AN ELECTRON MICROSCOPE STUDY OF ISOLATED MITOCHONDRIA

Method and Preliminary Results

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Plates 4 and 5

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The work of Altmann (1) and of Benda (2), and the subsequent investigations of Meves (3), Duesberg (4), Guilliermond (5, 6), and many others established that mitochondria are constant constituents of living cells (7). It was found that the size and shape of mitochondria are characteristic for a given cell type and that these features may vary considerably from one cell type to another: in some cases, as in lymphoid cells, mitochondria have the form of distinct granules; frequently, as in many glandular cells, they appear as rods or more or less slender filaments, the width of the latter being remarkably uniform along the entire element. Elongated mitochondria are known to break up into shorter segments or to resolve themselves into a series of individual granules, especially when the cell is subjected to injury. As regards the constitution of mitochondria, the complex composition of these cytoplasmic bodies seems to have been recognized as early as 1861 when Max Schultz, in his celebrated paper on protoplasm, described the cell substance surrounding the nucleus as being filled with granules of a protein and lipid nature (8). From tests on the solubility and staining properties of mitochondria in microscopic preparations a number of authors, among them Regaud (9), Fauré-Frémiet (10), Giroud (11), and Milovidov (12) concluded that these bodies contain both lipids and proteins. Mayer, Rathery, and Schaeffer suggested that mitochondria contain phospholipids with unsaturated fatty acid radicals (13). In recent work in which mitochondria were subjected to direct chemical analysis it was possible to ascertain that these elements contain, as major components, phospholipids and ribonucleoproteins, and these substances appear to be associated in the mitochondria in definite proportions (14, 15).

The problem of the derivation and growth of mitochondria has been the object of repeated investigations and the view most generally held is that these bodies represent independent entities capable of self-duplication and autonomous growth (5–7). Occasionally, observations are taken to indicate that mitochondria may arise *de novo* in the ground substance of the cell (16, 17). The latter view is supported by Bensley (18) who accepted the concept of Bungenberg de Jong (19) that mitochondria are coacervates and thus apt to appear or be resorbed in living cells, depending on changes of equilibrium in the protoplasm.

That the problems of the origin and morphology of mitochondria have remained undecided must be ascribed to their small size, and to limitation in the resolving power of the microscope using visible light, so that it has not been possible to investigate properly their fine structure or to ascertain their position with respect to the surround-

ing cytoplasm. The development of the electron microscope, with a resolving power 50 to 100 times greater than that of the ordinary microscope (20) has recently made the finest structures of the cell accessible to morphological study and it will be possible, with the aid of the new instrument, to reinvestigate the various problems bearing on the organization and development of mitochondria.

The present work constitutes a preliminary study of the morphology of mitochondria by means of electron microscopy. In this investigation, advantage was taken of the fact that mitochondria can be separated from the other components of the cell by a method of differential centrifugation (14, 15, 21) and thus made directly available for experimentation and examination in the electron microscope. The mitochondria used in this work were obtained from the neoplastic cells of a lymphosarcoma of the rat (22) and a biochemical study of these elements has appeared in a preceding paper (15). In the purified form the mitochondria of the rat lymphosarcoma appear as regular, spherical bodies, approximately 0.5 to 1.5 μ in diameter (15) and therefore clearly visible under the ordinary microscope. With the full definition of the ordinary light microscope, however, it has not been possible to disclose the existence of a differentiated structure within these elements. Such a structure, if present, would fall within the power of definition of the electron microscope and could then be perceived either directly, or with the aid of appropriate techniques.

Because of limitation in the penetrating power of electrons, the present technique of electron microscopy requires that specimens of organic material prepared for examination be no more than $\simeq 0.1$ μ in thickness, and preferably less. It would seem therefore that free mitochondria from rat lymphosarcoma are sufficiently thick to scatter the electron beam completely and thus are not suited for differential study in the electron microscope. It has been found, however, that the isolated mitochondria are apt to flatten out during preparation of the mount or may lose part of their substance during purification so that specimens can be obtained which, as regards thickness, are adequate for electron microscope studies. Observations made with this material are recorded in the present paper.

Material and Methods

The material selected as the source of mitochondria was a neoplastic cell of the rat, which proliferates rapidly when introduced into new hosts, producing a leukemic condition or giving rise to localized tumors, depending on the route of inoculation (22). The lymphosarcoma form of the disease is marked by rapid neoplastic growth, the subcutaneous injection of leukemic cells giving rise locally to 10 to 15 gm. tumors within 10 to 12 days. The tumor is uniform in cell type, the characteristic element being a lymphoid cell with a large nucleus and an abundant and highly basophilic cytoplasm. Many of the large granules visible in the cytoplasm by means of microscopy with ordinary light are spherical mitochondria, staining typically with Janus green (15).

Preparation of Extract.—The method employed for the separation and purification of mito-

chondria was essentially that used in previous work and described in a preceding paper (15). The freshly removed tumors were chilled, passed through a 1 mm. mesh masher, and the resulting pulp was ground alone in a mortar for about 3 minutes. The solvent, consisting of a 0.85 per cent NaCl solution buffered at pH 7.2 by the addition of the proper phosphate mixture to a final concentration of 0.005M, was added, very slowly at first, to a total volume equivalent to five times the weight of the pulp.[1] The tissue suspension was then submitted to two successive runs in the centrifuge; *i.e.*, one centrifugation of 4 minutes, followed by a centrifugation of 10 minutes at 1500 × *g*.[2] This procedure removes from the suspension practically all the debris, the unbroken cells, and the nuclei that were set free during extraction, leaving in the supernate, hereafter referred to as the extract, the free mitochondria and other cytoplasmic constituents of equal or smaller size (15).

Separation of Mitochondria.—The mitochondria were separated from the extract by 25 minutes centrifugation at 2400 × *g*, in an angle centrifuge, or else by 4 minutes at 18,000 × *g*.[2] The supernate was discarded and all, save the lowest portion, of the sediment was resuspended in buffered or alkaline saline, the final volume of the suspension being about one-fifth the original volume of the extract. Washing of the mitochondria was accomplished by submitting the sediment to two or three cycles consisting of sedimentation in the centrifuge and resuspension in buffered or alkaline saline. Each time, as just mentioned, a small amount of the material at the bottom of the deposit was left undisturbed thus eliminating the larger elements, such as red blood cells or cell nuclei, that might have been carried along with the mitochondria. The final deposit was resuspended in a small amount of saline. This fraction, known from previous work (15) to be composed almost exclusively of granules, approximately 0.5 to 1.5 μ in diameter, was the standard mitochondria preparation used in the electron microscope studies.

Fixation of Mitochondria.—The fixatives used in connection with the present work were neutral or alkaline solutions of formaldehyde at various concentrations, a neutral, 3 per cent, potassium dichromate solution, and a 2 per cent solution of osmium tetroxide.[3] Fixation was performed either by adding one part of the fixative solution to nine parts of the mitochondria suspension, or else by providing an excess of reagent, mixing one part of the mitochondria preparation to nine parts of the fixative solution. In some cases, Altmann's or Regaud's fluid, previously neutralized, was used as fixative. When dilution of the stock solution was resorted to, a neutral 4 per cent formaldehyde solution was used for formalin or dichromate fixed-material, and distilled water for osmium tetroxide-fixed preparations.

Neutral solutions of formaldehyde, when used as fixative, were found to result in an acid reaction when added to the mitochondria. This effect can be explained by the well known capacity of formaldehyde to unite with the amino groups of proteins (Sörensen reaction) with the concomitant release of acidic groups. Such an acid reaction is not desirable since, as al-

[1] Occasionally, a salt solution brought to pH 9.5 by the addition of NaOH was used in place of the buffered solution. As a rule, the reaction of material prepared with such alkaline solution is approximately neutral. As pointed out in other papers (14, 15), the various cytoplasmic elements of the cell will clump together if the medium is allowed to become even slightly acid, and thereafter cannot be properly separated. Unfractionated tissue extracts tend to become progressively acid and enough alkalinity should be provided to insure a neutral or slightly alkaline reaction.

[2] The centrifuge used throughout was the type SB, size 1, manufactured by the International Equipment Company, Boston, Massachusetts. The preliminary centrifugation (1500 × *g*) was done by means of the horizontal yoke No. 233 or 242; purification of the mitochondria was carried out in the conical head No. 823 (2400 × *g*) or with the use of the multispeed attachment and No. 295 head (18000 × *g*).

[3] The pH of the solutions was determined by means of a glass electrode.

ready mentioned, it would result in excessive clumping of the mitochondria. This was avoided by determining empirically, in each case, the amount of acid produced by the formaldehyde and adding beforehand enough NaOH to the fixative to insure a neutral reaction of the mixture.

When the reaction of the medium was kept neutral, mitochondria suspended in formaldehyde or in dichromate solutions remained fairly well dispersed, the rate of precipitation observed on standing being about equal to that produced in neutral saline, control suspensions, under the action of gravity. Osmium tetroxide fixation caused a certain amount of clumping, even in neutral or slightly alkaline solutions.

Preparation of Mounts for Electron Microscopy.—The instrument used in the present work was the electron microscope, Model B, manufactured by Radio Corporation of America, Camden, New Jersey.

The supporting membrane for the specimen was a "formvar" film[4] held on a 200 mesh screen of stainless steel. Preparation of the mounts for microscopic examination was as follows: A drop of the mitochondria suspension, appropriately diluted by means of neutral, 4 per cent formaldehyde, 0.85 per cent NaCl, or distilled water, was placed on the screen and allowed to remain in contact with the film for about 30 seconds. The material was then withdrawn as completely as possible and the preparation permitted to dry. When washing was resorted to, a drop of distilled water was placed on the screen immediately after removal of the mitochondria suspension and prior to drying, left on the film for a few seconds and withdrawn. This washing procedure was done once, or repeated several times in succession. Rinsing once or twice in this manner was usually sufficient to dissolve away the crystalline material present in the suspension, leaving insoluble mitochondrial bodies adherent to the formvar film.

An alternate method used in washing the preparation was to rest the screen on a gauze pad during the procedure, and increase the size of the drop of water until it touched the gauze at the periphery causing then a sudden and rather violent suction of the water into the mesh of the fabric. This washing of the preparation by flushing was found simple and effective.

Potassium dichromate is considered a choice fixative for mitochondria, and the fixing fluids habitually recommended contain this reagent (1, 9). Mitochondria kept in potassium dichromate solution were found to be fairly well preserved, as judged by their appearance under the light microscope. However, the elements were found to disintegrate on the film when an attempt was made to remove the excess of reagent by means of distilled water (Fig. 19). Drying the mount before rinsing resulted in a better preservation of the material but heavy deposition of dichromate crystals prevented a study of mitochondria fixed with this reagent (Fig. 18). These observations fall in with the belief that potassium dichromate is a fixative of the lipid portion of the mitochondria, not of the water-soluble elements (13).

Difficulties were encountered in the early experiments in that ring-shaped artifacts appeared on the film (Fig. 13). This alteration was avoided by allowing more time for the specimen to dry: in routine experiments, complete dehydration was insured by keeping the mounts 1 to several days in a desiccator, over P_2O_5, prior to examination in the electron microscope.[5]

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[4] "Formvar" (grade No. 15-95) is the trade name for a polyvinyl formal plastic manufactured by the Shawinigan Products Corp., New York.

[5] The relatively large amount of lipids that mitochondria contain (15) may interfere with rapid transfer of water, and retard complete desiccation of the mitochondrial body: expansion of residual moisture when the material is placed in the vacuum of the electron microscope, and circular displacement of the mitochondrial substance, may explain the formation of ring-shaped artifacts that were observed with incompletely dried specimens (Fig. 13). It is probable that the "bizarre structures" noted by Green, Anderson, and Smadel in electron micrographs of sedimentable material derived from rabbit kidney were altered remnants of mitochondria (23).

OBSERVATIONS

Size and Shape.—Present findings by means of electron microscopy are in general agreement with previous light microscope observations which indicate that mitochondria of the rat lymphosarcoma, in purified preparations, are spherical bodies varying in size roughly from 0.5 to 1.5 μ in diameter (15). Measurements made on the various electron micrographs available from 22 experiments showed that the majority of the mitochondrial elements present ranged in size from 0.6 to 1.3 μ in diameter; a number of elements encountered were as small as 0.3 μ and as large as 1.8 μ in diameter; large bodies measuring as much as 2.5 μ in diameter were occasionally seen. The relative size of mitochondria is illustrated in the various electron micrographs presented in Figs. 1 to 21. Fig. 1 represents a mitochondrion exactly 1 μ in diameter. Figs. 2 and 3 illustrate the size range in which mitochondria are most frequently found.

When viewed in fluid media, and under a light microscope, mitochondria[6] appear to have a perfect spherical shape. In electron microscope preparations, however, the mitochondria rest on a solid plane and flatten out over the supporting film, so that their apparent size is increased, and their thickness is appreciably decreased. In the case of Fig. 4 the film supporting a heavy preparation of mitochondria ruptured and folded back affording, in the new position, a side view of the elements. The profile of the mitochondria shown at the extreme left and right of the micrograph is not semicircular but is spread out at the base; the thickness at the center appears to be less than one-third the apparent diameter of the granules. It appears, therefore, that the area occupied by a mitochondrion, as seen in the electron microscope, is not strictly an indication of absolute size but is affected by the degree of flattening of the granule on the supporting film.

The volume of mitochondria may be considerably affected by the nature and the concentration of the solution in which they are suspended. They swell in hypotonic salt solutions and disintegrate completely in distilled water (14, 15). They appear smaller when suspended in hypertonic salt solutions. In their response towards salt concentration mitochondria behave as though they were invested with a semipermeable membrane. Although isolated mitochondria retain their general shape and dimension, size variations encountered in the electron microscope appear to be more pronounced than in the living cell, indicating that even the isotonic salt solution employed is not without effect.

Examination of mitochondrial preparations in the electron microscope indicated that, within certain limits, the apparent density of the granules is inversely proportional to their diameter. This is visible in Fig. 11 and, to a

[6] In this paper, the term mitochondria refers to "mitochondria derived from rat lymphosarcoma."

certain extent, in Fig. 12. The point is likewise illustrated by a comparison of Figs. 14 to 17 in which the density of the preparation appears to diminish as the diameter of the mitochondria increases. Some of these elements were sufficiently flattened to permit a differential transmission of the electrons which revealed certain details of structure.

Constitution.—In general, mitochondria appear in the electron microscope as relatively opaque bodies, with no apparent differentiated structure (Figs. 1 and 2). When in process of disintegration, amorphous substance can be seen streaming out at the periphery (Fig. 3).

Mitochondria appear to be capable of losing part of their substance while retaining their shape. Fig. 11 represents mitochondria that were mounted on formvar film soon after formaldehyde fixation. The elements appear uniformly opaque and the background is relatively free of debris. Fig. 12 represents mitochondria similarly fixed in formaldehyde solution but left in the fixative, at ice box temperature, for 5 months before mounting on the formvar film. The shape of the mitochondria in this preparation is well preserved and has remained regularly circular. Loss of substance is indicated by lessened density, uneven distribution of substance within the mitochondria, and the presence between the elements of a considerable amount of debris, some of it finely granular.

The formation that appears in Fig. 7 was produced by the juxtaposition of improperly fixed mitochondria agglutinated and so massed as to simulate a honeycomb structure. The outline of each element is preserved but that most of the substance normally present has been removed is indicated by the very low absorption exhibited by the mitochondrial body. Some absorbing material, probably of mitochondrial origin, has accumulated between the mitochondria. In connection with the latter observations it may be noted that the structural arrangement presented by mitochondria in Fig. 7 resembles artifacts frequently seen in the protoplasm of fixed cells. It is the appearance of structures of this sort in microscopic preparations which has contributed to the opinion that protoplasm is essentially an emulsion, or is formed of an accumulation of globules, and thus has led to the formulation of the alveolar or foam theory of protoplasm (24).

Limiting Membrane.—The existence of a differentiated membrane limiting the mitochondrial bodies could be postulated on the basis of their behavior as osmotic systems, the presence of a permanent, well defined, boundary, and the persistence of these elements as individual units through the process of centrifugation and suspension in artificial media. Examination of numerous mitochondria preparations in the electron microscope, and inspection of electron micrographs, have failed to detect the presence of a differentiated membrane in well preserved specimens (Figs. 1, 2, and 6). If such a membrane exists we must assume it to be extremely thin or the atomic density, reflecting

the power to scatter electrons, must be very similar both at the boundary and in the interior of the mitochondria.

The mitochondrion shown in Fig. 5 presents an opaque mass bordered at one side by a semitransparent margin. This area of less density may correspond to the empty portion of a differentiated membrane, which separated from the mitochondrial body at the time of drying. That this is the case cannot be decided, however, since a similar appearance could conceivably be caused by the persistence of a circular imprint of mitochondrial substance, left on the film after lateral displacement of the mitochondrial body.

In some cases, loss of substance seems to release the tension which keeps the mitochondrial body spherical and, in such circumstances, the impression is gained that mitochondria possess a differentiated covering which may become more or less completely separated from the mitochondrial mass. The formation found in Fig. 9 appears as a collapsed membrane, or sac, with two lateral folds at the left, and wrinkles across the upper surface. The scattering material at the lower right corner of the picture may represent some substance extruded from the mitochondrial cavity. The mitochondrion represented in Fig. 10 appears to be partially collapsed and folded once, the doubling over of the "membrane" producing an area of greater density and an angular outline at the extremities of the fold. The mitochondrion in Fig. 8 is crumpled, possibly with a major fold across the middle. The arrangement presented by mitochondria in Fig. 7 could be explained by assuming that these elements are enclosed in a continuous membrane, semirigid when fixed, and capable of retaining its original shape, even when most of the scattering mitochondrial substance has been removed, leaving then a clear area on the micrograph.

Mitochondria show no special tendency to coalesce or to fuse, even when brought into intimate contact by high speed centrifugation or by prolonged centrifugation at moderate speed. Bridges of substance between mitochondria, to be seen in Figs. 6 and 21 were produced during desiccation by the adherence to adjacent mitochondria or to the supporting film with incomplete retraction of the mitochondrial body.[7] In some cases mitochondria may be fragmented yet retain their general outline, indicating that, in fixed conditions, mitochondria may behave as a solid mass. Fig. 14 shows a broken mitochondrion with the displaced fragment lying at some distance from the gap.

Inner Structure.—As a rule, the body of the mitochondrion is uniformly absorbing and inner structures cannot be discerned by electron microscopy. Occasionally, however, it is possible to detect within the mitochondria small elements of greater density, usually of uniform size. These elements can be

[7] The elements which appear in Fig. 6 are, at the left, two mitochondria, at the right, a Golgi body. Observations on these bodies will be found in a subsequent paper. They can be differentiated by a relatively greater density with respect to the electron beam, and by a characteristic angular shape contrasting with the uniformly circular shape of the mitochondria.

seen in Fig. 15, where they are regularly arranged within the mitochondria. Similar elements appear also in Figs. 16 and 17. The dimension of the small particles visible in the three pictures just mentioned is approximately 80 to 100 mμ in diameter. Close inspection of Fig. 12, with the aid of a magnifying glass, will reveal the presence of small particles of this size within and outside the mitochondrial bodies. Detection of small elements 80 to 100 mμ in mitochondria is of particular interest in view of the findings, previously reported (15), that small particles, comprising this range of sizes can be obtained from mitochondria after the latter have been caused to disintegrate in distilled water.

DISCUSSION

In the present work mitochondria were separated from the cell, suspended in a salt solution, and subjected to fixation and drying prior to examination in the electron microscope. The effect that this treatment may have on the morphology of mitochondria cannot be estimated until an analysis of the various factors involved can be made, or until mitochondria can be examined directly in their normal environment; *i.e.*, the intact cytoplasm.

In evaluating the results of these studies it must be kept in mind that the mitochondria were derived from leukemic cells and some of the features encountered may therefore be the reflection or the outcome of the malignant condition. Furthermore, the method adopted involved an arbitrary selection of mitochondria of a certain size range since extremely small and unusually large elements, if present, were systematically excluded by the method of purification in the centrifuge. For this reason, the problem of the possible derivation of mitochondria by a process of progressive evolution from submicroscopic elements cannot be considered in this phase of the investigation.

The fact that mitochondria retain their individuality when removed from their normal, cytoplasmic, environment and that they persist as discrete bodies in aqueous media as long as the presence of some salt insures the proper tonicity of the solutions would seem to disprove the assumption that mitochondria may be formed by the concretion of substances preexisting in the cytoplasm or that they may disappear and reappear in living cells because of changes of equilibrium occurring in the surrounding protoplasm (18).

No appreciable affinity seems to exist between the outer surface of the mitochondrial elements as shown by the fact that they fail to coalesce or fuse together when forced into close contact by high speed centrifugation. Some evidence presented in this paper would indicate that the mitochondrial mass is surrounded by a differentiated membrane, the presence of which may be responsible for the persistence of these elements as individual units. The existence of a membrane with selective and semipermeable properties would explain the fact that mitochondria respond by variations in volume to changes in the tonicity of the medium.

It has been shown by previous work (15) that mitochondria will disintegrate completely when suspended in distilled water, yielding then minute elements indistinguishable thus far from ordinary microsomes. It must be assumed either that these small particles derive from a fragmentation of the mitochondrial substance, or that they are preformed in the mitochondria and are released under the action of distilled water. In this respect it is interesting that the electron microscope has revealed the presence of small granules, 80 to 100 mμ in diameter, within certain mitochondria of the leukemic cells. Further work will be needed to determine whether this type of granule is of exceptional occurrence, or is a normal constituent of mitochondria. In the latter case a study of the relation between these intramitochondrial granules and the ordinary microsomes will be indicated.

SUMMARY

1. The present paper constitutes a preliminary study of the morphology of mitochondria by means of electron microscopy.

2. The mitochondria that were the subject of this investigation were obtained from a lymphosarcoma of the rat. They were separated from the other components of the leukemic cells by a method of differential centrifugation, and thus made available for direct examination in the electron microscope.

3. In the purified form the mitochondria appeared as spherical bodies, the majority of them varying in size approximately from 0.6 to 1.3 μ in diameter.

4. Certain aspects of mitochondria in the electron microscope suggest that these elements are surrounded by a differentiated membrane. In some cases the limiting membrane seemed to be responsible for maintaining the general shape of the mitochondria, even when most of the mitochondrial substance had been lost.

5. By means of the electron microscope, it is possible to distinguish small elements, 80 to 100 mμ in diameter, within the body of certain mitochondria. Further work is suggested to establish whether these small granules are normal constituents of mitochondria, and what relation may exist between them and ordinary microsomes.

6. The nature of mitochondria as morphological units is discussed. Present evidence indicates that mitochondria constitute definite physical entities which can persist in the absence of the cytoplasm.

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PLATES

EXPLANATION OF PLATES

Except for Fig. 13, all illustrations were prepared with material obtained from rat lymphosarcoma.

PLATE 4

FIG. 1. Mitochondrion, exactly 1 μ in diameter. Element completely opaque to electrons, except for some diffuse scattering at the rim. No internal structure or differentiated membrane visible. Extraction and two washings carried out with alkaline saline;[1] fixation in 4 per cent formaldehyde, 8 days at 4°C.; mount on formvar film, 28 days of desiccation over P_2O_5. Magnification, 4750, enlarged to 35,000.

FIG. 2. Mitochondria, 0.6 and 1.1 μ in diameter, respectively. No differentiated structure indicated. Smooth outline suggests that the elements are relatively well preserved; material and mounts as in Fig. 1. Magnification, 4750, enlarged to 15,000.

FIG. 3. Mitochondria, 0.7 and 1.2 μ in diameter, respectively. That these elements are disintegrating is indicated by "streaming out" of substance at the periphery, but some of the processes seen may have resulted from adherence of the mitochondria to the supporting film, and subsequent contraction of the mass during desiccation. Extraction in buffered saline, pH 7.4; material: unfractionated extract; fixation: extract mixed with equal volume of 4 per cent formaldehyde—0.01M phosphate buffer, at pH 7.4; dilutions made with 1 per cent formaldehyde solution at pH 7.0. Magnification, 4750, enlarged to 15,000.

FIG. 4. The formvar film supporting the mitochondria preparation ruptured and curled during examination in the electron microscope, bringing the elements on the film at right angles with the electron beam. Parabolic profiles of mitochondria at extreme left and right of the photograph measure 0.65 μ at the base. Material: purified preparation of mitochondria fixed in 4 per cent formaldehyde; drying in desiccator over P_2O_5. Magnification, 4750, enlarged to 20,000.

FIG. 5. Mitochondrion, 1.2 μ in diameter. It has been displaced sideward, possibly at the time of drying. The circular zone of lesser density at the left either represents the emptied fold of a membrane, or corresponds to an imprint on the film left by the displaced mitochondrion. Material: mitochondria washed three times in alkaline saline; fixed in 4 per cent formaldehyde, pH 7.2, 24 hours; 15 days in desiccator. Magnification, 9300, enlarged to 30,000.

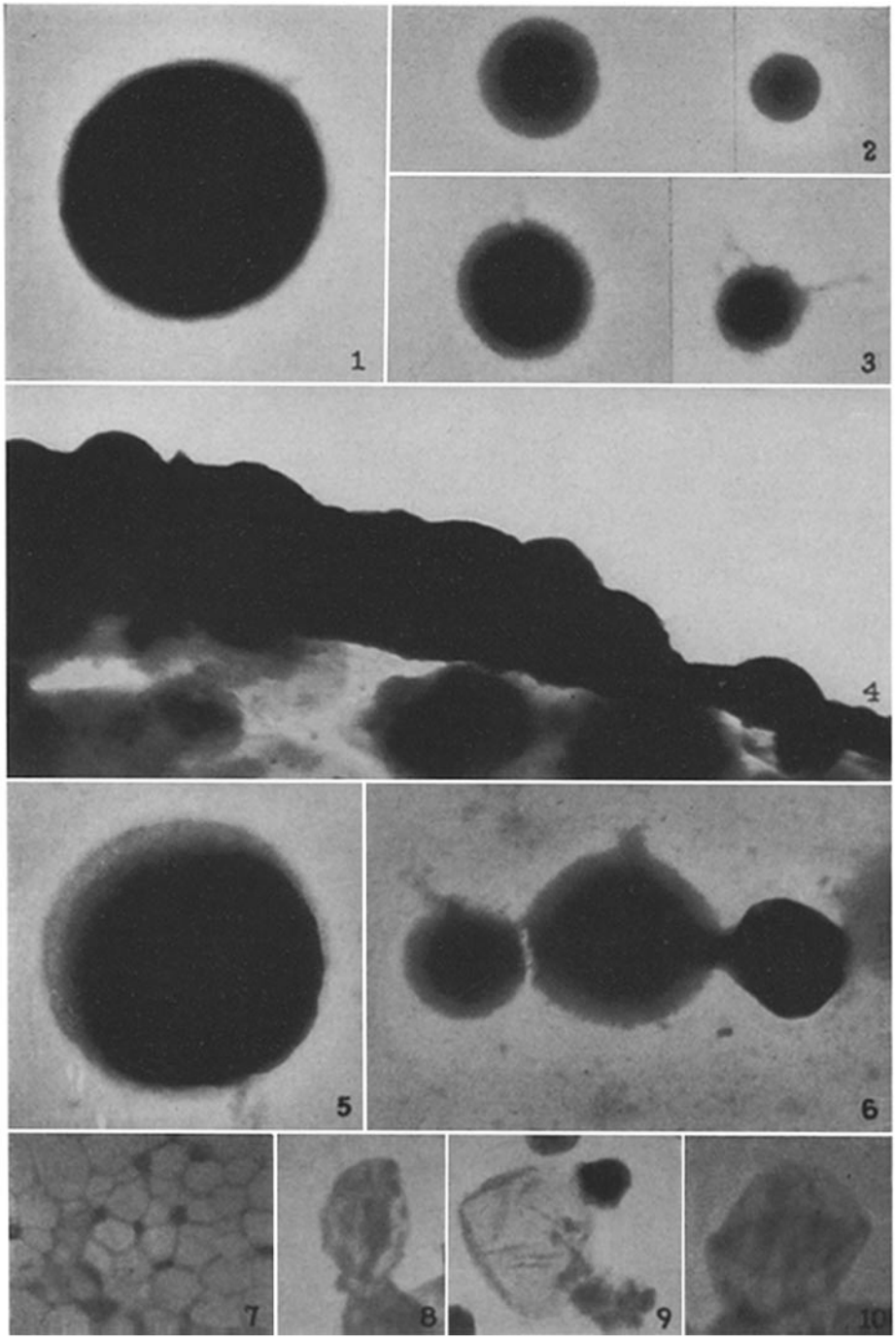
FIG. 6. Left: mitochondrion, 1.0 μ in diameter. Center: mitochondrion, 1.8 x 1.6 μ. Right: Golgi body. Mitochondria are circular, and of moderate density. Space between the two elements was probably produced by retraction during desiccation. The Golgi body is opaque, and angular in shape.[7] Material: whole extract, fixed in osmium tetroxide; mounts flushed with water; dried in desiccator over P_2O_5. Magnification, 3900, enlarged to 15,000

FIG. 7. Honeycomb formation produced by juxtaposed mitochondria. The substance is rarefied within the elements as shown by considerable transparency to electrons, but material has accumulated at the interface between the mitochondria. Size of elements, 0.5 to 2.2 μ in diameter. Material: mitochondria extracted, and washed twice, in alkaline saline. Fixation in 6 per cent formaldehyde, pH 9.2; reaction of mixture, pH 7.0; dilution in 6 per cent formalin, pH 8.0; mount on formvar film 4 days in desiccator over P_2O_5. Magnification 4100.

FIG. 8. Irregularly shaped mitochondrion, 1.2 x 1.6 μ. Low scattering power suggests that the element is flattened out or that some of its substance has been lost. The appearance suggests the presence of a membrane, partly collapsed and folded across the middle. The absorbing material is granular. Mitochondria extracted and washed in alkaline saline; fixed in 4 per cent neutral formaldehyde; mounts in desiccator, P_2O_5. Magnification, 2550, enlarged to 10,000.

FIG. 9. The formation looks like an empty sac, completely collapsed, with lateral folds on two sides, and minor folds across the upper surface. The position of the absorbing material at the lower, right corner of the picture suggests that it might have been extruded from the body of the mitochondrion. Dimension of the sac-like structure: 2.0 μ in diameter. Material: mitochondria separated and washed twice in buffered saline, pH 7.2; fixed 48 hours in Altmann's fluid; mount rinsed with neutral distilled water, 24 hours in desiccator over P_2O_5. Magnification, 4100, enlarged to 10,000.

FIG. 10. Mitochondrion appears to be folded over itself, indicating that internal tension is absent. Mitochondria extracted and washed in alkaline saline; fixed in neutral 4 per cent formaldehyde; screen mount desiccated over P_2O_5. Magnification, 2550, enlarged to 15,000.



(Claude and Fullam: Electron microscope study of isolated mitochondria)

PLATE 5

FIG. 11. Group of mitochondria, 0.4 to 1.0 μ in diameter: one exceptionally large body measures 2.0 μ on diameter.

The electron micrograph illustrates extreme variations in sizes. The appearance is more or less typical for formaldehyde-fixed mitochondria. See also Fig. 21. Magnification, 5000.

FIG. 12. Mitochondria extracted and washed three times in alkaline saline; fixed in 4 per cent formaldehyde (final reaction, pH 7.0); suspension in fixative stored 5 months at 4°C., before mounting on formvar film; screen mount rinsed with neutral distilled water, then kept in desiccator over P_2O_5 for 10 days.

The electron micrograph shows elements ranging in size from 0.4 to 1.6 μ in diameter and regularly circular in shape. As a rule, the small elements are denser than the larger ones. Details of structure suggest the presence of folds on the surface. Small granules, approximately 0.1 μ in diameter can be seen outside and within the mitochondria. Debris, some of it finely granular, is abundant throughout the preparation. General appearance of the preparation suggests considerable loss of substance, and extreme flattening of the mitochondria. Magnification, 5000.

FIG. 13. Microsomes (14, 15) from guinea pig liver, extracted and washed in buffered, 0.85 per cent NaCl solution, pH 7.2; mounted on formvar film, dried in air, and examined immediately in the electron microscope. The micrographs illustrate the production of ring-shaped artifacts when incompletely dried preparations are used. Magnification 35,000.

FIG. 14. Torn mitochrondrion, with slightly displaced fragment in front of the gap. Disposition indicates that, after fixation, the substance of a mitochondrion behaves as a solid mass. Mitochondria separated by centrifugation of osmic acid-treated tissue extract; sediment washed in neutral saline; mitochondria fraction suspended in distilled water, pH 7.0; screen mounts desiccated 3 days over P_2O_5. Magnification, 4100, enlarged to 12,000.

FIG. 15. Mitochondria showing differentiated internal structures consisting in small granules of uniform size, approximately 0.1 μ in diameter. (Small granules of that size appear also in Figs. 12, 16, and 17.) Mitochondria were extracted and washed in alkaline saline, fixed in 4 per cent formaldehyde pH 7.0; screen mounts dried over P_2O_5. Magnification, 2550, enlarged to 12,000.

FIG. 16. Mitochondrion beginning to disintegrate and showing denser structures in the form of small granules, about 0.1 μ in diameter. Extraction and washings in buffered saline, pH 7.2; fixation in Altmann's fluid; screen mount rinsed with neutral distilled water. Magnification, 4100, enlarged to 12,000.

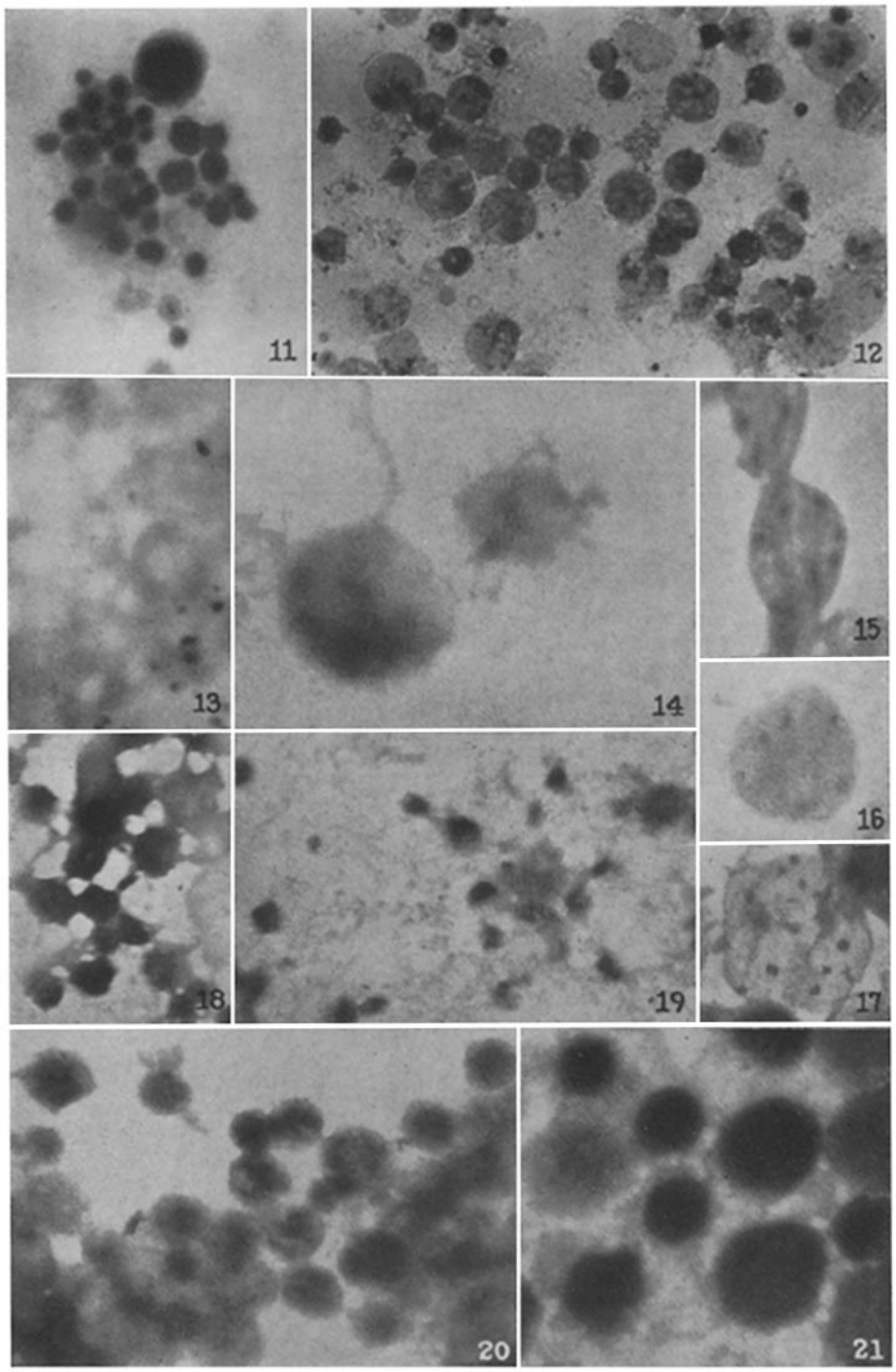
FIG. 17. Thinly extended mitochondrion, with sharp contour preserved. Discrete granules, about 0.1 μ in diameter, are visible. Purified mitochondria were fixed 5 days in 2 per cent osmium tetroxide, sedimented once, and resuspended in distilled water, pH 7.0; screen mounts were not rinsed; drying done for 24 hours over P_2O_5. Magnification, 4100, enlarged to 12,000.

FIG. 18. Mitochrondria separated and washed in buffered saline, pH 7.2; fixed 3 days in 3 per cent potassium dichromate, pH 6.4. The suspension was placed on the formvar film for 30 seconds, withdrawn, and the preparation allowed to dry in air. The film was then flushed with neutral distilled water and the mount kept 24 hours over P_2O_5. The clear, angular areas indicate the spaces occupied by potassium dichromate crystals subsequently removed from the dried preparation by washing. The mitochondrial substance is fairly well preserved, but crystallization of the fixative has altered the shape of the elements. Magnification, 4100, enlarged to 10,000.

FIG. 19. Material and preparation as for Fig. 18 except that flushing of the film with distilled water was done before the material was permitted to dry. Mitochondria appear considerably damaged, with the dispersion of debris, partly finely granular. Differences between Figs. 18 and 19 suggest that drying may be a factor in fixing mitochrondrial material. Magnification, 4500, enlarged to 10,000.

FIG. 20. Mitochondria separated and washed in buffered saline, pH 7.2. Fixed in insufficient amount of osmium tetroxide (final concentration, 0.2 per cent); fixed elements washed twice in neutral distilled water; mount 24 hours in desiccator, over P_2O_5. Size of mitochondria in preparation: 0.5 to 0.9 μ in diameter. Magnification 4100, enlarged to 10,000.

FIG. 21. Mitochondria extracted, and washed twice in alkaline saline; fixed 2 hours in 4 per cent formaldehyde pH 9.3 (reaction of mixture, pH 6.5); preparation on formvar film rinsed three times with neutral water; mount 26 days in desiccator, over P_2O_5. Size of elements in preparation about 0.6 to 1.0 μ in diameter. Clear spaces and connecting strands between mitochondria probably produced by retraction of substance during desiccation. Magnification, 5000, enlarged to 15,000.



(Claude and Fullam: Electron microscope study of isolated mitochondria)