Mitochondria Isolated from Rat Brown Adipose Tissue and Liver

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ABSTRACT

Mitochondrial fractions, relatively free from contamination by other cytoplasmic structures, have been isolated by differential centrifugation from homogenates of brown adipose tissue from starved rats. It was possible in such fractions to distinguish two types of mitochondria in this tissue.

Type I mitochondria, when morphologically intact, are limited by a bilaminar membrane and show regular parallel cristae. In isolated fractions, a proportion of these mitochondria are swollen, vacuolation occurring within the cristae between their limiting membranes.

Type II mitochondria are distinguished from the more numerous type I bodies by the opaque appearance of their matrix. They are limited by a membrane which is in part single, and in part double. They show a few, but crisply outlined internal membranes. Vacuolation of this type of mitochondrion has not been observed.

Vacuolation comparable to that in brown fat mitochondria was also observed between the two laminae of the enclosing membrane and within the cristae of liver mitochondria.

The cells of the brown fat tissue of starved rats are almost free of lipide droplets and are tightly packed with mitochondria (1). Extremely regular internal cristae and obvious bilaminar limiting membranes are apparent in electron micrographs of these mitochondria. Since the endoplasmic reticulum in brown adipose tissue occupies a small part of the cell volume and is relatively inconspicuous, it was considered, therefore, that this tissue would lend itself to the isolation of relatively pure mitochondrial fractions, free from microsomal contamination. This paper describes the appearance of the mitochondria isolated by differential centrifugation from brown fat tissue and makes comparison with corresponding studies on liver preparations. In the present study two types of brown fat mitochondria have been observed *in vitro*.

Materials and Methods

The material for this investigation was removed, under ether anesthesia, from the subcutaneous interscapular mass of brown fat of albino rats (1) starved for 7 to 10 days; and from the normal rat liver. For the study of the whole tissue small pieces were cut from the main mass, which was used for the isolation of mitochondria, and immediately fixed in Dalton's (2) buffered saline—1 per cent osmic acid solution for 1 to 2 hours at 5°C. After brief washing, dehydration in ethanol, and embedding in methacrylate, thin sections (silver and gold colored) were cut with a Porter-Blum microtome.

Mitochondria were isolated from the adipose tissue by homogenization in 0.44 M-sucrose and differential centrifugation, employing a method which has been elaborated by Chappell and Greville (3) for rat liver mitochondria. The once-washed particles were sedimented by centrifugation to form a thin pellet, which was then fixed and examined in the same way as was the intact tissue. A Siemens electron microscope was used.

OBSERVATIONS AND DISCUSSION

Brown Fat Mitochondria:

One of the most satisfactory results of the present study has been the reproducible production of mitochondrial fractions relatively free from microsomal and other detectable contamination (Fig. 4). However two varieties of mitochondria

(designated types I and II) were present in the fractions studied.

Type I Mitochondria.—These mitochondria, which far outnumber the other type, are either vacuolated or show signs of becoming so in the isolated state. Vacuolation is here taken to imply the presence within a mitochondrion of a space or spaces filled by a light material indistinguishable from the embedding plastic (Figs. 4 to 6). At one extreme of structural variation, mitochondria appear grossly distended, with very irregular cristae and often with a ruptured external membrane. At the other end of the spectrum, mitochondria appear to be well preserved and resemble closely those seen in the intact tissue (Figs. 1 to 3). The vast majority of isolated mitochondria lie between these two extremes. It is of interest that mitochondria corresponding to these partially vacuolated structures are seen in the intact cell in the final stages of starvation (Fig. 2).

By carefully examining vacuolated mitochondria *in situ* and after isolation, it was found that the vacuoles are located within the cristae, being limited by the two membranes which demarcate each crista and representing the distended light spaces of these internal folds (Fig. 2).

If, as is likely, the double membrane of a mitochondrial crista is continuous with the inner lamina of the limiting membrane of the mitochondrion, then the light space contained by the crista is continuous with the "outer mitochondrial chamber" between the two limiting (enclosing) membranes. It is to be expected, therefore, that in the more advanced stages of swelling the vacuolar space would extend to the outer enclosing membrane and this, in fact, can be seen to occur (Figs. 6 and 7). Of further note is the fact that the outer lamina of the limiting membrane of the vacuolated mitochondrion *in vitro* occasionally extrudes as a small bleb (Fig. 6).

Type II Mitochondria.—This body is referred to as a mitochondrion largely because it resembles structures presumed to be mitochondria in the cells of the brown fat of rats starved and injected with thyroxine (Fig. 3). It contains an evenly distributed and semiopaque material in which a variable number of crisply outlined double membranes are irregularly disposed (Fig. 5, *E*). There is a remarkable resemblance between these bilaminar membranes and the cristae of the type I mitochondria. The limiting membrane is in places single and in places double, and so cannot properly be described as bilaminar. In no instance has vacuolation (in the sense defined above) been observed in this type II mitochondrion.

It is not clear whether such structures as *B* in Fig. 7 are built up of smaller simpler units which have coalesced (*C*, Fig. 7), or are an unvacuolated form of mitochondrion (similar to the type II mitochondrion) undergoing fragmentation. Bearing on the first alternative, it may be remarked that not infrequently small bodies of doubtful identity have been observed (*e.g. B* in Fig. 5) with single limiting membranes and containing a semiopaque matrix: in density and texture this matrix resembles that within a type II mitochondrion. While the identity of these small bodies is obscure, their possible structural relationship to mitochondria is of interest, in view of Novikoff's (4) observation that the bodies he has identified as "lysosomes" in rat liver fractions were small, membrane-bound, and biochemically associated with the mitochondria.

Paigen (5) has suggested the existence of more than one type of mitochondrion in order to explain enzyme distribution in mitochondrial centrifugates: whether or not there proves to be substance to his theory remains to be determined. Nevertheless the terms "type I" and "type II" as used in the present context have an obvious descriptive value in stressing the marked morphological differences between the grossly vacuolated and the unvacuolated mitochondrion observed *in vitro*. It is important to note that both these types of mitochondria have morphological counterparts within the intact adipose cells (Figs. 2 and 3), and this might indicate that these types represent different functional stages in the mitochondrial life span.

Liver Mitochondria:

In marked contrast to the mitochondrial fractions of brown adipose tissue, those obtained from rat liver were always contaminated by elements of the endoplasmic reticulum (Fig. 8). As previously reported by Watson and Siekevitz (6), liver mitochondria suspended in 0.44 M sucrose are more compact (Fig. 9) than those in 0.25 M sucrose (Fig. 8): even in greatly swollen mitochondria the outer lamina of their enclosing membrane is commonly intact. Mitochondrial vacuolation apparently occurs between the two laminae of

this enclosing membrane and extends into the light spaces contained by the cristae (Novikoff, 4) in a manner similar to that observed in brown fat mitochondria.

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Explanation of Plates

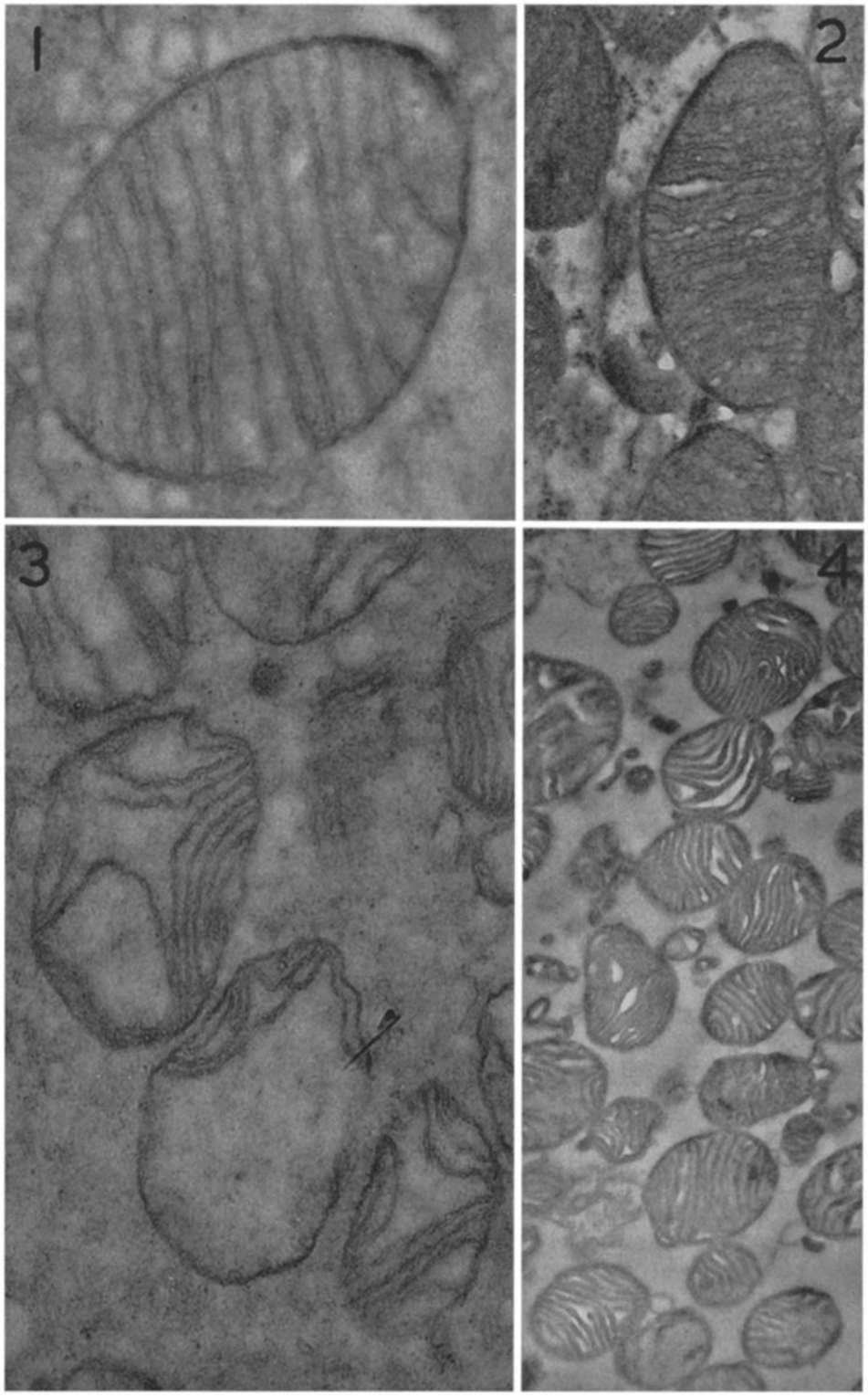
Plate 157

Fig. 1. An intracellular brown fat mitochondrion, showing near parallel bilaminar cristae and evidence of the double nature of the limiting membrane. × 65,000.

Fig. 2. In animals starved to a state of inanition, many of the intracellular mitochondria of brown fat show a vacuolation that commences intracristally, *i.e.* between the two membranous components of the cristae. × 40,000.

Fig. 3. In many intracellular brown fat mitochondria in starved, thyroxine-treated rats, the internal membranes are scanty and irregularly disposed. Compare with the unvacuolated type II isolated mitochondria in Fig. 6. × 40,000.

Fig. 4. Relatively pure mitochondrial fractions such as that depicted here can be produced by centrifugation of brown adipose tissue taken from starved animals. × 16,500.



(Lever and Chappell: Mitochondria from adipose tissue)

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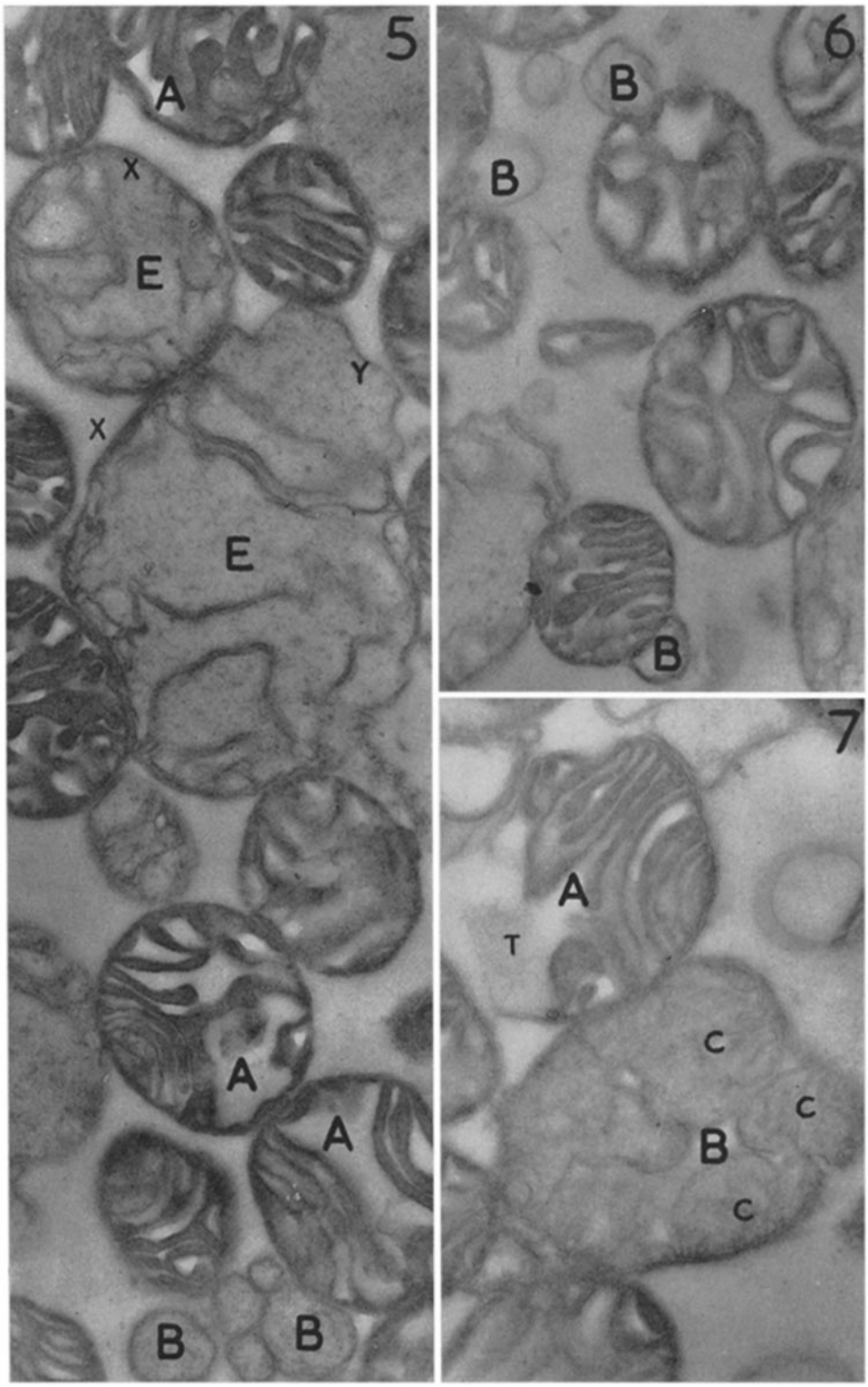
Figs. 5 to 7 show mitochondria isolated from rat brown adipose tissue.

FIG. 5. In type I mitochondria (*A*), the limiting membrane is seen to be bilaminar in some regions. Absolute definition of the double membranes defining the internal cristae in *A* is problematical, but the lighter areas are very likely the *intracristal* intervals (compare Fig. 2), and within these intervals vacuolation, to a varying degree, can be observed at *A*. That this vacuolation is intracristal is again suggested by the fact that the vacuolar space can be occasionally seen to extend to the outer enclosing membrane (see Fig. 7, *A*). An *unvacuolated* body, *E*, closely resembles certain intracellular mitochondria depicted in Fig. 3. These unvacuolated type II mitochondrial forms may be limited by a double membrane (*X*) or a single membrane (*Y*). They contain an evenly distributed matrix material in which crisp bilaminar sheets or cristae are disposed at random. Bodies (*B*) of equivocal nature contain a material comparable to that within unvacuolated mitochondria (*E*). × 30,000.

FIG. 6. The outer enclosing membrane of some type I vacuolated mitochondria may extrude as a surface bleb (*B*). × 30,000.

FIG. 7. The outer enclosing membrane in the vacuolated type I mitochondrion (*A*) is tangentially cut at *T*. The composite body *B* may be undergoing division or, alternately, may be in the process of formation by coalescence of individual bodies, *C*. × 30,000.



(Lever and Chappell: Mitochondria from adipose tissue)

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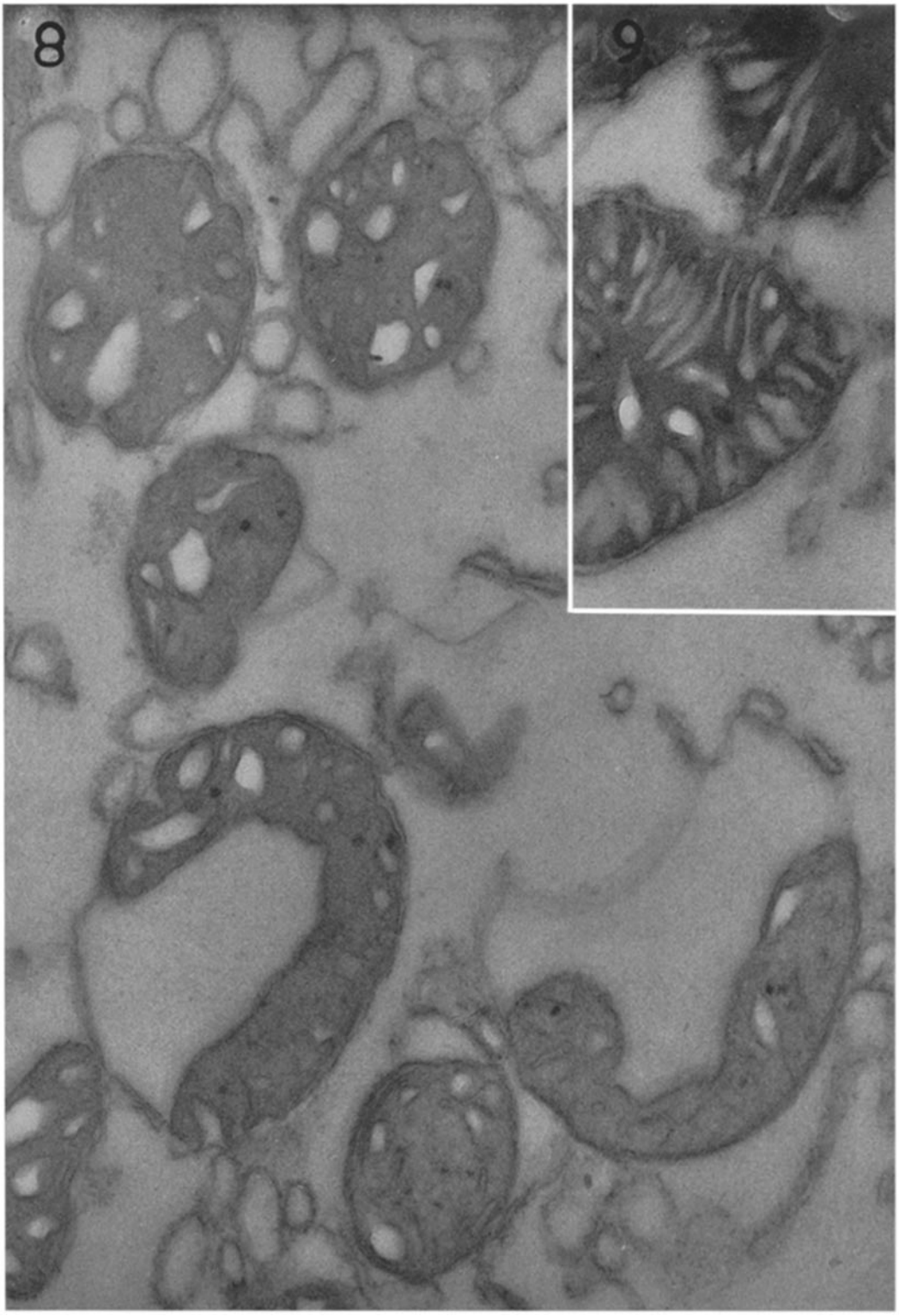
FIGS. 8 AND 9. These photographs of isolated rat liver mitochondria are included for comparison with Figs. 5 to 7. It will be seen that the site of vacuolation is similar in brown fat and liver mitochondria, being within the internal cristae and between inner and outer limiting membranes. Fig. 8. Liver mitochondria isolated in 0.25 M-sucrose. Note considerable microsomal contamination. Fig. 9. Mitochondria isolated in 0.44 M-sucrose. × 40,000.



(Lever and Chappell: Mitochondria from adipose tissue)