

## PHYSIOLOGICALLY INDUCED CHANGES IN ADRENOCORTICAL MITOCHONDRIA

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PLATES 98 AND 99

When the fine structure of the mitochondrion was first described (1, 2), Palade commented that the internal membranes of adrenocortical mitochondria were arranged as filaments and not as cristae. Such membranous filaments appear oval in oblique section, sausage-shaped in longitudinal section, and near-circular in cross-section (Figs. 1 and 3). In this connection the terms "folds" and "villi" should only be used with caution with the adrenal cortex since seldom can it unequivocally be shown that an inner lamina of the mitochondrial enclosing membrane does, in fact, fold inwards villus-fashion. There is considerable diversity in the appearance of the mitochondrial internal membranes at various cortical levels. In the zona glomerulosa these membranes are frequently seen as long filaments in parallel alignment while in the outer fasciculata (Fig. 1) the filaments are shorter and more irregularly disposed. In the inner zona fasciculata and the zona reticularis a predominance of circular cross-sectional outlines suggests that mitochondrial internal membranes there are saccular or vesicular rather than filamentous in shape (Fig. 2). These observations, initially made in the rat adrenal (3), have been borne out in the hamster (4).

That a filamentous or saccular arrangement of mitochondrial membranes presents a larger internal surface area has been previously stressed (3, 5). It is perhaps rewarding to speculate on a possible functional significance in the difference between the filamentous mitochondrial internum of the zona glomerulosa and outer fasciculata, and the saccular form in the zona reticularis. Relevant to this it is currently held that the zona glomerulosa is a less active secretory zone than the zona reticularis. From recent publications it would seem that the filamentous and saccular form of mitochondrial membranes is more widespread than was earlier supposed. They have been described as well in some mitochondria in the grasshopper kidney (6) and in certain protozoa (7).

As already reported in the adrenal medulla (8) and the corpus luteum (9), light and dark cells are also seen in electron micrographs of the adrenal cortex (3). This heterogeneity is most marked in the inner zona fasciculata and the zona reticularis in rat, mouse, and human adrenals (Fig. 4). The over-all electron opacity of the dark cell is high, its cytoplasm consisting very largely of tightly packed mitochondria of compact internal structure. The light cell, with a lower

electron density, has on the other hand fewer mitochondria per unit cross-sectional area and these appear generally larger in outline than in the dark cell and have loosely arranged internal membranes. Moreover, between many of the light-cell mitochondria are clusters of thin-walled and predominantly agranular vesicles. Any attempted interpretation of the light-dark diversity in cell appearance immediately raises the question as to whether this is merely a product of fixation of doubtful significance. In this connection it is worth recording that it was a constant feature of preparations fixed both by immersion and by perfusion with both Dalton's (10) and Palade's (11) fluids. Chapman (12) observed "differences in the state of preservation" of mitochondria within the same insect flight muscle cell: some of these mitochondria contained "blebs and vacuoles" but others did not. He postulated that differences in local physiology and hence in chemical activity within individual mitochondria accounted for this diversity in appearance.

On a larger (cellular) scale it is possible that light and dark cells in the adrenal cortex (Fig. 4) are in different states of secretory activity. Since the presence of intracellular osmiophilic materials is an accepted indication of the ability of an adrenocortical cell to secrete, and since during the process of secretion some at least of these materials are visibly depleted, it may be that the light cell is a discharging or discharged form, while the dark cell is in an undischarged state. That differences in secretory activity are reflected by cyto-morphological change is further supported by the finding of numbers of cortical cells intermediate in appearance between the light- and dark-cell extremes (Fig. 4). Whether or not a light cell may revert to the dark form is not certain. Particularly in the zona reticularis many of the light cells contain a preponderance of mitochondria with little or no recognizable internal membranes (Fig. 7): an appearance which strongly suggests an expendability of adrenocortical mitochondria.

The suggestion has already been made (3) that lipide material within the adrenal cortex appears within the mitochondria. In Fig. 5 from the rat zona fasciculata a series of bodies is shown ranging from mitochondria *per se* to bodies indistinguishable from lipide droplets. In the intermediate forms the content of osmiophilic material and the clear definition of internal membranes are reciprocally related. These findings in the rat adrenal have been sustained in the hamster adrenal, in which, bodies identifiable by their internal membranes as mitochondria have also a marked content of osmiophilic material (4).

If the term "mitochondrial vacuolation" is taken to mean a space or spaces within a mitochondrion, then two varieties of vacuolation are observed: (a) intercommunicating irregular spaces (or a single compound space) between mitochondrial internal membranes, and (b) well circumscribed spaces. The first variety is typically seen in many light-cell mitochondria while the second and less frequently occurring variety might be seen in any adrenocortical mitochondrion. Cautious interpretation of these appearances prompts the obvious

comment that saccular organelles in juxtaposition to mitochondria may indent, or be enwrapped by these bodies: thus in certain planes of section a mitochondrion would appear to contain a well circumscribed vacuole.

Miller (13) has recently shown that the mitochondrial count in the rat zona fasciculata is proportional to the level of cell activity. This finding is supported here although no quantitative estimates have been made. Thus after hypophysectomy it is well known that, concurrent with the depression of adrenocortical secretory activity, degenerative changes occur primarily in zona fasciculata and reticularis (14, 15). Electron micrographs of these zones in the rat suggest a considerable reduction in the numbers of mitochondria 6 weeks after hypophysectomy (16). Conversely if hypophysectomised animals are treated with ACTH there is an increased number of mitochondria, particularly in the zona fasciculata and reticularis, and to a lesser extent in the zona glomerulosa (16). Whereas ACTH treatment of the hypophysectomised rat results in a full-thickness repair of the adrenal cortex, a localized proliferation of the zona glomerulosa ensues if the Na/K ratio is lowered (16). This proliferated tissue consists of cells varying in their over-all electron opacity (Fig. 6). The darker cells contain frank lipide droplets and a number of compact mitochondria with saccular internal membranes. Many of the lighter cell mitochondria are irregularly vacuolated.

In electron micrographs of the rat and hamster adrenal cortex after stimulation (16, 4) by ACTH or by lowering the Na/K ratio (zona glomerulosa), many of the mitochondria are seen to have incomplete enclosing membranes (Figs. 6 and 8). Indeed in such situations there is free continuity between mitochondrial interna and the cytoplasm at large. Such open form mitochondria, a marked feature of the activated gland, are also to be found in the unstimulated cortex though not in such numbers. Powers and Ehret (7) have claimed continuity between the internal "tubules" of *Paramecium* mitochondria and the cytoplasm at large, and they have also questioned the integrity of a mitochondrial limiting membrane in their preparations.

A further notable feature of the hamster adrenal cortex is particularly well seen in the ACTH-treated animal (4). Within 2 hours of the commencement of treatment, the limiting membranes of a number of the mitochondria appeared (in electron micrographs) to be polylaminar in part of their extent (Figs. 8 and 9). These appearances while numerous in the zona fasciculata and reticularis are seen in fewer number in the zona glomerulosa. It must be recorded that this mitochondrial feature can occasionally be detected in the normal hamster adrenal. Such polylaminar membranous collections closely simulate the lamellar component of the Golgi apparatus, as described by Dalton and Felix (17). Multilaminar membranous fascicles are also found within mitochondrion-like bodies (4). It is highly probable that these layered membranes are in fact collections of bilaminar sheets having a tubular outline

in cross-section. If they were tubules *per se* their outlines would probably not appear in such uninterrupted length in so thin a section (150 to 200 Å). In a strongly stimulated adrenal cortex new mitochondria are almost certainly formed in large number. With this in mind, appearances such as those in Figs. 8 and 9 might suggest a mode of mitochondrial genesis for the adrenal cortex at any rate. Possibly a fascicle of the bilaminar sheets (already described as a likely Golgi component) curls upon itself inscribing a certain volume of cytoplasm. Then perhaps, by a budding process from these sheets (Figs. 8 and 9), a system of filaments or sacs is shed inwards to occupy the space contained by the new enclosing membranes.

Such an hypothesis of mitochondrial formation lends weight to the earlier observations on open form mitochondria. Clearly if mitochondria arise by the curling and subsequent modification of a collection of sheets they must be freely open to the cytoplasm in some part of their circumference. It may be that such an opening may seal up or become exceedingly small. The concept of a closed mitochondrion is in accord with observations that mitochondria behave as osmometers (18) and also contain a high enzyme concentration. However, it is not inconceivable that mitochondria are osmometers with a leak; this leak may be a variable quantity according to the size of the pore or deficiency in the enclosing membrane. The size of such a pore might be controlled by such unknown factors as the intramitochondrial pressure, the regional cytoplasmic pressure, and surface tension effects.

Finally, if the bodies depicted in Figs. 8 and 9 are indeed equivocally Golgi-mitochondrial in nature, then a more plastic concept of both organelles is clearly desirable.

#### SUMMARY

1. Adrenocortical mitochondria contain filamentous or saccular internal membranes and are probably the sites of lipide elaboration or accumulation. They are numerous in the stimulated gland and scanty after hypophysectomy. There is evidence that they are expendable: light cells contain numbers of irregularly vacuolated and broken mitochondria with disorganized internal membranes.

2. Openings have been observed in some mitochondrial limiting membranes, particularly in the stimulated hamster and rat adrenal cortices, but to a lesser extent in the unstimulated glands.

3. Similarities between mitochondrial and Golgi membranes suggest a possible mode of mitochondrial formation.

4. These findings have been interrelated and discussed.

#### REFERENCES

1. Palade, G. E., *J. Histochem. and Cytochem.*, 1953, **1**, 188.
2. Sjöstrand, F. S., *Nature*, 1953, **171**, 30.

3. Lever, J. D., *Am. J. Anat.*, 1955, **97**, in press.
4. Lever, J. D., data to be published.
5. Sedar, A. W., and Porter, K. R., *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 583.
6. Beams, H. W., Tahmisian, T. N., and Devine, R. L., *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 197.
- 7 Powers, E. L., Ehret, C. F., and Roth, L. E., *Biol. Bull.*, 1955, **108**, 182.
8. Lever, J. D., *Endocrinology*, 1955, **57**, 621.
9. Lever, J. D., *Anat. Rec.*, 1956, **124**, in press.
10. Dalton, A. J., *Anat. Rec.*, 1955, **121**, 281.
11. Palade, G. E., *J. Exp. Med.*, 1952, **95**, 285.
12. Chapman, G. B., *J. Morphol.*, 1954, **95**, 237.
13. Miller, R. A., *Am. J. Anat.*, 1953, **92**, 329.
14. Smith, P. E., *Am. J. Anat.*, 1930, **45**, 205.
15. Deane, H. W., and Greep, R. O., *Am. J. Anat.*, 1946, **79**, 117.
16. Lever, J. D., *Endocrinology*, 1956, **58**, 163.
17. Dalton, A. J., and Felix, M. D., *Am. J. Anat.*, 1954, **94**, 171.
18. Claude, A., and Fullam, E. F., *J. Exp. Med.*, 1945, **81**, 51.

## EXPLANATION OF PLATES

## PLATE 98

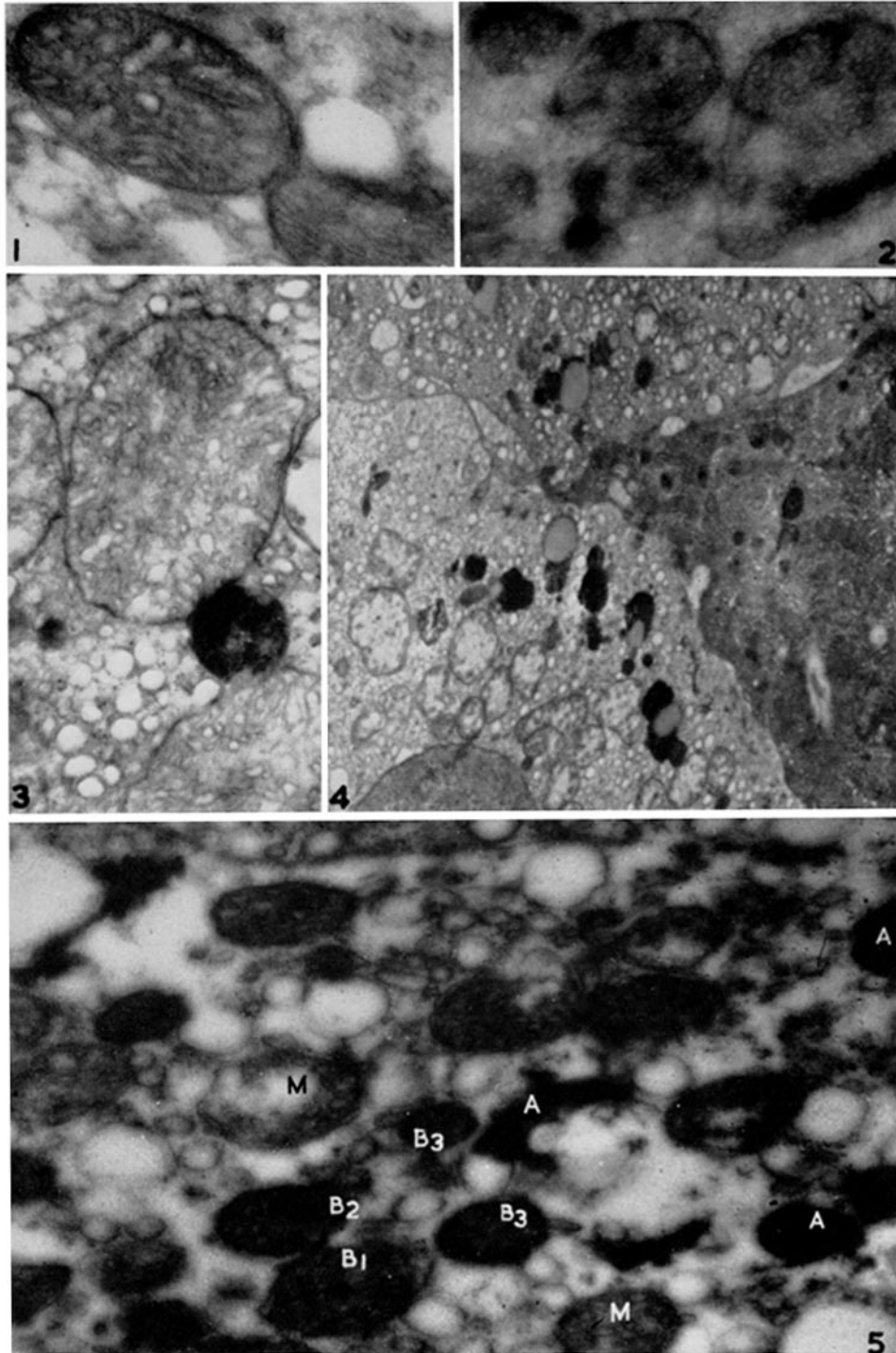
FIG. 1. Mitochondrial sections in the rat outer zona fasciculata. The variegated outlines of the internal membranes suggest a filamentous form.  $\times 36,000$ .

FIG. 2. Mitochondria in the rat zona reticularis. The cross-sectional outline of the internal membranes is almost exclusively near-circular which suggests a saccular morphology.  $\times 27,000$ .

FIG. 3. The mitochondrial internal membranes are clearly filamentous in this field from the hamster inner zona glomerulosa during a course of ACTH treatment.  $\times 27,500$ .

FIG. 4. In this human zona reticularis, examples of dark, light, and intermediate cells are depicted. Dark cells contain a large number of compact osmiophilic mitochondria per unit area of section and few obvious saccular organelles. Light cells have fewer mitochondria per unit area and these are loosely vacuolated: these cells and also the intermediate cells possess numbers of thin-walled sacs. Intensely opaque lipide bodies are seen in all three cell types.  $\times 10,000$ .

FIG. 5. From the rat zona fasciculata. Mitochondria (*M*), frank lipide droplets (*A*), and bodies intermediate in appearance (*B*) are depicted. There is an inverse relationship between the clarity of outline of the internal membranes and the content of osmiophilic material in these intermediate bodies (*B*<sub>1</sub>, *B*<sub>2</sub>, *B*<sub>3</sub>).  $\times 25,000$ .



(Lever: Adrenocortical mitochondria)

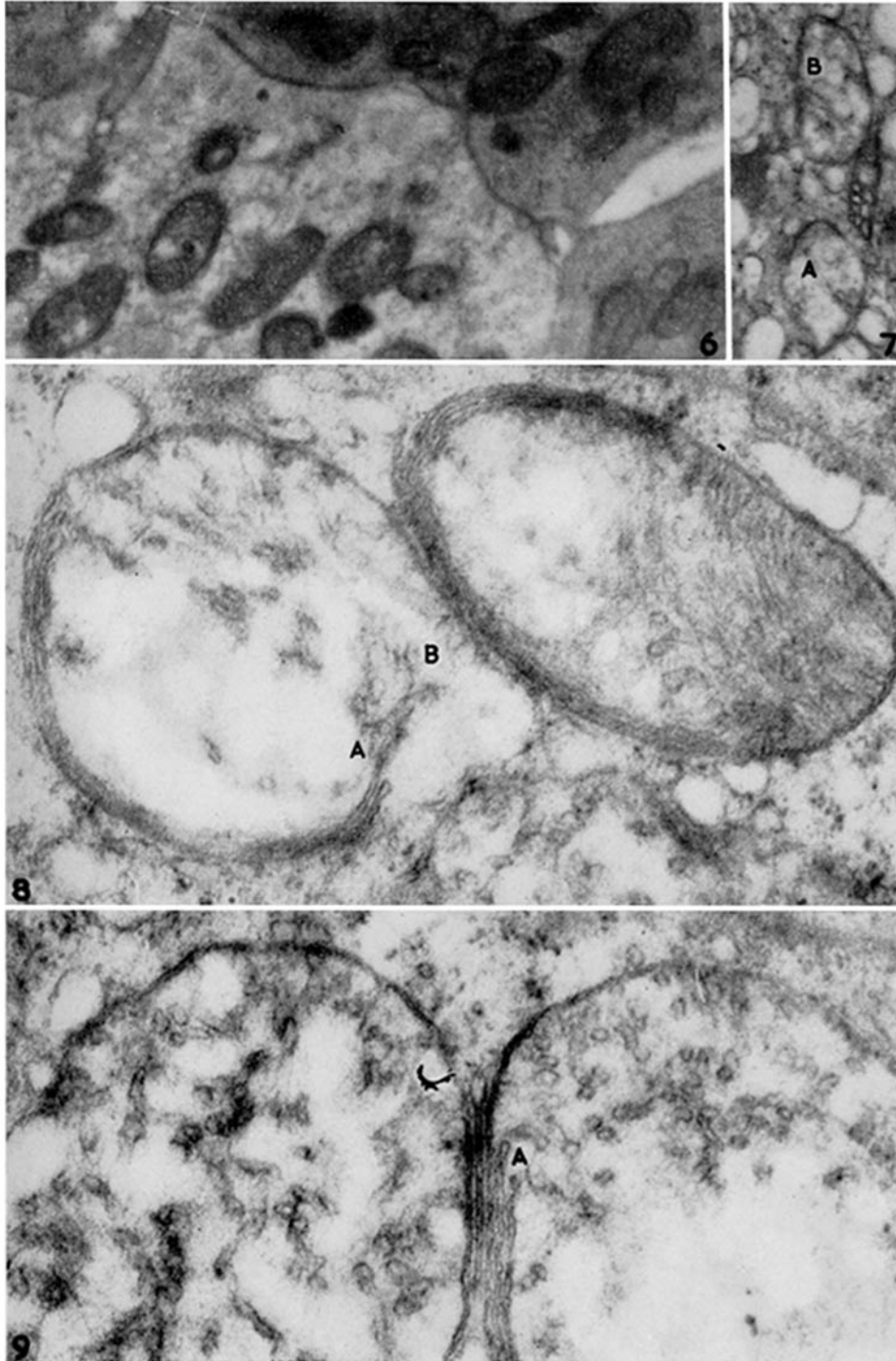
PLATE 99

FIG. 6. From the zona glomerulosa of an hypophysectomized (6 weeks) rat in which the Na/K ratio has been lowered. The zone becomes hyperplastic and numbers of light and dark cells are produced. Note that some mitochondria in both cell types have deficient limiting membranes allowing continuity between their interna and the general cytoplasm.  $\times 22,500$ .

FIG. 7. Portions of mitochondria in a light cell from the juxta-medullary zona reticularis in the rat. The lower body (*A*) is just recognizable as a mitochondrion by its scant content of internal sacs while in the upper body (*B*) recognizable internal membranes are lacking. The regional cytoplasm contains numbers of granular and agranular sacs.  $\times 22,500$ .

FIGS. 8 and 9. Both figures are from the outer zona fasciculata of the ACTH-treated hamster. Mitochondrion-like bodies are seen to have poly laminated limiting membranes over some of their perimeter. In Fig. 9 the appearance of these laminated membranes suggests their identity with the lamellar component of the Golgi apparatus. It may be that sacs and filaments bud inwards from these membranes at regions like *A* (both figures). In Fig. 8 note that the left mitochondrion is freely open to the cytoplasm along one part of its perimeter (*B*).  $\times 80,000$ .





(Lever: Adrenocortical mitochondria)