On Mitochondrial Inclusions in Granulosa Lutein Cells of Pregnant Cows

The granulosa lutein cells from human[1–3], porcine[4], and ovine[5,6] corpora lutea contain mitochondria with homogeneous osmiophilic inclusions very similar in density to lipid inclusions seen in the same cells. These intramitochondrial inclusions were observed in cyclic stages, but were more frequent in granulosa lutein cells of pregnancy. In the lutein cells from the period of delayed implantation and early postimplantation stages of the armadillo, mink and rat, the mitochondria were also found to contain granules but with less density than the frank lipid droplets in the cytoplasm[7].

From 5 cows at 80–240 days of pregnancy, 1 mm³ cubes of luteal tissue were fixed in 2.5% glutaraldehyde buffered with cacodylate at pH 7.4 and/or 1% osmium tetroxide with the same buffer. After dehydration and embedding in TAAB (TAAB Laboratories) 1 μm sections were stained with toluidine blue and thin sections of 70 nm were stained with uranyl acetate and lead citrate and examined in a Siemens Elmiskop 101.

The mitochondria from the granulosa lutein cells contained osmiophilic inclusions, which seemed to develop when the cristae in the central part of the mitochondria were filled with a homogeneous osmiophilic substance. Then the cristae were obscured or disappeared leaving a round homogeneous mass (Figure 1), which grew and finally was seen as an electron dense inclusion surrounded by the outer mitochondrial membrane (Figure 2). These intramitochondrial inclusions were very similar in density to lipid inclusions.

Intramitochondrial inclusions have been observed in mitochondria from corpora lutea in different species[1–9] and these mitochondria were often seen in relation to lipid inclusions. In a 7-day human corpus luteum, mitochondria were seen in juxtaposition to a lipid dropelet and the mitochondrial membrane was disrupted where it was related to the lipid droplet[8]. In the interstitial cells from the human foetal testis, the mitochondria were regularly found to contain osmiophilic inclusions[10].

In isolated mitochondria from the rat testis[11], the cholesterol side chain cleavage enzyme has been localized. This enzyme system needs a pyridin nucleotide, nicotinamide adenine dinucleotide phosphate, NADPH, to

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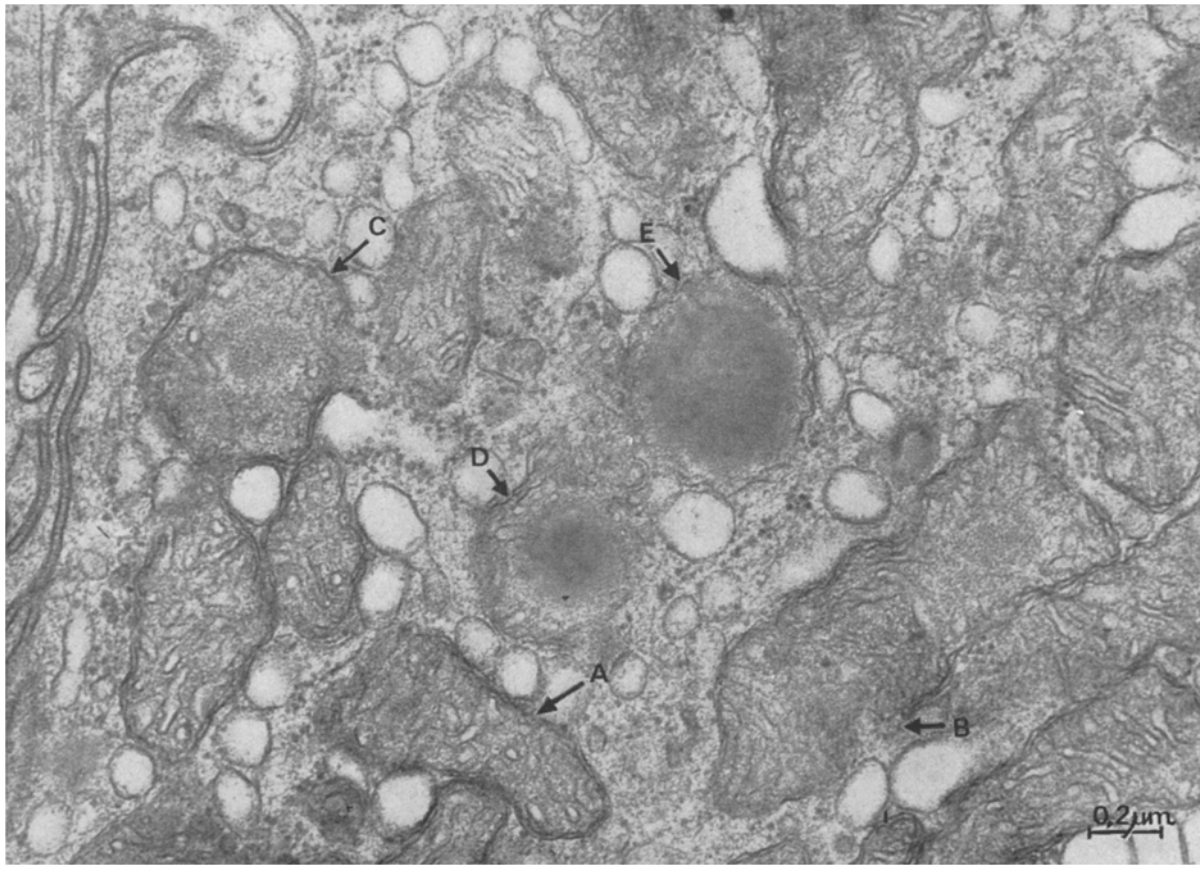

Fig. 1. Mitochondria with developing osmiophilic inclusions. The content of the cristae shows a gradual increasing of the density from A) to E), where an osmiophilic inclusion nearly fills the central part of the mitochondrion. 135 days of pregnancy. Electron micrograph. × 400,000.

convert cholesterole to pregnanolone. Histochemistry on human corpora lutea from cyclic stages has shown NADPHase activity on the mitochondrial cristae[12]. Mitochondria in guinea-pig ovaries have been seen in close relationship with rough endoplasmic reticulum[13]. Since the mitochondria contain NADPHase, the electron dense inclusions may be a condensation of some metabolic products from the steroid synthesis. So the inclusions in mitochondria from bovine granulosa cells of pregnancy may be a morphological feature closely related to steroid synthesis.

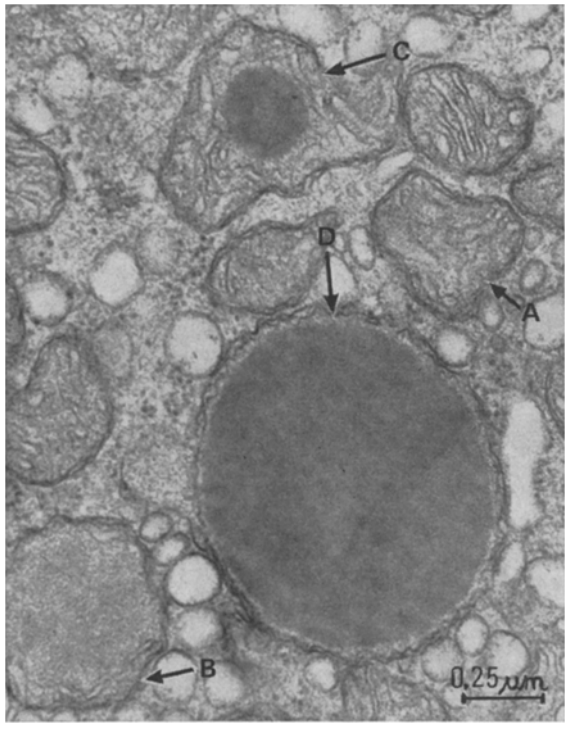

Fig. 2. A) Mitochondrion with obscured cristae. B) Mitochondrion where only few cristae are preserved. C) Mitochondrion with a round osmiophilic inclusion in a central position. D) A mitochondrion filled with an osmiophilic inclusion with the same appearence as a lipid inclusion. 135 days of pregnancy. Electron micrograph. × 40,000.

Zusammenfassung. Im Gelbkörper von Kühen wurden während der Periode von 80–240 Trächtigkeitstagen in den Granulosaluteinzellen Einschlüsse gefunden, die beinahe ganze Mitochondrien ausfüllten. Die Elektronendichte war der von Fett-Tröpfchen ähnlich. Die Einschlüsse könnten morphologisch dahin deuten, dass die Mitochondrien zur Steroidsynthese der Granulosazellen enge Beziehungen haben.

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The Occurrence of Filamentous Banded Elements as Components of *Mytilus galloprovincialis* Byssus

In the course of an extensive research on the ultrastructure of the byssal apparatus of Mytilus galloprovincialis, the presence has been discovered of periodic filamentous elements within the byssus structure.

The presence of such elements had remained undetected in the previous ultrastructural observations of Mercer[1], Randall et al.[2], Jackson et al.[3] and Bairati[4], and appears to be of some interest from the standpoint of the presence and location of the collagen protein in byssus.

Samples of *M. galloprovincialis* byssus obtained from live animals were fixed with a 3% glutaraldehyde solution buffered with s-collidine EM (TAAB) to a pH of 7.2 with the addition of 30 mg sucrose per ml (470 mOsm), then post-fixed in a 1% osmium tetroxide solution. They were embedded in Durcopan-ACM (Fluka) and sectioned, special care being devoted to the location of the cutting surfaces. The sections were contrasted with uranyl acetate and lead citrate, and were then examined with a Siemens Elmiskop 101 electron microscope.

Figure 1 shows what is the most frequent appearance of these periodic filamentous elements: prevalently anisodiametric bands approximately 0.2 µm in diameter. While their thickness is fairly constant, their length within the sections varies considerably, probably in relation to their wavy course. As their boundaries with the material forming the byssus matrix are never sharp, they look more like specific portions of the matrix itself than independent elements proper, this being the reason why 'filamentous banded elements' (FBE) would seem a more appropriate term to describe them rather than 'fibres' in the true meaning of the word, the definition being based more on their structure than on their shape.

From Figure 2 the FBE appear to consist of protofilaments approximately 75 Å in diameter, longitudinally arranged and clearly distinguishable in the less dense portion of the period.

The period itself is made up of a denser portion (A) and a markedly lighter area (B): 3 bands being at times recognizable in A, 2 of them (a, a) along the borders and one (b) forming the central, and lighter, zone. The whole period measures an average of 1000 Å, the extensions of the 2 portions being somewhat variable. As will be seen, the period is centrosymmetrical and non-polarized. As to the FBE, so far they have been identified mostly in the

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