Mitochondria

II. The Nuclear-Mitochondrial Relationship in *Pelomyxa carolinensis* Wilson (*Chaos chaos* L.)*

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ABSTRACT

This study has demonstrated that in the ameba, *Pelomyxa carolinensis* Wilson (*Chaos chaos* L.) the limiting membranes of mitochondria and postdivision nuclei are often continuous. The morphological relationship may be functional in that it permits an exchange of material resulting directly or indirectly in an increased enzyme content of the mitochondria. It is suggested that through a series of progressive foldings of its envelope, the nucleus may be a site of formation of mitochondria.

INTRODUCTION

The development of isolation procedures for mitochondria has permitted extensive biochemical analysis of these organelles. The information derived from these studies has firmly established the importance of the mitochondria in the oxidative release of energy by the cell (1, 2). In several other respects, however, mitochondria are still perplexing. We know little of their interrelationships with other cell constituents, nor do we know where they or their enzymes are synthesized in the cell.

Nearly every cell organelle has at one time or another been implicated in the formation of the mitochondria. There is evidence from light and electron microscope studies that the mitochondria can both fuse and divide. There are no indications however that this division results in an increase in total volume of mitochondria or in their enzyme content. Rouiller and Bernhard (3) suggested that the mitochondria develop from dense cytoplasmic organelles or "microbodies" as described by Rhodin (4). From observations on living cells in tissue culture, Gey *et al.* (5) have described pinocytic vesicles as developing into mitochondria. The Golgi complex (6, 7) and the endoplasmic reticulum (microsome fraction, 8) have also been proposed as the site of origin of the mitochondria.

Various degrees of nuclear-mitochondrial relationships have been suggested. Chévremont and Frederic (9–11) demonstrated from observations on living cells that the mitochondria are occasionally adherent to the nucleus. They postulated that an exchange of material occurred at this time between the two structures. A similar hypothesis was suggested by Ornstein (12) based on studies of fixed oocytes. Several authors have proposed that the nucleus may be the site of origin of the mitochondria (13–15). Although the electron microscope observations upon which this latter hypothesis is founded demonstrated that the nuclei and mitochondria were close, it was not clear whether the mitochondria and nucleus were still separated by their respective membranes or joined in some fashion.

From the work reported to date it is not possible to decide whether or not a direct exchange of material could occur between the nucleus and the mitochondria. It is felt, therefore, that further

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investigations are necessary in order to determine the nature of the morphological association, if any, between the nucleus and the mitochondria. The following study with the electron microscope was undertaken particularly with the purpose of examining the association of the nuclear envelope with the mitochondrial membranes during the time these two structures are in close contact.

Materials and Methods

Agrell (16) demonstrated an increase in the number of mitochondria in the sea urchin embryo following nuclear division. Similarities in dimensions between evaginations of nuclei in prophase and mitochondria have been reported in *Amoeba proteus* (17). Therefore it appeared that the nuclear-mitochondria relationship could be advantageously investigated in dividing ameba nuclei. A related ameba, *Pelomyxa carolinensis* (*Chaos chaos*), was chosen as the experimental cell. It is favorable for this study since it is a giant ameba containing 50 to 1,000 nuclei which divide synchronously (18). For the purposes of fixation, a 1 per cent solution of osmic acid in 1/1400 M veronal acetate stock (pH 8.6) was used for 5 minutes at 4°C. The cells were subsequently dehydrated in alcohol and embedded in *n*-butyl methacrylate. Sections 1 micron thick were cut, and mounted on glass slides for examination in the phase contrast microscope. Thinner sections (40 to 80 mμ) were cut on the Porter-Blum microtome for examination in the RCA EMU 3C electron microscope. Two mounting techniques were used for the electron microscope study. Sections were mounted either on carbon-coated grids or on formvar-coated grids and then "sandwiched" with carbon. In all other respects the techniques used were similar to those reported previously (19).

RESULTS

A large number of individually embedded amebas were examined in the light microscope in order to find cells in which mitochondria appeared to be in close association with the nuclei. When found, such favorable blocks were sectioned for examination in the electron microscope. Fig. 1 is a phase contrast photomicrograph of a 1 micron section of an ameba in which the nuclei have just divided. In this ameba some mitochondria can be seen clustered about one of the nuclei. The mitochondria do not appear homogeneous. Projections of the nuclei which are similar in density to the mitochondria can be seen (at arrows). Such close nuclear-mitochondrial associations most commonly appear at and shortly after nuclear division.

Figs. 2 and 3 are electron micrographs of thin sections from the same ameba shown in Fig. 1. They demonstrate that this close association actually consists of a continuity between the nucleus and the mitochondrion. The mitochondrial matrix is continuous with the nucleoplasm, and the nuclear envelope appears to be continuous with the two limiting membranes of the mitochondrion (Fig. 2). The relationship of the nucleus to the mitochondrial matrix is less clear in Fig. 3 because the membranes of the two structures are so closely interwoven.

Various degrees of nuclear-mitochondrial interconnections can be seen in Figs. 4 and 5. Processes of the outer membrane of the nuclear envelope appear to be connected with the mitochondrial membranes in Fig. 4. At the left in Fig. 5, irregularities of the margin of the mitochondrion are interdigitated with processes of the nuclear envelope. In the stroma of the mitochondrion at the right, fibrillar material (*F*) cut in cross-section can be seen. It is similar in density to the obliquely cut fibrillar material directly under the nuclear envelope (F^1). The fibrillar nature of the material in the stroma of the mitochondria (19) and under the nuclear envelope (20) has been described previously.

In Fig. 6, an oval structure (*M*) in the cytoplasm is seen adjacent to a club-shaped nuclear evagination (*NE*). The oval structure is bounded by a double limiting membrane and infoldings of the inner of the two membranes can be seen (at arrows). The club end of the nuclear evagination appears roughly similar in density, content, and dimensions to the adjacent oval structure.

DISCUSSION

In time-lapse motion picture studies of cells in tissue culture, Frederic and Chévremont (9–11) described the mitochondria as occasionally coming into close contact with the nucleus. A similar close association has been found in ameba. The electron micrographs presented here suggest that not only are the mitochondria occasionally adherent to the nucleus but their membranes may be continuous (Figs. 2 and 3).

The continuity of the mitochondria with the nucleus may be of little functional significance or it may be fundamental and provide for an exchange of material between the two organelles. Furthermore, depending on the degree and di-

rection of the exchange, it may represent either a stage in a process whereby the mitochondria may be said to "originate" from the nucleus, or a time when the mitochondria may be adding their substance to the nucleus. If the nucleus exchanges material with the mitochondria for example, we might find evidence for this in the morphological resemblance between some parts of the two structures. The presence in the mitochondria of fibrillar material (19) which is similar to that found directly under the nuclear envelope (20) suggests an interrelationship of nuclear and mitochondrial material. In Fig. 5 this fibrillar material is seen in cross-section in the mitochondrion and in oblique section under the nuclear envelope There are also similarities in thickness and spacing between the nuclear membranes and the two limiting membranes of the mitochondria (Figs. 4 and 5).

If the nucleus is the site of formation of the mitochondria, Figs. 2 and 3 could be interpreted as late stages in the process. In this connection, Fig. 6 is an electron micrograph of structures which can be construed as being in transitional stages between the nucleus and the mitochondria. The oval structure (*M*) in Fig. 6 has a few infoldings (at arrows) of the inner of the two limiting membranes. Lund *et al.* (21) and Buvat (22) have recently described similar structures in embryonic plant cells as "immature mitochondria." The nuclear evagination (*NE*) in Fig. 6 has similar dimensions to the mitochondrion (*M*). Similarities of dimensions between nuclear evaginations and the mitochondria of *Amoeba proteus* have been previously described (17). This evidence suggests that the nucleus, through progressive folding of its envelope, may provide for the formation of mitochondria.

There is other evidence which lends support to the concept that the nucleus directly contributes material to the mitochondria. One factor necessary to mitochondrial function has been shown to be synthesized in the nucleus. Hogeboom and Schneider (23) have demonstrated that the coenzyme DPN is synthesized by a nuclear enzyme, while Baltus (24) has localized this enzyme in the starfish oocyte nucleoli. Frederic and Chévremont (10, 11), from observations on living cells, described the nucleolus as moving to the side of the nucleus where the mitochondria have attached. They suggest that this relationship is functional in that it reflects an exchange of material between the nucleolus and the mitochondria.

Evidence from studies by Brachet (25) on enucleate and nucleate halves of amebas suggests that some mitochondrial factors are dependent on the nucleus. While Brachet reports no difference in the oxygen uptake of the two halves during starvation, there was a difference in the substrates they could oxidize. In 3 days the enucleate halves lost their ability to oxidize fats and glycogen and, therefore, were rich in these substances when compared with the nucleate halves. The enucleate halves also lost 50 per cent of their protein in 5 days while the nucleate halves lost only 15 per cent. Apparently the enucleate halves maintained their oxygen consumption by burning protein and sparing fats and glycogen. This evidence suggests that the absence of the nucleus may directly cause a deficit in the mitochondrial enzymes, since a close relationship exists between the mitochondria and fat metabolism (26). Furthermore, many steps in carbohydrate metabolism are also associated with the mitochondria (1, 2).

The biochemical evidence against a hypothesis of nuclear origin for the mitochondria is based, in the main, on negative findings. Siekevitz and Watson (27) have demonstrated that the succinoxidase and cytochrome oxidase systems are tightly bound to the mitochondrial membranes. These two enzyme systems have not been detected in the nucleus of mammalian tissue. It is difficult to reconcile the apparent lack of these enzymes in the nucleus with the evidence presented here which suggests that the nuclear envelope contributes to the mitochondrial membrane. However, modification of the nuclear envelope may take place after its incorporation into the mitochondria.

Another theory of the formation of mitochondria is that they increase by division. There are two general methods which can be postulated whereby the mitochondria can increase in this fashion. They can increase either by the uptake of enzymes available in the cytoplasm, or by the synthesis of these enzymes. There is evidence against the first postulate, that the mitochondria depend upon the cytoplasm for their enzymes. The mitochondria appear to be partitioned off from the cytoplasm by membranes (28) which limit free diffusion and cause them to behave as osmometers in sucrose solutions (29). Indeed

the usual isolation procedures (1, 2) for mitochondria, which include several washes in aqueous media before enzyme analysis, suggest that the soluble enzymes at least are unable to penetrate the limiting membranes. Furthermore certain mitochondrial enzymes do not appear to be in the cytoplasm (1, 2).

On the other hand, there is evidence that the mitochondria can increase by synthesis. Simpson reports that the mitochondria contain RNA, incorporate amino acid, (30, 31) and are capable of net synthesis of cytochrome C (32). However, in the absence of genetic evidence that the mitochondria are capable of indefinite self-duplication, it is reasonable to suggest that some of their content, perhaps their RNA, comes from some other source such as the nucleus. The mitochondria might, therefore, be capable of enzyme synthesis and limited division. From the morphological evidence presented in this report it is reasonable to speculate that the mitochondria may be able to return to the nucleus perhaps to renew their RNA (Figs. 2 and 3). Evidence is presented which also suggests the possibility that the nucleus may be capable of forming mitochondria (Fig. 6).

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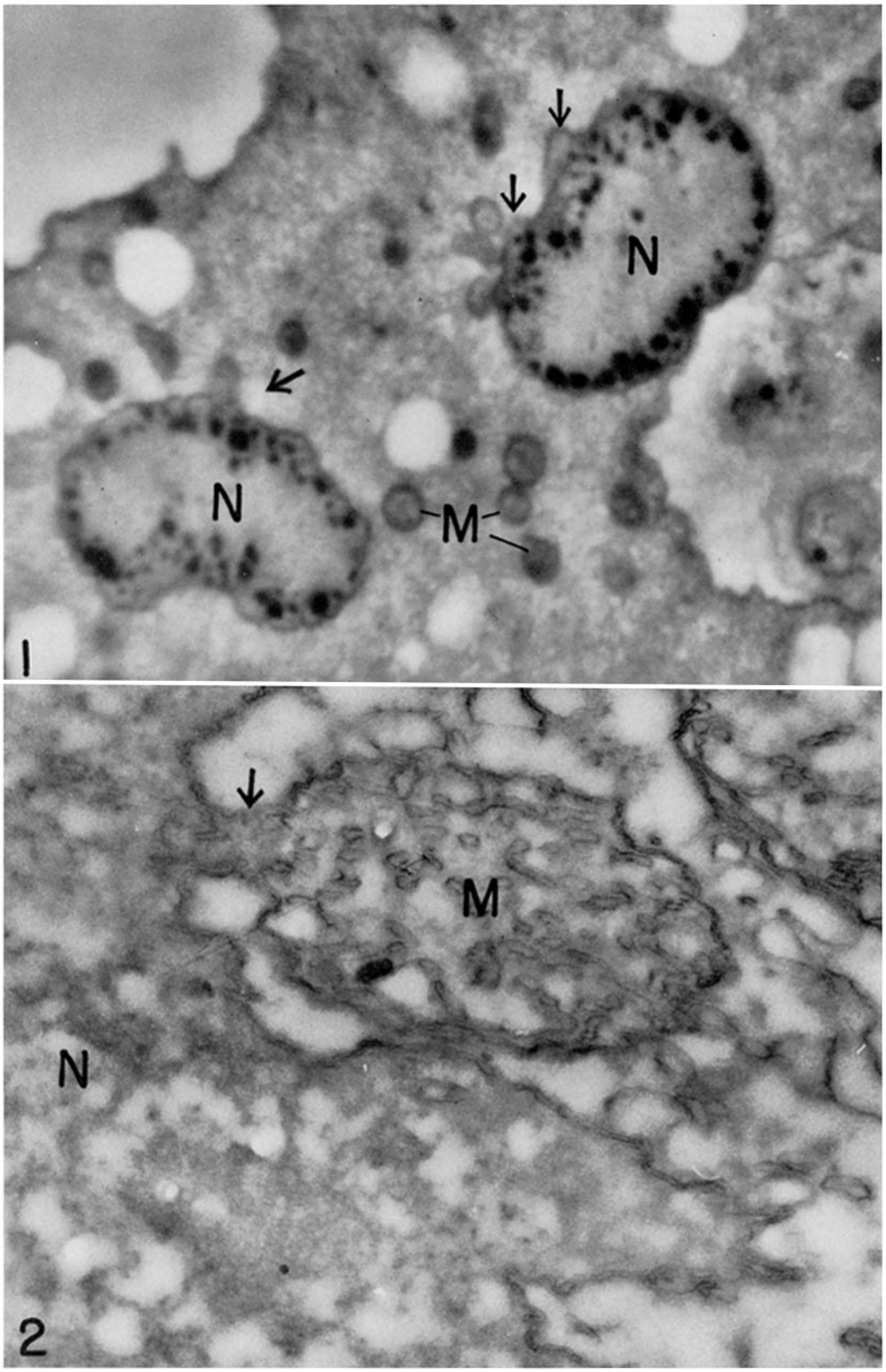
EXPLANATION OF PLATES

PLATE 55

FIG. 1. A photomicrograph of a 1 μ section of *Pelomyxa carolinensis* taken through the phase contrast microscope. The mitochondria do not appear homogeneous. Several mitochondria (*M*) can be seen clumped near one of the nuclei (*N*). Projections of the nuclei (at arrows) which appear similar in density to the mitochondria can be seen. × 2,100.

FIG. 2. Electron micrograph of a thin section from the same ameba as in Fig. 1. The mitochondrion (*M*) appears connected to the nucleus (*N*). It can be seen that the nuclear envelope is continuous with the outer limiting membranes of the mitochondrion (at arrow). × 53,000.

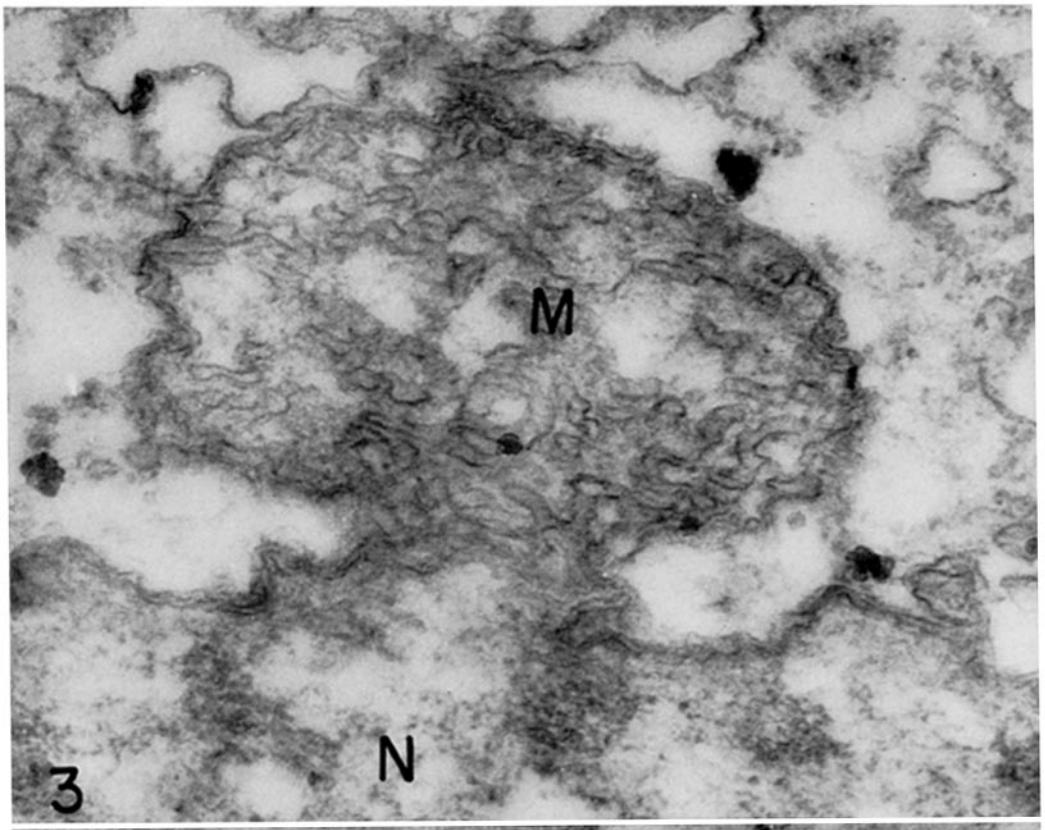


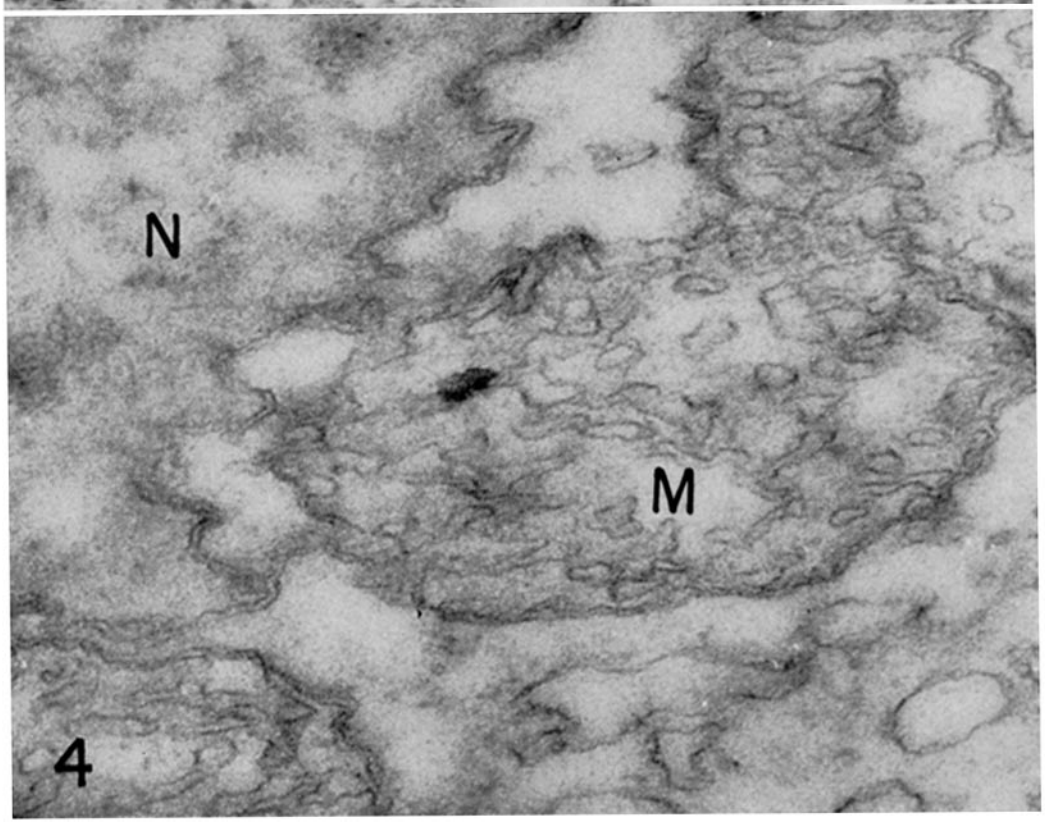
(Brandt and Pappas: Mitochondria. II)

PLATE 56

FIG. 3. The relationship between the nucleus (*N*) and mitochondrion (*M*) shown in this electron micrograph is more complex than that of Fig. 2. The membranes of the two structures are closely interwoven. × 65,000.

FIG. 4. There are continuities of the nuclear envelope with the limiting membranes of the mitochondrion (*M*) in this electron micrograph. The packing of the cristae of the mitochondrion attached to the nucleus (*N*) appears to be less dense than that of the mitochondria free in the cytoplasm. Similarities between the thickness and spacing of the two membranes of the nuclear envelope and the two limiting membranes of the mitochondria can also be seen. × 75,000.



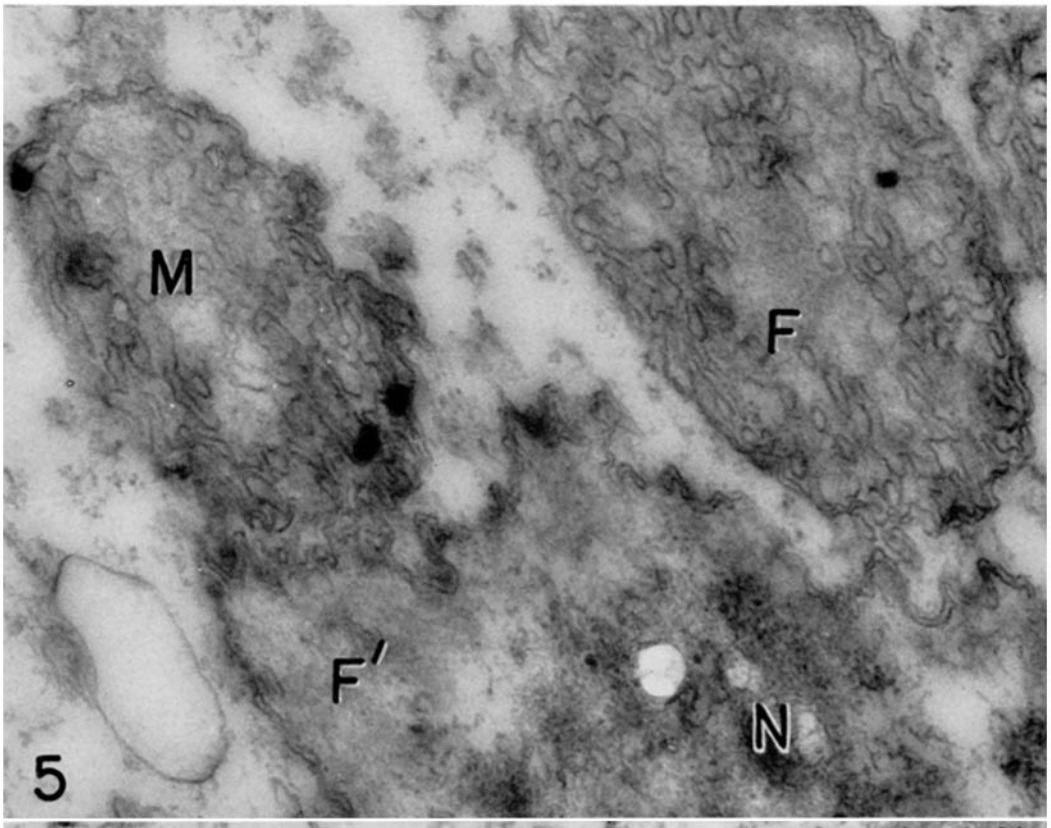


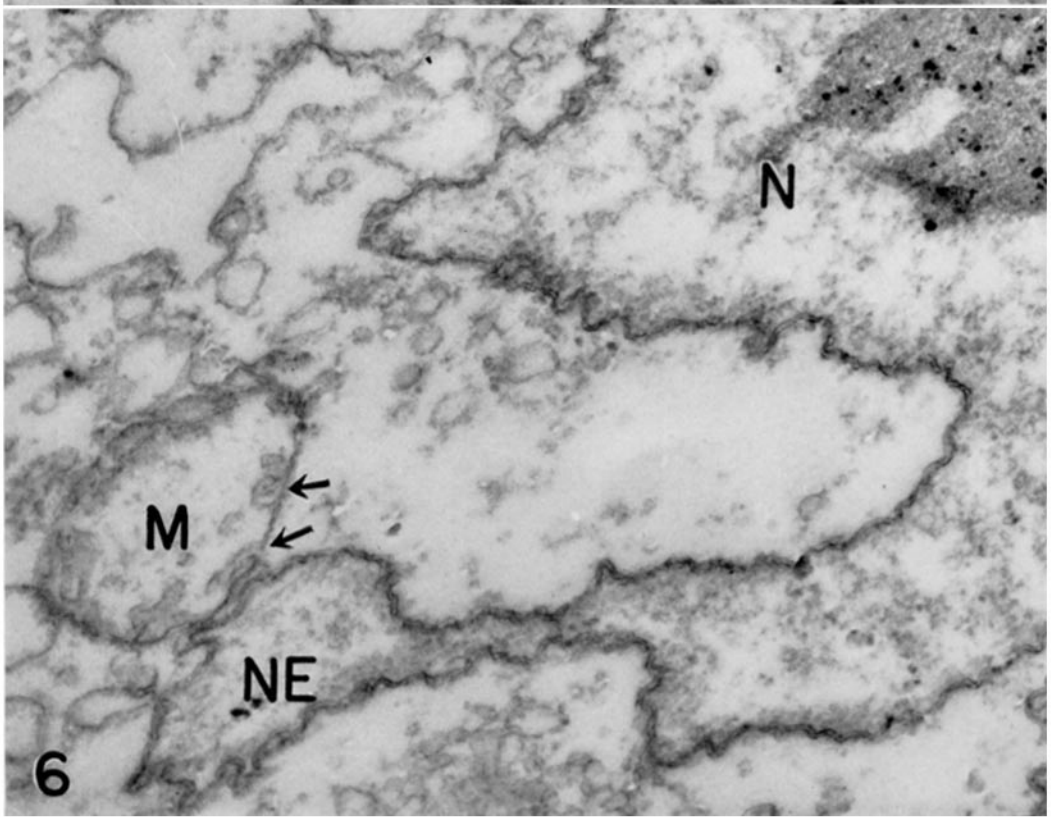
(Brandt and Pappas: Mitochondria. II)

PLATE 57

FIG. 5. In this electron micrograph the membranes of the mitochondrion (*M*) at the left of the field appear to be interdigitated with folds of the nuclear envelope. A fine fibrillar material (*F'*) can be seen in the nucleus (*N*) immediately under the nuclear envelope. It appears similar in density and structure to the fibrillar material (*F*) seen in the matrix of the mitochondrion at the right of the field. × 54,000.

FIG. 6. Electron micrograph showing a club-shaped evagination (*NE*) of the nucleus (*N*). Adjacent to the nuclear evagination is an oval structure (*M*), presumably a mitochondrion and containing a few "cristae" (at arrows). × 30,000.





(Brandt and Pappas: Mitochondria. II)