

## INVOLVEMENT OF VESICLE COAT MATERIAL IN CASEIN SECRETION AND SURFACE REGENERATION

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### ABSTRACT

The ultrastructure of the apical zone of lactating rat mammary epithelial cells was studied, with emphasis on vesicle coat structures. Typical 40–60-nm ID “coated vesicles” were abundant, frequently associated with the internal filamentous plasma membrane coat or in direct continuity with secretory vesicles (SV) or plasma membrane proper. Bristle coats partially or totally covered membranes of secretory vesicles identified by their casein micelle content. This coat survived SV isolation. Exocytotic fusion of SV membranes and release of the casein micelles was observed. Frequently, regularly arranged bristle coat structures were identified in those regions of the plasma membrane that were involved in exocytotic processes. Both coated and uncoated surfaces of the casein-containing vesicles, as well as typical “coated vesicles,” were frequently associated with microtubules and/or microfilaments. We suggest that coat materials of vesicles are related or identical to components of the internal coat of the surface membrane and that new plasma membrane and associated internal coat is produced concomitantly by fusion and integration of bristle coat moieties. Postexocytotic association of secreted casein micelles with the cell surface, mediated by finely filamentous extensions, provided a marker for the integrated vesicle membrane. An arrangement of SV with the inner surface of the plasma membrane is described which is characterized by regularly spaced, heavily stained membrane to membrane cross-bridges (pre-exocytotic attachment plaques). Such membrane-interconnecting elements may represent a form of coat structure important to recognition and interaction of membrane surfaces.

The epithelial cell of the lactating mammary gland provides a unique and favorable system for studies of membrane formation. In contrast to most secretory cells which produce membrane material to maintain a steady state, this cell accomplishes a considerable net synthesis of surface membrane material to replenish the apical plasma membrane expended in envelopment of milk fat globules (MFG) during secretion (9–11, 44, 58, 70, 77, 93, 142, 151, 156, 160, 161). The membrane which envelops MFG is biochemically similar, if not identical to plasma membrane from mammary gland (26, 88, 122, 123, 151, 160). During the period of maximal lactation, it can be calculated that bovine mammary epithelial cells must replace plasma membrane material equivalent to their

entire apical surface within 8–10 h (estimated from data in 44, 88, 123; and from our unpublished data). Thus, an extraordinary high rate of plasma membrane synthesis is to be expected in these cells. We have thus studied the fine structure of the apical zone of lactating rat mammary epithelial cells to determine whether there are specific morphological forms of endomembranes which are involved in continuous replacement of apical surface components. In particular, we have focused on the possible involvement of bristle coat structures in plasma membrane formation (cf. reference 50).

## MATERIALS AND METHODS

Chemicals were purchased from Merck (Darmstadt) or Serva (Heidelberg); osmium tetroxide was obtained from Degussa (Hanau). Sprague-Dawley rats, 17–26 wk of age and mostly during the second parturition, were used between the 7th and 14th days of lactation. Pups were either removed from their dams immediately (*a*) or 2 (*b*) and 4 (*c*) h before sacrifice, or separated from dams for 4 h and then returned and allowed to suckle for 1–2 min before sacrifice of the dams (*d*). Animals were sacrificed by cervical dislocation, and small pieces of mammary tissue from the inguinal glands were removed immediately and fixed according to one of the following schedules.

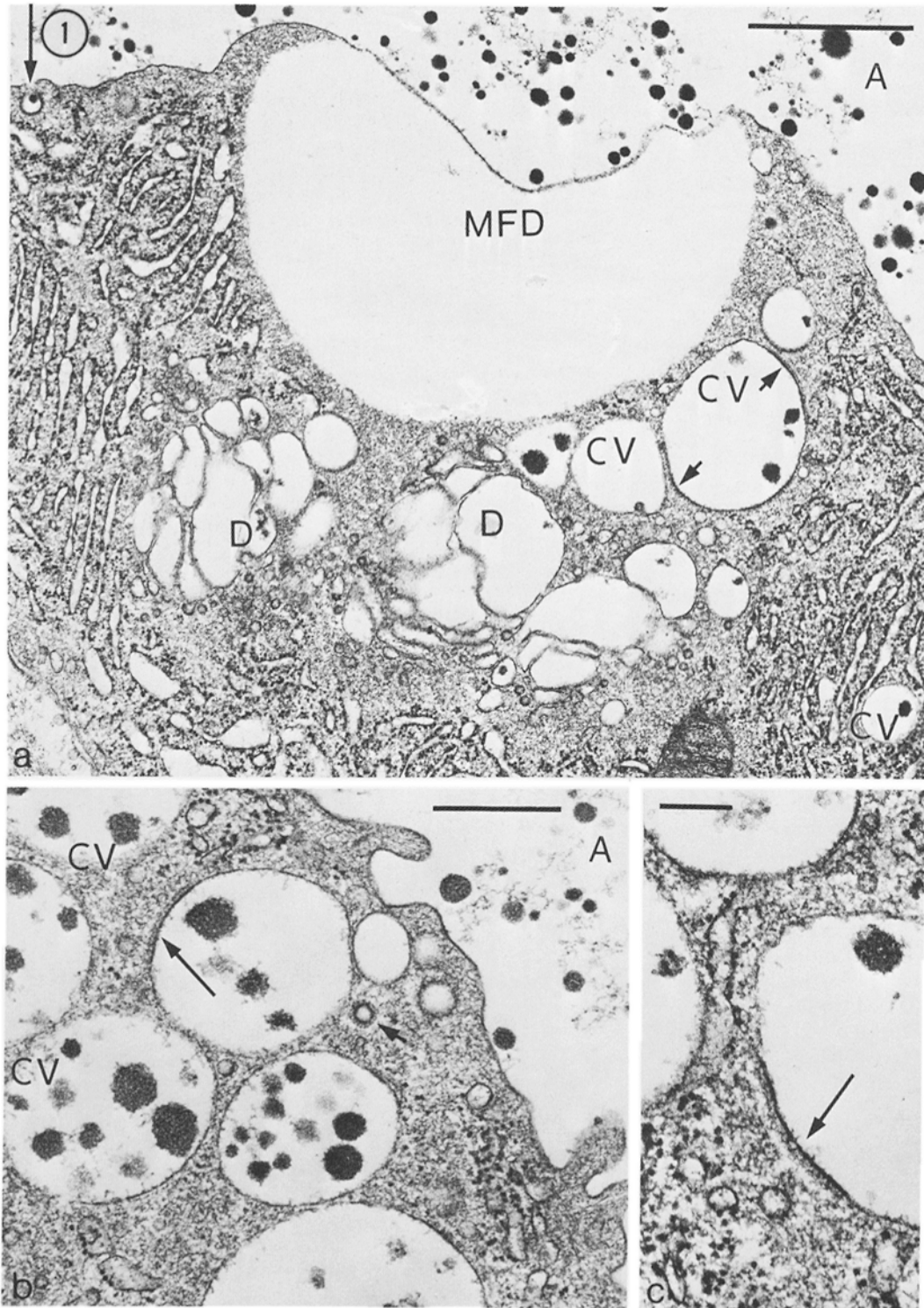
I Initial fixation in 2.5% glutaraldehyde buffered with 0.05 M sodium cacodylate (pH 7.2) containing 50 mM KCl, 2.5 mM MgCl<sub>2</sub>, and 1.25 mM CaCl<sub>2</sub> at room temperature or at 37°C for 30–60 min. Samples were then held in the cold (about 4°C) for an additional 15

min, washed repeatedly with cold cacodylate buffer, and postfixed in 4% aqueous osmium tetroxide for 4 h; II fixation as described under I, but with all steps performed at 0°–7°C; III fixation as described under I, but without ions in the initial fixative solution; IV fixation as under II, but without ions in the fixative solution; V initial fixation in a freshly prepared, cold mixture of osmium tetroxide and glutaraldehyde for 30 min as described (54); VI initial fixation in freshly prepared, cold mixtures of 0.55% glutaraldehyde and 3.6% osmium tetroxide without ions and then postfixation as under I; VII as described under VI, but with ions included in the fixative solution as indicated under I; VIII initial fixation in freshly prepared, cold mixtures of 1.1% glutaraldehyde and 3.6% osmium tetroxide without ions; IX as described under VIII; but with ions included as under I; X initial fixation in warm 2.5% glutaraldehyde with ions added for 10 min, followed by brief cooling (ice-bath to a final temperature of 5°–8°C) and addition of osmium tetroxide to a final concentration of 1.1%. After a further fixation period of 20 min the samples were processed as described under VIII and IX; XI fixation for 1–3 h in cold 4% osmium tetroxide buffered with sodium cacodylate and with ions as under I; XII fixation as under X, but at room temperature. The simultaneous fixation procedures (cf. 34, 54, 74, 152) which apparently do not specifically improve the preservation of proteins (78) appeared to favor the preservation of large lipidic structures and of the large secretory vesicles which are well known for their fragility (76).

Specimens were washed with distilled water and soaked overnight in aqueous 1% uranyl acetate at 4°C, dehydrated in graded ethanol solutions, transferred to propylene oxide, and embedded in Epon 812 (46, 102). Ultrathin sections were double stained with uranyl

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FIGURE 1 *a–c* Electron micrograph presenting the apical cone arrangement in the lactating rat mammary epithelium cell. The lower region of this zone is usually occupied by a group of dictyosomes (*D*) constituting a Golgi apparatus (Fig. 1 *a*; cf. reference 110) and numerous, mostly peridictyosomal and relatively large vesicles (*CV*; diameters from 0.6 to 1.5  $\mu$ m; Fig. 1 *a–c*) which contain structures morphologically recognizable as casein micelles (28, 32, 35, 141, 158, 159) and resemble the “condensing vacuoles” of pancreatic acinar cells (79; cf. the “forming secretory vesicles” in reference 108, and the “larger vesicles closely associated with dictyosomes” described in reference 20). Distal to the Golgi apparatus one usually observes an irregular accumulation of milk fat droplets (*MFD*, Fig. 1 *a*), small (inner diameters of 40–60 nm) “coated vesicles,” and casein-containing vesicles (e.g., arrows, upper left) which are usually somewhat smaller in this region than the vesicles occurring in the vicinity of dictyosomes (cf. Fig. 3). All these structures are contained in a fibrillar-textured ground cytoplasm which appears to constitute a zone of exclusion for mitochondria (a rare exception is shown in Fig. 6 *a*), endoplasmic reticulum, and also free polysomes (for occasional exceptions, see Fig. 2). This zone of exclusion extends into the peripheral subsurface cortex which varies widely in thickness (from about 50 to 300 nm) and underlies the entire apical plasma membrane as well as large portions of the lateral cell surfaces. The arrowheads in Fig. 1 *a* denote caps of bristle coat on surfaces of large casein vesicles (*CV*) which are shown at higher magnification in Fig. 1 *b* and *c* (arrows). The arrowhead in Fig. 1 *b* indicates one of the typical small “coated vesicles.” Many casein micelles are recognized in the alveolar space (*A*). (*a*)  $\times$  25,000; (*b*)  $\times$  39,000; (*c*)  $\times$  54,000. Scales represent 1  $\mu$ m (*a*), 0.5  $\mu$ m (*b*), and 0.2  $\mu$ m (*c*).



acetate (2% aqueous or 5% methanolic) and lead citrate (prepared according to references 129 and 153) and examined in a Siemens Elmiskop 101 or Zeiss EM 10 electron microscope.

For preparation of a crude secretory vesicle fraction, pieces of lactating mammary tissue were incubated at room temperature in isolation medium (0.3 M sucrose, 2% wt/vol purified gum arabic [reference 49], 30 mM Tris-maleate, pH 6.6, 1 mM MgCl<sub>2</sub>, and 1 mM CaCl<sub>2</sub>) and then homogenized in a Polytron PT 20 homogenizer (Luzern, Switzerland) at low speed. After filtration through several layers of gauze cloth<sup>1</sup> the homogenate was centrifuged for 10 min at 1,000 g. The supernate was layered over a linear gradient of 25–50% sucrose (with gum arabic, buffer, and ions as in the isolation medium) and centrifuged in swinging bucket rotors for 3 h at 100,000 g. A crude secretory vesicle fraction, recovered near the tube bottom, was fixed as described under fixation schedule I, collected by centrifugation, and processed for ultrathin sectioning.

## RESULTS

### *General Organization of the Apical Zone*

Fine-structural features of the apex of mammary epithelial cells during lactation have been described for a number of species in various stages of lactation (e.g., 7, 9–11, 21, 44, 68–70, 75, 77, 93, 124, 125, 137, 142, 151, 156, 158, 160, 161, 162). In the fully lactating rat this apical zone is normally characterized by a bowl-like cytoplasmic region confined basally by the nucleus and laterally by the densely stacked rough endoplasmic reticulum. A typical survey is given in Fig. 1. The height of the apical cone and the cortex was somewhat variable from cell to cell and also varied in relation to the stage of lactation and the presence or absence of suckling stimulus before sacrifice of the animals (for details on ultrastructural changes in rat mammary gland with respect to nursing stimuli, see 101, 124, 130).

### *Occurrence of Bristle Coats on Secretory Vesicles*

Typical, small coated vesicles without structurally distinct contents are commonly observed in a diversity of animal and plant cells (22, 24, 25, 27, 33, 37, 43, 57, 61–64, 72, 81, 94, 114, 119, 120, 135, 136, 146) and have been noted in some previous studies of lactating mammary epithelium in

both the apical (10, 28) and the more basal regions of the cells (11, 125, 160). With all fixation procedures used in this study and in all lactational stages examined, we found such small coated vesicles to be abundant in the apical regions of the cells (Figs. 1 *a, b*, 3, 8 *f*, 9 *a*). The origin, function, and fate of these small coated vesicles is unknown. In addition, many of the casein-containing vesicles had also a pronounced bristle coat which either covered the entire vesicle circumference traced in the specific section or was limited to parts of the vesicle surface (Figs. 1–3, 8 *a–e*, 9 *d, h*; as to the ultrastructure of casein micelles, see references 32, 35, 141, 159). The relative amount of the vesicle surface which was covered with the bristle coat was much higher in the smaller casein vesicles enriched at the cell apex in comparison with the larger peridictyosomal casein “vacuoles” (Figs. 1, 3, and 8 *a–e*). The ultrastructure of the coat was virtually identical to that described for the typical small coated vesicles in other cell types (22, 61–64, 72, 81, 136) and is perhaps best defined in terms of length of the bristles or ridges (140–200 Å) which, in grazing sections, revealed a hexagonal pattern (Fig. 9 *d*), the lateral periodicity of the bristles (180 Å) and the presence of a fine thread (about 40-Å thick) which connects the terminal knobs of the bristles and ridges. Direct comparison of the coat structure in a small coated vesicle and in a casein secretory vesicle is shown in Fig. 3 *b*. With appropriate isolation conditions, the coat organization was maintained in isolated casein-containing vesicles (Fig. 4; for isolated small coated vesicles, see references 81, 140).

Formation of localized, small coated outpocketings from casein vesicle surfaces was also occasionally observed, suggesting either fusion of small coated vesicles with smooth regions of casein vesicles (28), or bleb formation of coated vesicles from the secretory vesicles. These two possibilities cannot be distinguished at present.

Exocytotic secretion of casein micelles (Fig. 5 *b*) has been recognized previously (9–11, 14, 69, 70, 75, 93, 157). In the context of the present study, we wish to emphasize that the coat pattern, or a fuzzy layer which is probably derived from that coat material, was frequently recognized on indented regions of the apical surface. These indented regions represent sites of exocytotic release of caseins by fusion of secretory vesicles with the plasma membrane (Figs. 5 *b, c*, 6 *a, b*). This origin

<sup>1</sup> Franke, W. W., T. W. Keenan, J. Stadler, E.-D. Jarasch, R. Genz, and J. Kartenbeck. Submitted for publication.



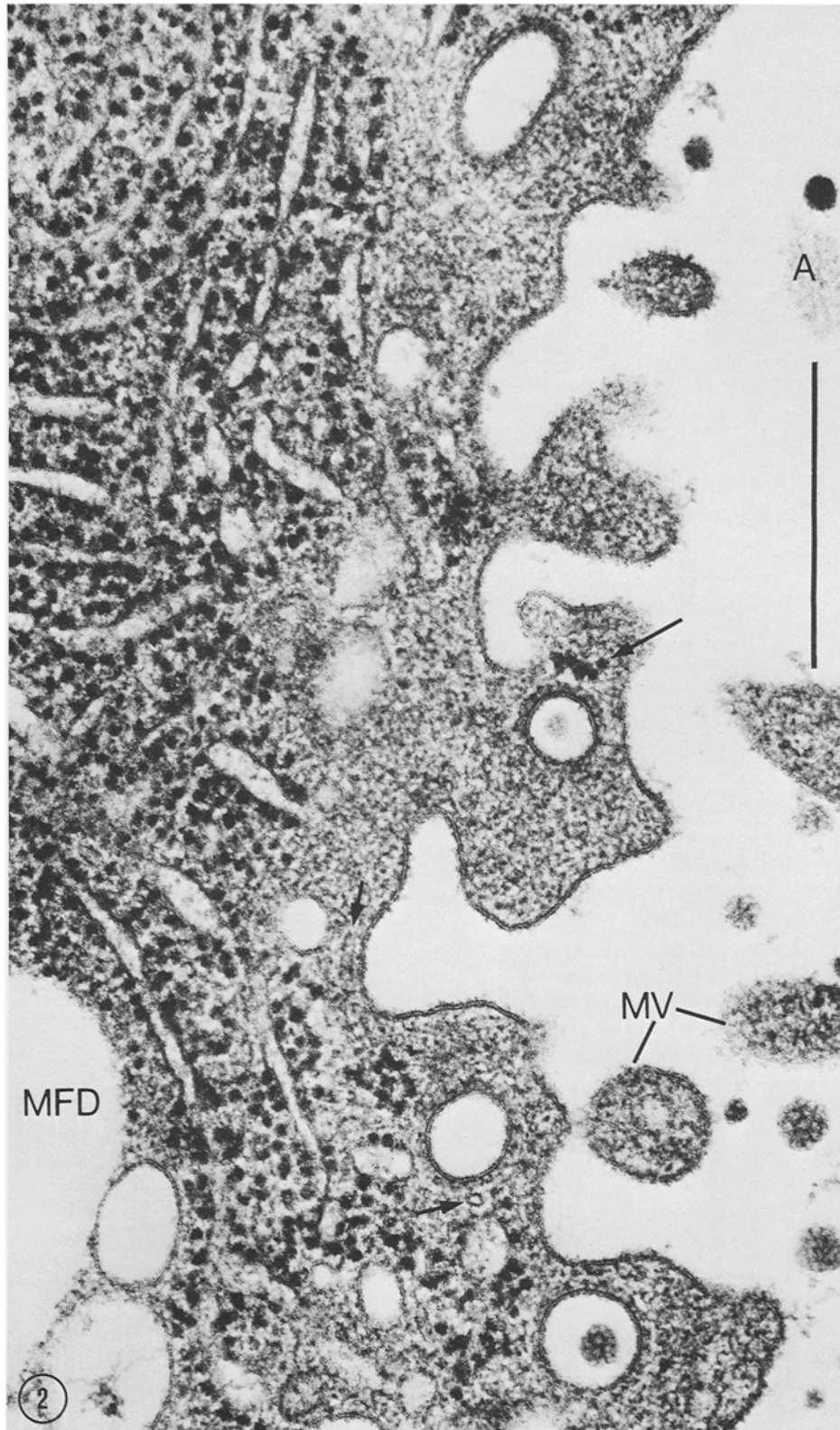
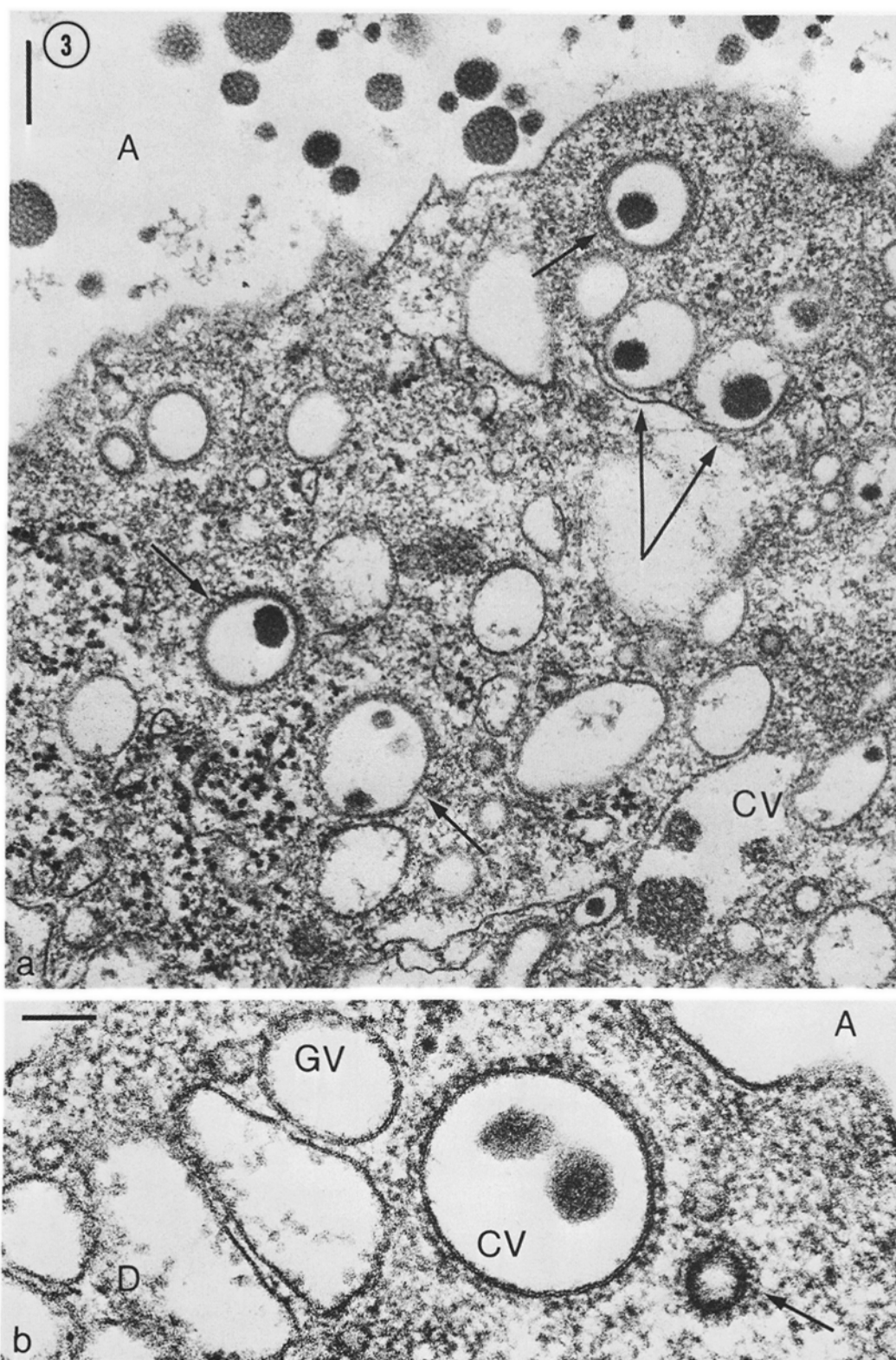


FIGURE 2 Electron micrograph showing the cortical region of a lactating epithelial cell as it appears outside of the apical cone. The entire apical layer is characterized by the presence of a filamentous subsurface web (note the occasional occurrence of polyribosomes in this zone as indicated by the arrow). Embedded in the microfilamentous network are casein secretory vesicles (most of them reveal a bristle coat), microtubules (arrowheads), and the small typical coated vesicles *sensu stricto*. Note the filamentous linkage of the microtubules to adjacent membranes. *MV*, microvilli; *A*, alveolar space; *MFD*, milk fat droplet.  $\times 90,000$ . Bar represents  $0.5 \mu\text{m}$ .



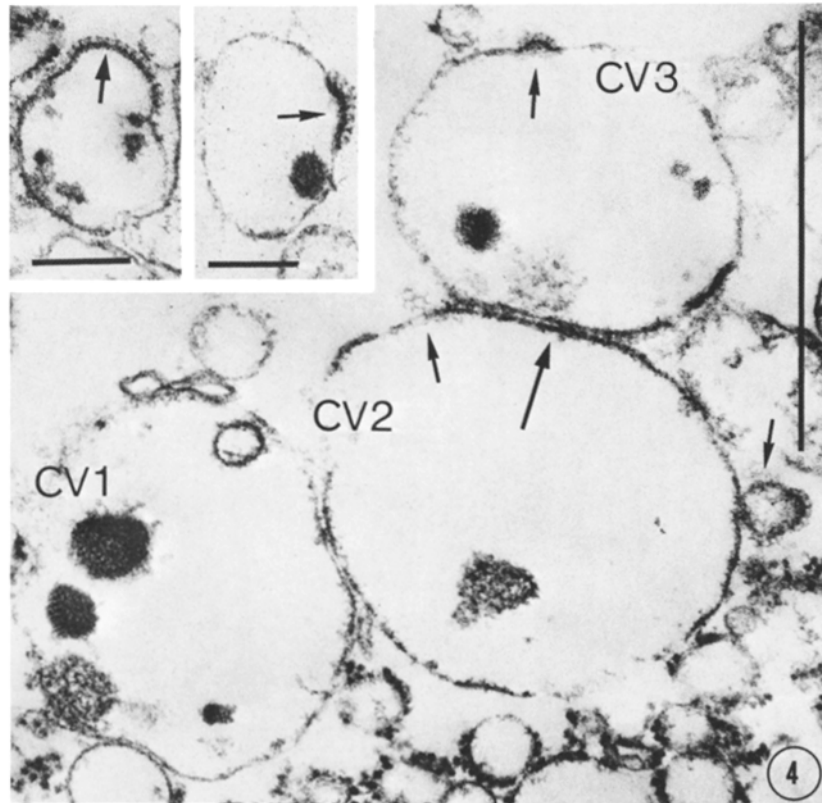


FIGURE 4 Appearance of isolated secretory casein vesicles (CV1–CV3) from rat mammary gland (crude fraction). Note the presence of casein micelles in these vesicles and the persistence of bristle coat regions which cover large (arrows) or small (arrowheads) membrane areas (compare, in particular, the two insets). Note also the indistinctiveness of vesicle membranes in some regions, perhaps indicative of a more fragile character.  $\times 57,000$ ; left inset,  $\times 55,000$ ; right inset,  $\times 60,000$ . Bars represent  $1 \mu\text{m}$  and  $0.2 \mu\text{m}$  (insets), respectively.

of surface membrane regions by insertion of secretory vesicle membrane was further indicated in those surface regions where the attachment of casein micelles via fine, root-like extensions to a more or less distinctly coated section of plasma membrane was revealed. Since such membrane-casein connections were commonly observed in the usually somewhat eccentrically located casein micelles inside secretory vesicles (Figs. 5 b, 6 a, c, 11),

it seems reasonable to interpret them as being due to a relationship which was preserved during the extrusion of casein micelles.

In addition to the exocytotic transport of caseins into the alveolar lumen by vesicle fusion, we observed indications of a different mechanism of casein extrusion. In a great many cells bulbous protrusions of the apical surface which contained a casein vesicle and a small rim (16–35-nm broad) of

FIGURE 3 a, b Details of the upper part of the apical cone region as revealed in a slightly oblique section. Note the abundance of casein secretory vesicles which are clearly identified when their micellar contents are included in the section (arrows). Most of the surface area of these vesicles shows a typical bristle coat. Note also the presence of many small coated vesicles in this region. Fig. 3 b presents a direct comparison of three major vesicle types of this cell: (i) smooth-surfaced Golgi apparatus-associated vesicles (GV); (ii) coated casein secretory vesicles (CV), and typical small coated vesicles *sensu stricto* (arrow). A, alveolar space characterized by the presence of many free casein micelles; D, dictyosome. (a)  $\times 65,000$ ; (b)  $\times 115,000$ . The bars denote  $0.2 \mu\text{m}$  (a) and  $0.1 \mu\text{m}$  (b).

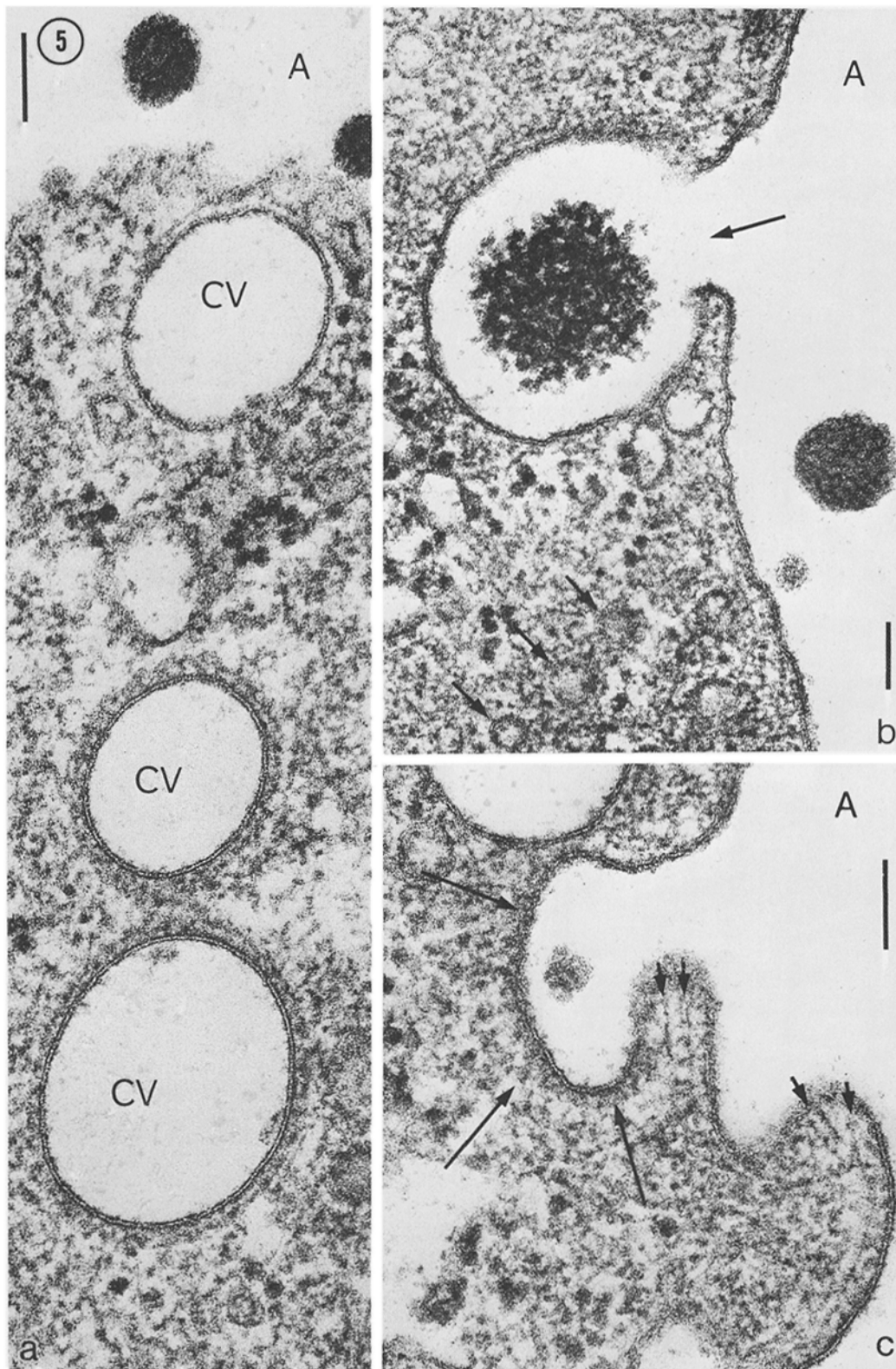


FIGURE 5 Sequence of apical secretory vesicles (SC) as revealed in the cortical cytoplasm ([a] sections somewhat oblique with respect to the cell surface) or during the process of exocytosis (b, c). Note the occurrence of prominent bristle coats in some of the membrane profiles (e.g., in the two lower vesicles of [a] and in [c]; the coat in the caveolar membrane in [c] is denoted by arrows) but not in others (upper vesicle in [a] and in the exocytotic caveola denoted by the arrow in [b]). The arrowheads in (b) point to some of the very small, frequently coated cortical vesicles. The arrowheads in (c) denote regular arrays of the (actin-like) microfilaments. (a)  $\times 135,000$ ; (b)  $\times 105,000$ ; (c)  $\times 135,000$ . Bars indicate  $0.1 \mu\text{m}$ .



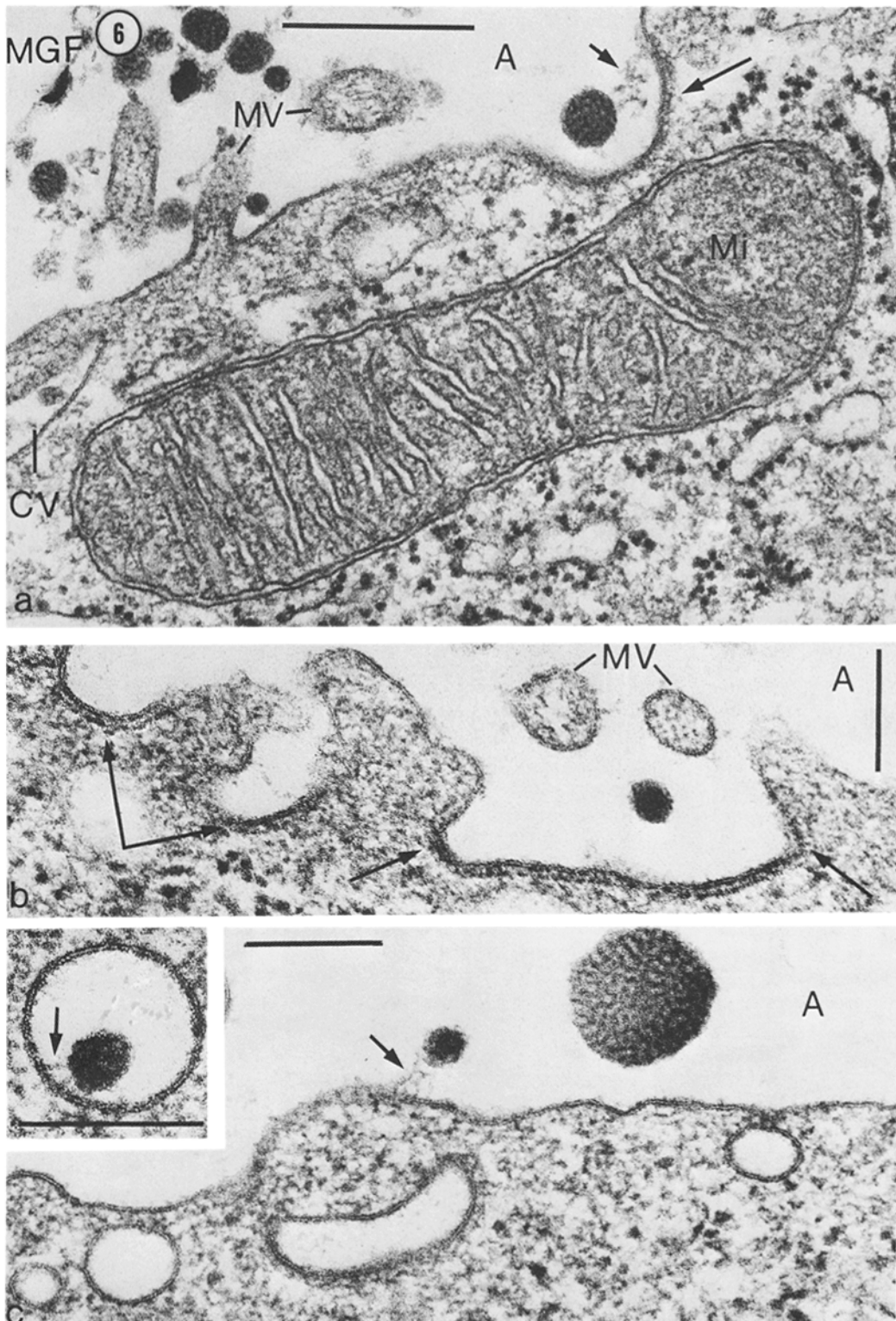


FIGURE 6 *a-c* Details of the apical subsurface region of lactating mammary epithelial cells. The whole plasma membrane is lined with an apparently irregularly coiled microfilamentous web but reveals in certain limited regions, especially in secretory caveolae, the specific bristle coat appearance (denoted by the arrows in [*a*] and [*b*]). The microfilamentous material extends into the microvilli (*MV*, [*a*, *b*]) and seems to connect apical vesicles ([*c*], and the casein vesicle *CV* in the very left of [*a*]) with the inner aspect of the surface membrane. Note also the rootlet-like connections (arrowheads) between casein micelles and the internal face of secretory vesicles (insert in Fig. 6 *c*) as well as the outer surface of the plasma membrane (*a* and *c*). *Mi*, mitochondria; *A*, alveolar space. (*a*)  $\times 59,000$ ; (*b*)  $\times 95,000$ ; (*c*)  $\times 105,000$ ; inset in (*c*)  $\times 150,000$ . Bars denote  $0.5 \mu\text{m}$  (*a*) and  $0.2 \mu\text{m}$  (*b* and *c*), respectively.

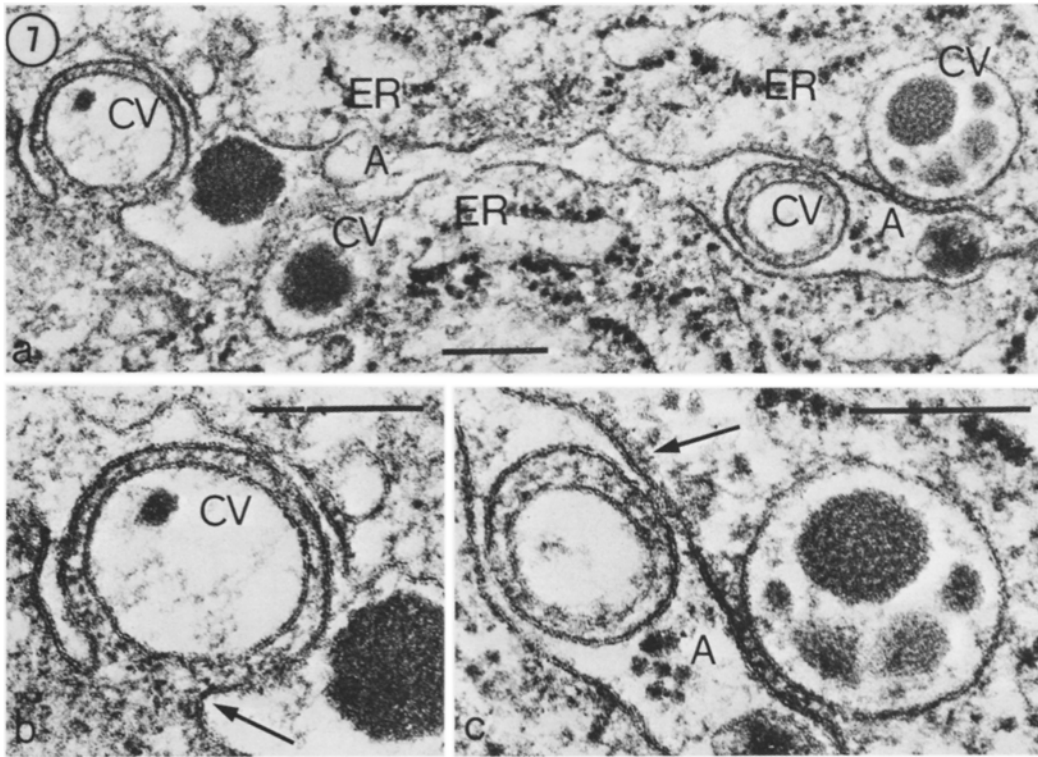
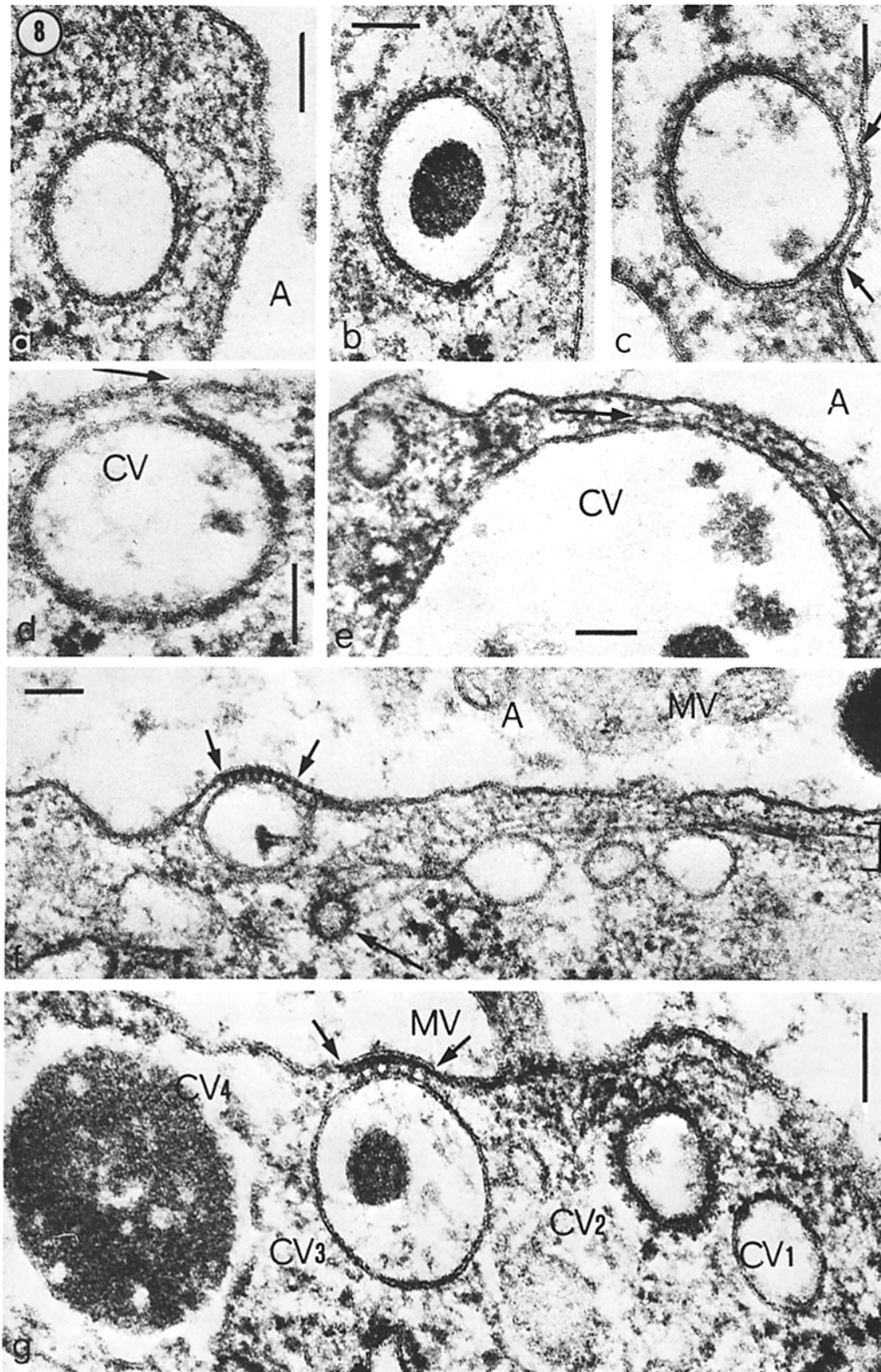


FIGURE 7 *a-c* Apical cell surface of lactating mammary epithelial cells in a state of almost collapsed alveolar spaces (*A*). Note, besides the usual subsurface association of casein secretory vesicles (*CV*) as seen in the very right of (*a*) and at higher magnification in (*c*) (note the attachment plaque), the occurrence of bulbous outpocketings of the cell surface which closely invest casein vesicles. The only other structures that are enclosed in such surface blebs are the microfilaments (for higher magnifications, see [*b*] and [*c*]). The situation suggests that such apical bulbs detach from the cell body by constriction in the neck region (indicated by the arrow in [*b*]) and give rise to the formation of milk granules that contain double membrane-bounded casein, a process that is similar to the formation of the milk fat globules. Such casein vesicle-containing granules that are obviously derived from apical surface are occasionally found in milk samples (see text). The arrow in (*c*) indicates a bristle-coated section of apical plasma membrane. *ER*, endoplasmic reticulum. (*a*) 69,500; (*b*, *c*)  $\times 115,000$ . Bars indicate  $0.2 \mu\text{m}$ .

FIGURE 8 *a-g* Electron micrographs illustrating various forms of connections between the surface of casein secretory vesicles (*CV*) and the inner aspect of the apical plasma membrane. Frequently, the bristle coat of the secretory vesicle seems to be interrupted at the apical pole (*a-d*). The situation suggests that the bristle coat structures disintegrate and merge into the microfilamentous web of the cell cortex (*b* and *c*; see in particular the site denoted by the arrows in the relatively thick—golden interference color—section in [*d*] and the apical poles of vesicles *CV1* and *CV2* in [*g*]). Also, cortical microfilaments occurring singly or in bundles are frequently seen to abut tangentially upon large (*CV* in [*c*]) or small (*f*) vesicles. Close membrane to membrane approximation (denoted by the pairs of arrows in [*c*], [*f*], and [*g*]) is often characterized by the formation of vesicle to surface pre-exocytotic attachment plaques (*PAP*; see [*f*] and [*g*]). The arrow in the bottom part of [*f*] denotes one small coated vesicle *sensu stricto*. *A*, alveolar space; *MV*, microvilli. (*a*)  $\times 125,000$ ; (*b*)  $\times 105,000$ ; (*c*)  $\times 88,000$ ; (*d*)  $\times 125,000$ ; (*e*)  $\times 95,000$ ; (*f*)  $\times 88,000$ ; (*g*)  $\times 135,000$ . Scales represent  $0.1 \mu\text{m}$ .



the cortical ground cytoplasm, including microfilamentous arrays, were noted (Fig. 7). The occasional occurrence of such surface-derived droplets containing casein vesicles in freshly collected rat, cow, and human milk samples (101; cf. references 12, 150, 163) suggests that these formations represent an alternate route for casein release.

#### *Attachment of Casein-Containing Vesicles to the Plasma Membrane*

Both coated and uncoated regions of casein-containing vesicles were frequently identified in close association with microfilamentous structures of the apical ground cytoplasm (Figs. 2, 3, 6 *a, c*, 8, 9 *a*, 10). Such intimate relationships between the microfilamentous network of the cytoplasmic matrix and the typical small coated vesicles have been noted in other cell types (for special hypotheses on the origin and functions of this relationship, see references 62–64). Frequently, direct continuity of the fuzzy coats of the vesicles and the inner side of the plasma membrane was suggested (Figs. 8 *b–d, g*, 10 *e*). Occasionally, special strands of cortical microfilaments appeared to abut on the surface of casein vesicles, mostly tangentially, and to separate the vesicles from the plasma membrane (Figs. 8 *e–g*). With many vesicles, on the other hand, both the microfilamentous material and the vesicle surface coat appeared to be partially removed at their lumenally oriented poles (Figs. 8 *b–d*), similar to the situation noted with secretory vesicles in a variety of cell types (119; for reviews on the fuzzy coat barrier hypothesis, see also references 3, 39, 104). Most secretory vesicles, however, showed a different form of attachment to the cell surface (Figs. 8 *f, g*, 9 *a–i*, 10 *e*). A

conspicuous intermembrane attachment plaque appeared to connect the vesicle with the corresponding area of the plasma membrane at a rather constant membrane to membrane distance. This plaque was made up of a series (1–10 per plaque) of heavily stained cross-bridge elements (12–15 nm long and 9–12 nm wide) which were regularly spaced with a lateral periodicity of about 18 nm. Occasionally, the individual cross-bridge elements were not clearly resolved. This might be due to their oblique orientation in the section or might represent stages of disintegration of these elements during or before membrane fusion. These plaque structures, which to our knowledge have not been reported for any exocytotic vesicle-surface membrane association (however, see plate 20 *f* of reference 138), resemble other forms of intermembrane cross-bridges which we have described earlier for a variety of membrane relationships (52, 53, 56). It may be that such structures represent a special (perhaps artificially induced) organization of membrane-associated coat material and that the coassembly of coat structures of both the vesicle and plasma membranes contributes to recognition and specific interaction of two different membrane faces. Interestingly, bristle coat structures are usually less frequent or less developed in the other regions of such surface membrane-associated vesicles.

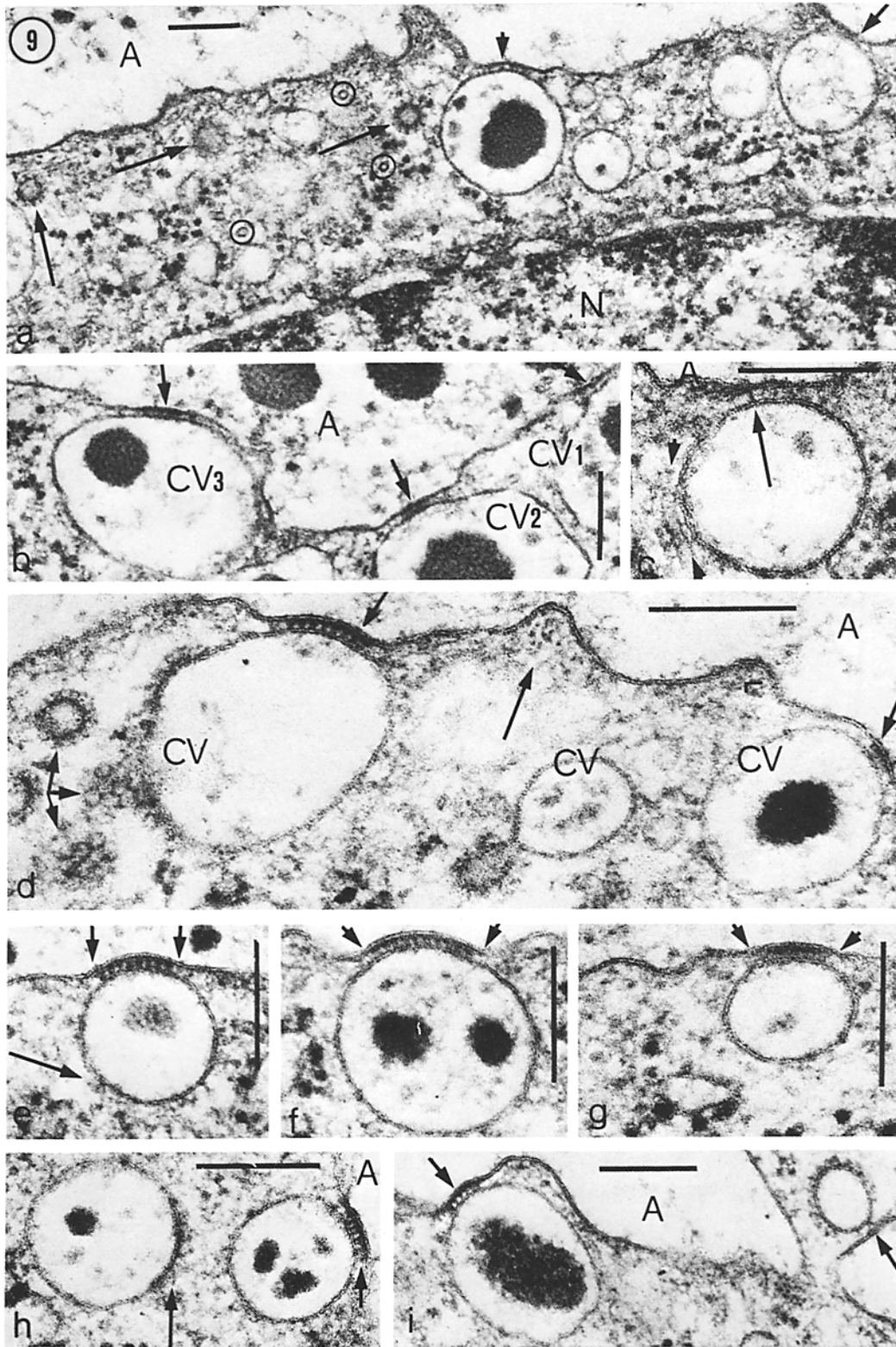
#### *Association of Casein Vesicles with Microtubules and Microfilaments*

Microtubules are prevalent in mammary epithelial cells, particularly in the apical regions (Figs. 9 *a*, 10 *a*; reference 125). With respect to orientation, two different classes of microtubules may be

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FIGURE 9 *a–i* Details of the attachment plaques of casein vesicles and apical surface membrane. Fig. 9 *a* and *b* present typical cell cortex surveys and illustrate the frequency of the pre-exocytotic attachment plaques (PAPs; denoted by the arrowheads in Fig. 9 *a–i*). Such PAPs are noted with casein vesicles of all size classes and are characterized by the presence of either a relatively indistinct, heavily stained intermembranous matrix, or regularly spaced, membrane to membrane cross-bridge elements (e.g., [*c–e*]; for detailed description see text). The number of identifiable membrane linkages as well as the whole size of the PAP is variable (compare, e.g., the PAPs shown in Fig. 9 *c* and 9 *e*). Note that vesicles showing PAPs sometimes reveal bristle coats in other regions (e.g., Fig. 9 *d* and *i*; see also the arrows in Fig. 9 *e*). Note further the abundance of small coated vesicles *sensu stricto* (some are denoted, e.g., by arrows in Fig. 9 *a* and by the triple arrowhead in the very left of Fig. 9 *d*), microtubules (some are denoted by the circles in Fig. 9 *a*; see also the arrow in Fig. 9 *c*), and microfilaments (revealed in cross sections, e.g., in the surface ridge denoted by the arrow in [*d*]). *A*, alveolar space; *CV*, casein vesicles; *N*, nucleus. The situation shown in Fig. 9 *h* might suggest that a polar differentiation of vesicle coat material (arrow) takes place before the formation of a PAP. (*a*)  $\times 55,000$ ; (*b*)  $\times 69,000$ ; (*c, d*)  $\times 110,000$ ; (*e*)  $\times 100,000$ ; (*f*)  $\times 105,000$ ; (*g*)  $\times 110,000$ ; (*h*)  $\times 95,000$ ; (*i*)  $\times 75,000$ . Bars denote 0.2  $\mu\text{m}$ .





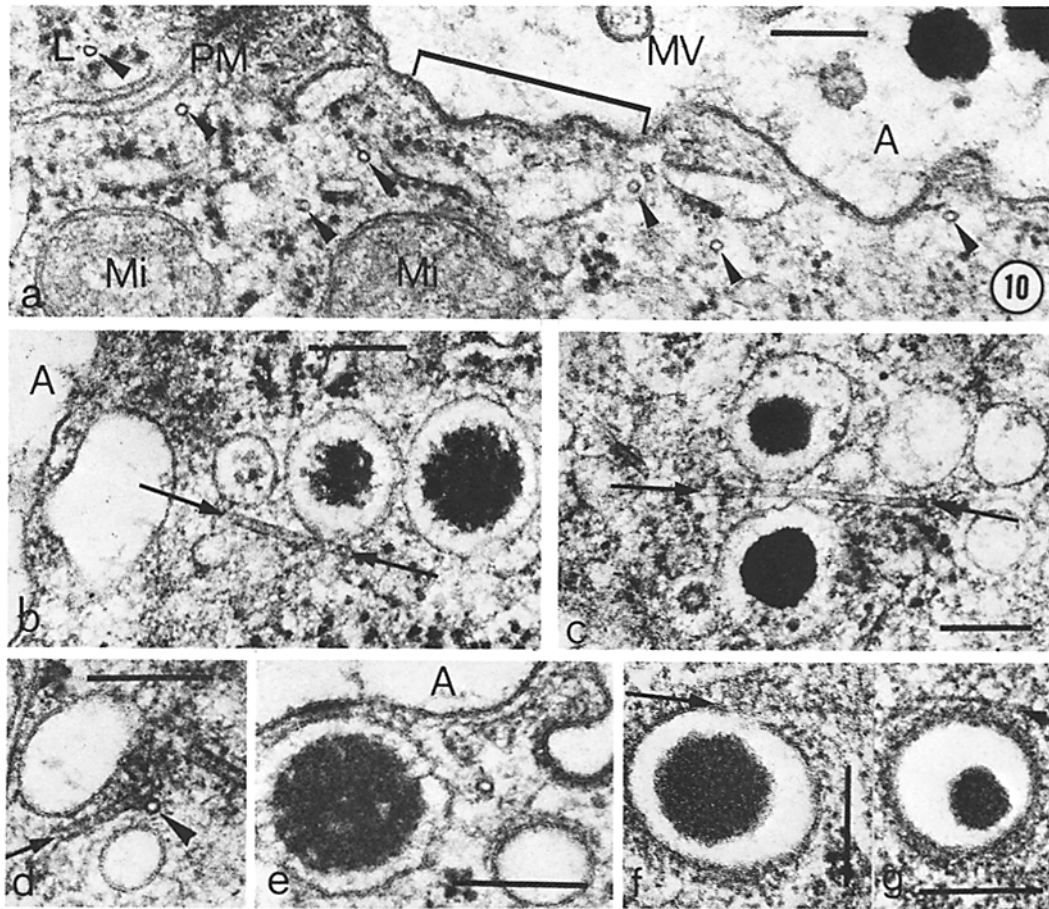


FIGURE 10 *a-g* Structural details of the interrelationships of casein secretory vesicles and microtubules and microfilaments as revealed in the apical zone of the lactating rat mammary epithelial cell. Microtubules are abundant in the whole apical region, especially in the cortex itself, and usually occur singly (arrows and arrowheads in Fig. 10 *a-e*). Intimate associations with casein secretory vesicles are frequently observed (Fig. 10 *b-d*) and might sometimes be mediated by fine, filamentous, lateral linkages (see, e.g., Fig. 10 *e*). Close associations between the casein vesicles and microfilaments are also common (e.g., as denoted by the arrows in [*f*] and [*g*]). The brackets in Fig. 10 *a* demarcate a surface membrane area that shows a fuzzy coat on its inner aspect. *Mi*, mitochondria; *A*, alveolar space; *MV*, microvillus. (*a*, *b*)  $\times 65,000$ ; (*c*)  $\times 60,000$ ; (*d*)  $\times 85,000$ ; (*e*)  $\times 90,000$ ; (*f*)  $\times 80,000$ ; (*g*)  $\times 85,000$ . Scales indicate  $0.2 \mu\text{m}$ .

distinguished, namely (*a*) those which are oriented nearly parallel to the apical surface (Figs. 9 *a*, 10 *a*, *e*), and (*b*) those which have a more polar (i.e. axial) orientation. Both smooth and coated surfaces of casein-containing vesicles were closely associated with microtubules, and this association was seemingly mediated by 6–30-nm long filamentous bridge connections (Figs. 2, 9 *c*, 10 *b-e*). Similar microtubule interactions with cytoplasmic vesicles and cisternae have been described in various other cell types (2, 8, 17, 47, 48, 51, 117, 132, 134, 145, 147). Frequently, two or more

casein vesicles were associated with the same microtubule (Fig. 10 *b, c*).

The cytoplasm of the apical cone and the cortical region continuous with this zone was abundantly packed with microfilamentous structures which occurred either in bundles (Fig. 8 *f*) or as single filaments (Fig. 10 *f, g*). Such microfilaments occurred in an especially dense and sometimes paracrystalline packing at the inner face of the plasma membrane and in the microvilli (Figs. 5 *c*, 6 *a-c*, 8 *f*, 9 *a, d*, 10 *e*). Close association of vesicles, including casein vesicles, with microfila-

ments was common, and the microfilaments were frequently observed to abut tangentially the vesicle surface and bristle coat (Figs. 3, 8 *e, f*, 10 *f, g*). Our observations suggested an intimate connection

between the microfilaments and the fuzzy coats of both the vesicles and the plasma membrane, thus resembling the connections of (actin-like) microfilaments to vesicle membranes seen in other

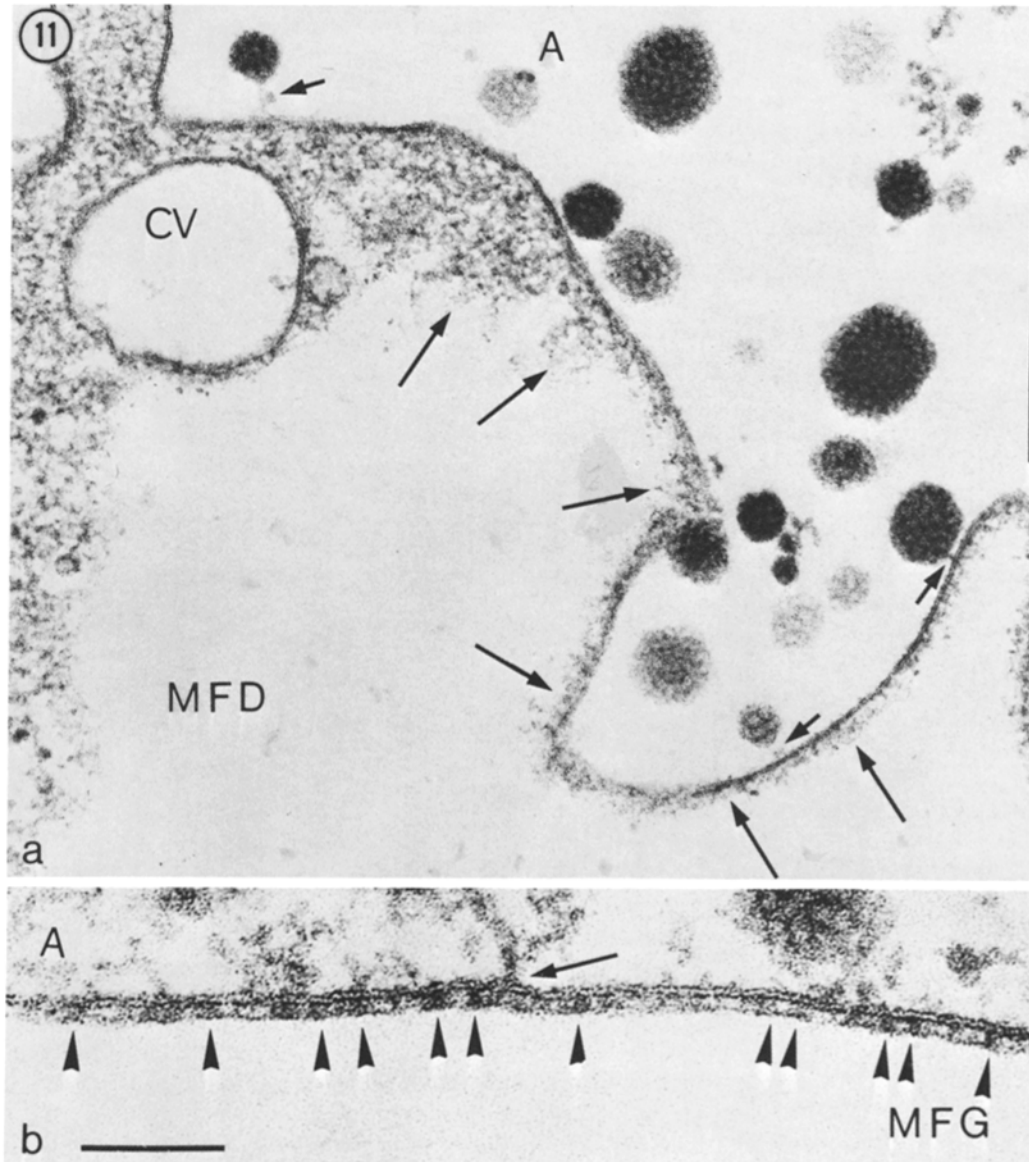


FIGURE 11 *a, b* Electron micrographs of the apical zone (Fig. 11 *a*) and the periphery of an intra-alveolar milk fat globule (*MFG*, Fig. 11 *b*) demonstrating that during the formation of the milk fat globule the milk fat droplet (*MFD* in [*a*]) does not come into direct contact with the inner aspect of the plasma membrane but remains spaced from it, over most of the surface, by the plasma membrane-associated microfilamentous web material (arrows in Fig. 11 *a* and arrowheads in Fig. 11 *b*). This plasma membrane-associated fuzzy coat material then frequently condenses and may appear in the form of somewhat regularly arranged knobs (see, e.g., arrowheads in [*b*]). Note also the rootlet connections between casein micelles and the cell surface (indicated by arrowheads in Fig. 11 *a* and by the arrow in Fig. 11 *b*). *A*, alveolus; *CV*, casein vesicle. (*a*)  $\times 85,000$ ; (*b*)  $\times 190,000$ . Bars represent  $0.5 \mu\text{m}$  (*a*) and  $0.1 \mu\text{m}$  (*b*), respectively.

cell types (for references see 1, 39, 90, 112, 117, 133).

#### *Association of Internal Plasma Membrane Coat Structures with the Milk Fat Globule Membrane*

During the process of milk fat globule discharge, the cytoplasmic milk fat droplet is pressed against the cell surface, causes the surface to bulge outward, locally alters the surface membrane plasticity, and finally detaches by pinching off as a result of plasma membrane rupture and refusion in the neck region. Such fat droplet-containing, surface-derived blebs may also contain a variable portion of apical cytoplasm (for references, see introductory paragraphs). We noted that in the forming milk fat globule the fat droplet in most regions does not directly contact the inner face of the plasma membrane, but rather is separated from this membrane by the membrane-associated coat material with a minimum distance of about 10 nm (Fig. 11; see also references 161 and 160). This seemingly argues against the hypothesis (122) that "the forces promoting the envelopment of the fat droplet by the plasma membrane are primarily the London dispersion forces attracting the membrane to the droplet." During the process of milk fat globule secretion, and further in the expelled fat globules observed in alveoli, this layer of plasma membrane coat material becomes strongly condensed and reveals both an increased staining intensity and a somewhat regular subunit pattern (Fig. 11 *b*). In alveolar fat globules, and, in particular, in freshly obtained milk samples, this inner surface coat-derived material remains associated with the milk fat droplet surface, even in regions where the plasma membrane proper has become disintegrated or delaminated. This retained inner coat material appears to be identical to the "zone of dense material" or "cytoplasmic layer" described by Wooding (161, 160).

#### DISCUSSION

Involvement of coated vesicles in membrane translocations has heretofore been demonstrated with clarity only in endocytotic processes, primarily by the use of exogenously added, electron-dense labels, the uptake of which could be directly demonstrated (6, 36-38, 41, 43, 57, 60, 72, 94, 119, 135, 136, 146). The involvement of coated vesicles in exocytotic transport has recently been indicated for the formation of new plasma mem-

brane during plant cell division (50, see also reference 71). The question as to whether bristle coat structures might also be involved in translocations of secretory vesicles has been raised repeatedly (20, 27, 47, 50, 71, 115, 118), but this question has remained unanswered due to the absence of identifiable secretory contents and bristle coat structures in the same vesicles. Relatively small areas of coat structures have been noted in the juxta-dictyosomal "condensing vacuoles" of pancreatic (79, 118) and hepatic (82) cells. However, these structures have not been reported in typical secretory vesicles. Micrographs presented in this paper illustrate the existence of typical bristle coats in secretory vesicles with a morphologically identifiable content. We thus conclude that bristle coat structures are not indicative, per se, of endocytotic translocations but also occur on secretory vesicles. Perhaps such structures are manifest only transiently, during migration of vesicles to sites of exocytotic discharge, and may be absent during periods of vesicle storage (see comments in reference 118; a coated appearance is also indicated in the Clara cells of rat bronchioles, see Fig. 11 in reference 92).

We believe that the highly ordered arrays of bristles and ridges of the coats on plasma membranes and vesicles are true, membrane-associated entities of a probably proteinaceous nature (see, however, 62-64). Speculations on the chemical nature and physiological roles of bristle coats have recently been summarized (50). On the basis of the finding that the fuzzy coats of the plasma membrane contain both actin and myosin (18, 19, 30, 31, 39, 59, 65, 66, 80, 103, 111, 126, 127, 143), and in view of the close association of the coat bristles with the microfilaments (95; and references cited above), one is led to suggest that this vesicle coat is structurally and functionally integrated into the actomyosin system and provides motility forces and/or guide elements for vectorial translocation (see also references 51 and 127). We view the bristles as being enriched in myosin-like proteins which intimately interact with both the actin-like microfilaments and tubulin (for references on membrane-associated tubulin, see references 18, 91, 148). The demonstrated effects of colchicine on a variety of secretory processes (40, 96, 113, 116, 128, 149), including lactation (121), implies an interaction of secretory vesicles with microtubules. Although the relationship of coat structures to the secretory vesicle-surface membrane attachment plaques (PAP) described herein is not clear, it is

tempting to speculate that both arrays represent different forms of similar, perimembranous structures. Interestingly, the arrangement of the PAP closely resembles the organization of the "neurostenin" complexes as hypothesized for the association of synaptic vesicles with the presynaptic plasma membrane (16). While in some vesicles the coat material seems to be involved in PAP formation (e.g. Fig. 9 *h*), other vesicles show an absence of bristles in the plasma membrane contact area (e.g., Fig. 8 *c-e*). Therefore, the involvement of coat structures in membrane contact does not seem to represent an obligatory transition stage of exocytosis.

Our observations suggest that large areas of the apical plasma membrane are produced from the incorporation of membrane and associated coat material of the casein-containing vesicles in accordance with the membrane flow concept, which predicts a contribution of secretory vesicle membrane to the formation and regeneration of plasma membrane. Several papers presenting arguments for (4, 5, 15, 55, 73, 83, 108-110, 131) and against (45, 106, 107, 118, 155) this concept have been published. In particular, it is supported by the recent demonstration of true membrane integration of secretory products such as immunoglobulins (29, 67, 89, 154) and procollagen (42, 116), as well as by studies on the formation of viral envelope glycoproteins (97). This concept is especially attractive for explaining the extensive net production of plasma membrane of the lactating mammary epithelial cell. In fact, the simplest mechanism for replenishment of the plasma membrane that is lost from the cell during envelopment of the fat globule would appear to be incorporation of casein secretory vesicle membrane into the plasma membrane. Biochemical studies of mammary gland fractions also favor the concept of membrane flow (84-88, 123). In addition to the morphologically recognizable casein micelles, evidence that these mammary secretory vesicles also serve as vehicles for the cellular discharge of the lactose (23, 86, 99, 100), water (100), and certain of the inorganic ions (13, 98, 100, 144) has been presented. Important to the concept of secretory vesicle membrane addition to the apical plasma membrane is our demonstration that not only the plasma membrane but also the associated fibrillar material is lost with the milk fat globule (for polypeptide patterns, see reference 105). This leads to the conclusion that the cell must accomplish extensive and coordinated production of both

the surface membrane and the microfilamentous terminal web.

*Note Added in Proof:* After completion of this manuscript we learned of an earlier article in which it was hypothesized that "coated vesicles play a role in the formation of intercellular junctions" (Sheffield, J. B. 1970. Studies on aggregation of embryonic cells: initial cell adhesions and the formation of intercellular junctions. *J. Morphol.* **132**:245-264). The association of actin and myosin with a secretory vesicle membrane has recently been reported for the chromaffin granules from bovine adrenal medulla (Burrige, K., and J. H. Phillips. 1975. Association of actin and myosin with secretory granule membranes. *Nature (Lond.)* **254**:526-529.).

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## REFERENCES

1. ALLEN, R. D. 1973. Structures linking the myonemes, endoplasmic reticulum, and surface membranes in the contractile ciliate *Vorticella*. *J. Cell Biol.* **56**:559-579.
2. ALLEN, R. D. 1975. Evidence for firm linkages between microtubules and membrane-bounded vesicles. *J. Cell Biol.* **64**:497-503.
3. ALLISON, A. C., and P. DAVIES. 1974. Mechanisms of endocytosis and exocytosis. In *Transport at the Cellular Level*. M. A. Sleight and D. H. Jennings, editors. Cambridge University Press, London. 419-446.
4. AMSTERDAM, A., J. OHAD, and M. SCHRAMM. 1969. Dynamic changes in the ultrastructure of the acinar cell of the rat parotid gland during the secretory cycle. *J. Cell Biol.* **41**:753-773.
5. AMSTERDAM, A., M. SCHRAMM, J. OHAD, Y. SALOMON, and Z. SELINGER. 1971. Concomitant synthesis of membrane protein and exportable protein of the secretory granule in rat parotid gland. *J. Cell Biol.* **50**:187-200.
6. ANDERSON, E. 1972. Oogenesis. J. D. Biggers and A. W. Schnetz, editors. University Park Press, Baltimore, Md. 87-117.
7. ANDERSON, W. A., J. TRANTALIS, and Y.-H. KANG. 1975. Ultrastructural localization of endogenous mammary gland peroxidase during lactogenesis in the rat. Results after tannic acid-formaldehyde-glutaraldehyde fixation. *J. Histochem.*

- Cytochem.* **23**:295–302.
8. BARDELE, C. F. 1972. A microtubule model for ingestion and transport in the suctorian tentacle. *Z. Zellforsch. Mikrosk. Anat.* **126**:116–134.
  9. BARGMANN, W., K. FLEISCHHAUER, and A. KNOOP. 1961. Über die Morphologie der Milchsekretion. II. Zugleich eine Kritik am Schema der Sekretionsmorphologie. *Z. Zellforsch. Mikrosk. Anat.* **53**:545–568.
  10. 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.
  11. BARGMANN, W., and U. WELSCH. 1969. On the ultrastructure of the mammary gland. In *Lactogenesis*. M. Reynolds, and S. J. Folley, editors. University of Pennsylvania Press, Philadelphia, Pa. 43–52.
  12. BAUER, H. 1972. Ultrastructural observations on the milk fat globule envelope of cow's milk. *J. Dairy Sci.* **55**:1375–1387.
  13. BAUMRUCKER, C. R., and T. W. KEENAN. 1975. Membranes of mammary gland. X. Adenosine triphosphate-dependent calcium accumulation by Golgi apparatus-rich fractions from bovine mammary gland. *Exp. Cell Res.* **90**:253–260.
  14. BEERY, K. E., L. F. HOOD, and S. PATTON. 1971. Formation of casein micelles in Golgi vesicles of mammary tissue. *J. Dairy Sci.* **54**:911–912.
  15. BERGERON, J. J. M., W. H. EVANS, and J. J. GESCHWIND. 1973. Insulin binding to rat liver Golgi fractions. *J. Cell Biol.* **59**:771–776.
  16. BERL, S., S. PUSZKIN, and W. J. NICKLAS. 1973. Actomyosin-like protein in brain. *Science (Wash. D. C.)* **179**:441–446.
  17. BIKLE, D., L. G. TILNEY, and K. R. PORTER. 1966. Microtubules and pigment migration in the melanophores of *Fundulus heteroclitus* L. *Protoplasma* **61**:322–345.
  18. BLITZ, A. L., and R. E. FINE. 1974. Muscle-like contractile proteins and tubulin in synaptosomes. *Proc. Natl. Acad. Sci. U.S.A.* **71**:4472–4476.
  19. BLUEMINK, J. G. 1972. Cortical wound healing in the amphibian egg: An electron microscopical study. *J. Ultrastruct. Res.* **41**:95–114.
  20. BONNETT, H. T., and E. H. NEWCOMB. 1966. Coated vesicles and other cytoplasmic components of growing root hairs of radish. *Protoplasma* **62**:59–75.
  21. BOUSQUET, M., J. E. FLECHON, and R. R. DENAMUR. 1969. Aspects ultrastructuraux de la glande mammaire de lapine pendant la lactogenese. *Z. Zellforsch. Mikrosk. Anat.* **96**:418–436.
  22. BOWERS, B. 1964. Coated vesicles in the pericardial cells of the aphid (*Mysus persicae* Sulz.) *Protoplasma* **59**:351–362.
  23. BREW, K. 1969. Secretion of alpha-lactalbumin into milk and its relevance to the organization and control of lactose synthesis. *Nature (Lond.)* **222**:671–672.
  24. BRIGHTMAN, M. W. 1962. An electron microscopic study of ferritin uptake from the cerebral ventricles of rats. *Anat. Rec.* **142**:219.
  25. BRUNI, C., and K. R. PORTER. 1965. The fine structure of the parenchymal cell of the normal rat liver. *Am. J. Pathol.* **46**:691–756.
  26. BRUNNER, J. R. 1974. Physical equilibria in milk: The lipid phase. In *Fundamentals of Dairy Chemistry*. B. H. Webb, A. H. Johnson and J. A. Alford, editors 2nd edition. Avi Publishing Company, Westport, Conn. 474–602.
  27. BRUNS, R. R., and G. E. PALADE. 1968. Studies on blood capillaries. I. General organization of blood capillaries in muscle. *J. Cell Biol.* **37**:244–276.
  28. BUCHHEIM, W., and U. WELSCH. 1973. Evidence for the submicellar composition of casein micelles on the basis of electron microscopical studies. *Neth. Milk Dairy J.* **27**:163–180.
  29. CHOI, Y. S., P. M. KNOPF, and E. S. LENNOX. 1971. Intracellular transport and secretion of an immunoglobulin light chain. *Biochemistry* **10**:668–679.
  30. CLARKE, M., G. SCHATTEN, D. MAZIA, and J. A. SPUDICH. 1975. Visualization of actin fibers associated with the cell membrane in amoeba *Dictyostelium discoideum*. *Proc. Natl. Acad. Sci. U.S.A.* **72**:1758–1762.
  31. COMLY, L. T. 1973. Microfilaments in *Chaos carolinensis*. Membrane association, distribution, and heavy meromyosin binding in the glycerinated cell. *J. Cell Biol.* **58**:230–237.
  32. COWIE, A. T., and J. S. TINDAL. 1971. The physiology of lactation. Edward Arnold (Publishers) Ltd. London. 1–392.
  33. CRONSHAW, J., and K. ESAU. 1968. Cell division in leaves of *Nicotiana*. *Protoplasma* **65**:1–24.
  34. DANEEL, S., and N. WEISSENFELS. 1965. Besseres Fixierungsverfahren zur Darstellung des Grundplasmas von Protozoen und Vertebratenzellen. *Mikroskopie* **20**:89–93.
  35. DEWAN, R. K., A. CHUDGAR, R. MEAD, V. A. BLOOMFIELD, and C. V. MORR. 1974. Molecular weight and size distribution of bovine milk micelles. *Biochim. Biophys. Acta* **342**:313–321.
  36. DOUGLAS, W. W., J. NAGASAWA, and R. A. SCHULZ. 1971. Coated microvesicles in neurosecretory terminals of posterior pituitary glands shed their coats to become smooth "synaptic" vesicles. *Nature (Lond.)* **232**:340–341.
  37. DROLLER, M. J., and T. F. ROTH. 1966. An electron microscope study of yolk formation during oögenesis in *Lebistes reticulatus guppyi*. *J. Cell Biol.* **28**:209–232.
  38. DUMONT, A. 1969. Ultrastructural study of the maturation of peritoneal macrophages in the ham-

- ster. *J. Ultrastruct. Res.* **29**:191-209.
39. DURHAM, A. C. H. 1974. A unified theory of the control of actin and myosin in nonmuscle movements. *Cell.* **2**:123-136.
  40. EHRLICH, H. P., and P. BORNSTEIN. 1972. Microtubules in transcellular movement of procollagen. *Nat. New Biol.* **238**:257-260.
  41. ERICSSON, J. L. 1965. Transport and digestion of hemoglobin in the proximal tubule. II. Electron microscopy. *Lab. Invest.* **14**:16-39.
  42. FAULK, W. P., L. B. CONOCHIE, A. TEMPLE, and M. PAPAMICHAIL. 1975. Immunobiology of membrane-bound collagen on mouse fibroblasts. *Nature (Lond.)*. **256**:123-125.
  43. FAWCETT, D. W. 1965. Surface specializations of absorbing cells. *J. Histochem. Cytochem.* **13**:75-91.
  44. FELDMAN, J. D. 1961. Fine structure of the cow's udder during gestation and lactation. *Lab. Invest.* **10**:238-255.
  45. FLEISCHER, B., and S. FLEISCHER. 1971. Comparison of cellular membranes of liver with emphasis on the Golgi complex as a discrete organelle. In *Biomembranes*. L. A. Manson, editor. Plenum Publishing corporation, New York. **2**:75-94.
  46. FRANKE, W. W. 1970. On the universality of nuclear pore complex structure. *Z. Zellforsch. Mikrosk. Anat.* **105**:405-429.
  47. FRANKE, W. W. 1971. Membrane-microtubule-microfilament-relationships in the ciliate pellicle. *Cytobiologie.* **4**:307-316.
  48. FRANKE, W. W. 1971. Cytoplasmic microtubules linked to endoplasmic reticulum with cross-bridges. *Exp. Cell Res.* **66**:486-488.
  49. FRANKE, W. W., B. DEUMLING, B. ERMEN, E.-D. JARASCH, and H. KLEINIG. 1970. Nuclear membranes from mammalian liver. I. Isolation procedure and general characterization. *J. Cell Biol.* **46**:379-395.
  50. FRANKE, W. W., and W. HERTH. 1974. Morphological evidence for de novo formation of plasma membrane from coated vesicles in exponentially growing cultured plant cells. *Exp. Cell Res.* **89**:447-451.
  51. FRANKE, W. W., W. HERTH, W. J. VAN DER WOUDE, and D. J. MORRÉ. 1972. Tubular and filamentous structures in pollen tubes: Possible involvement as guide elements in protoplasmic streaming and vectorial migration of secretory vesicles. *Planta (Berl.)*. **105**:317-341.
  52. FRANKE, W. W., J. KARTENBECK, S. KRIEN, W. J. VAN DER WOUDE, U. SCHEER, and D. J. MORRÉ. 1972. Inter- and intracisternal elements of the Golgi apparatus. A system of membrane-to-membrane cross-links. *Z. Zellforsch. Mikrosk. Anat.* **132**:365-380.
  53. FRANKE, W. W., J. KARTENBECK, H. W. ZENTGRAF, U. SCHEER, and H. FALK. 1971. Membrane-to-membrane cross-bridges. *J. Cell Biol.* **51**:881-888.
  54. FRANKE, W. W., S. KRIEN, and R. M. BROWN, JR. 1969. Simultaneous glutaraldehyde osmium tetroxide fixation with postosmication. *Histochemie.* **19**:162-164.
  55. FRANKE, W. W., D. J. MORRÉ, B. DEUMLING, R. D. CHEETHAM, J. KARTENBECK, E.-D. JARASCH, and H.-W. ZENTGRAF. 1971. Synthesis and turnover of membrane proteins in rat liver: An examination of the membrane flow hypothesis. *Z. Naturforsch.* **26 b**:1031-1039.
  56. FRANKE, W. W., H. W. ZENTGRAF, U. SCHEER, and J. KARTENBECK. 1973. Membrane linkages at the nuclear envelope. *Cytobiologie.* **7**:89-100.
  57. FRIEND, D. S., and M. G. FARQUHAR. 1967. Functions of coated vesicles during protein absorption in the rat vas deferens. *J. Cell Biol.* **35**:357-376.
  58. 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.
  59. GOLDMAN, R. D., and D. M. KNIPE. 1973. Functions of cytoplasmic fibers in non-muscle cell motility. In *The mechanisms of muscle contraction*, Cold Spring Harbor Symp. Quant. Biol. **37**:523-534.
  60. GRAHAM, R. C., and M. J. KARNOVSKY. 1966. The early stages of absorption of injected horse radish peroxidase in the proximal tubules of mouse kidney: Ultrastructural cytochemistry by a new technique. *J. Histochem. Cytochem.* **14**:291-302.
  61. GRAY, E. G. 1961. The granule cells, mossy synapses and Purkinje spine synapses of the cerebellum: Light and electron microscope observations. *J. Anat.* **95**:345-356.
  62. GRAY, E. G. 1972. Are the coats of coated vesicles artifacts? *J. Neurocytol.* **1**:363-382.
  63. GRAY, E. G. 1973. The cytonet, plain and coated vesicles, reticulosomes, multivesicular bodies and nuclear bodies. *Brain Res.* **62**:329-335.
  64. GRAY, E. G. 1975. Synaptic fine structure and nuclear, cytoplasmic and extracellular networks. The stereoframework concept. *J. Neurocytol.* **4**:315-339.
  65. GRUENSTEIN, E., A. RICH, and R. R. WEILING. 1975. Actin associated with membranes from 3T3 mouse fibroblast and HeLa cells. *J. Cell Biol.* **64**:223-234.
  66. GWYNN, J., R. B. KEMP, B. M. JONES, and M. GROESCHEL-STEWART. 1974. Ultrastructural evidence for myosin of the smooth muscle type at the surface of trypsin-dissociated embryonic chick cells. *J. Cell Sci.* **15**:279-289.
  67. HAUSTEIN, D., J. J. MARCHALONIS, and M. J. CRUMPTON. 1974. Immunoglobulin of T lymphoma cells is an integral membrane protein. *Nature (Lond.)*. **252**:602-603.
  68. HEALD, C. W. 1974. Hormonal effects on mam-



- mary cytology. *J. Dairy Sci.* **57**:917-925.
69. HEALD, C. W., and R. G. SAACKE. 1972. Cytological comparison of milk protein synthesis of rat mammary tissue in vivo and in vitro. *J. Dairy Sci.* **55**:621-628.
  70. 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.
  71. HEPLER, P. K., and E. H. NEWCOMB. 1967. Fine structure of cell plate formation in the apical meristem of *Phaseolus* roots. *J. Ultrastruct. Res.* **19**:498-513.
  72. HEUSER, J. E., and T. S. REESE. 1973. Evidence for recycling of synaptic vesicle membrane during transmitter release at the frog neuromuscular junction. *J. Cell Biol.* **57**:315-344.
  73. HIRANO, H., B. PARKHOUSE, G. L. NICOLSON, E. S. LENNOX, and S. J. SINGER. 1972. Distribution of saccharide residues on membrane fragments from a myeloma-cell homogenate: its implications for membrane biogenesis. *Proc. Natl. Acad. Sci. U.S.A.* **69**:2945-2949.
  74. HIRSCH, J. G., and M. E. FEDORKO. 1968. Ultrastructure of human leukocytes after simultaneous fixation with glutaraldehyde and osmium tetroxide and "postfixation" in uranyl acetate. *J. Cell Biol.* **38**:615-627.
  75. HOLLMANN, K. H. 1959. L'ultrastructure de la glande mammaire normale de la souris en lactation. Etude au microscope électronique. *J. Ultrastruct. Res.* **2**:423-443.
  76. HOLLMANN, K. H. 1966. Sur des aspects particuliers des protéines élaborées dans la glande mammaire. Etude au microscope électronique chez la lapine en lactation. *Z. Zellforsch. Mikrosk. Anat.* **69**:395-402.
  77. HOLLMANN, K. H. 1974. Cytology and fine structure of the mammary gland. In Lactation. B. L. Larson, and V. R. Smith, editors. Academic Press, Inc., New York. Vol. I. 3-95.
  78. HOPWOOD, D. 1970. The reactions between formaldehyde glutaraldehyde and osmium tetroxide and their fixation effects on bovine serum albumin and on tissue blocks. *Histochemie.* **24**:50-64.
  79. JAMIESON, J. D., and G. E. PALADE. 1967. Intracellular transport of secretory proteins in the pancreatic exocrine cell. I. Role of the peripheral elements of the Golgi complex. *J. Cell Biol.* **34**:577-596.
  80. JØRGENSEN, N.-C. 1972. Actomyosin-like ATPase activity at the surface of fish eggs. *Exp. Cell Res.* **71**:460-464.
  81. KANASEKI, T., and K. KADOTA. 1969. The "vesicle in a basket." A morphological study of the coated vesicle isolated from the nerve endings of the guinea pig brain, with special reference to the mechanism of membrane movements. *J. Cell Biol.* **42**:202-220.
  82. KARTENBECK, J. 1974. Kontinuierliche und diskontinuierliche Membranbeziehungen in der Rattenhepatocyte und in Hepatom-Zellen. Ph.D. thesis. University of Freiburg. Germany. 1-233.
  83. KAWASAKI, T., and J. YAMASHINA. 1971. Metabolic studies of rat liver plasma membranes using D-1-<sup>14</sup>C-glucosamine. *Biochim. Biophys. Acta.* **225**:234-238.
  84. KEENAN, T. W., and C. M. HUANG. 1972. Membranes of mammary gland. VI. Lipid and protein composition of Golgi apparatus and endoplasmic reticulum from bovine mammary gland. *J. Dairy Sci.* **55**:1586-1596.
  85. KEENAN, T. W., C. M. HUANG, and D. J. MORRÉ. 1971. Membranes of mammary gland. III. Lipid composition of Golgi apparatus from rat mammary gland. *J. Dairy Sci.* **55**:51-57.
  86. KEENAN, T. W., D. J. MORRÉ, and R. D. CHEETHAM. 1970. Lactose synthesis by a Golgi apparatus fraction from rat mammary gland. *Nature (Lond.)* **228**:1105-1106.
  87. KEENAN, T. W., D. J. MORRÉ, and C. M. HUANG. 1974. Membranes of mammary gland. In Lactation. A Comprehensive Treatise. B. L. Larson and V. R. Smith, editors. Academic Press, Inc., New York. **2**:191-233.
  88. KEENAN, T. W., D. J. MORRÉ, D. E. OLSON, W. N. YUNGHANS, and S. PATTON. 1970. A biochemical and morphological comparison of the plasmalemma and milk fat globule membrane from bovine mammary gland. *J. Cell Biol.* **44**:80-93.
  89. KENNEL, S. J., and R. A. LERNER. 1973. Isolation and characterization of plasma membrane associated immunoglobulin from cultured human diploid lymphocytes. *J. Mol. Biol.* **76**:485-502.
  90. KOMNICK, H., U. STOCKEM, and K. E. WOHLFARTH-BOTTERMANN. 1973. Cell motility: mechanism in protoplasmic streaming and amoeboid movement. *Int. Rev. Cytol.* **34**:169-249.
  91. KORNGUTH, S. E., and E. SUNDERLAND. 1975. Isolation and partial characterization of a tubulin-like protein from human and swine synaptosomal membranes. *Biochim. Biophys. Acta.* **393**:100-114.
  92. KUHN, C., III, L. A. CALLAWAY, and F. B. ASKIN. 1974. The formation of granules in the bronchiolar Clara cells of the rat. I. Electron microscopy. *J. Ultrastruct. Res.* **49**:387-400.
  93. KUROSUMI, K., U. 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.
  94. LAGUNOFF, D., and D. E. CURRAN. 1972. Role of bristle-coated membrane in the uptake of ferritin by rat macrophages. *Exp. Cell Res.* **75**:337-346.
  95. LAZARIDES, E., and K. WEBER. 1974. Actin antibody: the specific visualization of actin filaments in non-muscle cells. *Proc. Natl. Acad. Sci. U.S.A.* **71**:2268-2272.



96. LEMARCHAND, Y. A. SINGH, F. ASSIMACOPOULOS-JEANNET, L. ORCI, C. ROUILLIER, and B. JEANRENAUD. 1973. A role for the microtubular system in the release of very low density lipoproteins by perfused mouse liver. *J. Biol. Chem.* **248**:6862-6870.
97. LENARD, J., and R. W. COMPANS. 1974. The membrane structure of lipid-containing viruses. *Biochim. Biophys. Acta.* **344**:51-94.
98. LINZELL, J. L., and M. PEAKER. 1971. Intracellular concentrations of sodium, potassium and chloride in the lactating mammary gland and their relation to the secretory mechanism. *J. Physiol. (Lond.)*. **216**:683-700.
99. LINZELL, J. L., and M. PEAKER. 1971. The permeability of mammary ducts. *J. Physiol. (Lond.)*. **216**:701-716.
100. LINZELL, J. L., and M. PEAKER. 1971. Mechanism of milk secretion. *Physiol. Rev.* **51**:564-597.
101. LÜDER, M. R. 1976. Ultrastruktur von Endo- und Plasmamembranen in der laktierenden Mamma-Epithelzelle. Diploma thesis, University of Freiburg, Germany. 1-72.
102. LUFT, J. H. 1961. Improvements in epoxy resin embedding methods. *J. Biophys. Biochem. Cytol.* **9**:409-414.
103. MABUCHI, J. 1973. A myosin-like protein in the cortical layer of the sea urchin egg. *J. Cell Biol.* **59**:542-547.
104. MALAISSE, W. J. 1972. Role of calcium in insulin secretion. *Isr. J. Med. Sci.* **8**:244-251.
105. MATHER, J. H., and T. W. KEENAN. 1975. Studies on the structure of milk fat globule membrane. *J. Membr. Biol.* **21**:65-85.
106. MELDOLESI, J. 1974. Secretory mechanisms in pancreatic acinar cells. Role of the cytoplasmic membranes. *Adv. Cytopharmacol.* **2**:71-85.
107. MELDOLESI, J. 1974. Dynamics of cytoplasmic membranes in guinea pig pancreatic acinar cells. I. Synthesis and turnover of membrane proteins. *J. Cell Biol.* **61**:1-13.
108. MORRÉ, D. J., T. W. KEENAN, and C. M. HUANG. 1974. Membrane flow and differentiation: origin of Golgi apparatus membranes from endoplasmic reticulum. *Adv. Cytopharmacol.* **2**:107-125.
109. MORRÉ, D. J., and H. H. MOLLENHAUER. 1974. The endomembrane concept. In *Dynamics of plant ultrastructure* A. W. Robards, editor. McGraw-Hill Publishing Co. Ltd., London, 84-137.
110. MORRÉ, D. J., H. H. MOLLENHAUER, and C. E. BRACKER. 1971. Origin and continuity of Golgi-apparatus. In *Origin and continuity of cell organelles*. J. Reinert and H. Ursprung, editors. Springer-Verlag KG., Berlin. 82-126.
111. MUKHERJEE, T. M., and L. A. STAEHELIN. 1971. The fine-structural organization of the brush border of intestinal epithelial cells. *J. Cell Sci.* **8**:573-599.
112. MURRAY, R. L., and M. W. DUBIN. 1975. The occurrence of actin-like filaments in association with migrating pigment granules in frog retinal pigment epithelium. *J. Cell Biol.* **64**:705-710.
113. NÈVE, P., C. WILLEMS, and J. E. DUMONT. 1970. Involvement of the microtubule-microfilament system in thyroid secretion. *Exp. Cell Res.* **63**:457-460.
114. NEWCOMB, E. H. 1967. A spiny vesicle in slime-producing cells of the bean root. *J. Cell. Biol.* **35**:C17-C22.
115. O'BRIEN, T. P. 1972. The cytology of cell-wall formation in some eukaryotic cells. *Bot. Rev.* **38**:87-118.
116. OLSEN, B. R., and D. J. PROCKOP. 1974. Ferritin-conjugated antibodies used for labeling of organelles in the cellular synthesis and transport of procollagen. *Proc. Natl. Acad. Sci. U.S.A.* **71**:2033-2037.
117. ORCI, L., A. A. LIKE, M. AMHERDT, B. BLONDEL, Y. KANAZAWA, E. B. MARLISS, A. E. LAMBERT, C. B. WOLLHEIM, and A. E. RENOLD. 1973. Monolayer cell culture of neonatal rat pancreas: an ultrastructural and biochemical study of functioning endocrine cells. *J. Ultrastruct. Res.* **43**:210-297.
118. PALADE, G. 1975. Intracellular aspects of the process of protein synthesis. *Science (Wash. D.C.)*. **189**:347-358.
119. PALADE, G. E., and R. R. BRUNS. 1968. Structural modulations of plasmalemmal vesicles. *J. Cell Biol.* **37**:633-649.
120. PALAY, S. L. 1963. Alveolate vesicles in Purkinje cell of the rat's cerebellum. *J. Cell Biol.* **19**:(2, Pt. 2) 89 a-90 a (Abstr.).
121. PATTON, S. 1974. Reversible suppression of lactation by colchicine. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **48**:85-87.
122. 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.
123. PATTON, S., and T. W. KEENAN. 1975. The milk fat globule membrane. *Biochim. Biophys. Acta.* **415**:272.
124. 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.
125. PITELKA, D. R., P. R. KERKOF, H. T. GAGNÉ, S. SMITH, and S. ABRAHAM. 1969. Characteristics of cells dissociated from mouse mammary glands. I. Method of separation and morphology of parenchymal cells from lactating glands. *Exp. Cell Res.* **57**:43-62.
126. POLLARD, T. D., and S. ITO. 1970. Cytoplasmic filaments of *Amoeba proteus*. I. The role of filaments in consistency changes and movement. *J. Cell Biol.* **46**:267-289.
127. REAVEN, E. P., and S. G. AXLINE. 1973. Subplas-

- malemmal microfilaments and microtubules in resting and phagocytizing cultivated macrophages. *J. Cell Biol.* **59**:12-27.
128. REDMAN, C. M., D. BANERJEE, K. HOWELL, and G. E. PALADE. 1975. Colchicine inhibition of plasma protein release from rat hepatocytes. *J. Cell Biol.* **66**:42-59.
  129. REYNOLDS, E. S. 1963. The use of lead citrate at high pH as an electronopaque stain in electron microscopy. *J. Cell Biol.* **17**:208-212.
  130. RICHARDSON, K. C. 1949. Contractile tissues in the mammary gland, with special reference to myoepithelium in the goat. *Proc. R. Soc. Lond. Ser. B Biol. Sci.* **136**:30-45.
  131. RIORDAN, J. R., M. MITRANIC, M. SLAVIK, and M. A. MOSCARELLO. 1974. The incorporation of L-[<sup>14</sup>C]fucose into glycoprotein fractions of liver plasma membranes. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **47**:248-251.
  132. ROBISON, W. G., and J. S. CHARLTON. 1973. Microtubules, microfilaments, and pigment movement in the chromatophores of *Palaemonetes vulgaris* (Crustacea). *J. Exp. Zool.* **186**:279-290.
  133. ROHRlich, S. T. 1974. Fine structural demonstration of ordered arrays of cytoplasmic filaments in vertebrate iridophores. A comparative survey. *J. Cell Biol.* **62**:295-304.
  134. ROTH, L. E., D. J. PIHLAJA, and Y. SHIGENAKA. 1970. Microtubules in the heliozoan axopodium. I. The gradion hypothesis of allosterism in structural proteins. *J. Ultrastruct. Res.* **30**:7-37.
  135. ROTH, T. F., and K. R. PORTER. 1963. Membrane differentiation for protein uptake. *Fed. Proc.* **22**: No. 2. (Abstr.).
  136. ROTH, T. F., and K. R. PORTER. 1964. Yolk protein uptake in the oocyte of the mosquito *Aedes aegypti* L. *J. Cell. Biol.* **20**:313-332.
  137. 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. Vol. 2. 147-189.
  138. SATIR, B. 1974. Membrane events during the secretory process. In *Transport at the cellular level*. M. A. Sleight and D. H. Jennings, editors. Cambridge University Press, London. 399-418.
  139. SATIR, B., C. SCHOOLEY, and P. SATIR. 1973. Membrane fusion in a model system. *J. Cell Biol.* **56**:153-176.
  140. SCHJEIDE, O. A., R. I. LIN, E. A. GRELLERT, F. R. GALEY, and J. F. MEAD. 1969. Isolation and preliminary chemical analysis of coated vesicles from chicken oocytes. *Physiol. Chem. Phys.* **1**:141-163.
  141. SCHMIDT, D. G., P. BOTH, B. W. VAN MARKWIJK, and W. BUCHHEIM. 1974. The determination of size and molecular weight of casein micelles by means of light scattering and electron microscopy. *Biochim. Biophys. Acta.* **365**:72-79.
  142. SEKHRI, K. K., D. R. PITELKA, and K. B. DE OME. 1967. Studies of mouse mammary glands. I. Cytomorphology of the normal mammary gland. *J. Natl. Cancer Inst.* **39**:459-490.
  143. SELMAN, G. G., and M. M. PERRY. 1970. Ultrastructural changes in the surface layers of the newt's egg in relation to the mechanism of its cleavage. *J. Cell Sci.* **6**:207-227.
  144. SILCOCK, W. R., and S. PATTON. 1972. Correlative secretion of protein, lactose and Kt in milk of the goat. *J. Cell. Physiol.* **79**:151-154.
  145. SLAUTTERBACK, D. B. 1963. Cytoplasmic microtubules. I. *Hydra. J. Cell Biol.* **18**:367-388.
  146. SLAUTTERBACK, D. B. 1967. Coated vesicles in absorptive cells of *Hydra*. *J. Cell Sci.* **2**:563-572.
  147. SMITH, D. S. 1971. On the significance of cross-bridges between microtubules and synaptic vesicles. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **261**:395-402.
  148. STADLER, J., and W. W. FRANKE. 1974. Characterization of the colchicine binding of membrane fractions from rat and mouse liver. *J. Cell Biol.* **60**:297-303.
  149. STEIN, O., and Y. STEIN. 1973. Colchicine-induced inhibition of very low density lipoprotein release by rat liver in vivo. *Biochim. Biophys. Acta.* **306**:142-147.
  150. STEWART, P. S., D. L. PUPPIONE, and S. PATTON. 1972. The presence of microvilli and other membrane fragments in the non-fat phase of bovine milk. *Z. Zellforsch. Mikrosk. Anat.* **123**:161-167.
  151. STOCKINGER, L., and J. ZARZICKI. 1962. Elektronenmikroskopische Untersuchungen der Milchdrüse des laktierenden Meerschweinchens mit Berücksichtigung des Saugaktes. *Z. Zellforsch. Mikrosk. Anat.* **57**:106-123.
  152. TRUMP, B. F., and R. E. BULGER. 1966. New ultrastructural characteristics of cells fixed in glutaraldehyde-osmium tetroxide mixture. *Lab. Invest.* **15**:368-379.
  153. VENABLE, J. H., and R. COGGESHALL. 1965. A simplified lead citrate stain for use in electron microscopy. *J. Cell Biol.* **25**:407-408.
  154. VITETTA, E. S., and J. W. UHR. 1975. Immunoglobulins and alloantigens on the surface of lymphoid cells. *Biochim. Biophys. Acta.* **415**:253-271.
  155. WALLACH, D., N. KIRSHNER, and M. SCHRAMM. 1975. Nonparallel transport of membrane proteins and content proteins during assembly of the secretory granule in rat parotid gland. *Biochim. Biophys. Acta.* **375**:87-105.
  156. WELLINGS, S. R. 1969. Ultrastructural basis of lactogenesis. In *Lactogenesis*. M. Reynolds and S. J. Folley, editors. University of Pennsylvania Press, Philadelphia, Pa. 5-25.

157. WELLINGS, S. R., and K. B. DEOME. 1961. Milk protein droplet formation in the Golgi apparatus of the C3H-Crg1 mouse mammary epithelial cells. *J. Biophys. Biochem. Cytol.* **9**:479-485.
158. WELLINGS, S. R., K. B. DEOME, and D. R. PITELKA. 1960. Electron microscopy of milk secretion in the C3H/Crg1 mouse. I. Cytomorphology of the prelactating and the lactating gland. *J. Natl. Cancer Inst.* **25**:393-421.
159. WELLINGS, S. R., and J. R. PHILP. 1964. The function of the Golgi apparatus in lactating cells of the BALB/c Crg1 mouse. An electron microscopic and autoradiographic study. *Z. Zellforsch. Mikrosk. Anat.* **61**:871-882.
160. WOODING, F. B. P. 1971. The mechanism of secretion of the milk fat globule. *J. Cell Sci.* **9**:805-821.
161. WOODING, F. B. P. 1971. The structure of the milk fat globule membrane. *J. Ultrastruct. Res.* **37**:388-400.
162. WOODING, F. B. P. 1973. Formation of the milk fat globule membrane without participation of the plasmalemma. *J. Cell Sci.* **13**:221-235.
163. WOODING, F. B. P. 1974. Milk fat globule membrane material in skim-milk. *J. Dairy Res.* **41**:331-337.