MITOCHONDRIAL STRUCTURE IN SITES OF STEROID SECRETION*

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Plates 126 and 127

INTRODUCTION

In an extended study of the adrenal gland of the rat, peculiarities of the mitochondria of the adrenal cortex were noted. Since the mitochondria of this steroid secretor appeared unique, a brief comparative survey was made to include the mitochondria of other steroid glands. The results are of sufficient interest to warrant a preliminary report at this time devoted solely to mitochondria in steroid-producing organs, including in addition to the adrenal cortex, the ovarian follicle and corpus luteum, the testis, and the placenta.

Materials and Methods

The observations in this report are based on tissue from rats of the Wistar and Long-Evans strains. All operations were performed under nembutal anesthesia.

For study of the adrenal cortex, placenta, and testis, the organs were exposed, bisected *in vivo*, and cold 2 per cent osmic acid fixative, buffered in the manner of Palade (6), was dropped on the cut surfaces. The surface film of blood which formed at the instant of cutting was flushed off vigorously with fixative. A fixative drip was continued for 20 minutes, after which time the halves were removed from the animal and transferred to a bath of cold fixative for 1 hour and 40 minutes, so that the total elapsed time of fixation was 2 hours. In a few instances in the case of testis and adrenal cortex, fixation was continued for as long as 48 hours at refrigerator temperatures so that extraction would enhance the apparent contrast of mitochondrial membranes.

Ovarian structures (graafian follicles and corpora lutea) were obtained by immersing the ovary, still in its periovarial sac, into cold fixative. The periovarial sac then was removed while so immersed, after which the structures desired were removed by blunt dissection with the aid of a dissecting microscope. The follicle or corpus luteum was then split to permit easy access of the fixative. Fixation was continued for 2 hours. To obtain maturing graafian follicles at proestrus vaginal smears were done.

RESULTS

In the adrenal cortex, especially the deeper half, the mitochondria are unusually large and notably abundant (Fig. 1). Here, the mitochondria are dense spheres of highly variable size and it is not unusual to see a cytosome so packed with these organelles, that they appear to occupy the greater part

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of its volume. Measurements of the larger mitochondria show that they frequently exceed 2 μ in diameter. The size variation, even within any one given cell, is quite marked, contrary to the usual observation of the light microscopist (review by Greep and Deane (4)). This is not attributable to parasagittal sectioning of the spheres for small mitochondria can be found which show sharp limiting membranes such as can be obtained only with near equatorial sections, while larger ones with indefinite outlines can also be seen, indicative of parasagittal sectioning (Fig. 1).

The internal architecture of the mitochondria of the zona fasciculata and zona reticularis (Fig. 2) is formed by tubular inward reflections of the internal mitochondrial membrane. These tubules pursue a rather tortuous course at random orientation to each other and it is relatively uncommon to see a parallel array of tubular profiles. While in transverse sections the structures appear round like small vacuoles, a sufficient number has been seen in other aspects to indicate that they are indeed tubules, and that they are in direct continuity with the inner mitochondrial membrane as an inward tubular reflection of it.

The diameter of the tubules is 40 $\pm$ 5 mμ, which is about twice the 18 to 22 mμ width quoted for cristae (8). While in some instances the tubules virtually fill each mitochondrion, there appears to be some variation in their number. They are embedded in a matrix of moderate electron density. Prolonged fixation of 12 to 24 hours decreases the density of the matrix by extraction. This frequently occurs differentially, so that the matrices of the smaller mitochondria are extracted first.

In the zona glomerulosa (Fig. 3), especially in the outer layers, the mitochondria differ from those just described in their size, abundance, shape, and internal morphology. They are fewer, smaller, and frequently rod-shaped. In contrast to the internal tubular make-up of the mitochondria of the deeper portion of the gland, the mitochondria here do contain cristae, although occasional tubules are also present. In all zones of the cortex, the mitochondria are randomly oriented.

In the theca interna (Fig. 4) of the graafian follicle at proestrus and in the corpus luteum (Fig. 5) the mitochondria are qualitatively quite similar to those of the zona fasciculata and reticularis of the adrenal gland as regards their basic architecture, although there are quantitative differences. These mitochondria again are predominantly spherical with only occasional elongate forms. The inner mitochondrial membrane is reflected inward to form tubules (35 mμ). However, the tubules are not present in as great abundance as in the adrenal gland and the matrix into which they project has a low electron density. Compared to the mitochondria of the adrenal cortex, the mitochondria here are fewer in number and smaller in size. They measure about 0.6 μ in the corpus luteum and about 0.7 μ in the theca interna. In both sites occasional

elongate forms reaching 1 to 2 μ are found, but giant spheroids comparable to those seen in the adrenal cortex are extremely rare.

The mitochondria of the interstitial cells of the testis (not illustrated) are smaller than those encountered in the other sites reported, ranging from about 0.3 to 0.6 μ with considerable size variability. Internally, they contain sparse numbers of tubules and cristae embedded in a matrix of high density, which nearly obscures internal detail. Occasionally, very dense granules are present in the matrix.

In the two other sites investigated (not illustrated), the granulosa cells of the graafian follicle and the placenta, filamentous forms of mitochondria were seen which contained cristae of the usual type. In these sites, however, an occasional tubule was seen among the cristae.

DISCUSSION

Mitochondria have been intensively studied by electron microscopy and their characteristic internal structure well defined (7, 8, 10), but the peculiarities of the mitochondria of the adrenal cortex have received thus far little attention. Palade (8) noted the "filamentous" internal structure of mitochondria of the adrenal cortex and also of protozoa. The latter have been studied more intensively recently by Sedar and Porter (9). Lever (5) made a reference to "microvillous inward projections" in mitochondria of the adrenal cortex and also noted that they exhibited a considerable variation in size. The truly unique features of these mitochondria have not been emphasized.

Unusual structures have been reported in the adrenal cortex of the rat, as seen by conventional microscopy. Deane and Greep (2) and Greep and Deane (4) used the term "liposome" in a restrictive sense to refer only to what they considered a lipide droplet in the rat adrenal which stained in mitochondrial preparations after prolonged mordanting in potassium dichromate. It seems likely that the structures observed were in reality giant mitochondria, rather than fatty droplets of a different chemical composition. Since these giant mitochondria are out of the ordinary size range, they might be confused with other structures when conventional microscopy is used.

Although the mitochondria of some of the other sources of steroid hormones are not so large, they possess in general the same tubular internal make-up. At first, this led the authors to consider the tubular structures as a mitochondrial specialization that might be related to steroid production, but such is apparently not the case. Tubules have been reported as occasional constituents of mitochondria in other sources in addition to the adrenal cortex and protozoa. Beams *et al.* (1) reported a tubular component in the mitochondria of the kidney of the grasshopper, and Fawcett (3) noted the same in mitochondria of hepatic cells. From a careful scrutiny of micrographs that have been produced in this laboratory over the last several years, it is concluded

that mitochondria from many diverse sites contain at least some tubular components. However, the presence of tubules and no conventional cristae in the corpus luteum, theca interna, and in most of the adrenal gland suggests that this is truly a specialization, possibly related to function. The functional significance of tubular inward extensions of the inner mitochondrial membrane is elusive, but then so is that of cristae. Tubules would furnish a considerably greater surface area per unit volume than would cristae. This, coupled with the number, size, and abundance of tubules of the mitochondria of the adrenal cortex, signifies that there is a great surface area presented by the mitochondrial tubules, probably greater than in any other cell. If the cristae do serve as the framework for insoluble oxidative enzymes, as Palade (8) suggests, then the adrenal gland must possess a remarkable potential for enzymatic activity.

SUMMARY

1. Mitochondria of the adrenal gland, corpus luteum, theca interna and granulosa of the graafian follicle, interstitial cells of the testis, and placenta of the rat have been studied with the electron microscope.

2. In most sites of steroid secretion, the internal structure of the mitochondria is in the form of tubular reflections of the internal mitochondrial membrane, rather than plate-like cristae.

3. The mitochondria of the adrenal gland have a highly variable size, even within a single cell; they may reach 2 to 3 μ in diameter, and are nearly filled with tubules.

4. It is suggested that while mitochondria in many places have a tubular component, those in the adrenal gland are highly specialized by virtue of their giant size and their internal structure.

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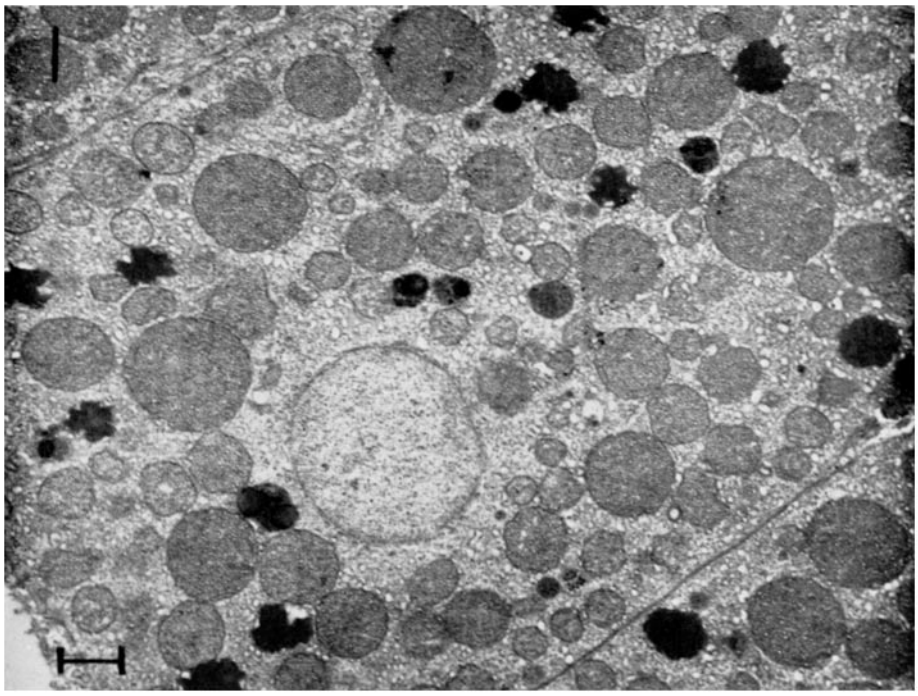
PLATES

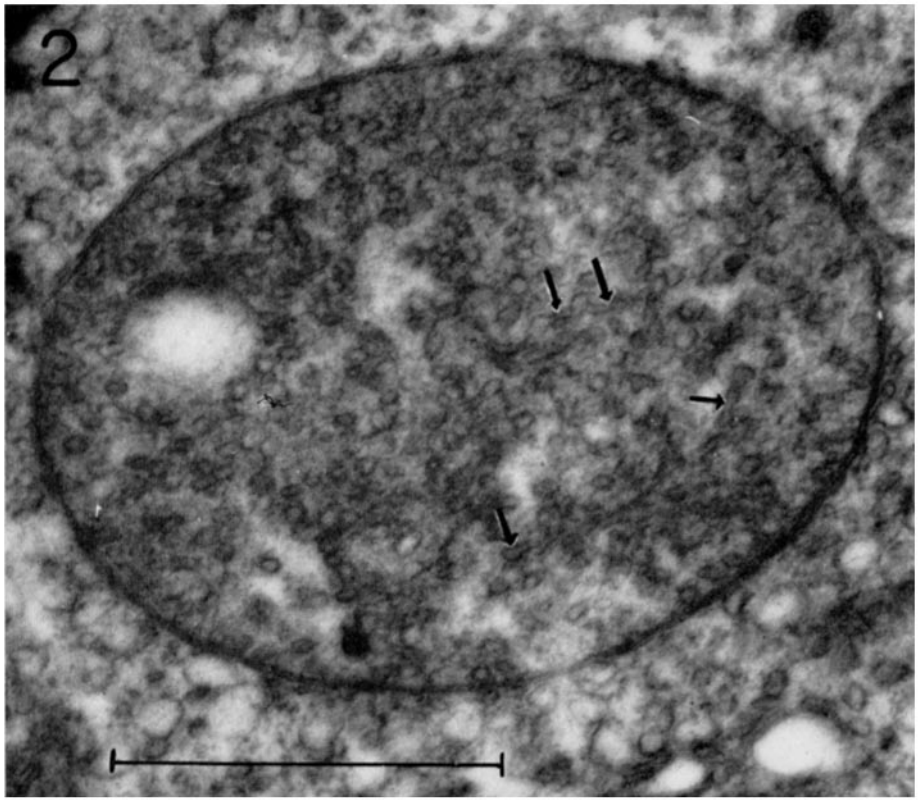
EXPLANATION OF PLATES

PLATE 126

FIG. 1. Adrenal cortex, deep zona fasciculata, 16 hour fixation. Mitochondria sectioned equatorially with sharp limiting membranes have variable size. Fuzzy outlines of some are indicative of a parasagittal section, and this makes the judgment of their size difficult. Irregular outline of lipide droplets (dense bodies) is artifactual. × 8,560.

FIG. 2. Adrenal cortex, large mitochondrion of zona fasciculata. Tubular internal structure evident. While most of the tubules are sectioned transversely or only slightly obliquely, some are sectioned longitudinally and seen as slits (arrows) for variable distances, showing the tubular character of these structures. × 51,000.





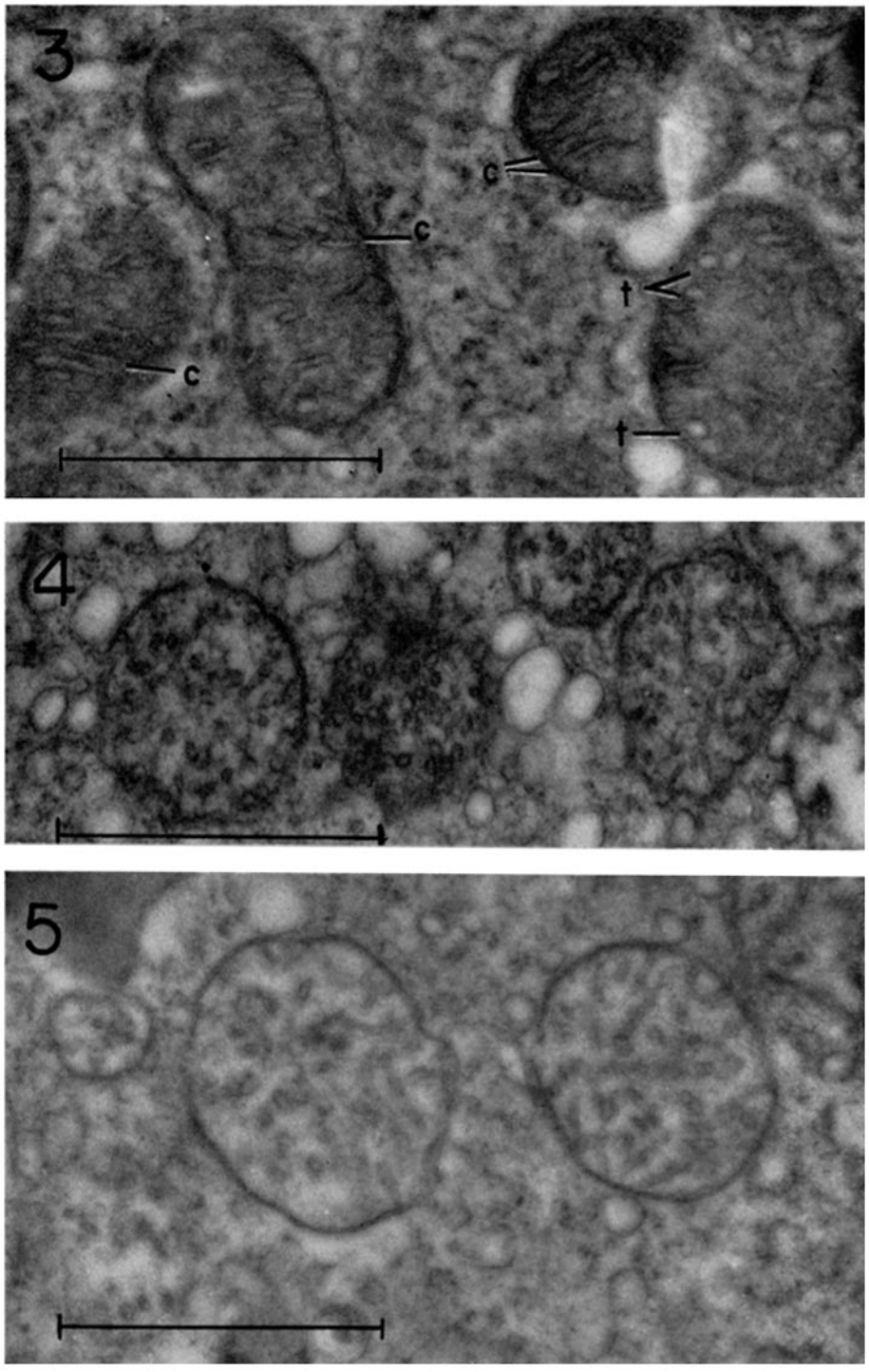
(Belt and Pease: Mitochondria in steroid secretors)

PLATE 127

FIG. 3. Adrenal cortex, zona glomerulosa, 16 hour fixation. Mitochondria are frequently elongate. Cristae (*c*) are common, but some tubules (*t*) are seen. That the slits are cristae rather than sections through oriented tubules is suggested by the mitochondrion at extreme left, which is sectioned parasagittally not far inside the limiting membranes. Compare to a similar situation in Fig. 4. × 44,300.

FIG. 4. Theca interna from graafian follicle at preostrus. 2 hour fixation. Several tubules can be seen sectioned longitudinally for short distances, especially in the mitochondrion at far right. The middle mitochondrion sectioned parasagittally just inside the limiting membranes shows perfectly transverse sections of tubules just after being reflected. × 44,300.

FIG. 5. Corpus luteum, mitochondria, 2 hour fixation. Tubules are shown in transverse as well as in various degrees of oblique section. Continuity of tubules with the internal mitochondrial membrane is shown. × 44,300.



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