MITOCHONDRIA AND MITOCHONDRIA-TONOFILAMENT-DESMOSOMAL ASSOCIATIONS IN THE MAMMARY GLAND SECRETORY EPITHELIUM OF LACTATING COWS

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SUMMARY
The lactating cow mammary secretory epithelial cell is very active synthetically and contains numerous very pleomorphic mitochondria. Cup- and ring-shaped mitochondria are frequent and many are extremely elongated. Preferential localization of mitochondria in the basal region, or at the lateral margins of the secretory cell adjacent to intraepithelial monocytes have been observed. Occasional mitochondria show one or several very densely staining cristae with a lattice pattern. Mitochondria are frequently seen closely associated with the tonofilament-desmosomal complex. These structures and associations are present after immersion or perfusion fixation of mammary gland from several breeds of cow; their possible significance is discussed.

INTRODUCTION
The lactating mammary gland secretory epithelial cell is long lived and very active synthetically. Among the wide range of mammalian species the ultrastructure is remarkably uniform (Wooding, 1977) considering the range of carbohydrate, protein and fat concentrations and the variations in the molecular proportions making up those 3 main categories in the milks of the various species. To fuel this synthetic capacity there is a considerable mitochondrial population which increases in amount with the onset of lactation correlated with the expansion of cellular volume (Hollman, 1974).

We report here for the cow, the animal bred for an extreme capacity for milk synthesis, a variety of unusual shapes, distributions and associations of the mitochondria which, as far as we are aware, have not been reported from lactating mammary cells of other species. Similar structures within the mitochondrial cristae have been noted in ischaemic skeletal muscle mitochondria (Hanzlikova & Schiaffino, 1977) and pregnant rat hepatocytes (Tuchweber, Kovacs, Khandekar & Garg, 1972). Association of mitochondria with desmosomes has been reported in a range of other tissues (for a full list see Guillouzo, Guillouzo & Boisnard, 1978) but not in a mature secretory tissue such as the mammary gland except for a brief mention, without comment, in a preliminary report on keratinization in the lactating mammary gland (Zerban & Franke, 1977).

MATERIALS AND METHODS

Unstressed Dexter (1), Friesian (2) or Jersey (4) cows in full lactation were used. The cows were either shot or killed with an overdose of pentobarbitone injected rapidly intravenously, the udder was then cut off, the mammary artery dissected out and ligated and the blood washed out of the mammary tissue by perfusion with 0.5 % procaine in Dulbecco phosphate saline at room temperature for 2 min. This was followed by 4 % glutaraldehyde in 0.1 M NaH₂PO₄-Na₂HPO₄ buffered pH 7.2 with 2 % sucrose added, for 15 min at room temperature. The best perfused, i.e. the most solid, yellowest tissue was then cut up into 1-mm cubes in the fixative and left for a further 1–2 h at room temperature.

For immersion fixation fresh tissue was cut up into 1-mm pieces in the above fixative and fixed for 1–2 h at room temperature.

Postfixation was for 1 h in 1 % osmium tetroxide in 0.1 M veronal acetate buffer at pH 7.2, the tissue blocks were stained in 2 % aqueous uranyl acetate for 1 h, dehydrated in ethanol and propylene oxide and embedded in Araldite. All processing was carried out at room temperature. Sections were stained with uranyl acetate and lead citrate.

RESULTS

At peak lactation (3 months after parturition) and at later stages the mammary glandular epithelial cell cytoplasm consists of a rich network of rough endoplasmic reticulum, membrane-bound casein granules, well-developed Golgi apparatus and lipid droplets (Fig. 1). There are many mitochondria which are usually found scattered in the cytoplasm, but occasionally a greater number are seen clustered at the basal region of the cells (Fig. 1). In some cells mitochondria are sometimes seen aligned along the lateral plasma membrane with variable amounts of intervening cytoplasm. In certain regions where adjacent epithelial cells show a much more definite mitochondrial margination, a pale mononuclear cell (cell of monocyte/macrophage series) is seen located between them (Fig. 2). This gives the impression that the monocyte is surrounded by a chain of mitochondria. Closer examination reveals that the lateral surfaces of the mitochondria are closely applied to the lateral plasma membrane of the epithelial cell with very little intervening cytoplasm.

In the lactating mammary glandular epithelial cells a high proportion of the mitochondria are long and slender, and some are seen with a markedly attenuated middle segment and 2 sacculated extremities (Figs. 3, 7). U- and ring-shaped mitochondria are also frequently seen, and the cytoplasm located within the confines of their concave surfaces is much less dense than the surrounding cytoplasm (Fig. 3).

Mitochondria with a lattice structure within one or two cristae are occasionally seen (Figs. 4, 8). Such structures are usually in the narrower parts of a mitochondrion and appear to constrain the mitochondrial shape (Figs. 5–7). The mitochondria with these

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Fig. 1. Mammary secretory epithelial cells at a late stage of lactation, with cytoplasm consisting of a rich network of rough endoplasmic reticulum (r), Golgi vesicles containing casein granules (c) and lipid droplets (l). Note the cluster of mitochondria (m) located at the basal region of the cell, and a mitochondria-tonofilament-desmosomal complex (arrow). × 9900.

Fig. 2. A cell of the monocyte-macrophage series (ma) located between 2 epithelial cells (e). Note the mitochondrial margination in the adjacent epithelial cells (arrows) which gives the impression that the monocyte is surrounded by a chain of mitochondria. A mitochondria-tonofilament-desmosomal complex is located close to the apical surfaces of the cells (arrowhead). × 19800.
Fig. 3. U- and ring-shaped mitochondria. Note that the cytoplasm located within the ring and the confines of the concave surfaces is less dense than the surrounding cytoplasm (arrowheads). × 28,400.

Figs. 4–6. Mitochondria with one or more densely stained cristae (arrows). Fig. 4 shows higher magnification of 4 cristae which are characterized by lattice structures. Fig. 4, × 47,500; Fig. 5, × 11,500; Fig. 6, × 15,700.
peculiar morphological characteristics do not appear to have any preferential distribution in the cytoplasm of the cells.

A very striking feature in the secretory epithelium is the frequent occurrence of mitochondria with an apparently specific association with the tonofilament-desmosomal complex. Desmosomes are present in most lactating cells just below the tight junctions, and are rare below this. In some sections through the epithelium just below the tight junction all the desmosomes have mitochondria in close association (Fig. 9). Desmosomes lower down the lateral surface show this less frequently.

In any one cell, 1 mitochondrion may be associated with 1, or stretched between 2 (Fig. 10), or several (Fig. 9) adjacent desmosomes. Occasionally a Golgi vesicle is also seen apparently as part of the association (Fig. 11).

A desmosome may have mitochondria associated with both ends (Figs. 12, 13) in contiguous cells, and 2 desmosomes may share 2 mitochondria (Figs. 9, 10). Desmosomes with a mitochondrion on only one side are far less frequent (Figs. 9, 14).

Higher magnification of the association shows that the mitochondria are associated with the tonofilaments and not the dense material of the desmosome itself. The mitochondrial outer membrane is within 5 nm of one or more tonofilaments originating from the plaque. Tilting the section reveals no point of contact, nor any strand-like connexion between the surfaces. Where a tonofilament runs alongside a mitochondrial outer membrane the separation is constant (Figs. 12, 13). Mitochondria are also occasionally seen associated with bundles of tonofilaments close to the lateral plasma-lemma (Fig. 14) but well away from any desmosomal plaque.

In the ductal epithelia of the lactating gland and the secretory and ductal epithelia of the mid pregnant cow, mitochondria-tonofilament-desmosomal associations are rarely observed, and their mitochondria show none of the peculiar shapes and structures seen in the secretory cells.

DISCUSSION

The reality of the various unusual mitochondrial shapes and internal structures and associations found in the lactating cow mammary gland rests largely on their reproducible demonstration in early and late lactation with both immersion and perfusion fixation techniques. They seem to be peculiar to the cow; nothing similar has been seen in sheep, goat, rat, guinea-pig, domestic pig, wallaby or elephant seal mammary glands fixed by equivalent procedures (Wooding, 1977). The increased density shown by some mitochondrial cristae membranes is sometimes correlated with a lattice-like substructure. From the angular outlines of some of the mitochondria containing such structures it seems possible that their formation may cause or be the result of the extreme attenuation of the mitochondrion. Similar structures have been found in ischaemic skeletal muscle mitochondria (Hanzlikova & Schiaffino, 1977). Since at least 10 min elapsed between death of the perfused cows and the start of our perfusion fixation it is possible that formation of the lattice structure within the crista is a pathological response to low oxygen concentrations. However, mammary glands of other species showed perfectly normal mitochondria after a similar preparation. The
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The presence of similar structures in the mitochondria of a wide variety of tissues, including plants (Newcomb, Steer, Hepler & Wergin, 1968) suggests that it is one of a range of structures that can be assumed by the basic building blocks formed by the assemblies of respiratory proteins.

The epithelial cells of lactating mammary gland have a very high energy demand for its massive active transport, synthetic and secretory activities. It therefore seems unlikely that there would be any consistent preferential localization of mitochondria at the base of the cells as was suggested by some observations, but conclusive evidence for or against this observation would require extensive morphometric investigations.

Evidence for margination of the mitochondria of the mammary epithelial cells seemed significant only where secretory cells were contiguous with intraepithelial leukocytes. The function of this margination may be related to transport of some kind between the 2 types of cells. There was no evidence for any gap (communicating) or other junctions between such cells.

The most striking feature of the mitochondrial population is the close association with the tonofilament-desmosomal complex. Pitelka (1978) found that in the mouse there were few if any desmosomes left at full lactation; the situation seems very different in the cow, since even late in lactation desmosomes of conventional structure are usually present. A large number of these desmosomes have mitochondria alongside, but the mitochondria are most closely associated with the tonofilaments that run into the desmosomal plaque. No continuity between the attached tonofilaments and the mitochondrial outer membrane has been clearly demonstrated. However, the uniformity of the separation between tonofilament and mitochondrion suggests there may be specific adhesion.

The function of the association is obscure. It seems unlikely that it is essential to gland function because there seems to be nothing unique about the ultrastructural mechanism of secretion in the cow, but the association has not been observed in the mammary gland of any other species. Similar mitochondrial associations with desmosomes have been reported from a variety of other tissues under both physiological and pathological conditions, but there appears to be no other feature or function common to all other reports. All the previous reports, where it is possible to discern detail, show that the mitochondria are associated more directly with tonofilaments originating from the desmosomal plaque than with the plaque itself. Initial suggestions that the association was peculiar to foetal, neonatal or developing

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Fig. 7. A mitochondrion with a markedly attenuated middle segment and 2 sacculated extremities. Note the presence of a densely stained crista which extends along the entire attenuated segment (arrows). ×15350.

Fig. 8. Details of 2 cristae (arrows) in the mitochondrion in Fig. 5. The cristae show a lattice structure which changes abruptly into longitudinal streaks. ×90500.

Fig. 9. Transverse section of mammary secretory epithelium close to the apical surface, showing complexes of various configurations (arrows). Note that almost all the desmosomes are associated with mitochondria. ×7750.

Fig. 10. Details of a mitochondria-tonofilament-desmosomal complex shown in Fig. 9 (arrowhead). Note that 2 desmosomes and 2 mitochondria are involved. ×112000.
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epithelia (Deane, Wurzelmann & Kostellow, 1966) do not fit in with subsequent results for thyroid (Bernstein & Wollman, 1975) or our observations here on an obviously mature actively secreting tissue.

The correlation with young cells was thought to indicate that the mitochondria were involved in the formation or breakdown of desmosomes, possibly by their regulation of the local calcium concentration. Since Pitelka (1978) has indicated that desmosomes do decrease in number at the onset of lactation in the mouse and rabbit it is possible that the association with mitochondria observed in the cow is related to changes in tonofilament and/or desmosomal distribution. However, we could find no indication of any decrease in individual tonofilament and desmosomal substance or frequency whatever the stage of lactation.

It is unlikely that there could be any intercellular transport function for the association because there is no evidence that the desmosome is anything but an impermeable adhesive patch between cells (Staehelin, 1974).

Since the mitochondria seem to be closer to the tonofilaments than the desmosomal plaque material it could be that the association represents a purely fortuitous bottleneck in the cellular transport system. Although there are no reports of movements of intracellular organelles from direct observations of living mammary secretory cells it is clear that there must be a continuous traffic of lipid droplets from the basal regions of the cell where they form, and of Golgi vesicles from the Golgi body, to the apical plasmalemma where they are continuously released.

There is evidence from other cell systems that Golgi vesicles move along microtubule surfaces (Lacy & Malaisse, 1973; Smith, Jarlfors & Cameron, 1975). In the mammary cell the Golgi vesicles associate with the lipid droplets (Wooding, 1977), so both may move on the same system.

Tonofilaments (or intermediate filaments) have been implicated in intracellular movements in cultured cells (Goldman, 1971). If mitochondria moved along filament surfaces they would be expected to stop when the filament inserts into the desmosomal plaque. In the mammary secretory cell cytoplasm tonofilaments usually run singly and no unequivocal association with the mitochondrial outer membrane has been observed. When tonofilaments group into small bundles near the lateral plasmalemma, mito-

Fig. 11. A Golgi vesicle (arrowhead) in association with the mitochondria-tonofilament-desmosomal complex. Tonofilaments (arrows). × 70,000.

Fig. 12. Details of a desmosome having mitochondria associated with both ends. Note the nearest tonofilament (arrows) maintains a uniform distance of approximately 5 nm from the mitochondrion outer membrane (arrowhead). × 101,000.

Fig. 13. Details of the association of 2 mitochondria with 1 desmosome. Note tonofilaments in cross-section (arrow) between the mitochondrion outer membrane and the dense plaque of the desmosome. × 105,000.

Fig. 14. A mitochondrion associated with a bundle of transversely sectioned tonofilaments but with no sign of any desmosomal plaque. Note a tonofilament bundle (arrow) in the adjacent cell; presumably there was a desmosome above or below the plane of this section. × 115,000.
Mitochondria are occasionally seen in association with these bundles away from any desmosomal plaque. Tonofilaments are present throughout the cytoplasm of a wide variety of cells (Franke et al. 1978), and there is a recent report that they contain actin (Buckley, Raju & Stewart, 1978). This might enable the filaments to play a more active role in organelar movement as well as their more conventional structural or 'guy rope' function. We have seen an occasional Golgi vesicle in close association with the tonofilament-desmosomal complex, and both endoplasmic reticulum cisternae in thyroid cells (Bernstein & Wollman, 1975) and peroxisomes in liver cells (Tandler & Hoppel, 1970) have been reported in a similar association.

In cultured hepatocytes, Guillouzo et al. (1978) have recently shown that the mitochondria-tonofilament-desmosomal association can be reproducibly varied by changing the glucose or phenobarbital concentrations, without any equivalent change in desmosomal frequency or structure. Here the variations in the conditions could be affecting the frequency of attachment of the mitochondria to the filaments. If the filament system is seen as a pathway for slow fairly random intracellular movements primarily of cell organelles not destined for secretion then the various observations of organelle associations with desmosomes can be explained.

Thus the various forms and distribution of the mitochondria of the lactating bovine mammary epithelial cell could, possibly, most simply be accounted for as expressions of plastic deformations and cell movements, consequent on considerable essential intracellular traffic in a cell crowded with organelles. Why this should be only found in the bovine mammary gland remains unclear.

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REFERENCES


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