MITOCHONDRIAL STRUCTURE*

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The fine structure of mitochondria revealed by the electron microscope was first investigated by Palade (1). Since then a number of serious studies have been made (2 to 6) which have established a general concept of mitochondrial structure. Certain obvious features are agreed upon by all authorities, but there is some disagreement concerning finer details. In the course of an electron microscopic investigation of peripheral human blood conducted in this laboratory by James A. Freeman, it was found that the mitochondria of leucocytes were well suited to structural analysis. It therefore seemed worth while to analyze certain finer points in these preparations.

The following technique, which does not use anticoagulants and does not introduce any foreign substance into the blood prior to fixation, was found by Mr. Freeman to be the most satisfactory.

Materials and Methods

Normal human blood was obtained by venipuncture either by (*a*) sterile withdrawal with a 10 cc. silicon-coated[1] syringe fitted with an arquad-coated[2] 20 gauge needle and transference to a 10 cc. lusteroid centrifuge tube (International) precooled to 5–10°C. or (*b*) by needle drip directly into the tube. The ice-cooled blood sample was centrifuged at 1500 R.P.M. for 15 minutes at 0°C. (R.C.F. = 265: International model PR-2, refrigerated, angle head). The buffy coat was aspirated with a silicon-coated pipette and transferred to a glass tube containing 5 cc. of 1 per cent veronal buffered (pH = 7.4) OsO_4 at 5–10°C. It was fixed for ½ hour. Between each successive step (½ to 1 hour) of fixation, dehydration, and methacrylate infiltration the specimen was centrifuged for 1 to 1½ minutes at 1500 R.P.M. (R.C.F. = 385: Clay-Adams Safeguard) in glass tubes (alcohol dissolves lusteroid!). After each centrifugation the supernatant fluid was decanted. The next fluid was added and the tube manually agitated to produce a suspension. The last methacrylate suspension was permitted to settle by gravity in 00 gelatin capsules for ½ to 1 hour to avoid close packing. It was then polymerized overnight at 47°C. The remainder of the technique was routine.

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[1] Dow-Corning 200; 2 per cent in CCl_4. Glassware immersed and baked ½ to 1 hour at 450–550°C.

[2] Armour Monocote (tris-(2-hydroxyethyldodecyl)-NH_4Cl); 10 per cent aqueous. Metalware immersed, drained, and air-dried.

RESULTS

It is agreed by all investigators (1 to 6) that double membrane systems are characteristic of mitochondrial structure. These systems (Figs. 1 to 4) consist of two more or less parallel, dense lines separated by a clear space, usually of somewhat larger dimensions. The external mitochondrial membrane is recognized to be double (2 to 6) and this observation is confirmed in the present study (Figs. 1-*A*, 1-*B*, 1-*C*, and 2-*A*). The internal double membranes are larger systems. Their basic duality has been repeatedly observed and is obvious in the accompanying micrographs (Figs. 1 to 4). But their relationship to the external membrane is disputed. They have been named "cristae mitochondriales" by Palade (1, 2) who interpreted them as infoldings of the inner membrane of the surface double membrane system. But Sjöstrand and Hanzon (5) interpret the inner double membrane systems to be in contact only with the surface membrane, usually at one end. The two constituent membranes of the former are continuous around the perpendicularly cut edge. They conclude that the inner double membrane systems are "individual structures with only topographic relations to the outer membrane." This concept is supported by Rhodin (6). The structural situation described by Palade (2) has been repeatedly observed in the course of our work, and is illustrated in Figs. 1-*D*, 2-*B*, and 3-*A*.

The cristae mitochondriales have been reported to branch (2, 6), forming Y-shaped structures. This has been commonly observed in our material, as have arc-shaped cristae returning to connect with the outer membrane system on the same side. Y-shaped cristae are illustrated in Figs. 2-*C* and 4-*A*. In Fig. 4-*B* a more complex crista with four separate branches is shown.

There has been frequent mention of granules or particles in the mitochondrial matrix (1, 2, 4 to 6). Size measurements have varied with individual studies. Rhodin (6) described a granular matrix whose particles measured 45 to 50 A with opaque areas measuring 75 to 150 A. Sjöstrand and Hanzon (5) described "opaque spherical bodies" measuring 180 to 400 A, but previously Sjöstrand and Rhodin (4) had reported scattered dark areas varying from 200 to 700 A. The latter were interpreted as artifacts. In our preparations granular material in the matrix varies from the intensely opaque to the barely visible, with a size range sometimes as great as 300 A, but the majority of the granules measure about 65 to 100 A. These particles in the matrix are illustrated in Figs. 2-*D* and 4-*C* where the size can be observed to vary. In Fig. 3-*B* there is a group with more nearly average dimensions. Majority opinion interprets these inclusions as artifacts. But their appearance in tissue which shows no certain fixation artifacts suggests otherwise. The fact that they vary in size and density should not discourage interpretation of them as true structural or even functional units. The intense physiological activity known to be characteristic of mitochondria in general has not expressed itself recognizably in any other

mitochondrial structure. It therefore seems reasonable to speculate that these extremely variable inclusions may indeed be visualizations of a constantly changing functional process.

The sections used in the illustrations were cut by Max R. Clevenger. The RCA-EML-1-B electron microscope was maintained by Lucien G. Caro. James A. Freeman took the micrographs and did the photography.

REFERENCES

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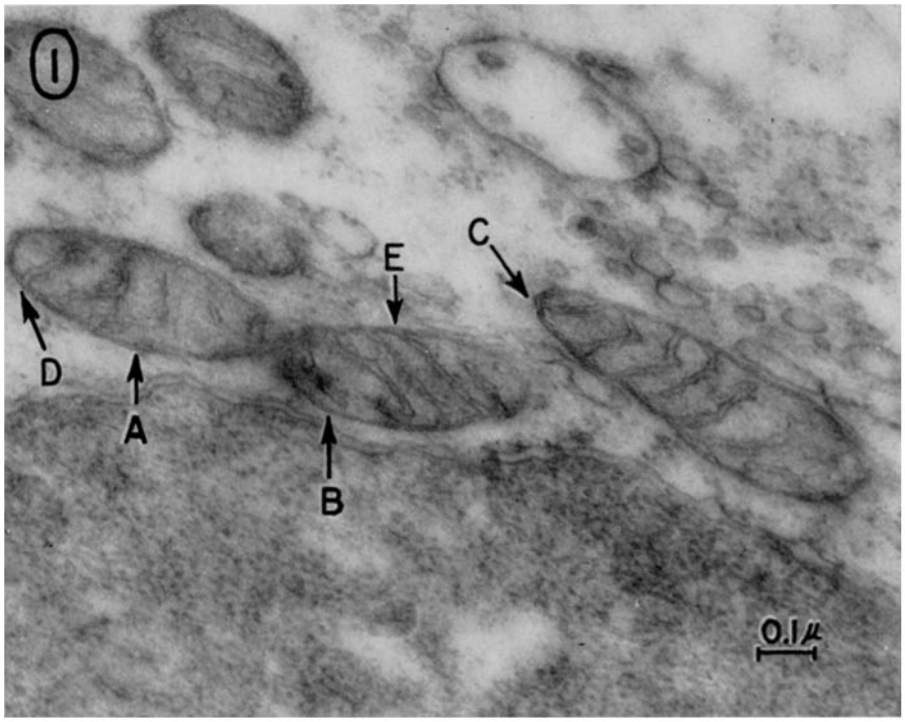
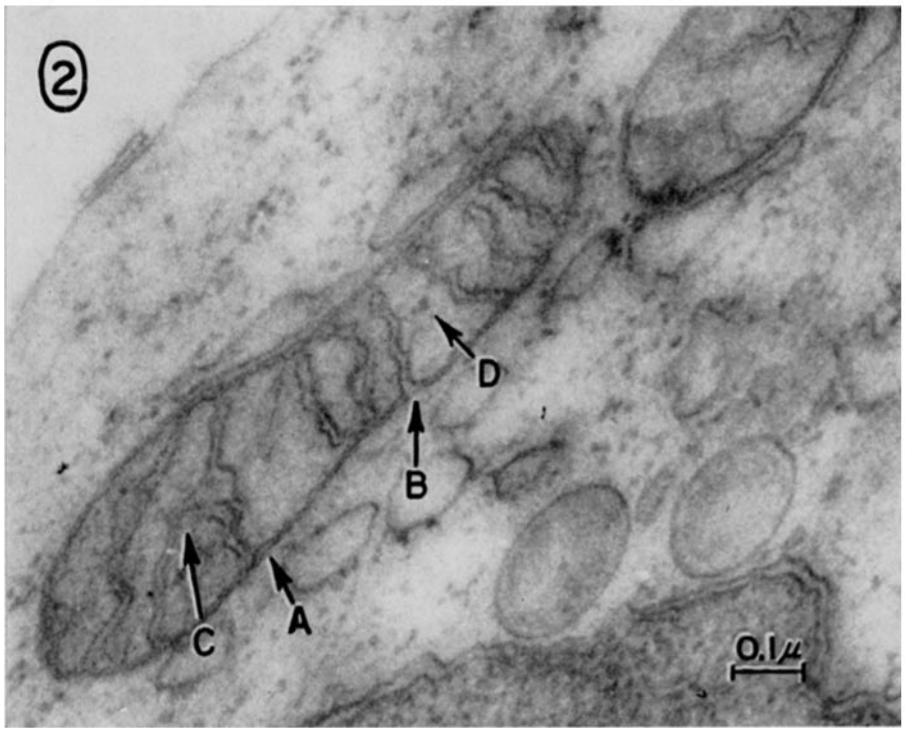
EXPLANATION OF PLATES

PLATE 111

FIG. 1. Mitochondria of normal human blood leucocyte. External double membranes are visible at *A*, *B*, and *C*. At *D* and *E* the cristae can be observed arising from the inner membrane of the surface double membrane system. × 74,000.

FIG. 2. Mitochondria of normal human blood leucocyte. At *A* the external membrane is seen to be double. At *B* a crista is derived from the inner membrane of the external double membrane system. A bifurcate crista is at *C*. At *D* there are granules of varying size and density in the matrix. × 94,000.





(Low: Mitochondrial structure)

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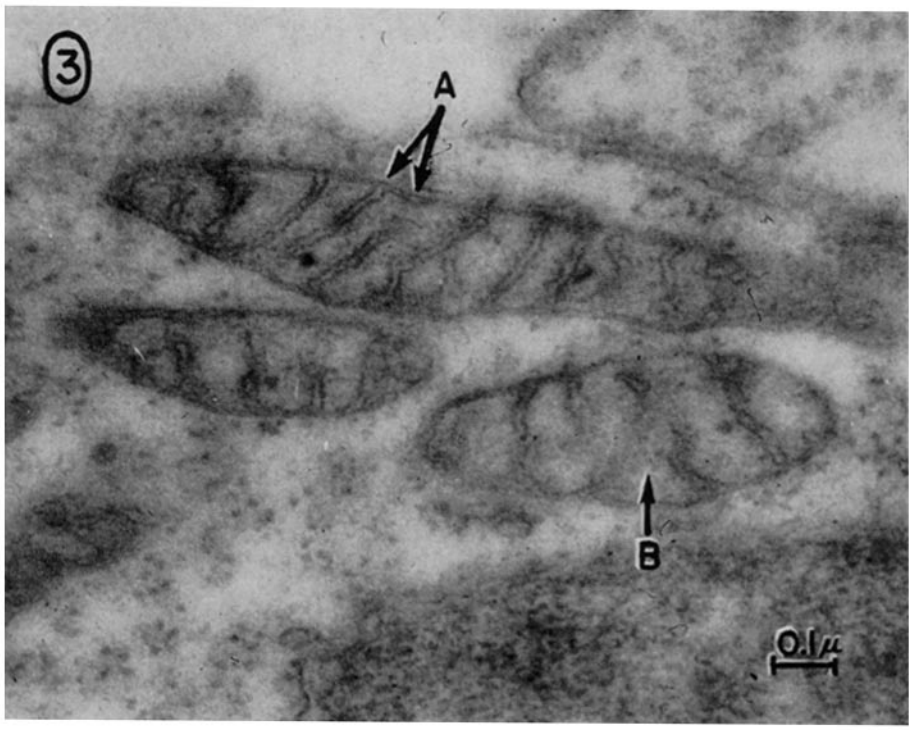
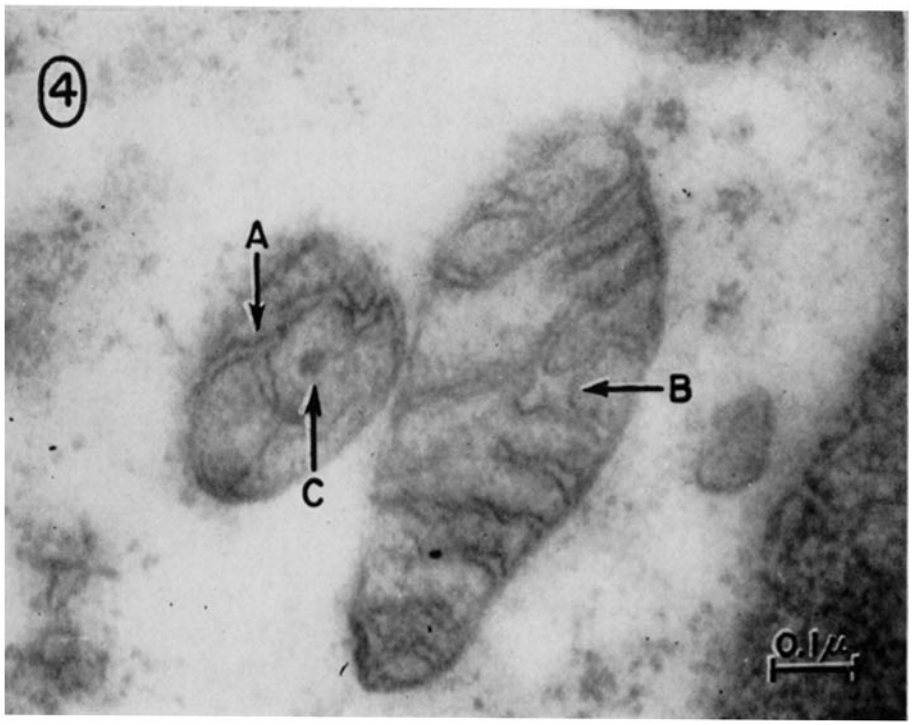
FIG. 3. Mitochondria of normal human blood leucocyte. At *A* a crista is derived from the inner membrane of the external double membrane system. The granules in the matrix at *B* are of average size, 65 to 100 A. × 84,000.

FIG. 4. Mitochondria of normal human blood leucocyte. At *A* a crista bifurcates and at *B* a crista with at least 4 branches is visible. At *C* there are granules of various sizes in the matrix. × 110,000.





(Low: Mitochondrial structure)