UNUSUAL MITOCHONDRIA OF THE BOVINE OOCYTE

P. L. SENGER and R. G. SAACKE. From the Department of Dairy Science, Virginia Polytechnic Institute, Blacksburg, Virginia 24061

Numerous reports have appeared in the literature that characterize a wide spectrum of mitochondrial forms in different animal cells. Typically, the descriptions of this organelle have shown that it ranges from filamentous mitochondria to variations of spherical organelles. This report describes a morphologically unusual type of mitochondrion revealed during an ultrastructural study of bovine oocytes.

MATERIALS AND METHODS

Oocytes were obtained from 12 cows in estrus via follicular aspiration at the time of slaughter. The mature Graafian follicle was of major interest since, in the bovine, this follicle is easily distinguished by its size from other antral follicles present on the ovary, and with reasonable certainty it contains the developing oocyte destined to be ovulated approximately 12 hr following cessation of estrus (8). Thus, 12 oocytes recovered from mature follicles were reasonably similar with respect to stage of maturation. For comparison, 25 oocytes from small antral follicles were recovered and examined, with recognition that many such follicles are in the process of atresia rather than development (4).

Oocytes were fixed for 30 min–1 hr in 1% osmium tetroxide buffered to pH 7.4 with Veronal-acetate (5). Sucrose was added to adjust for tonicity (1). Oocytes were dehydrated in ethanol baths (80, 95, and 100%) and embedded in Epon 812. For light microscopy, thick sections (0.5–1 μ) were cut on a Porter-Blum MT-2 ultramicrotome and stained with Azure II according to the method of Joen (3). Oil Red O was used to identify lipid in the oocyte cytoplasm. For electron microscopy, thin sections were cut on a Porter-Blum MT-2 ultramicrotome, stained with uranyl acetate and lead citrate (9), and examined in an RCA-EMU-3H electron microscope. Details concerning manipulation of ova from recovery to microscopy were carried out according to the procedure of Senger and Saacke (6).

RESULTS

Mitochondria that possessed a thin appendage arising from the surface of the organelle were abundant in all oocytes recovered from mature and small antral follicles. In thin sections, some mitochondria appeared to have an appendage that formed a full arch (Fig. 2), resulting in the formation of a cavity limited by the outer mitochondrial membrane. Other mitochondria, however, possessed a less complete arch which formed a cavity with an obvious opening to the cytoplasm (Figs. 2 and 3,*A*). Occasionally, the appendage extended from the mitochondrion in a linear manner rather than in the form of an arch. The appendage forming the arch was composed of both inner and outer mitochondrial membranes (Fig. 3,*A*), and contained a matrix having greater electron opacity than the matrix of the mitochondrial body (Fig. 3). While cristae could not

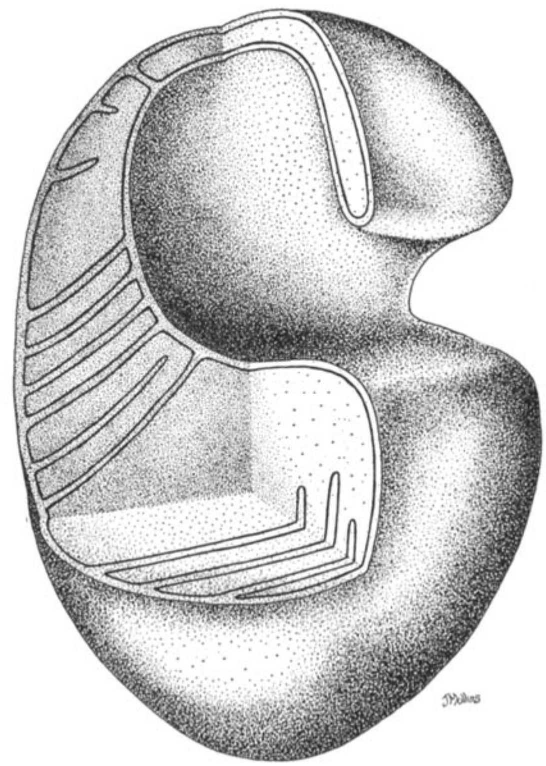

FIGURE 1 A three-dimensional schematic illustration of a hooded mitochondrion.

be clearly identified within the appendage, they were observed often near the junction of the appendage and the mitochondrial body (Fig. 3,*A*).

The appendages are interpreted as thin sections through a hood that is approximately as wide as the mitochondrion. A three-dimensional graphic interpretation of a hooded mitochondrion is presented in Fig. 1. Such an interpretation is based on examination of many thin sections and is intended to illustrate the type of hood envisioned, recognizing that a great degree of variation undoubtedly exists among mitochondria within the oocyte. Sections through the hood alone were often encountered. These appeared as arches or complete circles, depending upon plane of section (Fig. 2).

Mitochondria, predominantly hooded mitochondria, were distributed in the vicinity of large droplets in the cytoplasm (Fig. 2). The droplets were identified as lipid by means of Oil Red O staining of adjacent thick sections observed with the light microscope.

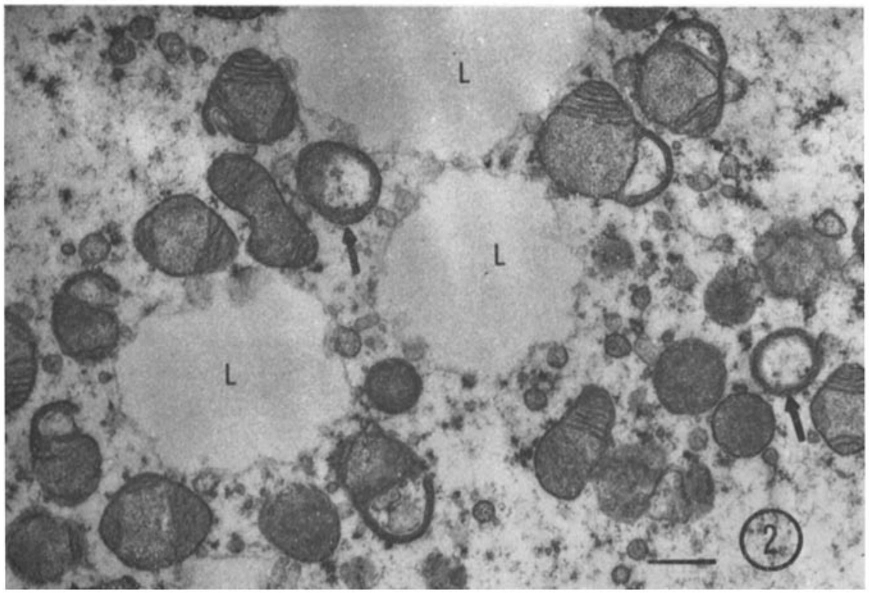

FIGURE 2 An electron micrograph showing variations in the hooded mitochondria in close proximity to the lipid droplets (*L*). In this section some mitochondria have short appendages while others have appendages forming a more complete arch. Circular structures (arrows) represent a section through the top or side of a hood formed by the arch. Scale line, 0.9 cm = 1 μ. × 9300.

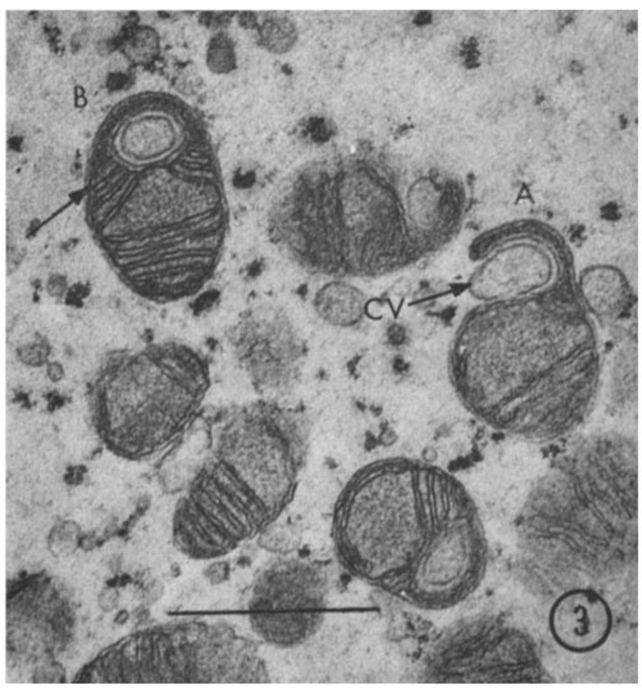

FIGURE 3 An electron micrograph showing various orientations of mitochondria within a single section. A mitochondrion is present with a distinct finger-like appendage (*A*); a cytoplasmic vesicle (*CV*) lies within the cavity formed by the hood. Basket-shaped mitochondria (*B*) are also present. These are believed to be hooded mitochondria cut in a plane perpendicular to the plane shown in mitochondrion. *A*. Cristae in the basket-shaped mitochondria are oriented into the direction of the cavity formed by the hood (arrow). Note difference in electron opacity within the matrix of the appenagde as compared to the matrix of the mitochondrial body. Scale line, 2.8 cm = 1 μ. × 28,000.

Frequently, membrane-bounded vesicles were closely associated with the inner surface of the mitochondrial hoods (Figs. 2 and 3). Similar vesicles were also abundant elsewhere in the cytoplasm. While it appears from these micrographs that the hooded mitochondria have an association with the cytoplasmic vesicles, the origin and function of the vesicles are not clear.

DISCUSSION

The functional significance of the hooded mitochondria observed in this study is not known. The abundance of these unusual organelles in bovine oocytes and their absence in oocytes from other mammalian species studied to date are likewise without explanation. Morphological evidence in this study suggests that the hood may represent a modification that results in an increase in the surface area of the organelle. Such a modification may be critical to a cell which presumably depends, at least in part, on previously accumulated energy sources. These accumulated materials undoubtedly play an important role in the survival of newly ovulated ova and the early embryo. The efficient utilization of these materials may be associated with the unusual hooded mitochondria observed in this study.

In Leydig cells of neonatal rat testis, Christensen and Chapman (2) reported the presence of cup-shaped mitochondria which engulfed portions of the cytoplasm. Stephens and Bils (7), studying normal hepatic tissue, reported the occurrence of atypical mitochondria containing a cavity in a very limited number of adjacent cells. On the basis of serial sections, they (7) showed that the cytoplasm within the mitochondrial cavity is continuous with extramitochondrial cytoplasm through a small pore.

While the mitochondria observed by Christensen and Chapman (2) and Stephens and Bils (7) contained a cavity continuous with the cytoplasm, only those described by the latter workers (7) bore close resemblance to those reported in the present study. Both sets of workers postulated that the unusual mitochondria may serve the cell through an increase in the surface area of the organelle. Stephens and Bils (7) further speculated that the hooded mitochondria were associated with the onset of a pathological condition in hepatic tissue.

Due to the abundance of hooded mitochondria observed in all oocytes in this study, we believe that these organelles characterize the bovine follicular oocyte. Recent studies in our laboratory,[1] in which glutaraldehyde fixation was used, have shown the presence of hooded mitochondria in 19 additional oocytes from mature Graafian follicles. These observations provide further confidence that the hooded mitochondria are normally-occurring organelles in this cell.

Appreciation is expressed to Mrs. K. J. Mullins for the art work required in the three-dimensional graphic interpretation. Appreciation is also expressed to Dr. R. F. Kelley and Mr. Leon Linkous for their cooperation in the use of the University slaughter facilities for collection of the oocytes used in this study.

Received for publication 23 December 1969, and in revised form 6 February 1970.

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