphia, Pa. 5–25.
43. WELLINGS, S. R., K. B. DEOME, and D. R. PITELKA. 1960. Electron microscopy of milk secretion in the C3H/Crgl mouse. I. Cytomorphology of the prelactating and the lactating gland. *J. Natl. Cancer Inst.* **25:**393–421.
44. WOODING, F. B. P. 1971. The structure of the milk fat globule membrane. *J. Ultrastruct. Res.* **37:**388–400.
45. WOODING, F. B. P. 1971. The mechanism of secretion of the milk fat globule. *J. Cell Sci.* **9:**805–821.
46. WOODING, F. B. P. 1972. Milk microsomes, viruses and the milk fat globule membrane. *Experientia (Basel).* **28:**1077–1079.
47. WOODING, F. B. P. 1973. Formation of the milk fat globule membrane without participation of the plasmalemma. *J. Cell Sci.* **13:**221–235.
48. WOODING, F. B. P., M. PEAKER, and J. L. LINZELL. 1970. Theories of milk secretion: evidence from the electron microscopic examination of milk. *Nature (Lond.).* **226:**762–764.