THE STRUCTURE OF PIGEON BREAST MUSCLE MITOCHONDRIA*

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PLATES 124 AND 125

The fine structure of mitochondria has been the subject of a number of recent electron microscope studies (11, 12, 16).[1] Though mitochondria exhibit distinct structural variations from one cell type to another, it has become generally recognized that they all have in common certain features which distinguish them from other cytoplasmic organelles or inclusions. These features are (*a*) a double-layered enveloping membrane, (*b*) a series of internal double-layered membranes usually roughly parallel to one other and transverse to the long axis of the mitochondrion, and (*c*) a matrix which usually appears structureless except for the presence of a few dense granules (diameter 20 to 30 mμ) in the mitochondria of certain cell types.

The exact relation of the internal membranes to the enveloping membrane is still in dispute. One view held by several investigators (2, 7, 12) is that the transverse membranes (termed "cristae" by Palade (11)) are infoldings of the inner layer of the enveloping membrane. In a few types of mitochondria the infoldings are in the form of villi rather than ridges or lamellae, and so they may appear in sections as circles rather than as parallel lines (15, 20). Another view held by other investigators (14, 17, 18) is that there is no continuity between the internal and enveloping membranes, and accordingly members of this group term these structures simply inner and outer double-lined membranes respectively.

Before the advent of electron microscopy it had been deduced from several lines of evidence that mitochondria are surrounded by membranes, and this view had become generally, but not universally, accepted. As long ago as 1888, Kölliker (10) explained the osmotic behavior of insect muscle mitochondria ("interstitial granules") as being dependent on their possessing enveloping membranes. Recent observations made on heart muscle along similar lines by Cleland and Slater (3) have been similarly interpreted. Early electron microscope studies of skeletal and heart muscle mitochondria (11) showed them to have enveloping membranes together with numerous internal membranes, and subsequent studies, with few exceptions, have confirmed these observations (2, 20).

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[1] See also several articles in this issue.

Recently, however, the commonly accepted concept of mitochondrial structure has been challenged, particularly in the instance of pigeon breast muscle by Harman (5) and Weinreb and Harman (19). These authors find no evidence for the existence of enveloping membranes in pigeon breast muscle mitochondria, and on the basis of their electron microscope observations, propose a "folded ribbon" theory for the internal structure of mitochondria. Other authors who have questioned the existence of a limiting mitochondrial membrane on electron microscope evidence are Hartmann (6) who studied nerve cells, and more recently Powers, Ehret, and Roth (13) who studied *Paramecium*. The conclusions of the latter authors have been criticized in a recent article by Sedar and Porter (15).

In view of the importance of mitochondria in cell activities and the conflicting opinions on their fundamental structure described above, it was thought that a further examination of pigeon breast muscle would be of value.

Materials and Methods

The investigation was carried out in conjunction with biochemical studies on mitochondrial fractions prepared by differential centrifugation from homogenates of pigeon breast muscle. Opportunity was taken to study by thin sectioning methods the fine structure of mitochondria in isolates as well as in intact tissue.

In addition to the electron microscope examination of sections of intact muscle and of mitochondrial pellets, bright field and phase contrast studies of teased muscle fragments and of the final mitochondrial preparation diluted with the suspending medium (0.25 M or 0.45 M sucrose), both fresh and after fixation in OsO_4, were also made.

All the tissue was obtained from the pectoralis major muscle of domestic pigeons. For electron microscopy the muscle was exposed, a small piece snipped off and immediately immersed in a drop of buffered OsO_4. Further trimming to obtain pieces of about 1 mm. cube was carried out under the surface of the fixative. The small pieces were then fixed further for 1 hour, washed briefly in distilled water, dehydrated in 70 per cent, 95 per cent, and absolute ethanol, and finally embedded in a mixture of 9 parts butyl to 1 part methyl methacrylate. Sections were cut with a Porter-Blum microtome and examined in an RCA EMU-2A electron microscope.

The mitochondrial fractions were prepared from homogenates of the muscle tissue in 0.25 M and 0.45 M sucrose solution by standard centrifugation procedures. The mitochondrial pellets were processed for sectioning in the manner described above for tissue blocks except that they were spun in a centrifuge before each change of fluid at a speed sufficient to sediment them.

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OBSERVATIONS

Intact Muscle Tissue.—As revealed by examination with bright field and phase contrast illumination, the general appearance of the tissue was found to be similar to that described by Kitiyakara and Harman (8). The muscle fibers are of two distinct types, those with a high content of mitochondria and those in which they are very sparse. In the former type the mitochondria are mostly disposed in long parallel rows between columns of myofibrils. Interspersed at fairly regular intervals between the mitochondria are small round refractile bodies which are densely stained with osmium tetroxide. These are most readily distinguished from mitochondria in fixed preparations by bright field illumination.

Electron micrographs of thin sections show the same features. Fig. 1 shows a small portion of a muscle fiber which is in a state of contraction. The myofibrils are arranged in columns. Individual fibrils are separated by thin layers of cytoplasm. Between the columns there are long rows of tightly packed mitochondria. The mitochondria have distinct enveloping membranes which in places can be seen to be double-layered. They also have large numbers of double-layered internal membranes which in many instances stretch across the whole breadth of the mitochondrion (Fig. 2).

At intervals between the mitochondria there are bodies which differ from them in several respects. They are smaller (about 0.5 micron in diameter) and rounder than mitochondria, and appear to be more rigid because the mitochondria on either side of one are usually indented (Fig. 1). They are bounded by a single membrane and in thin sections show evidence of a fibrillar internal structure. In thicker sections they are fairly dense, suggesting that they have a high lipide content. One such body is shown at the upper right in Fig. 1. Part of another appears in the lower left-hand corner. They are more numerous in some muscle fibers than in others and are most easily distinguished from mitochondria in tissue which is in a poor state of preservation (Fig. 3). In this preparation the mitochondria are greatly swollen but the lipide bodies retain their normal size and shape. As may be seen in this figure they are frequently (though not invariably) associated with a Z band.

Small regions of cytoplasm densely stained with OsO_4 are not infrequently seen in electron micrographs of many different cell types. Their outline is usually irregular or inkspot-shaped, and they bear no evidence of internal structure or of a bounding membrane. A few such regions were observed in the muscle cells. It is reasonable to suppose that they represent lipide inclusions and are to be distinguished from the more organized granules described above.

Isolated Mitochondria.—In sections of mitochondrial fractions obtained by differential centrifugation from sucrose homogenates of pigeon breast muscle, many bodies easily recognizable as mitochondria were present, together with some fragments of myofibrils and other cellular debris. The mitochondria were in various states of preservation. Some were almost unaltered, with intact enveloping membrane (seen to be double-layered in places) and an array of transverse membranes (Fig. 4). Others were greatly swollen and obviously damaged, and still others appeared shrunken and condensed. In preparations isolated in 0.45 M sucrose the state of preservation was improved but did not approach that observed in well fixed intact muscle tissue. It has not been ascertained at what stage in the preparative procedure the damage to the mitochondria occurs. Bodies resembling the lipide granules described earlier were occasionally observed, but positive identification was difficult.

DISCUSSION

The presence of granules in skeletal muscle tissue has been known for over a century. Kölliker (9) first described them in detail in 1857, calling them "interstitial granules." Later authors distinguished two types of granule—"true interstitial granules" and "fat granules"—and in describing these considerable confusion in terminology arose. It is clear that the "true interstitial granules" correspond to what are now called mitochondria and the "fat granules" to the lipide bodies described above. In recent reports Harman and his associates refer to the latter type of granule as sarcosomes, but this may lead to confusion as the term "sarcosomes" is used by many authors to describe the mitochondria of muscle tissue.

In discussing the fine structure of mitochondria, it is assumed that the double lines observed in electron micrographs around and within mitochondria represent true membranes and not merely interfaces between regions with different physical properties. This assumption appears to be made also by Weinreb and Harman (19) in interpreting their electron micrographs of pigeon breast muscle, in that the absence of continuous lines around the mitochondria seen in their preparations is taken as morphological evidence for the absence of a limiting membrane. On the basis of their observations on this material, these investigators have proposed a "folded ribbon" theory for the internal structure of mitochondria. Mitochondria are regarded as composed of two protein gels, a fibrous gel in the form of a folded ribbon, enclosed within a non-fibrous gel matrix. The folds of the ribbon are visible as a series of parallel lines but no membrane separates the mitochondrial gels from the surrounding sarcoplasm.

The present observations do not support the "folded ribbon" theory. All the mitochondria examined were surrounded by well defined membranes which in thin and favorably orientated sections were seen to be double-layered. This observation applied to mitochondria sectioned both *in situ* and in isolated cell fractions. The transverse internal membranes, likewise double-layered, were prominent, regularly spaced, and unusually closely packed. The relation of the internal membranes to the enveloping membrane was not as clear as in some other types of mitochondria, but instances of an apparent continuity between the inner layer of the enveloping membrane and transverse membranes were noted. The link between the internal and enveloping membranes is apparently a weak one, for in mitochondria which were swollen or poorly fixed there were frequently gaps at this point.

It is possible that the apparent absence of an enveloping membrane in Weinreb and Harman's micrographs is due to tissue damage occurring either during the preparative procedure or during exposure to the electron beam. The lack of continuity between the lipide bodies ("sarcosomes") and the surrounding cytoplasm is suggestive of such an interpretation.

The nature and function of the lipide granules are not yet clear. Harman (5) has presented biochemical evidence that they contain considerable nucleic acid and concludes that they are not identical with neutral fat particles. The present electron microscope evidence is in agreement with this conclusion as the granules differ morphologically from fat inclusions by having a limiting membrane and some internal structure. The presence of possibly homologous bodies has been observed in several other types of tissue. Rhodin (14) has described organelles in the proximal convoluted cells of mouse kidney which he calls "microbodies." These are described as being spherical or ellipsoid in shape, smaller than mitochondria, bounded by a single membrane, and having no well defined internal structure. Bodies of similar appearance have been observed by the present author in rat liver cells as well as in other tissues. Fig. 5 shows a small area of cytoplasm of a liver cell from a 20-day rat embryo. The large object in the upper right is a mitochondrion. In the lower left corner there is another body which differs from a mitochondrion in having a single limiting membrane and no cristae. Corresponding bodies are seen also in normal mature rat liver but are more easily visible in animals which have been starved for a few days. In such animals the liver mitochondria may be somewhat distended, but scattered among them and readily distinguished from them are small dense bodies which retain their original size (Fig. 6). It is suggested that these bodies may constitute the fraction isolated from rat liver in partially purified form by de Duve *et al.* (1, 4) and found to have biochemical properties different from either mitochondria or microsomes.

SUMMARY

The structure of pigeon breast muscle mitochondria was studied both in whole muscle tissue and in mitochondrial fractions separated by differential centrifugation. No evidence was found to indicate that the mitochondria of pigeon breast muscle differ in any fundamental way from those of other tissues. In particular the absence of an enveloping membrane, claimed in recent reports, was not confirmed.

The structure of the lipide bodies present in muscle has been described, and compared with that of similar bodies in other tissues.

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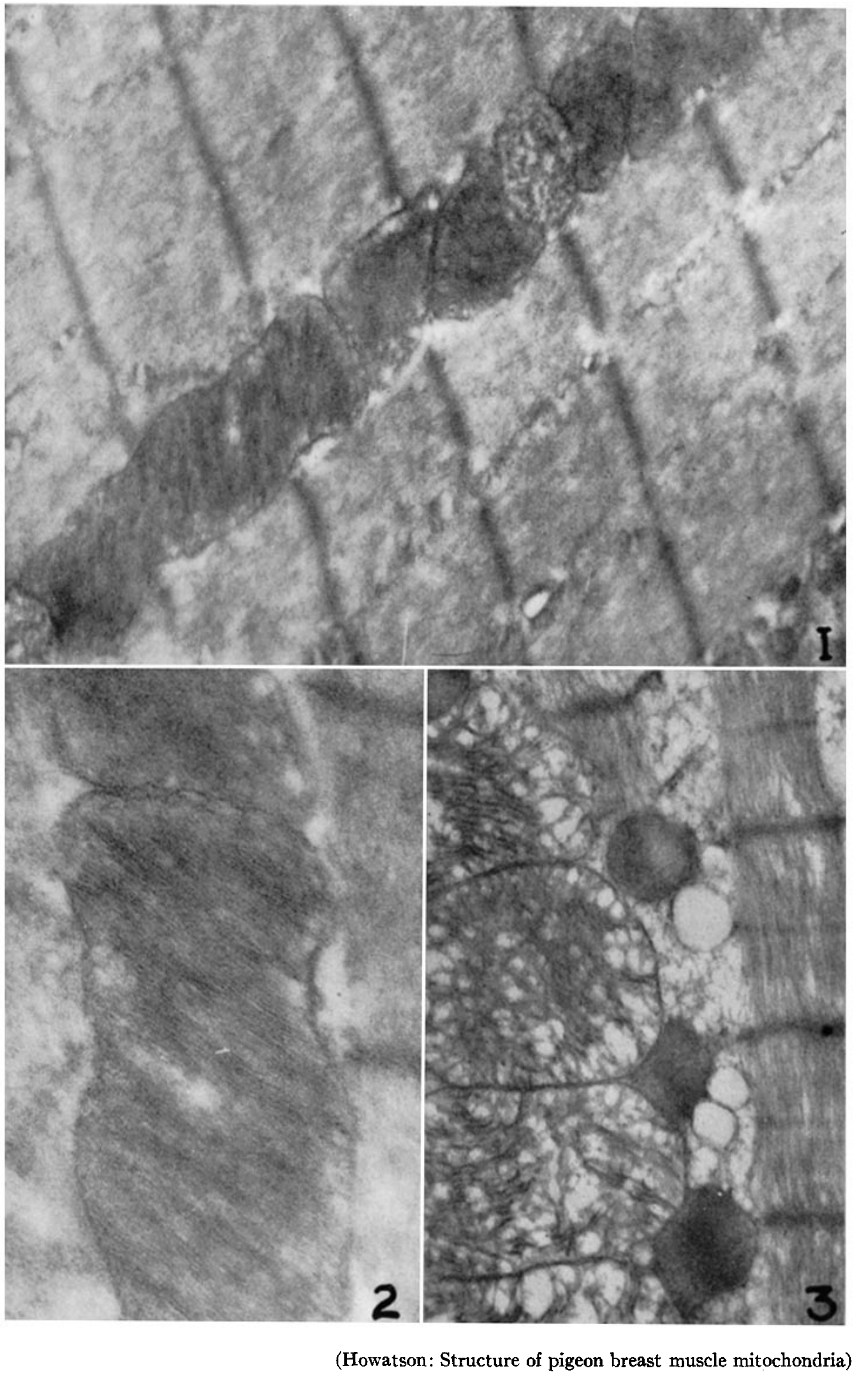
EXPLANATION OF PLATES

Plate 124

Fig. 1. Electron micrograph of a small portion of a pigeon breast muscle fiber. A number of myofibrils run diagonally across the picture from the lower left to the upper right. They are separated from one another only by thin strands of cytoplasm except for the middle pair between which there is a single row of mitochondria which appear compressed because the muscle is contracted. Near the upper right end of the row there is a lipide granule which can be distinguished from the mitochondria by the absence of internal membranes (cristae). Note that the granule indents the mitochondria on either side of it. Part of a second granule is just visible at the extreme lower left-hand corner of the picture. × 25,000.

Fig. 2. Part of Fig. 1 enlarged to show the structure of the mitochondria. Characteristic features are double-layered enveloping membranes and a series of closely packed double-layered internal membranes. × 67,000.

Fig. 3. Part of a poorly preserved muscle fiber showing greatly swollen mitochondria and three lipide bodies associated with the Z bands of a myofibril. × 25,000.



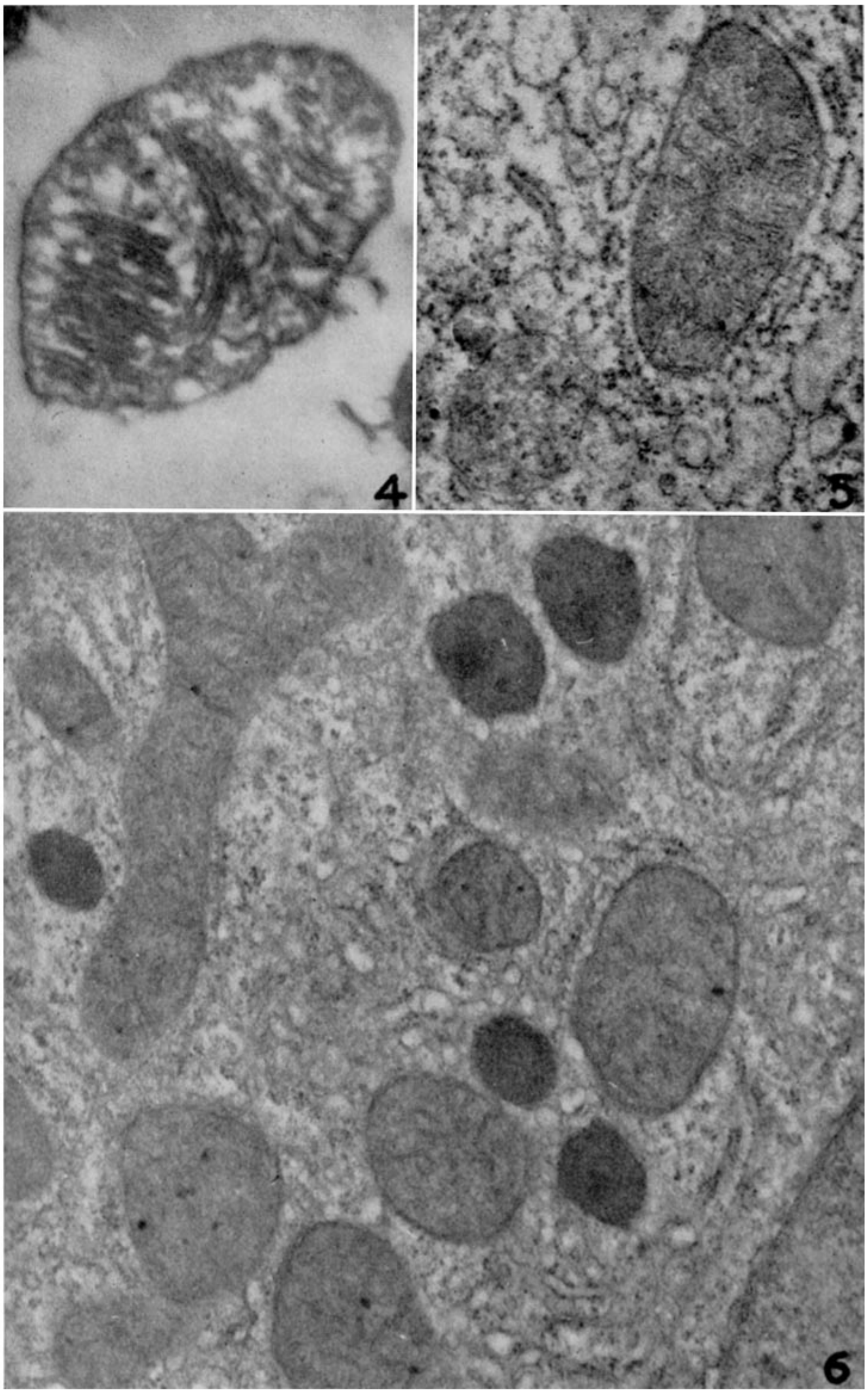
(Howatson: Structure of pigeon breast muscle mitochondria)

PLATE 125

FIG. 4. Section of a mitochondrion isolated by differential centrifugation from a homogenate of pigeon breast muscle in 0.25 M sucrose. The mitochondrion is considerably swollen but the enveloping membrane is complete and portions of the internal membranes are visible. × 31,000.

FIG. 5. A small region of the cytoplasm of a liver cell from a 20-day rat embryo. A mitochondrion is visible at the upper right. At the lower left there is a smaller body which can be distinguished from a mitochondrion by the absence of internal membranes (cristae) and the single-layered nature of its enveloping membrane. × 40,000.

FIG. 6. Part of a liver cell of a rat which had been fasted for 2 days. Double-layered enveloping and internal membranes distinguish the mitochondria. Interspersed between them are smaller, somewhat denser bodies with single-layered enveloping membranes and without internal membranes. × 30,000.



(Howatson: Structure of pigeon breast muscle mitochondria)