The Origin of Adrenal Cortical Mitochondria and Liposomes: a Preliminary Report.*

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INTRODUCTION

The origin of the lipide droplets or liposomes of the adrenal cortex has been the subject of controversy among cytologists for many years. Mulon (14) and Celestino da Costa (2, 3) suggested that they were derived from mitochondria by a direct transformation. Miller and Riddle (13) and Knouff and Hartman (7) have observed that mitochondria and liposomes of the adrenal cortex of birds are present in inverse proportions; Cowdry (4) made a similar observation concerning neurons as far back as 1914. While this gives indirect support to the concept of transformation, Knouff and Hartman considered such a transformation unlikely in the adrenal cortex. Hoerr (6), in denying the concept of a direct transformation, stated that the appearance of mitochondria with lipide in their centers was due to faulty technique. An approach utilizing the high resolving power of the electron microscope should be a means of settling this problem, since mitochondria can be identified so readily with that instrument (16, 17, 20), even though in the adrenal cortex and certain other steroid secretors the mitochondria present a slight variation of the basic plan (1, 8–10). While Lever (8, 10), following an electron microscopic study of the adrenal cortex, has reported that mitochondria do transform into liposomes, the conclusions do not seem completely justified in the light of this author's findings.

It has long been known that mitochondria of cells in tissue culture may arise by fission of existing mitochondria, and that they also fuse during their constant motion (11). Miller (12), by examining fixed preparations of adrenal cortex with the light microscope, found that mitochondria elongate and apparently divide following stimulation with ACTH or epinephrine. In electron micrographs of rat liver, Fawcett (5) showed mitochondria with an appearance suggestive of fission. In the liver of experimentally treated rats, Rouiller and Bernhard (19) found an increase in number of small, moderately dense objects, which they termed "microbodies" because of their resemblance to structures in the kidney which were given that name by Rhodin (18). Rouiller and Bernhard also observed structures, apparently related to "microbodies," which they interpreted as forms differentiating into mitochondria. They occasionally found other structures which they considered to be degenerative forms of microbodies which resulted in lipide droplets, rather than mitochondria. If a similar change were to occur in the adrenal cortex, it could be interpreted as a functional change, rather than a degenerative one, since in this organ lipide droplet formation is a normal feature.

The preliminary electron microscopic observations reported here are consistent with the observations of Rouiller and Bernhard (19), *i.e.*, that microbodies and variations thereof are present in the adrenal cortex of the rat. This author suggests that these structures may be interpreted as a common precursor to both mitochondria and liposomes.

The name "microbody" seems singularly inappropiate for the structure discussed here because of its obvious relation to microsome (Greek *soma*, body). However, it will be used here, since a new term would probably only increase confusion.

Materials and Methods

The adrenal glands were excised from male rats of the Wistar strain during nembutal anesthesia, halved, and fixed in cold 2 per cent osmium tetroxide buffered in the manner described by Palade (15). Fixation was effected from 2 to 24 hours. The longer times were sometimes used because of the enhanced contrast of mitochondria due to extraction of the mitochondrial matrix. Blocks from the exposed surface were rapidly dehydrated and embedded in butyl methacrylate, to which was added 2 per cent benzoyl peroxide. Sections were examined with an RCA-EMU2e electron microscope.

RESULTS

Small objects, microbodies, were encountered in the cytoplasm of adrenal cortical cells in the

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zona fasciculata (Figs. 1 and 2); they resembled those seen by Rhodin (18) and Rouiller and Bernhard (19). These structures were not found in close proximity to the Golgi apparatus or mitochondria. Those possessing the simplest structure were the smallest, commonly about 0.2 to 0.3 μ. They consisted of a limiting membrane surrounding a moderately dense, granular core. Between the core and the limiting membrane was a well defined zone of lower density. Other small structures have been found which were sufficiently similar to microbodies to be considered as related structures. Of these there were two sorts, one variety containing one or several dense granules, the other containing membranes. Those structures containing granules, presumed to be lipide (lip_1, lip_2 in Figs. 1, 2, and 4), were generally larger than microbodies. The largest encountered were homogeneously dense with no discernible limiting membrane (Fig. 3). In those bodies that contained rudimentary membranes, (Figs. 4 and 5), the membranes frequently appeared as circular profiles, which might be expected if they were cross-sections of tubules like those in the mitochondria of the zona fasciculata. A few structures have been found which contain both granules and membranes.

DISCUSSION

The pleomorphic structures just described can be used to construct two series of transition forms. The first series would begin as a microbody, which, by the accumulation of lipide, would terminate as a structure approaching a liposome. The second series, again starting as a microbody, would be interpreted as developmental stages of mitochondria by the formation of membranes in the microbody. In neither series have enough close stages been obtained to give unequivocal evidence, but a sufficient number of these "developing forms" have been encountered to suggest strongly that microbodies are the source of lipide droplets and also may be the source of mitochondria. In some instances during the earlying differentiation of the microbodies, accumulation of dense material appeared simultaneously with the development of the membranes. It is not readily apparent when the determination of subsequent differentiation occurs, but it is certainly quite early, because there has been seen no accumulation of lipide in a differentiated mitochondrion which would suggest that it was transforming into a lipide droplet.

It is concluded that the mitochondrial role in liposome formation is not that of a direct transformation. This is suggested by the usual uniform density of the mitochondria. Mitochondria do vary in density following a prolonged fixation, since the matrix of the smaller mitochondria seems to extract first. Density of mitochondria also varies in areas of poor preservation, but bizarre effects are then visible throughout the cell. These findings concerning the genesis of lipide droplets are not in agreement with those of Lever (8, 10), who illustrated a considerable range of density in "transition forms." This was in large measure Lever's basis for the conclusion that a direct transformation does occur. Fig. 6 of Lever (8) unquestionably shows a mitochondrion containing or surrounding a liposome, but the other figures showing subtler intergrades, chiefly as varying densities of mitochondria, may be the result of inadequate preservation. No evidence has been found which indicates that a direct transformation commonly occurs. The more frequent finding of small structures, which accumulate lipide, according to my interpretation, suggests that the liposomes arise from microbodies. No mitochondrial remnants are found in the smallest or "youngest" structures; it is, therefore, unlikely that microbodies are the result of mitochondrial degeneration. In addition, the size of the mitochondrion is considerably greater than that of the microbody.

It should be pointed out that if both mitochondria and liposomes arise from a common precursor, then one might expect to find them varying conversely in number. Thus, if there are more mitochondria, there should be fewer liposomes, and *vice versa*. This explains the repeated observation that the two cytoplasmic components are found in cells in inverse proportions as satisfactorily as does a theory which has a mitochondrion transforming into a lipide droplet.

While the occurrence of phenomena interpreted as mitochondrial fission and fusion is well documented by observations upon the living cell, it is certainly possible that two modes of formation could exist. This author is currently initiating a series of experiments which should clarify these relationships.

SUMMARY

1. A number of small (0.2 μ) moderately dense structures were observed in the cytoplasm of adrenal cortical cells which appeared similar to the

"microbodies" of Rhodin (18). This structure consisted of a limiting membrane surrounding a core of granular material. Apparently related structures, often slightly larger, contained an ill defined system of membranes, or one to several dense areas considered to be lipide.

2. An interpretation is suggested that mitochondria and liposomes may arise from a morphologically common precursor, the "microbody."

3. In the adrenal cortex of the rat, lipide accumulation in mitochondria was *not* encountered. This is evidence that mitochondria do *not* transform into liposomes.

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EXPLANATION OF PLATE 191

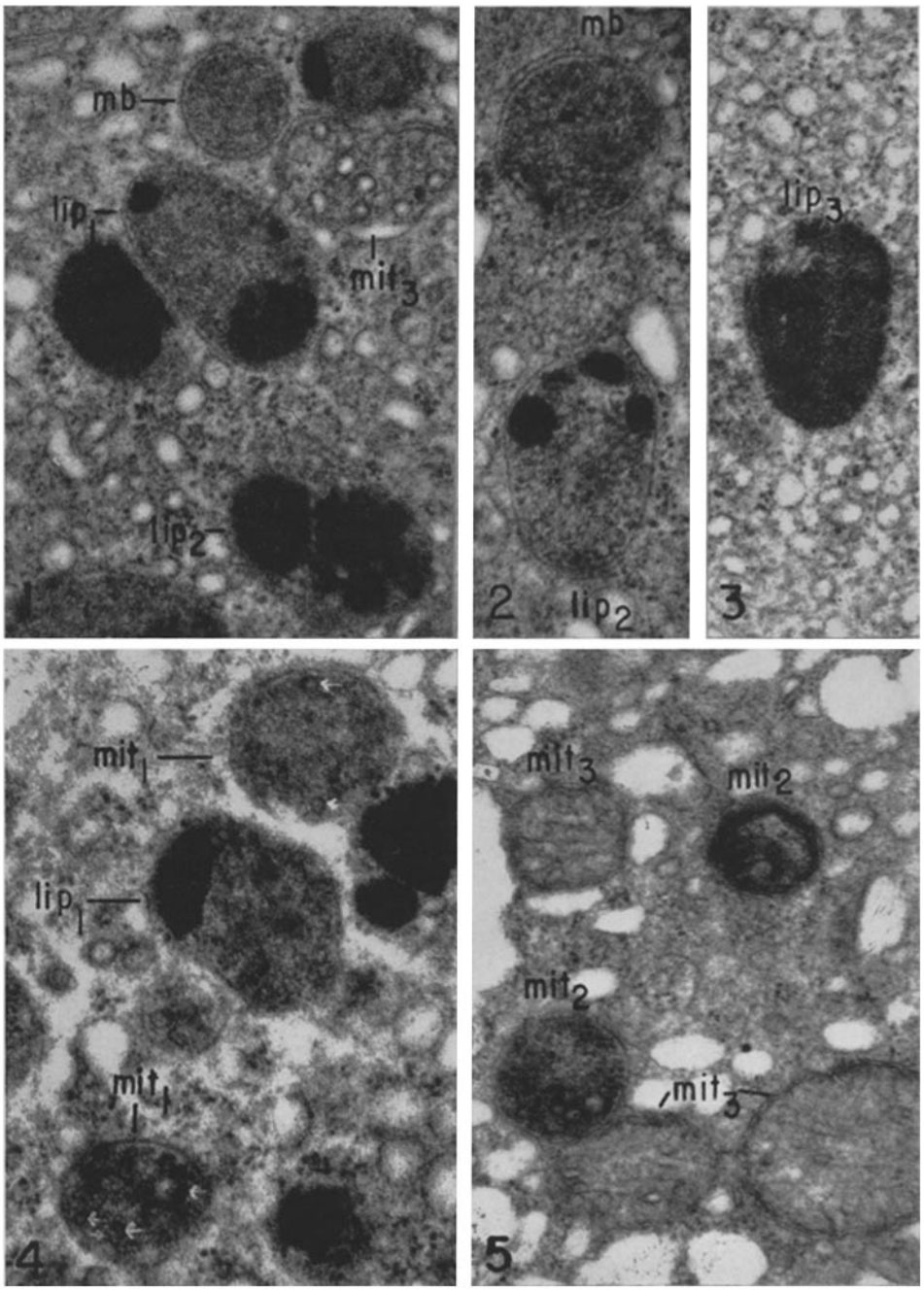
All figures are from the zona fasciculata of the adrenal cortex of the rat. Fig. 4 is shown at a magnification of 60,000; the rest of the figures at 45,000.

Abbreviations

mb, "microbody".
lip$_1$, early accumulation of dense material, presumably lipide, in developing microbody.
lip$_2$, more advanced stage of preceding; more lipide.
lip$_3$, structure virtually filled with lipide.
mit$_1$, interpreted as first elaboration of membrane-like structures in microbodies destined to be mitochondria.
mit$_2$, membrane formation better developed than preceding.
mit$_3$, mitochondrion, possibly immature.

FIGS. 1 to 3 show stages considered to be lipide droplets developing from microbodies by progressive accumulation of dense material.

FIGS. 4 and 5 show stages considered to be mitochondria developing from microbodies by formation of a membranous internum. The first profiles of the internal membranes are indicated by arrows, and are obvious on the original. The upper *mit*$_2$ in Fig. 5 shows a parallel array of membranes, while the lower *mit*$_2$ shows the profiles of the internal tubules cut transversely.



(Belt: Mitochondrial and liposomal origin)