THE CELL

FAWCETT

Chapter 7:
Mitochondria

Second Edition

THE CELL

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PREFACE

The history of morphological science is in large measure a chronicle of the discovery of new preparative techniques and the development of more powerful optical instruments. In the middle of the 19th century, improvements in the correction of lenses for the light microscope and the introduction of aniline dyes for selective staining of tissue components ushered in a period of rapid discovery that laid the foundations of modern histology and histopathology. The decade around the turn of this century was a golden period in the history of microscopic anatomy, with the leading laboratories using a great variety of fixatives and combinations of dyes to produce histological preparations of exceptional quality. The literature of that period abounds in classical descriptions of tissue structure illustrated by exquisite lithographs. In the decades that followed, the tempo of discovery with the light microscope slackened; interest in innovation in microtechnique declined, and specimen preparation narrowed to a monotonous routine of paraffin sections stained with hematoxylin and eosin.

In the middle of the 20th century, the introduction of the electron microscope suddenly provided access to a vast area of biological structure that had previously been beyond the reach of the compound microscope. Entirely new methods of specimen preparation were required to exploit the resolving power of this new instrument. Once again improvement of fixation, staining, and microtomy commanded the attention of the leading laboratories. Study of the substructure of cells was eagerly pursued with the same excitement and anticipation that attend the geographical exploration of a new continent. Every organ examined yielded a rich reward of new structural information. Unfamiliar cell organelles and inclusions and new macromolecular components of protoplasm were rapidly described and their function almost as quickly established. This bountiful harvest of new structural information brought about an unprecedented convergence of the interests of morphologists, physiologists, and biochemists; this convergence has culminated in the unified new field of science called cell biology.

The first edition of this book (1966) appeared in a period of generous support of science, when scores of laboratories were acquiring electron microscopes and hundreds of investigators were eagerly turning to this instrument to extend their research to the subcellular level. At that time, an extensive text in this rapidly advancing field would have been premature, but there did seem to be a need for an atlas of the ultrastructure of cells to establish acceptable technical standards of electron microscopy and to define and illustrate the cell organelles in a manner that would help novices in the field to interpret their own micrographs. There is reason to believe that the first edition of *The Cell: An Atlas of Fine Structure* fulfilled this limited objective.

In the 14 years since its publication, dramatic progress has been made in both the morphological and functional aspects of cell biology. The scanning electron microscope and the freeze-fracturing technique have been added to the armamentarium of the miscroscopist, and it seems timely to update the book to incorporate examples of the application of these newer methods, and to correct earlier interpretations that have not withstood the test of time. The text has been completely rewritten and considerably expanded. Drawings and diagrams have been added as text figures. A few of the original transmission electron micrographs to which I have a sentimental attachment have been retained, but the great majority of the micrographs in this edition are new. These changes have inevitably added considerably to the length of the book and therefore to its price, but I hope these will be offset to some extent by its greater informational content.

Twenty years ago, the electron microscope was a solo instrument played by a few virtuosos. Now it is but one among many valuable research tools, and it is most profit-

ably used in combination with biochemical, biophysical, and immunocytochemical techniques. Its use has become routine and one begins to detect a decline in the number and quality of published micrographs as other analytical methods increasingly capture the interest of investigators. Although purely descriptive electron microscopic studies now yield diminishing returns, a detailed knowledge of the structural organization of cells continues to be an indispensable foundation for research on cell biology. In undertaking this second edition I have been motivated by a desire to assemble and make easily accessible to students and teachers some of the best of the many informative and aesthetically pleasing transmission and scanning electron micrographs that form the basis of our present understanding of cell structure.

The historical approach employed in the text may not be welcomed by all. In the competitive arena of biological research today investigators tend to be interested only in the current state of knowledge and care little about the steps by which we have arrived at our present position. But to those of us who for the past 25 years have been privileged to participate in one of the most exciting and fruitful periods in the long history of morphology, the young seem to be entering the theater in the middle of an absorbing motion picture without knowing what has gone before. Therefore, in the introduction to each organelle, I have tried to identify, in temporal sequence, a few of the major contributors to our present understanding of its structure and function. In venturing to do this I am cognizant of the hazards inherent in making judgments of priority and significance while many of the dramatis personae are still living. My apologies to any who may feel that their work has not received appropriate recognition.

It is my hope that for students and young investigators entering the field, this book will provide a useful introduction to the architecture of cells and for teachers of cell biology a guide to the literature and a convenient source of illustrative material. The sectional bibliographies include references to many reviews and research papers that are not cited in the text. It is believed that these will prove useful to those readers who wish to go into the subject more deeply.

The omission of magnifications for each of the micrographs will no doubt draw some criticism. Their inclusion was impractical since the original negatives often remained in the hands of the contributing microscopists and micrographs submitted were cropped or copies enlarged to achieve pleasing composition and to focus the reader's attention upon the particular organelle under discussion. Absence was considered preferable to inaccuracy in stated magnification. The majority of readers, I believe, will be interested in form rather than measurement and will not miss this datum.

CONTENTS

MITOCHONDRIA

Mitochondria are ubiquitous organelles found in nearly all eukaryotic cells. Their major function is to provide the chemical energy necessary for the biosynthetic and motor activities of the cell. This is accomplished by a complex series of integrated chemical reactions. Carbohydrates, fatty acids, and amino acids from food are oxidized in the mitochondria to carbon dioxide and water and the free energy released is used to convert adenosine diphosphate (ADP) and inorganic phosphate to adenosine triphosphate (ATP) — a remarkable molecule responsible for most of the energy transfer involved in living processes. ATP is exported from the mitochondria into the surrounding cytoplasm. Wherever the energy stored in this molecule is required to drive synthetic or contractile processes, it is made available by splitting ATP to ADP and phosphate. These are then taken up again by the mitochondria, where ATP is again generated. To carry out this most important activity and a number of secondary functions, mitochondria contain 50 or more enzymes strategically located and precisely ordered in the various structural components of the organelle which are described in this chapter.

The assignment of priority for the observation of mitochondria is not possible. Many cytologists in the late 1800s observed granules, rodlets, and filaments in the cytoplasm, and surely some of these were mitochondria. Flemming was certainly among the first to recognize them as distinct cell organelles. He described granules and thread-like structures, which he called *fila*, in many cell types and devoted special attention to those of muscle. Indeed, in 1888 he teased some of these out of insect muscle, where they are unusually large, and observed that they swelled in water. He inferred from this that they were limited by a membrane — a suggestion that was not validated until some 75 years later.

Systematic study of this organelle did not begin until relatively selective staining methods were developed using acid fuchsin (Altmann, 1890) or crystal violet (Benda, 1898). Their form, relative abundance, and distribution were then described for many cell types. The term mitochondrion, from Greek roots meaning "thread" and "grain," was introduced by Benda but was not widely accepted at the time. A profusion of other terms appeared, of which the most enduring was chondriosome. This term persisted until very recent times, especially in the European literature, but it is now less commonly used.

Altmann looked upon the mitochondria as elementary organisms, or "bioblasts," and considered them the essential living units of protoplasm from which all other parts of the cell were derived. This extreme view was not widely shared. However, other leading cytologists of the time — including Benda (1903), Meves (1907), and Duesberg (1907) — noted that mitochondria increase before cell division, are distributed equally to the daughter cells, and are transferred by the sperm to the egg at fertilization. Their passage from cell to daughter cells and from generation to generation suggested to these authors that they might play a role in inheritance. Although erroneous, this conclusion does not seem unreasonable when we recall that the chromosomal theory of inheritance was originally based upon similar considerations of organelle continuity.

The literature of the early part of this century abounds with claims that mitochondria gave rise during differentiation to myofibrils, yolk, pigment, secretory granules, and many other cell components. These interpretations were based entirely upon circumstantial evidence such as the proximity of mitochondria to sites of formation of these structures and identification of elements which appear to be intermediate stages in transformation of mitochondria.

The development of methods for growing cells in vitro provided a new approach to the study of this organelle. Cells flattened against the glass substrate of the culture vessel offered ideal conditions for observation on the form and behavior of mitochondria in the living state (Lewis and Lewis, 1915–1920; Strangeways, 1924). They usually appeared as short rods or slender filaments but were capable of changing their shape under altered environmental conditions. Direct observations and lapse-time cinematography showed that they are constantly executing slow flexuous movements as they move about in the cytoplasm. It could not be ascertained whether they possessed an intrinsic capacity for locomotion or were passively transported by streaming of the cytoplasm. As mitochondria move, they usually maintain the shape characteristic for that cell type, but filamentous forms may be observed to branch or to break up into short rods. Such observations on living cells established that mitochondria are dynamic organelles displaying considerable plasticity and responding to changing physiological and environmental conditions.

Study of mitochondria and speculation as to their probable function were almost exclusively the prerogative of the morphologist, but there were two biochemical observations which later proved to be significant. Michaelis (1898) demonstrated that mitochondria of living cells reduced Janus green, an oxidation-reduction indicator dye, and Warburg (1913) showed that the capacity to consume oxygen resided in particulate components of the cell. The majority of biochemists that followed had little interest in the cell organelles, and they tended to regard particulate components of cells as an annoyance and obstacle to their efforts to isolate respiratory and other enzymes. The concept had evolved that oxygen is involved in cell metabolism through the action of an iron-containing catalyst, later called cytochrome (Warburg, 1925). The pyridine nucleotides were isolated and their structure determined and the principle was developed that oxidations in cell respiration take place by removal of hydrogen from the substrate. In the 1930s flavoproteins were found to be the link between dehydrogenases and the cytochromes in the flow of hydrogen in cell respiration.

A number of the dehydrogenation reactions by which oxidation of foodstuffs is accomplished were worked out in the years that followed. It was proposed that oxidation of carbohydrates took place in a cycle of reactions in which short carbon chains are continuously broken down to yield CO_2 — the Krebs cycle. It was also discovered that the oxidation of glyceraldehyde phosphate was somehow coupled to the synthesis of ATP (Warburg, 1938) and that generation of additional ATP is linked to the respiratory sequence of reactions that reduce oxygen to water. It became apparent that the coupling of ATP synthesis to respiration, called oxidative phosphorylation, is the principal source of ATP in the cell.

The cytologists Bensley and Hoerr (1934) first attempted to isolate mitochondria by differential centrifugation from homogenates of liver. This initial effort met with little success. However, when this approach was taken up again nearly a decade later (Claude, 1946), it was found that when 0.88 M sucrose was used as the medium, mitochondria could be successfully isolated. They retained near normal form and stained supravitally with Janus green (Hogeboom, Schneider and Palade, 1948). This extraordinarily important technical advance led to rapid expansion of knowledge on the function of mitochondria and other subcellular organelles. Mitochondria were found to contain nearly all of the cytochrome oxidase (Hogeboom et al., 1948) and fatty acid oxidase (Kennedy and Lehninger, 1948) of the homogenate from which they were isolated. They could carry out in vitro the oxidation of all the tricarboxylic acid cycle intermediates and generate ATP by oxidative phosphorylation (Kennedy and Lehninger, 1949). Thereafter mitochondria were regarded as the principal site for generation of energy for cellular activities.

The advent of the electron microscope and methods for preparation of ultrathin tissue sections permitted, for the first time, an examination of the interior of these cell organelles. Their typical internal structure was described independently by Palade (1953) and Sjostrand (1953). The mitochondrion was found to be limited by a continuous outer membrane and lined by an inner membrane that formed narrow folds, or *cristae*,

projecting inward. The interior of the organelle was occupied by a dense matrix that appeared homogenous except for occasional dense *matrix granules* 25 to 35 nm in diameter. Although subsequent examination of a great many cell types revealed considerable variation in size and shape of mitochondria and in the form and number of cristae, there was no significant departure from the basic structural plan described by Palade.

The fixatives routinely used for electron microscopy did not preserve all of their structural components. Examining fragments of unfixed mitochondria by the negative staining technique, Fernandez-Moran (1963) was able to visualize subunits of the inner membrane that had previously gone undetected. These were spherical particles 9 nm in diameter connected to the inner membrane by a slender stalk **3** to 4 nm wide and about 5 nm long. They were found only on the side of the membrane toward the matrix, and it was immediately suspected that they might consist of some of the membrane-associated mitochondrial enzymes. The subunits could be separated from the membranes of lysed mitochondria by sonication and isolated by centrifugation in a sucrose gradient. They did not contain cytochromes (Williams and Parsons, 1964), but they did catalyze oxidative phosphorylation and ATP hydrolysis (Racker et al., 1964). Moreover, when mitochondrial ATPase, purified by chemical means, was examined with the electron microscope in negatively stained preparations, it consisted of 9 nm particles'similar to the isolated *inner membrane subunits.* Thus the units projecting into the matrix from the inner membrane appear to contain the complement of enzymes responsible for most of the energy production in animal cells.

The energy-generating machinery of mitochondria is activated when the products of digestion of food are delivered to them in the form of pyruvate (from carbohydrate), amino acids (from protein), and fatty acids (from fats). These substrates are oxidized in the respiratory chain, a multienzyme system within the inner mitochondrial membrane. The members of this system are not randomly distributed in the membrane but are believed to be specifically arranged in assemblies that facilitate their functional interaction. These assemblies recur throughout the membrane (Lehninger, 1964). Their arrangement moves protons (H^+) vectorially from the matrix into the space between the inner and outer membranes, creating a concentration gradient and therefore a membrane potential. The ATPase associated with the inner membrane subunits then pumps protons back through the membrane into the matrix, resulting in a proton current that drives the formation of ATP (Mitchell, 1966). Thus the powerful combination of ultrastructural and biochemical analysis has given us considerable insight into the functions of this remarkably complex and precisely organized organelle.

An unexpected development in the 1960s was the discovery that mitochondria possess their own DNA and RNA and have the capacity to synthesize some of their own proteins. Early morphological studies on trypanosomes had identified a structure near the base of the flagellum called the *kinetoplast.* This stained with the Feulgen reaction and therefore seemed to contain DNA. When it became possible to examine this organelle with the electron microscope, it was found to have outer and inner membranes and a limited number of cristae and therefore had to be interpreted as a specialized mitochondrion. The dense mass of filaments in its matrix was further identified as DNA by the demonstration that it incorporated tritiated thymidine before cell division (Steinert, 1960). The kinetoplast was considered an exceptional case with little bearing upon mitochondria in general, until mitochondria and chloroplasts of certain unicellular algae were also found to contain a few 4 nm filaments (Ris, 1962). Simultaneously and independently, similar filaments were observed in pale areas of matrix in mitochondria of chick embryos and soon thereafter in many other cell types (Nass and Nass, 1962; Nass, Nass and Afzelius, 1963). In biochemical studies, amphibian eggs were shown to contain 300 to 500 times the amount of DNA present in other cells of the same organisms and this extra DNA was found in the mitochondrial fraction prepared from homogenates of eggs (Dawid, 1966).

When mitochondria were disrupted and spread upon a water surface, the liberated DNA appeared in electron micrographs as filaments about 4 nm in diameter, forming

circles about 5 μm in circumference. Denaturation of the DNA with heat separated the filament into single nucleotide chains, and it was concluded that *in vivo* it occurs as a double helix like that of the nucleus. The circular DNA strands closely resemble those of viruses and bacteria.

Also present in the mitochondrial matrix are small (10 to 15 nm) dense granules interpreted as ribonucleoprotein particles comparable to ribosomes of the general cytoplasm (André and Marinozzi, 1965). In size, they resemble the ribosomes of bacteria. It has now been shown that mitochondria contain all of the factors necessary for incorporation of nucleotides into DNA and RNA and for synthesis of proteins from amino acids. However, the limited information content of mitochondrial genomes makes it unlikely that they synthesize all of their own components. Thus, mitochondrial DNA probably codes for their DNA, RNA, and the protein of their ribosomes, for transfer RNA and for certain of their structural elements. However, mitochondria seem to be dependent upon the nuclear genome for synthesis of many, if not most, of their enzymes.

STRUCTURE OF MITOCHONDRIA

A mitochondrion in thin section exhibits the characteristic internal organization illustrated in the accompanying electron micrograph. It is bounded by a smooth-contoured outer membrane about 7 nm thick. Within this limiting membrane and separated from it by a space 8 to 10 nm wide is an inner membrane. The inner membrane has numerous infoldings, the cristae mitochondriales, that project into the interior of the organelle. These are of variable length and form a series of incomplete transverse septa. The membranes of the mitochondrion delimit two compartments. The narrow cleft between the outer and inner membrane and extending inward between the leaves of the cristae is called the membrane space, or intracristal space. Its content is of low density. The large cavity bounded by the inner membrane and its cristae is occupied by a homogenous or finely granular matrix, which varies in density from one cell type to another and with different degrees of extraction during specimen preparation. After freeze-substitution it is invariably quite dense. In most cell types dense matrix granules 30 to 50 nm in diameter are scattered at random in the inner compartment.

This pattern of internal structure with thin lamellar orfoliate cristae is described as the typical, or orthodox, configuration of the mitochondrion and is the one found in micrographs of the great majority of cells after routine specimen preparation. However, numerous modifications of this basic architectural plan are encountered in specific cell types and in different physiological states of the same cell type, as will be illustrated in later pages.
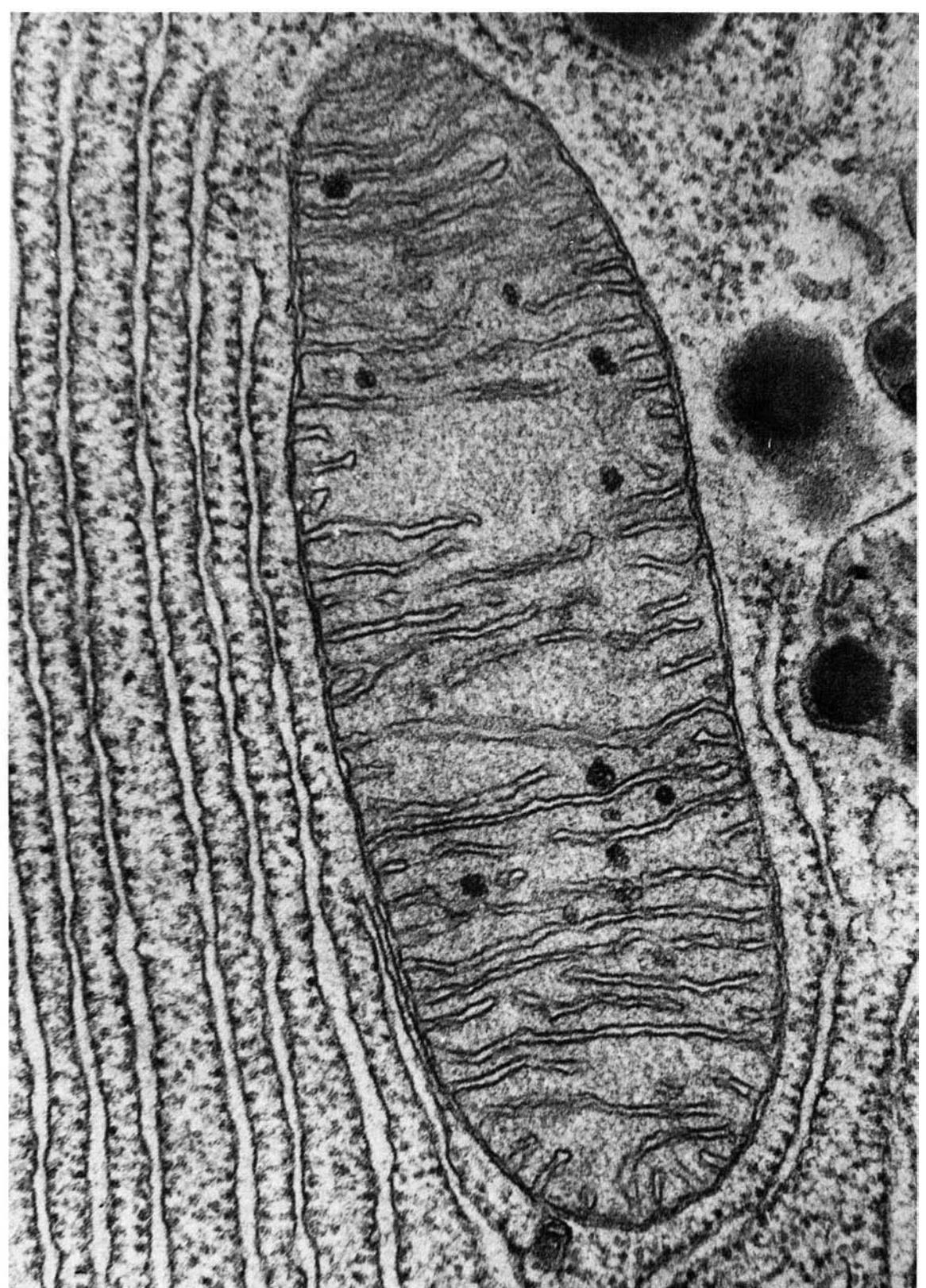

Figure 218. A longitudinal section of a mitochondrion and surrounding cytoplasm from pancreas of the bat, *Myotis lucifugus.*(Micrograph courtesy of Keith Porter.)

Figure 218

Electron micrographs of thin tissue sections are a poor basis for description of the external form of mitochondria. Their shape can be seen to better advantage in selectively stained histological sections or in living cells viewed with phase contrast microscopy. Such preparations are favorable because they are thick enough to include whole mitochondria. Electron micrographs, on the other hand, give a misleading impression of their shape because the section includes only thin slices through cell organelles. Randomly oriented mitochondria of uniform shape therefore present various round, elliptical, or elongated profiles, depending upon the orientation of the organelles in relation to the plane of the section.

In the micrograph shown here the majority of the mitochondria1 profiles are round cross-sections; only a few are elongated, oblique, or longitudinal sections. In fact, all of the mitochondria of this cell type are long flexible rods, but this is not apparent in thin sections.
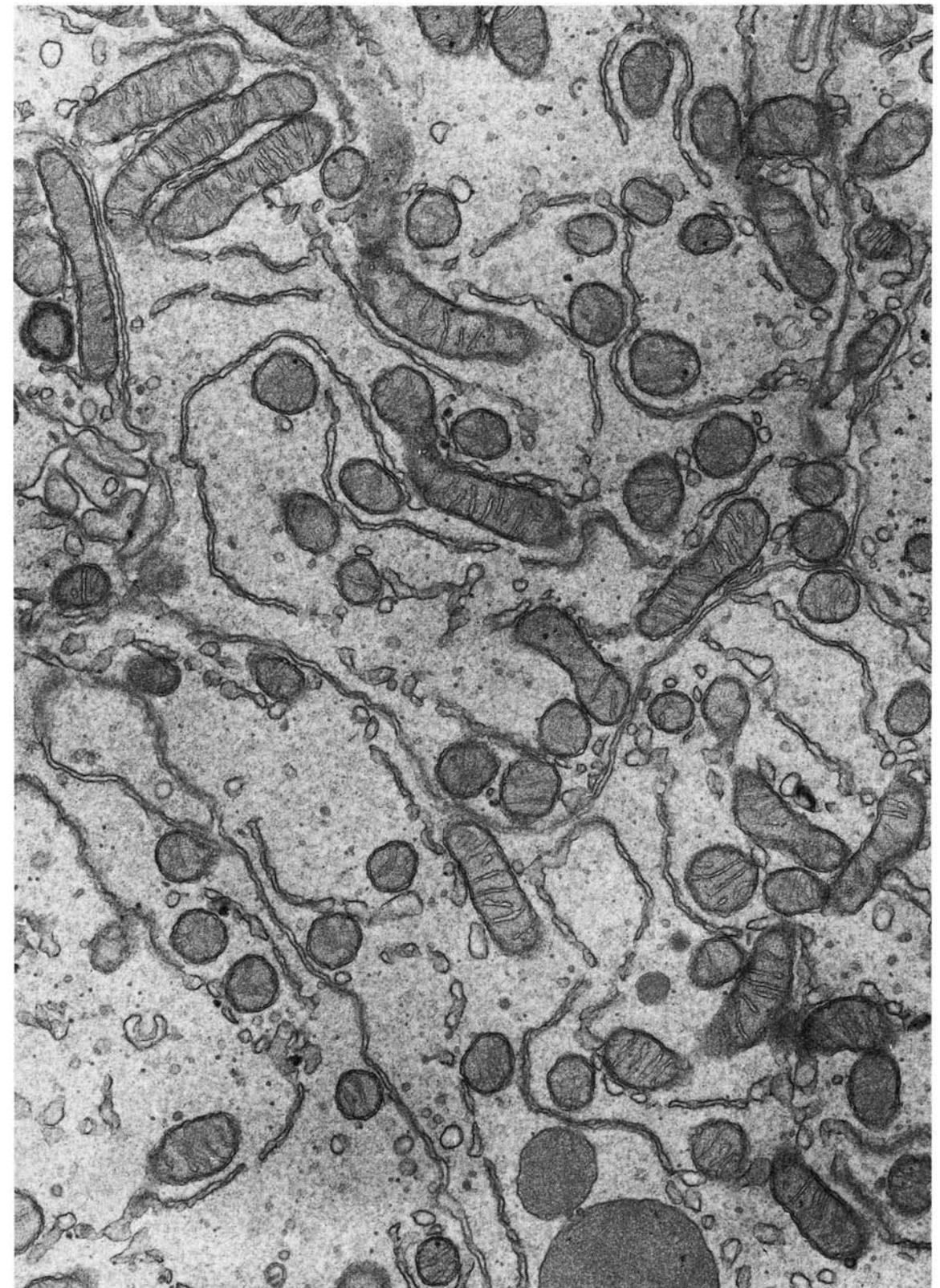

Figure 219. Micrograph of a cell from the ductulus efferens of the ground squirrel, *Citellus lateralis.* (Micrograph courtesy of Jeffrey Pudney.)

Figure 219

In general, the number of mitochondria per cell and the number of cristae per mitochondrion are related to the energy requirement for the function carried out by that cell type. The mitochondrion in the upper figure from moderately active epididymal epithelium has an average number of lamellar cristae. In the lower figure, the mitochondrion, from a very rapidly contracting skeletal muscle with high energy requirements, contains many long and closely packed cristae.
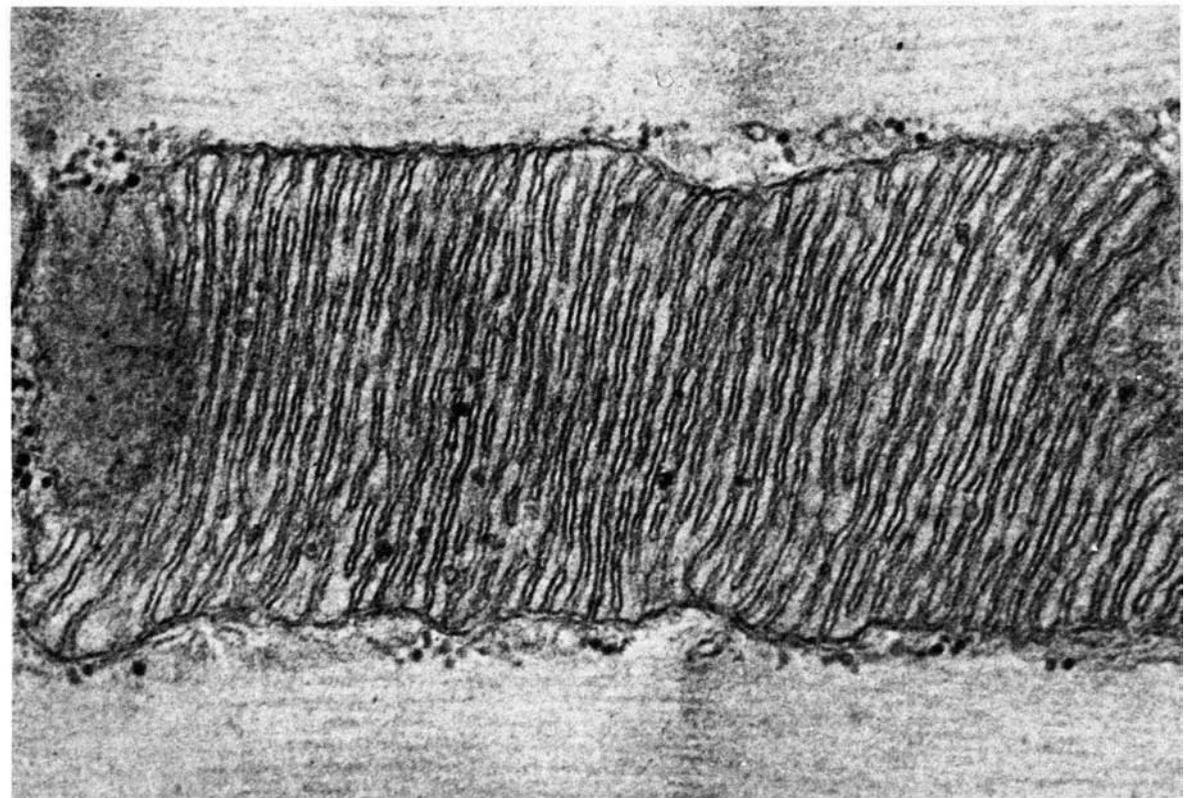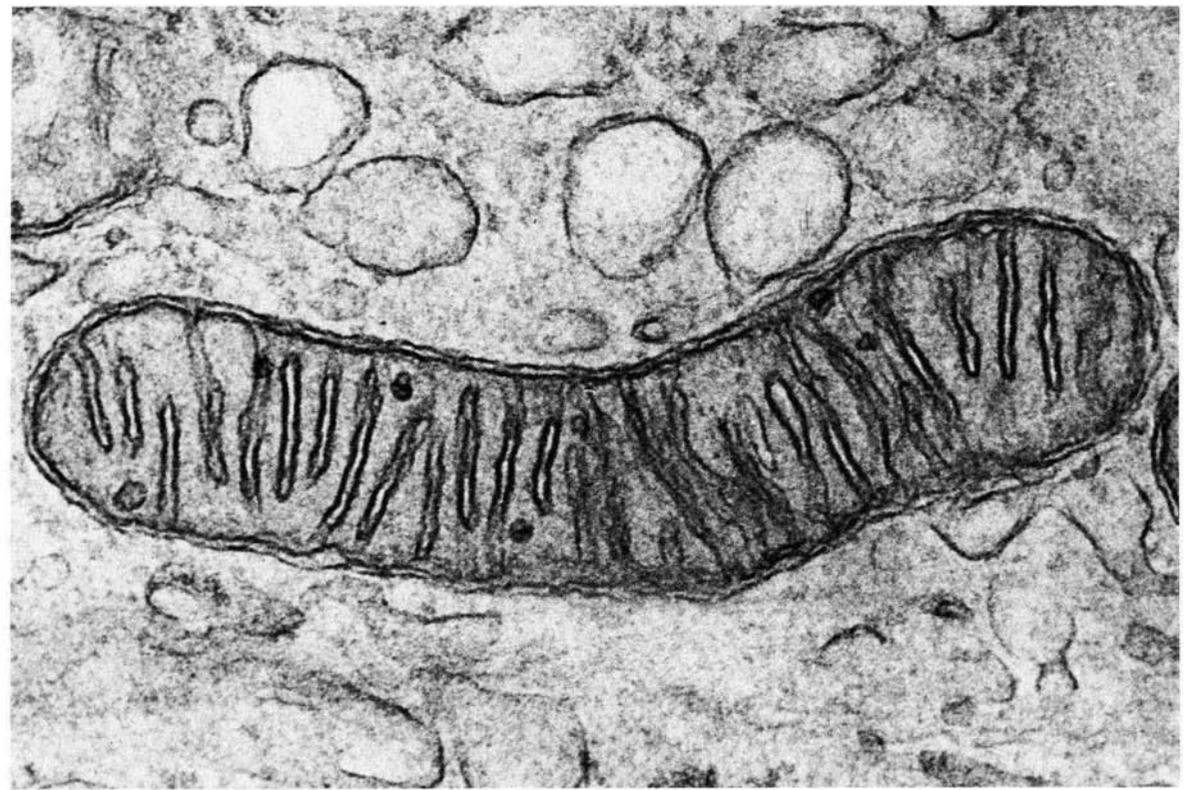

Figure 220. Mitochondrion from mouse epididymis.

Figure 221. Mitochondrion from cricothyroid muscle of the bat, *Myotis lucifugus.*

Figure 220, *upper*   **Figure 221,** *lower*

MATRIX GRANULES

The matrix granules are approximately spherical, osmiophilic inclusions located within the space bounded by the inner mitochondrial membrane. They may be free in the matrix but are often closely associated with the membranes of the cristae. Their density usually obscures any internal structure, but in very thin sections viewed at high magnification they appear to be subdivided by thin membrane-like septa into minute loculi or compartments. These membrane-like profiles may also assume concentric configurations.

The composition and function of the matrix granules continue to be subjects of controversy. When calcium or other divalent cations are present in high concentration in the fluid bathing isolated mitochondria, large dense granules are found in their matrix. It seemed likely that these conspicuous densities were not inorganic precipitates formed de *novo* but that cations had accumulated in the preexisting matrix granules. It was suggested therefore that these granules probably functioned in the regulation of the internal ionic environment of the mitochondrion (Peachey, 1964).

This interpretation was strengthened by the detection of calcium phosphate in the large matrix granules of cells in calcifying cartilage and bone by x-ray microprobe analysis (Sutfin et al., 1971). Although it was assumed from these observations that sequestration of calcium was probably a general property of mitochondrial matrix granules, it was difficult to exclude the possibility that the calcium detected had diffused into the mitochondria from the calcium-rich environment of these calcifying tissues during specimen preparation.

When mitochondria from several noncalcifying tissues were subjected to x-ray microanalysis after glutaraldehyde fixation, no calcium was found in the matrix granules. But when fixed in osmium supplemented with 5 mM Ca^{2+} they were found to bind detectable amounts of this element (Barnard and Ruusa, 1979). In most cell types the granules have no inherent electron density but only become dense after exposure to osmium-containing fixatives, indicating that their composition is mainly organic. Their affinity for osmium and their membrane-like ultrastructure in cells other than bone suggests that their major component is lipid (Barnard and Afzelius, 1972). If they do seed the deposition of calcium and other cations these evidently make up only a small proportion of their mass. Efforts to isolate a matrix granule fraction for biochemical analysis have met with some success and indicate that they are composed of a phospholipoprotein (Brdiczka and Barnard, 1980). The lipoprotein binds calcium under some *in vitro* conditions, but there is no convincing evidence that the matrix granules in nonmineralized tissues are involved in mitochondrial calcium sequestration *in vivo*. Their close association with the cristae and their membrane-like composition suggests that they may instead be involved in assembly of the mitochondrial inner membrane.
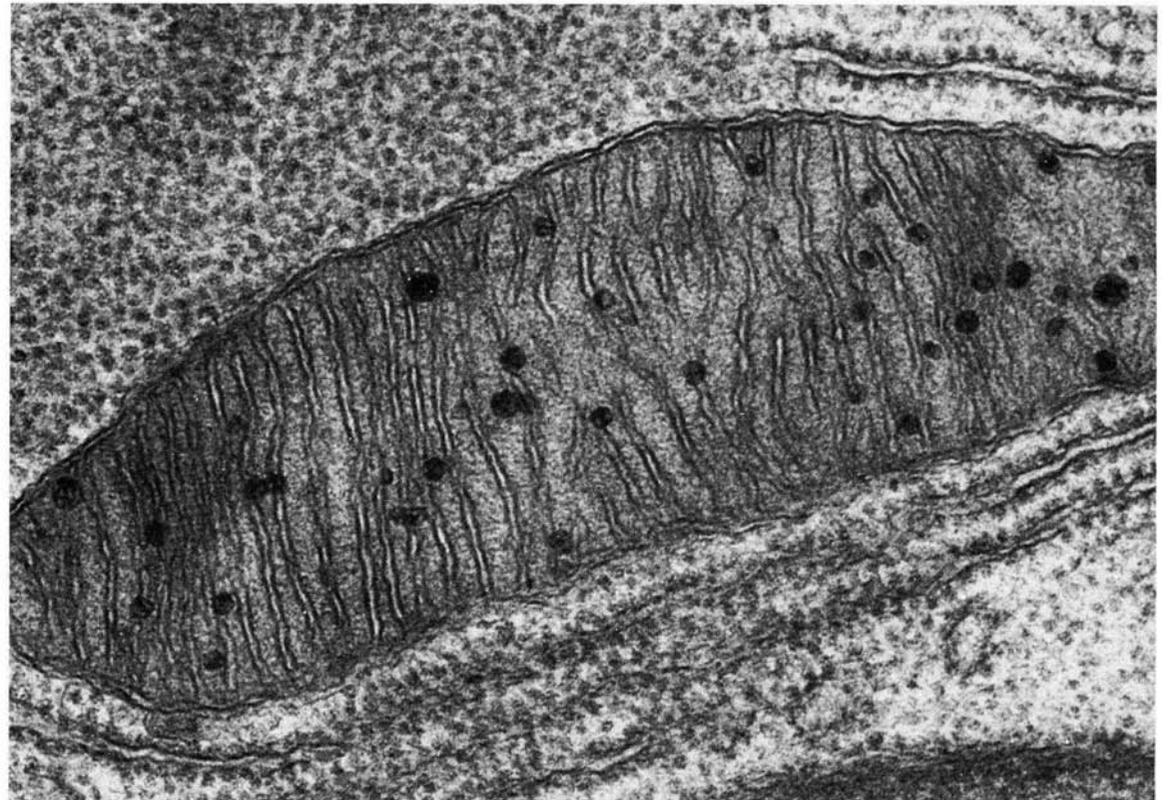
Figure 222.   Mitochondria from cat ventricular cardiac muscle with numerous matrix granules.

Figure 223.   Mitochondrion of bat pancreas.

Figure **222,** upper   Figure **223,** lower

Matrix granules have been reported to be sensitive to a great variety of physiological stimuli and to toxic agents, but from such observations no meaningful pattern has emerged that sheds light upon their origin or function.

Mitochondria1 granules range in size from 25 nm to 120 nm and vary greatly in number in different tissues and in different physiological states of the same tissue. The upper figure shows the typical small granules in liver mitochondria. The lower figure illustrates the less common, large matrix granules occasionally encountered. Those illustrated here are from certain cells in proximal convoluted tubule of an apparently normal guinea pig kidney.

The mitochondria of osteoclasts active in bone resorption often contain clusters of large matrix granules which exhibit small dense granules in their interior (Gonzales and Karnovsky, **1961**). The presence of calcium and phosphorus in granular deposits within mitochondria of bone cells has been confirmed by electron probe microanalysis (Sutfin et al., **1971**). Matrix granules of bone cells and duodenal cells have been shown to sequester calcium from the fixative in the presence of osmium (Martin and Mathews, **1969;** Sampson et al. **1970).** Thus matrix granules in these cell types appear to have the capacity to sequester calcium by a nonenergy-requiring mechanism. Whether this is their major role in *vivo* or whether their organic constituents have other important functions remains unsettled.
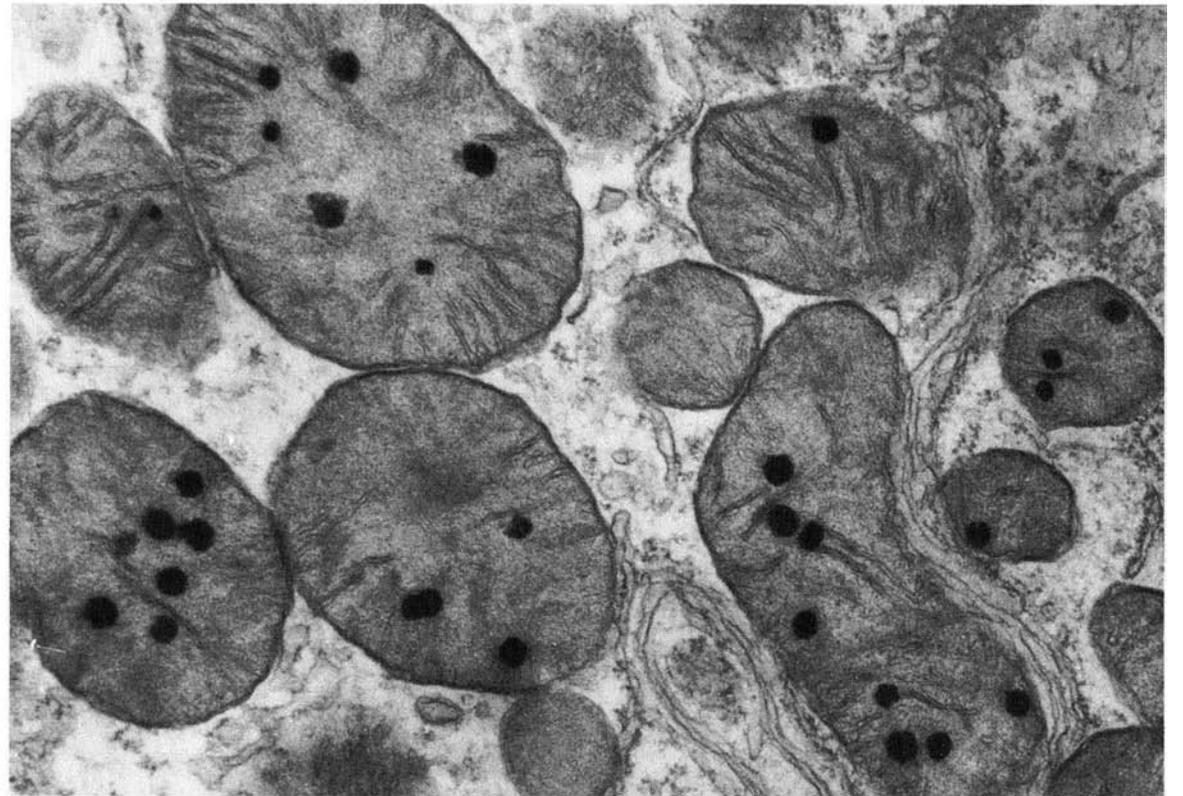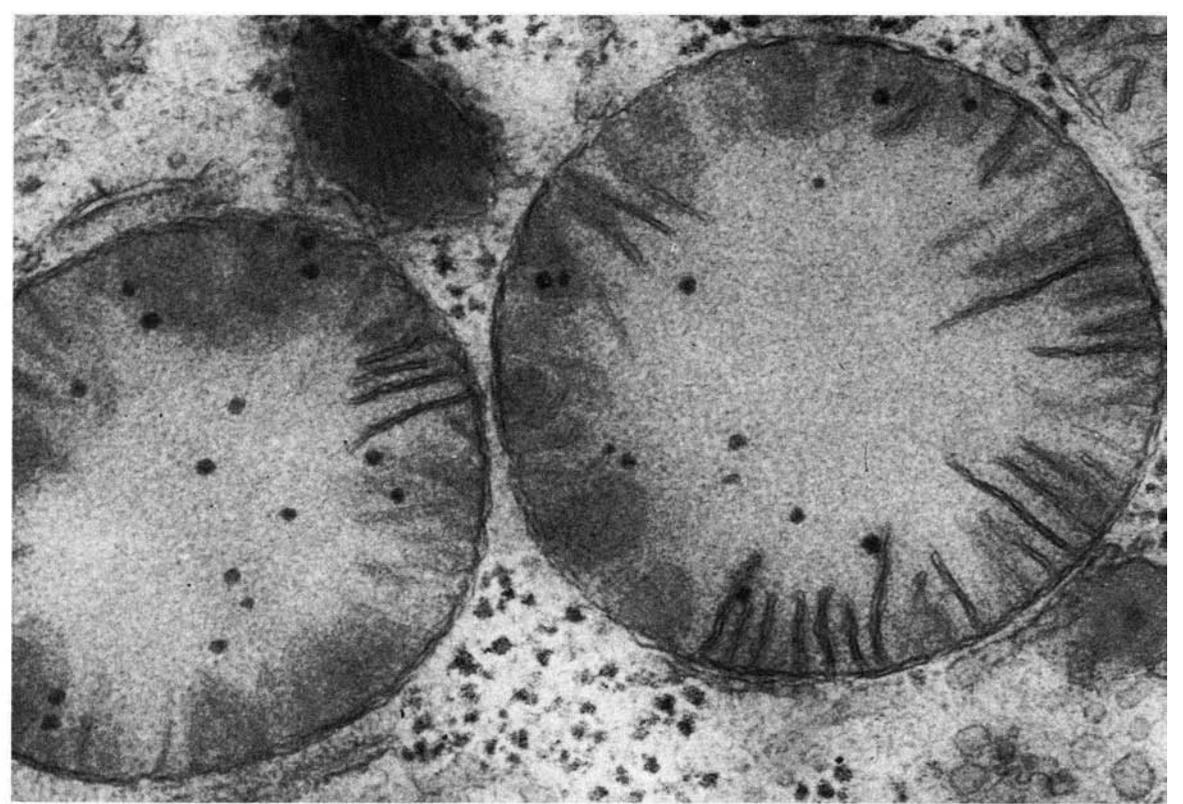

Figure *224.* Mitochondria from bat liver. (Micrograph courtesy of Susumu Ito.)

Figure *225.* Mitochondria from guinea pig proximal convoluted tubule.

Figure *224,* upper   **Figure** *225, lower*

MITOCHONDRIAL DNA AND RNA

DNA has been demonstrated in the mitochondria of many plant and animal cells. It is found in the form of filaments of varying thickness located in the matrix in circumscribed regions of lower density. Initially no significance was attached to these small pale areas of matrix because larger but otherwise similar areas occur as a common artefact of glutaraldehyde fixation. Microscopists have now learned to look for **DNA** in such areas. The thicker strands of **DNA** frequently appear to branch into more slender filaments. It is likely that the thicker strands are formed by lateral association of thin **DNA** filaments.

A mitochondrion from a plant cell is shown here in the upper figure. In addition to conspicuous **DNA** filaments in the matrix, there are a number of small dense particles of uniform size. These are **RNP** particles comparable in composition and function to cytoplasmic ribosomes. In mitochondria of animal cells these are usually obscured by a relatively dense matrix. **A** mitochondrion from adrenal cortex, shown in the lower figure, is crowded with tubular cristae, but short segments of **DNA** can be identified in two or three small areas of low density.

Figure 226.   Mitochondrion from the plant *Vica fabia.* (Micrograph courtesy of Hewson Swift.)

Figure 227.   Mitochondria from snake adrenal adjacent to a highly ordered array of endoplasmic reticulum. (Micrograph courtesy of John Long.)
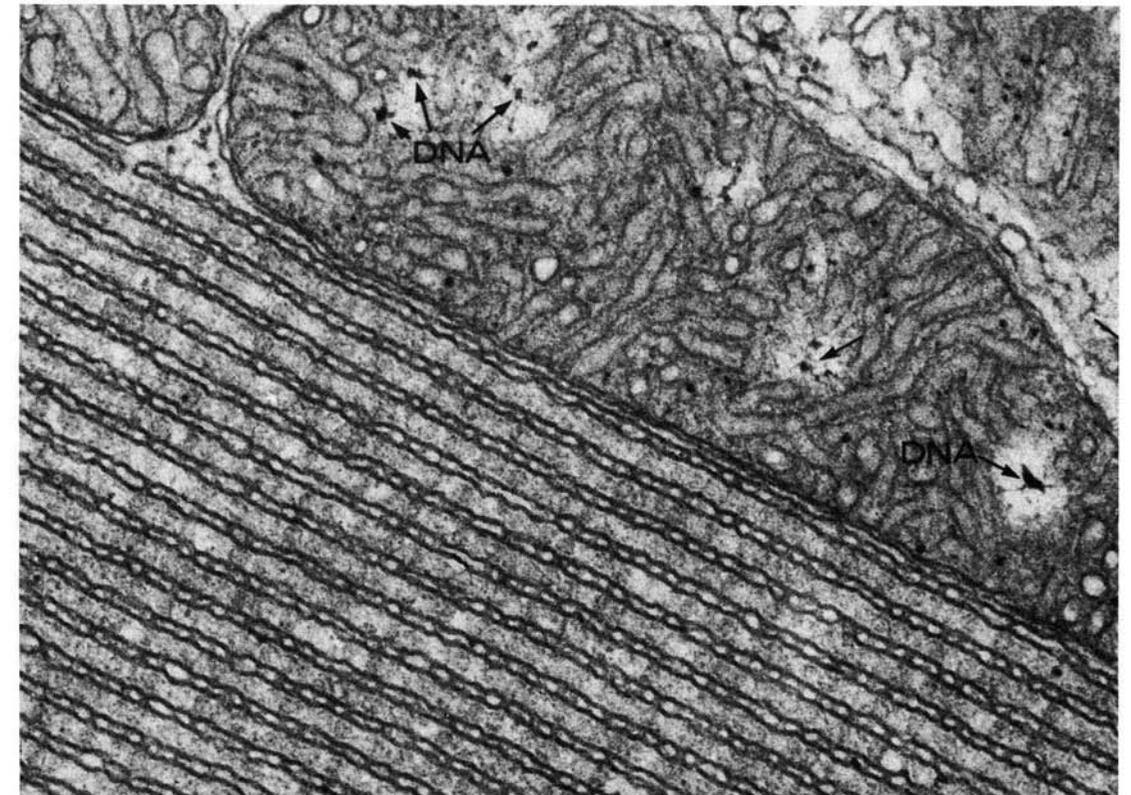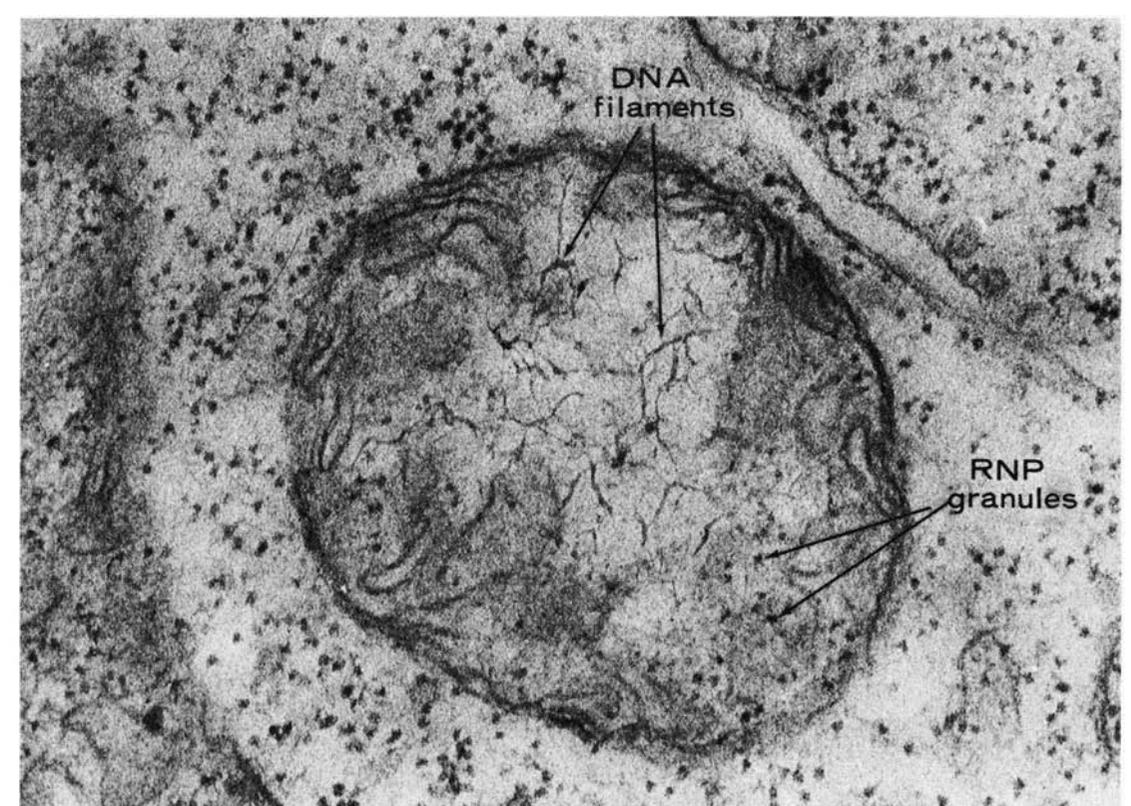
Figure 226, *upper*   **Figure 227,** *lower*

It is rare to observe more than two or three sites of DNA in a single thin section of a mitochondrion. The example on the facing page, from cultured adrenal, is unusually large and shows a greater than normal number of DNA filaments. The abnormal environment in *vitro* may have resulted in growth of the organelle and DNA replication without subsequent division.

When disrupted mitochondria are spread by surface tension on a film of water and the liberated DNA is collected on an electron microscope grid, it appears in the form of circular filaments such as that shown in the lower figure. These circular DNA molecules are 5 to 6 μm long, have a molecular weight of about 10 million and consist of 15 to 17 kilobase pairs. There are normally from **3** to 6 per mitochondrion. The maximum coding capacity of this relatively small amount of DNA is estimated to be only about 5000 amino acids (Borst et al., 1967). Mitochondrial DNA codes for two ribosomal RNAs and a set of transfer RNAs. Evidence for mitochondrial messenger RNAs is still indirect but it seems clear that each mitochondrion has all of the ingredients necessary for transcription of the genetic information carried in its DNA. However, it is apparent from the limited coding capacity of its small genome, that the mitochondrion must be dependent upon the nuclear genome to direct the synthesis of many of its constituents.
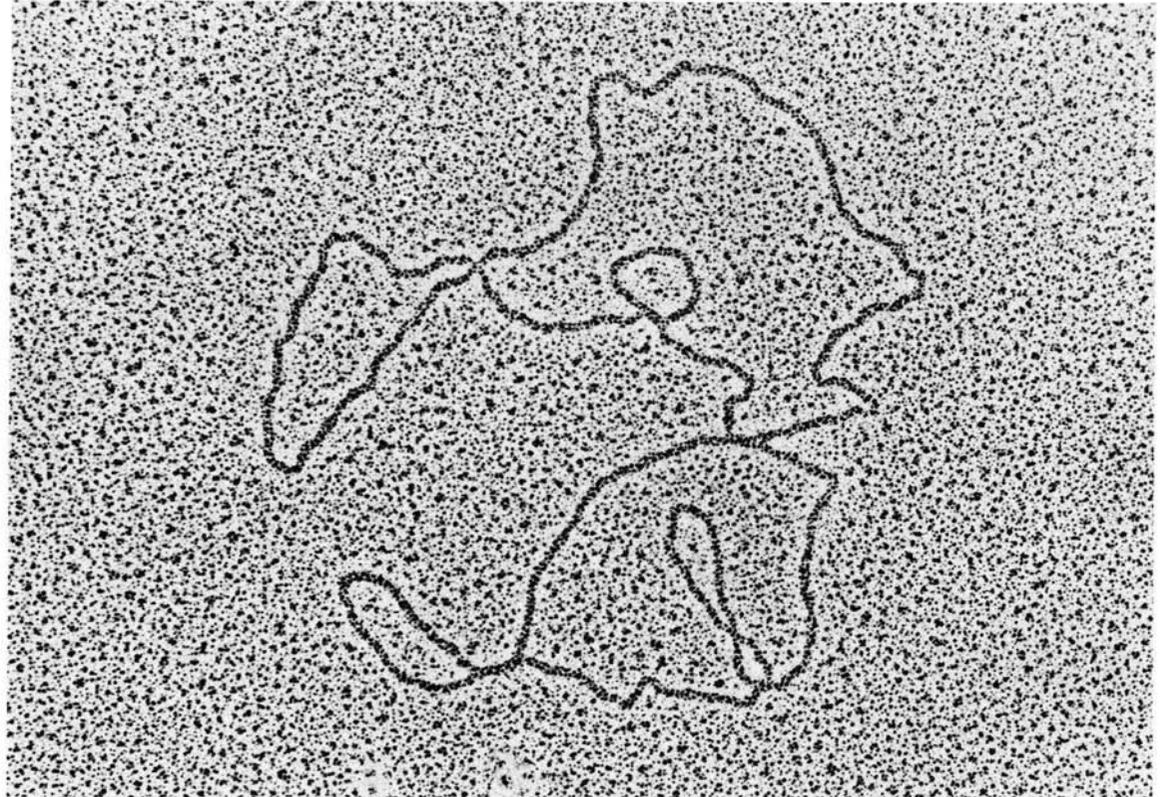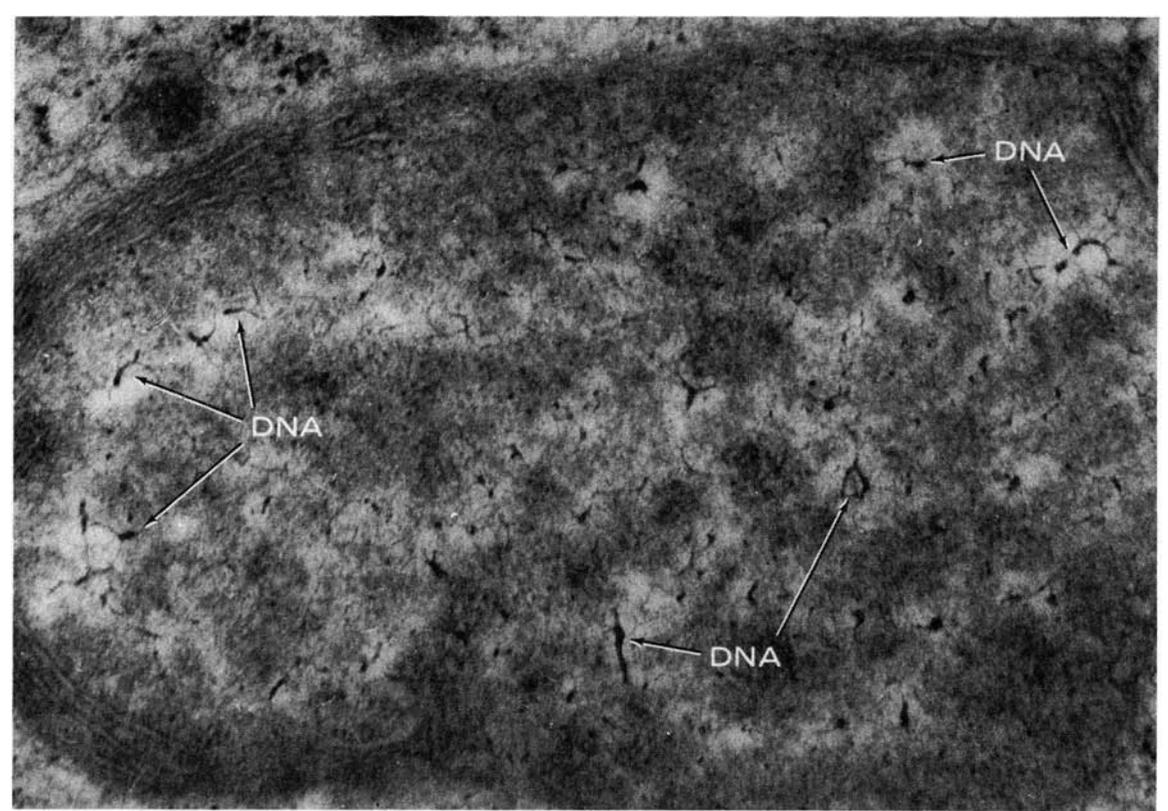

Figure 228. Mitochondrion from an organ culture of rat adrenal. (Micrograph courtesy of Arvi Kahri.)

Figure 229. Mitochondrial DNA molecule from an oocyte of Xenopus laevis. (Micrograph courtesy of Igor Dawid.)

Figure 228, upper   **Figure 229,** lower

The first suggestive evidence of mitochondrial **DNA** came from morphological and histochemical observations on trypanosomes over 50 years ago. In these organisms a unique organelle, the *kinetoplast,* is located near the base of the flagellum. Light microscopists suspected that this structure was a component of a modified mitochondrion because the organelle containing it could be stained with Janus green (Shipley, 1916). When the kinetosome was subsequently shown to give a positive Feulgen reaction (Bresslan and Scremin, 1924), the term *kinetonucleus* was preferred by some investigators. The paradoxical occurrence of Janus green and Feulgen staining of the same organelle was considered an exceptional situation limited to trypanosomes and therefore of little general interest.

The nature of the kinetoplast was clarified nearly 40 years later by the electron microscope, which showed that the Feulgen reactive component is a long rod or disc made up of intensely staining **DNA** filaments oriented transversely to its long axis, as illustrated in the lower illustration on the facing page. This structure is located in the matrix of a modified mitochondrion with outer and inner membranes and a few typical cristae (Steinert, 1960). The kinetoplast replicates and divides prior to cell division. Interference with **DNA** synthesis by treatment with acridine dyes results in a marked decrease in oxidative metabolism, which suggests that normal mitochondrial function is dependent upon kinetoplast **DNA** (Trager and Rudzinska, 1964).

In some stages of the life cycle of parasitic trypanosomes, the Krebs cycle enzymes appear to be absent but they become active again when the organism enters a more virulent phase. This transformation is accompanied by striking changes in size, shape and internal configuration of the trypanosome's single mitochondrion (Vickermann, 1962, 1965; Hecker et al., 1972).
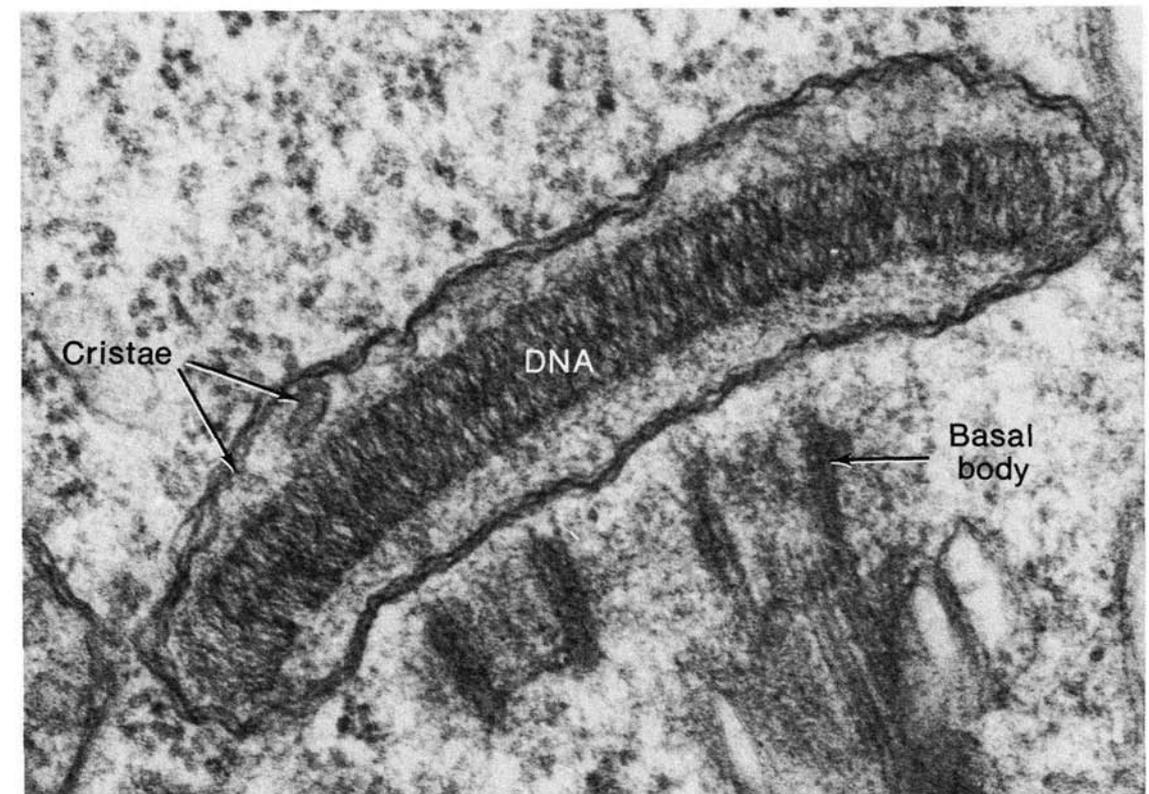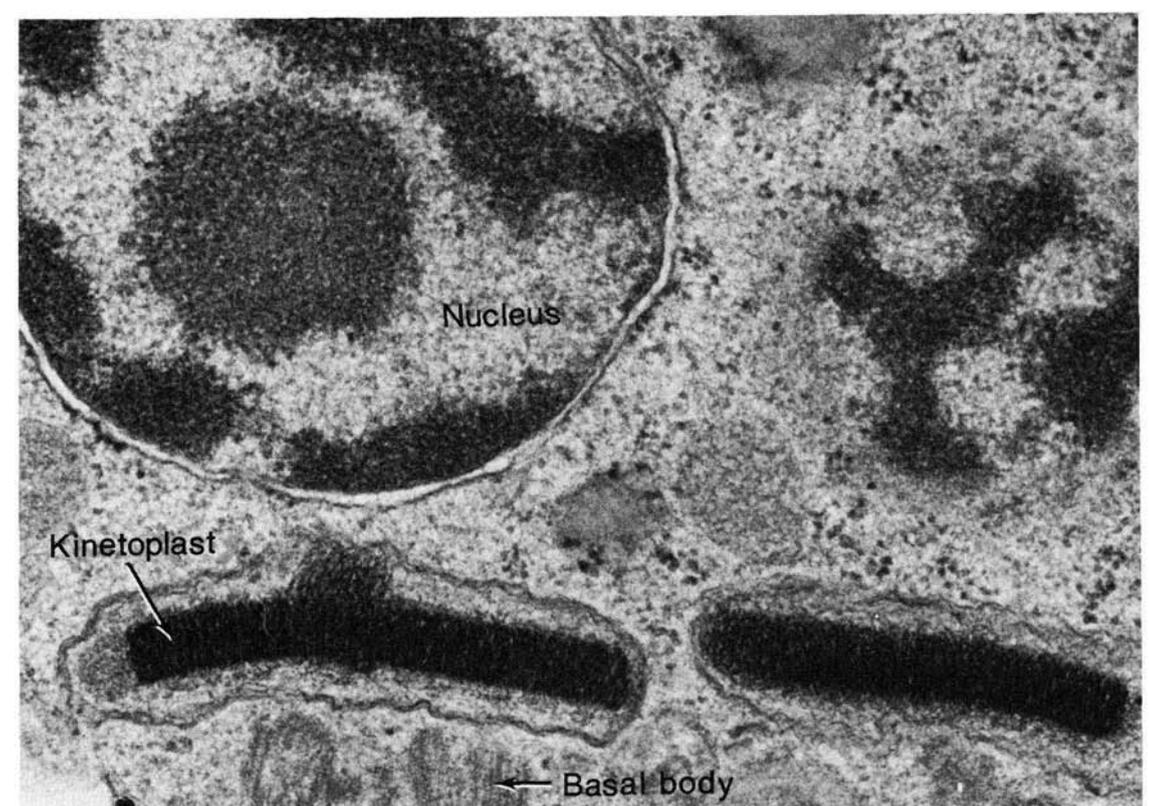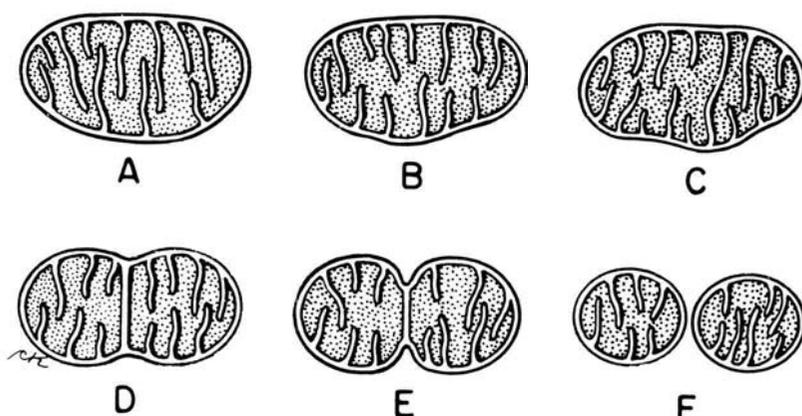
Figure 230.   Kinetoplast region of the *Trypanosoma mega.* **(Micrographs courtesy of Winston Anderson.)**

Figure 230, upper *and lower*

DIVISION OF MITOCHONDRIA

In the 1930s, time lapse cinematography of cells maintained in culture recorded frequent examples of apparent division or fragmentation of mitochondria. Some 20 years later, when the electron microscope was applied to the study of ultrathin sections of liver (Fawcett, 1955), mitochondria were occasionally observed in which a pair of membranes formed a partition extending across the organelle from the inner membrane on one side to that on the other. It was suggested that these probably represented stages in a process of mitochondrial division. However, from study of static images, the possibility that they were stages of mitochondrial fusion could not be excluded. This latter interpretation was rendered highly improbable by the subsequent observation of an increased frequency of septa under conditions in which rapid increase in mitochondrial number could be induced experimentally or was known to occur naturally. Hepatic cells of riboflavin-deficient mice have reduced numbers of exceptionally large mitochondria, but when riboflavin is added to the diet, their normal number and size are restored within two days. Electron micrographs of the liver during this period of rapid proliferation show large numbers of mitochondria with transverse partitions and associated constriction of the limiting membrane (Tandler et al., 1969). Many of the mitochondria of the fat body in certain insects undergo autolysis before pupation and then in a 12-hour period after emergence of the adult, there is a sevenfold increase in the number of mitochondria. During this period, partitioned and constricted mitochondria are very abundant (Larsen, 1970).

The most compelling evidence for the origin of new mitochondria from preexisting mitochondria came from autoradiographic studies on the distribution of isotopically labeled membrane precursors among mitochondria isolated from cultures of a strain of Neurospora requiring choline (Luck, 1963). Labeled choline was supplied to cultures in a logarithmic phase of growth, and at suitable intervals after transfer to nonradioactive medium, mitochondria were isolated and spread upon slides for autoradiography. If de novo formation of mitochondria had occurred under these conditions, there should have been two populations, one labeled and the other unlabeled. Instead the label was found to be uniformly distributed among all mitochondria. In comparable studies on Tetrahymena, mitochondrial DNA was labeled with tritiated thymidine (Parsons and Rustad, 1968). After transfer to unlabeled medium, the label remained evenly distributed among all mitochondria for four generations of logarithmic growth. Therefore it was concluded that the increase in mitochondrial mass in the course of the experiment had been due to growth and division of the preexisting labeled mitochondria.

As in the proliferation of bacteria, mitochondrial division involves more than simple fission. Filaments of DNA are seen most frequently in mitochondria of rapidly growing cells. When isolated, some of the circular molecules exhibit a segment that branches and rejoins in a configuration suggesting DNA replication by a mechanism comparable to that postulated for the circular DNA of bacteria. It is believed that in the initial stage of mitochondrial multiplication the genome is replicated and this is followed by DNA-directed synthesis of components of mitochondrial ribosomes and some structural proteins. However, since the coding potential of mitochondrial DNA is quite limited, most of its structural protein, its complement of enzymes, and its lipid components are probably synthesized in the cytoplasm under the direction of nuclear genes. Little is known about how these components enter the growing organelle or what controls their ordered assembly to create the specific properties of the outer and inner membranes.

Mitochondria are occasionally seen in the process of degradation within autophagic vacuoles and they apparently have a limited life span. Investigations of the turnover rate of mitochondrial DNA in liver indicate that they have a half-life of about 8 days compared to estimates of 350 days or more for the nucleus. Similar analyses on other tissues yield values ranging from 6 days in heart muscle to 31 days in brain (Gross et al., 1969; Fresco and Bendick, 1960).

Schematic representation of successive stages in mitochondrial division. (Redrawn after Larsen, J. Cell Biol. 47:373, 1970.)

The septum of dividing mitochondria consists of two membranes separated by a narrow interspace with dimensions similar to those of an intracristal space. This might suggest that partitioning occurs by extension of one of the preexisting cristae across the organelle and fusion of its edge with the inner membrane on the opposite side. However, the membranes of the septa are slightly thinner than those of the cristae and in freeze-fracture preparations, they have fewer intramembrane particles (Kanaseki, 1975). Therefore the septum is not simply a modified crista but a distinctive structure formed anew from the inner mitochondrial membrane for its specific partitioning role in division of the organelle. A circumferential fold of the outer membrane then invades the space between the septal membranes and when its advancing edges meet and fuse, the separation of the daughter mitochondria is completed.

The micrograph on the facing page shows an early stage of invasion of the septum by the outer mitochondrial membrane. The paucity of such images suggests that this event proceeds more rapidly than the earlier formation of the septum.
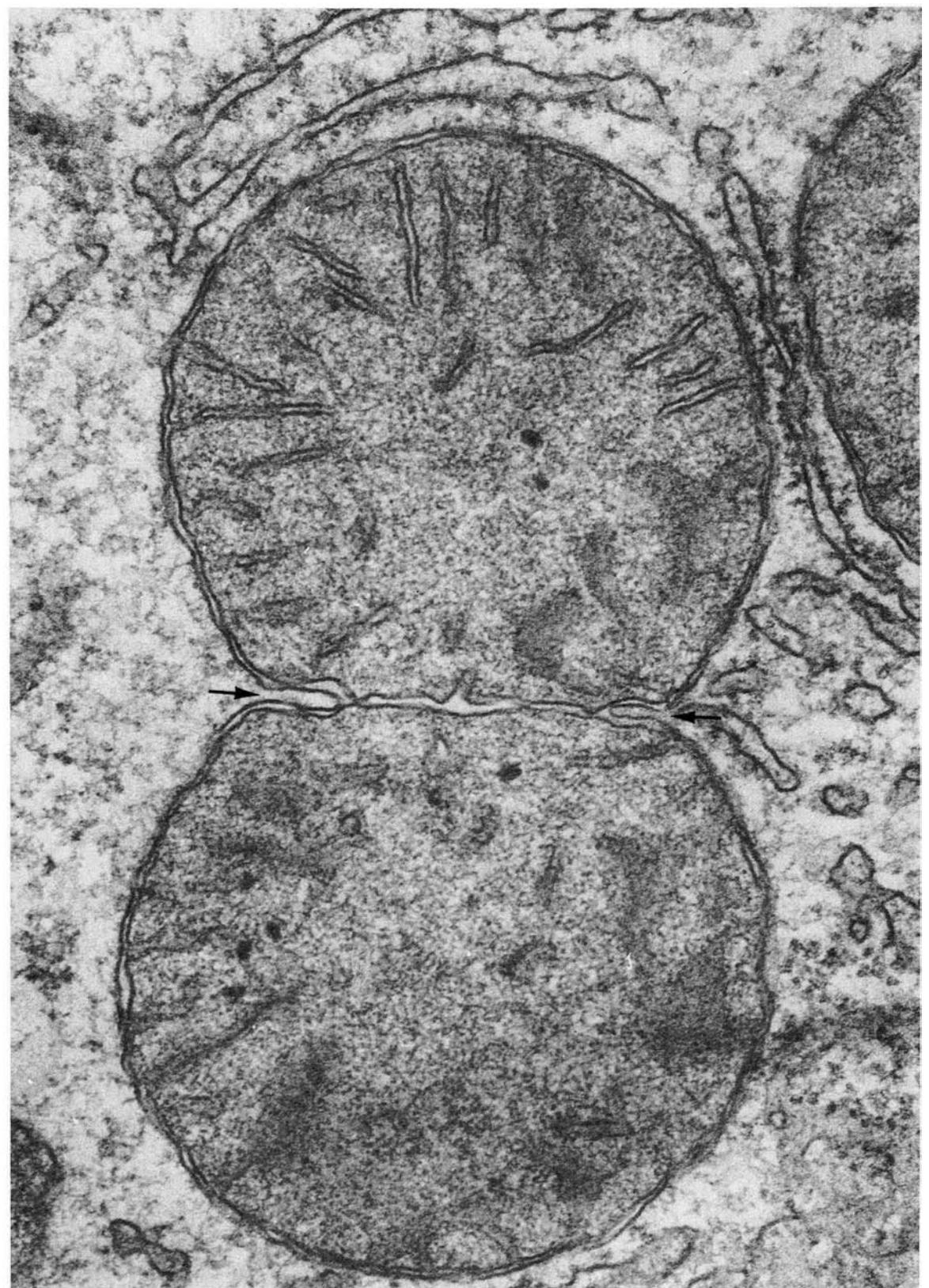
Figure 231. Dividing mitochondrion in rat liver. (Micrograph courtesy of Daniel Friend.)

Figure 231

In the upper figure of a dividing mitochondrion, the bilaminar septum has been formed but separation of the two compartments by invasion of a fold of the outer membrane has not yet begun. It is evident from examination of the lower figure that the outer membrane does not always form a circumferential invagination of uniform width. In the central portion of the septum (at star), the membranes are separated by a deep and slender invagination from a sector outside the plane of section, while the outer mitochondrial membrane in this plane (at arrows) has advanced only a short distance into the cleft.
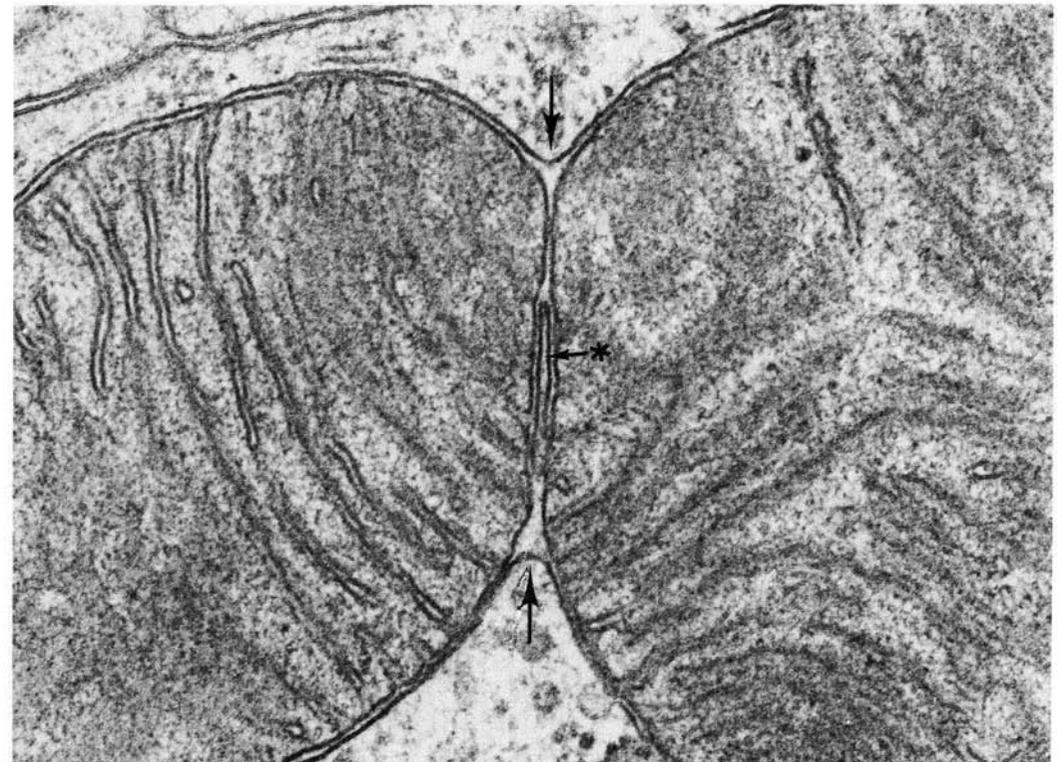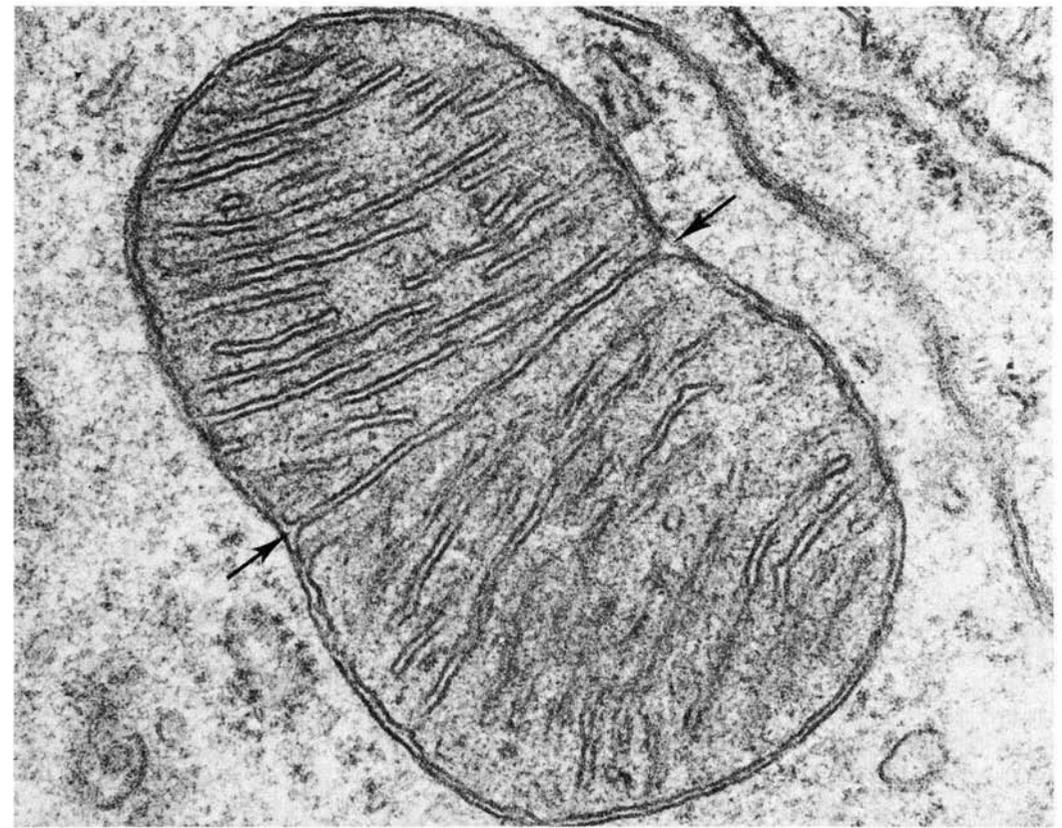

Figure **232.** Dividing mitochondria in gastric mucosa of a mole. (Micrographs courtesy of Toku Kanaseki.)

Figure *232, upper and lower*

The accompanying micrographs represent three of the stages in mitochondrial division. In A, the free edge of the developing septum has not yet reached the opposite side of the organelle. In B, the asymmetrical penetration of the fold of outer membrane is evident. In C, the separation of the daughter mitochondria is complete.
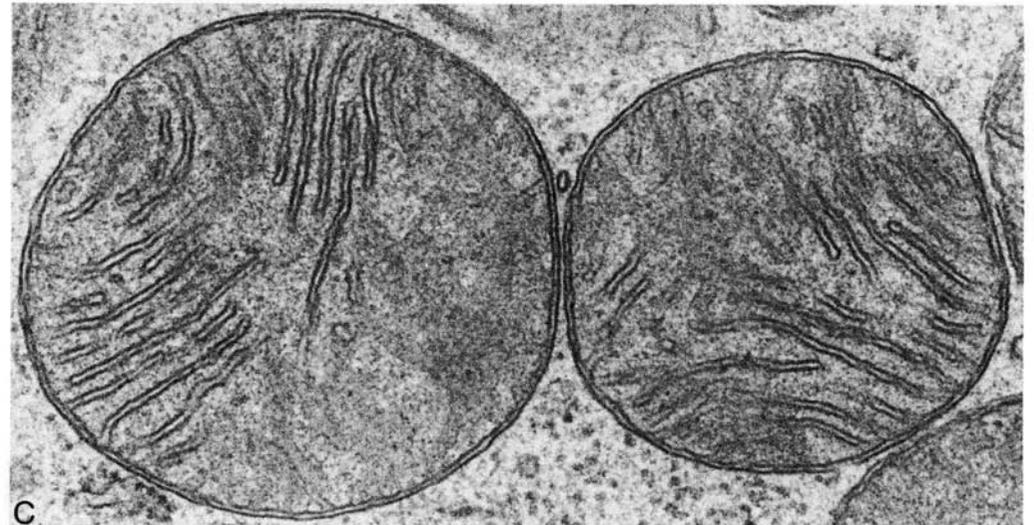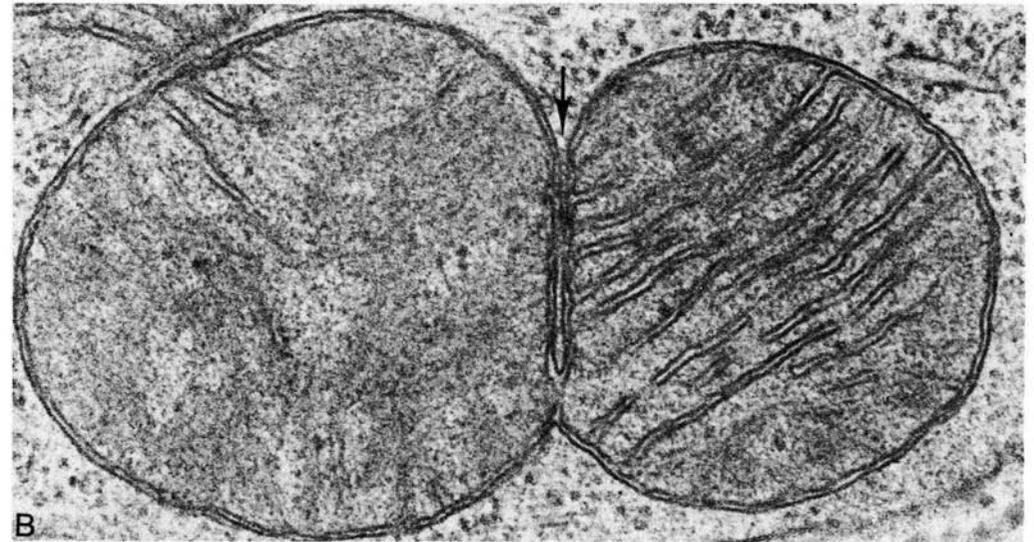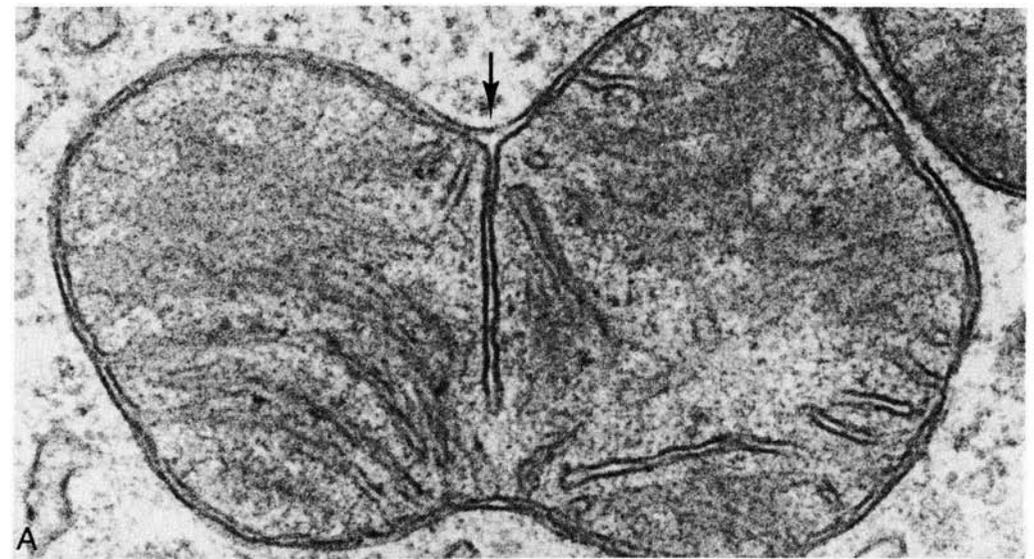

Figure 233. Dividing mitochondria from gastric mucosa of a mole. (Micrographs courtesy of Toku Kanaseki.)

Figure 233

FUSION OF MITOCHONDRIA

No doubt in many cell types from time to time two mitochondria may make contact and fuse into a single mitochondrion, but these are isolated events of little physiological significance. The most dramatic examples of mitochondrial fusion are those that occur in insect spermiogenesis. Soon after the second meiotic division, spermatid mitochondria aggregate, as shown in the upper figure on the opposite page. They then begin a process of fusion that results in the formation of a large spherical mass traditionally called the *nebenkern*. As shown in the lower figure the progressive fusion of mitochondria results in longer and thicker organelles that are interwoven in a remarkably complex pattern.
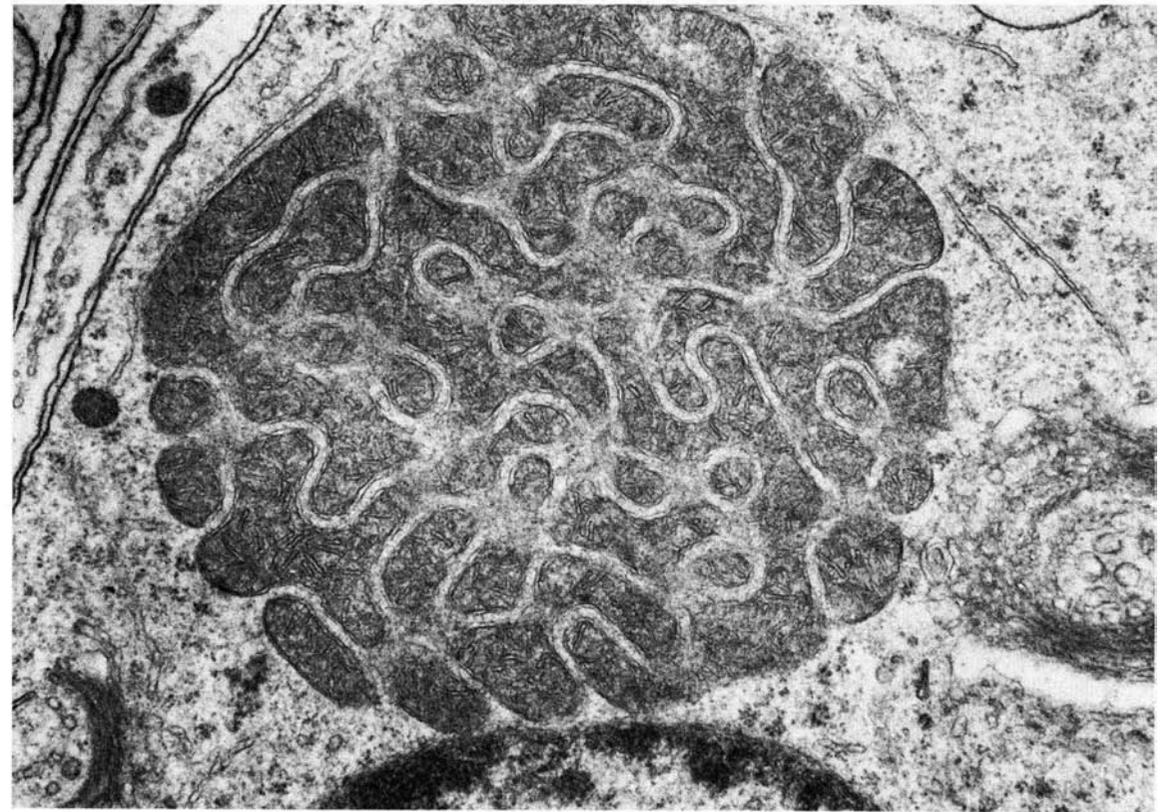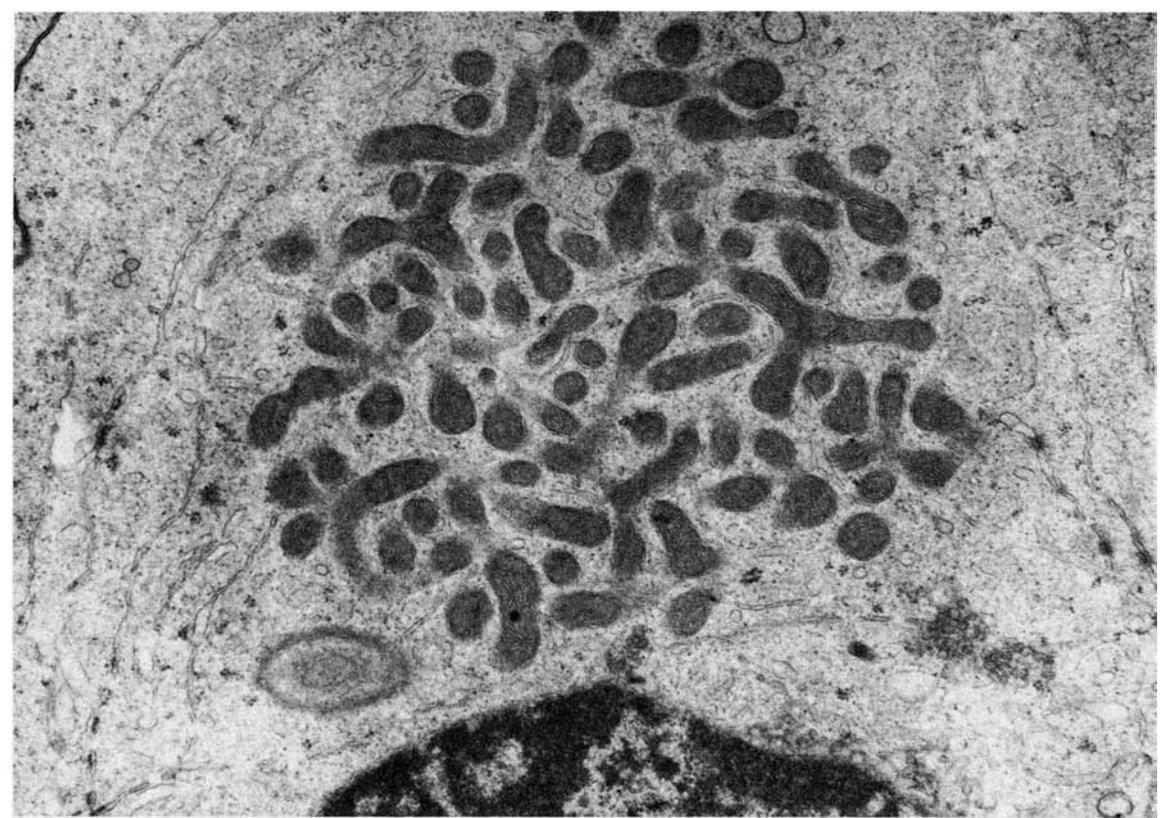
Figures *234* and *235*. Successive stages in the formation of the mitochondrial nebenkern in insect spermiogenesis. (Micrograph by David Phillips.)

Figure *234*, upper   Figure *235*, *l o w e r*

In the further development of the nebenkern beyond the stage illustrated here, the initial complexity of its labyrinthine pattern is reduced by continuing fusion (Pratt, 1968). Internal reorganization within the nebenkern then results in formation of two hemispherical masses that are situated on either side of the flagellum. These elongate to form two mitochondrial derivatives that may course parallel to the axoneme for nearly the full length of the sperm tail. In the process of elongation and internal differentiation, the mitochondria become extensively modified, with a loss of cristae and formation of one or more long crystalline inclusions (Phillips, 1970).

No counterpart of the nebenkern is observed in mammalian spermiogenesis. The mitochondria assemble directly around the proximal portion of the flagellum, where they form a helical sheath in which the mitochondria usually retain their individuality. However, in some reptiles and birds they may fuse end-to-end in the mitochondrial helix.
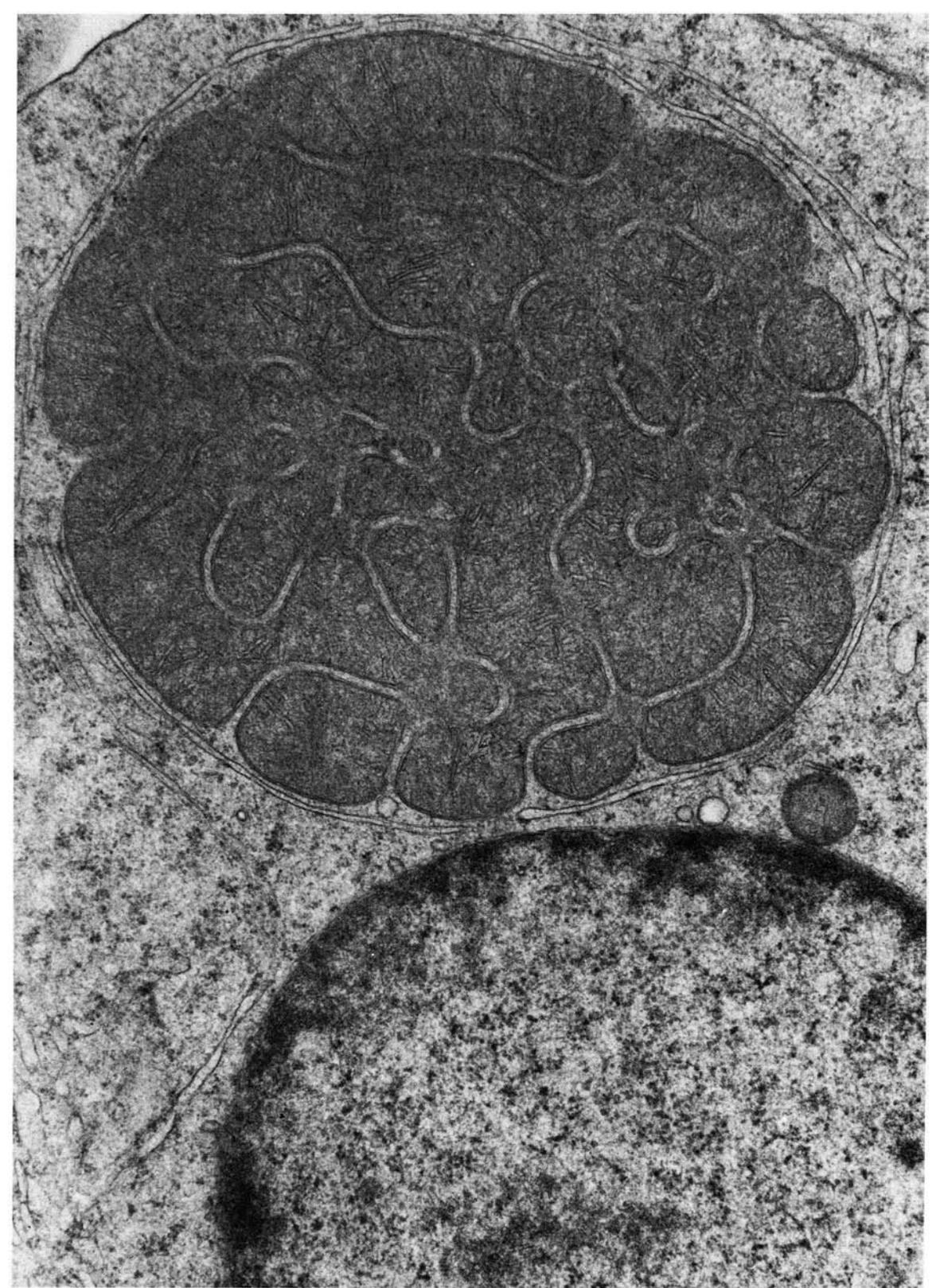

Figure 236

Figure 236. Fully formed nebenkern from an insect spermatid. (Micrograph courtesy of David Phillips.)

VARIATIONS IN
INTERNAL STRUCTURE

The mitochondria of brown adipose tissue are exceedingly numerous and have an extensive internal membrane surface. The accompanying micrograph shows part of an adipose cell from the interscapular region of a bat collected near the end of its period of hibernation. The cell is depleted of lipid and most of the cytoplasm is occupied by large spherical mitochondria. The unusual number of mitochondria in this tissue and their abundant cristae are believed to be related to the high energy requirements for the synthesis of fat from carbohydrate. In the mitochondria of this organ, oxidation is incompletely coupled to phosphorylation. Therefore during arousal from hibernation, much of the energy from oxidation of fat is dissipated in the form of heat which helps raise the body temperature to normal.
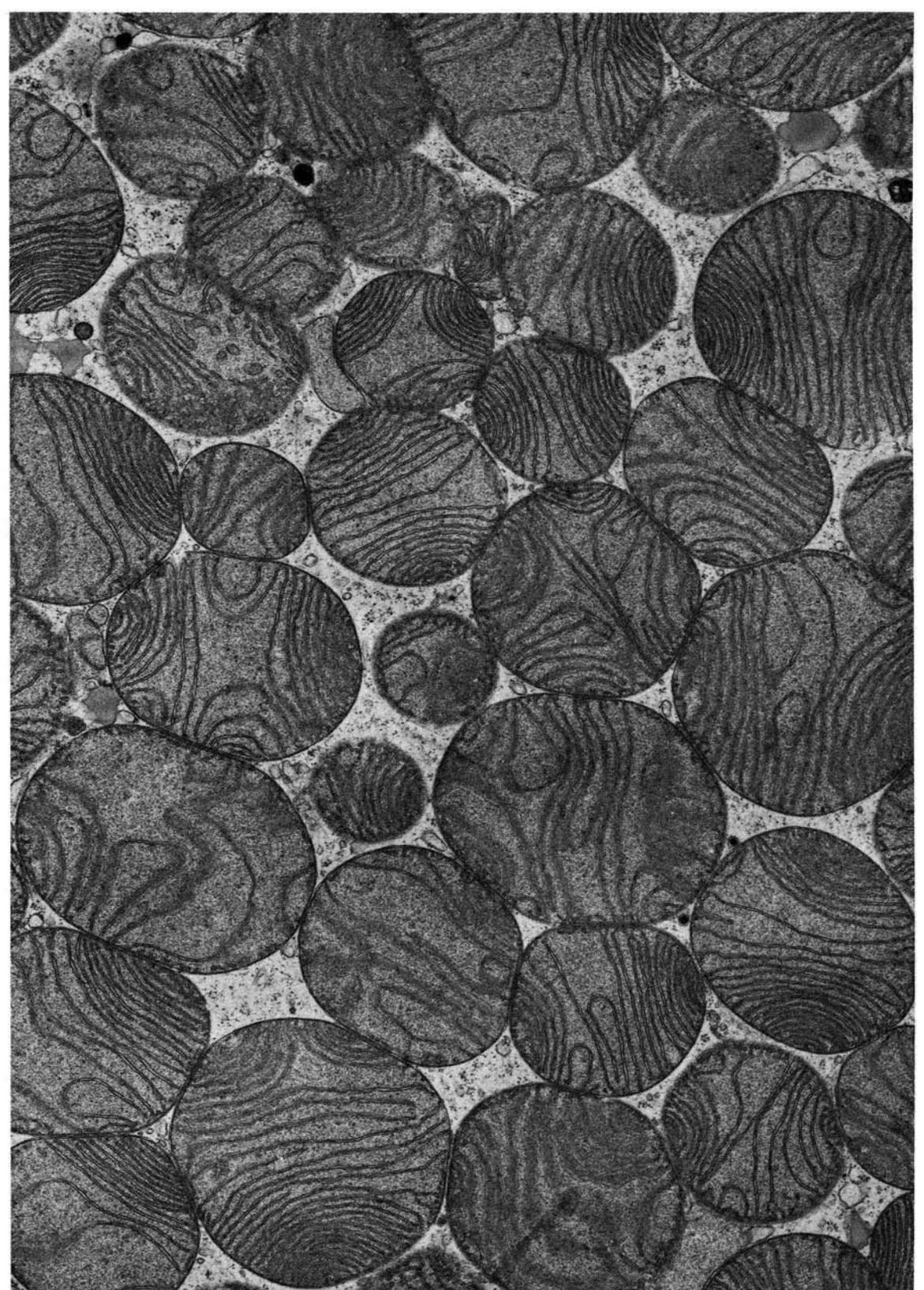

Figure 237. Interscapular brown adipose cell from the bat, *Eptesicus* **fuscus.**

Figure 237

Mitochondria of brown adipose tissue may attain a diameter of 5 to 7 μm. The large size of the three shown here can be appreciated by comparison with a sector of the cell nucleus at the left of the figure. Despite the large size of the mitochondria, the cristae traverse the entire width of the organelle. At high magnification it can be shown that the cristae have numerous small fenestrations near their junction with the inner membrane. These serve to prevent separation of the matrix into multiple isolated compartments.
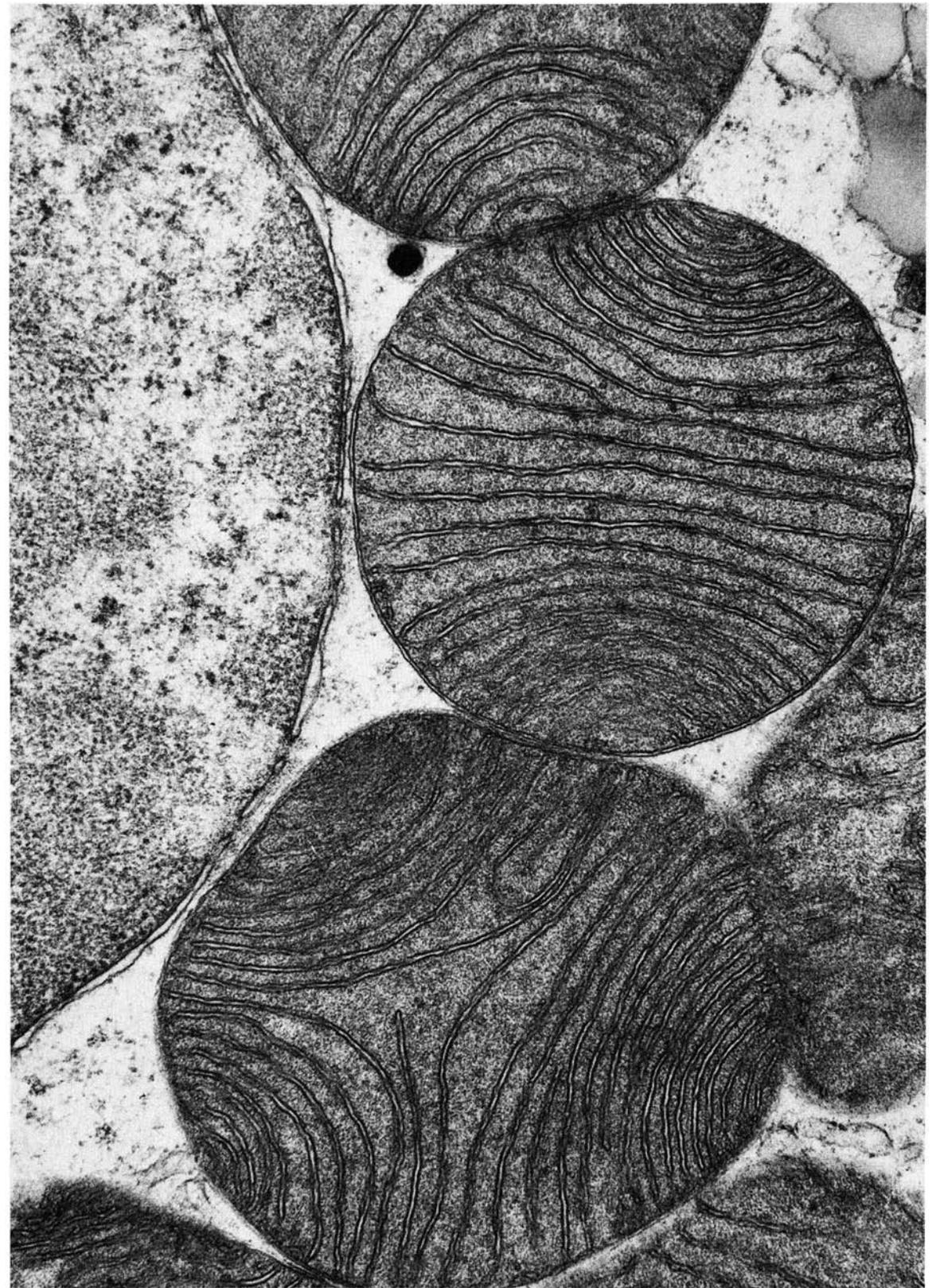

Figure 238

Figure 238. Mitochondria of brown adipose cell from a bat, *Myotis lucifugus.*

In mitochondria of well-preserved cells the cristae often exhibit sharp angulations of the membranes recurring at regular intervals along their length. The angulations frequently alternate in direction, occurring first on one leaf of the crista and then on the other. This gives the crista a zigzag course. Cristae with this angular configuration are commonly found in active tissues such as skeletal and cardiac muscle, but they have also been observed in a great variety of other cell types and in a wide range of species.

The examples illustrated in the accompanying figures are from cardiac muscle. The angular ridges on one crista are often opposite the concavities on the adjacent crista, but where this pattern is perturbed so that the angulations of neighboring cristae make contact they may coalesce to produce a honeycomb configuration of cristae outlining parallel cylindrical areas of matrix (see at arrows).
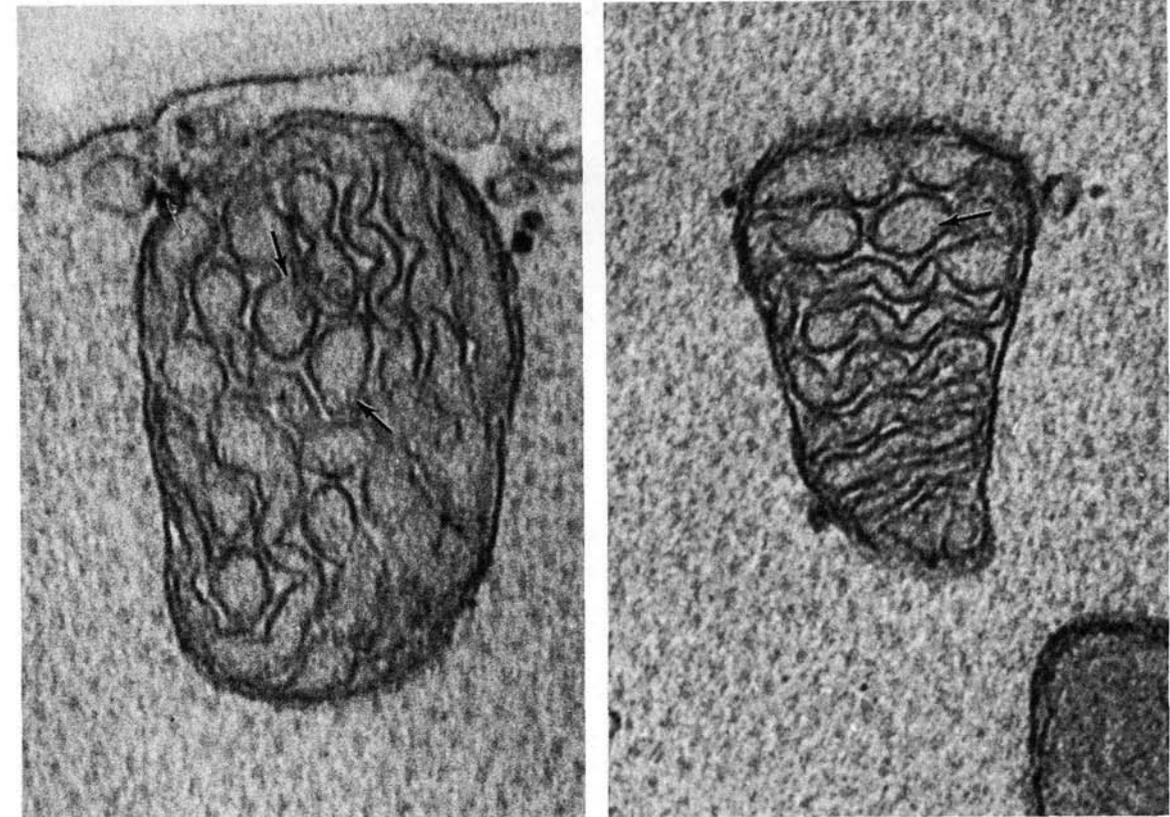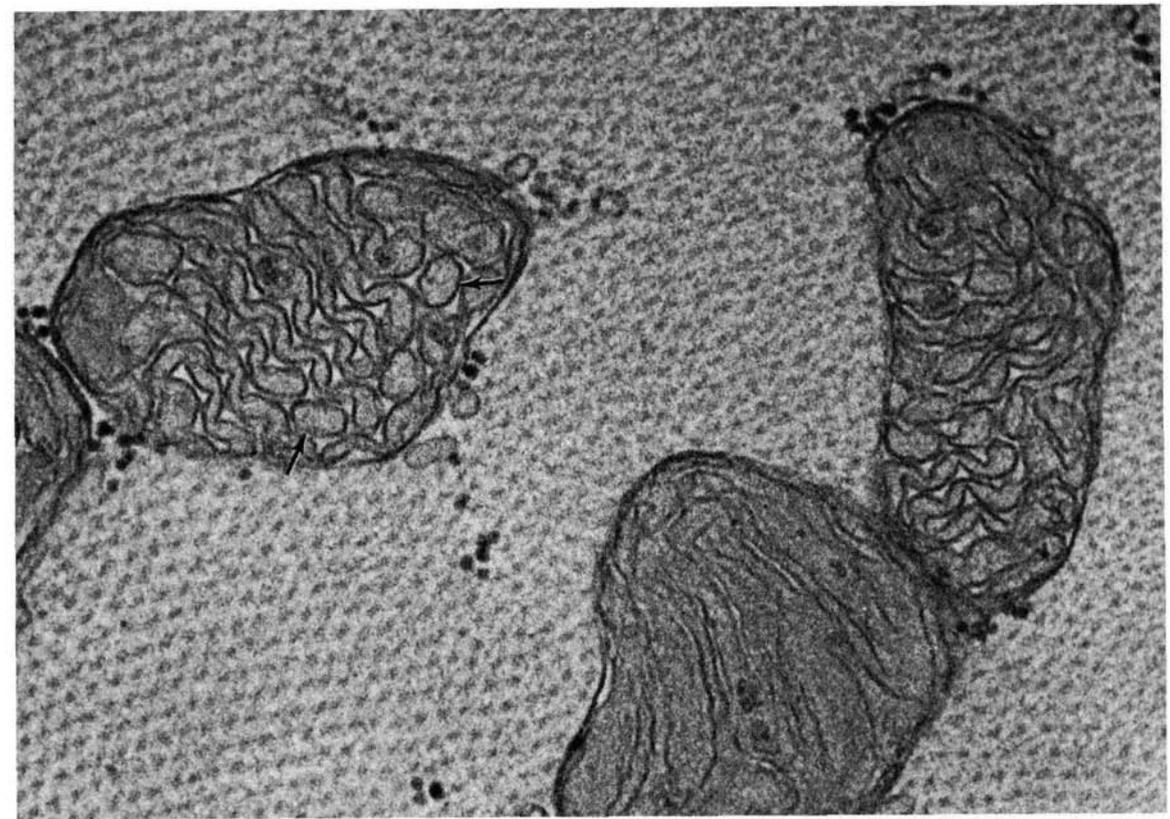

Figure 239. Mitochondria of cat ventricular papillary muscle.

Figure 239

A thin fold of the inner membrane is the commonest configuration of the cristae mitochondriales. An alternative form, found in many protozoa and in certain organs of metazoa, is a slender villus or blind-ending tubule. Among mammalian tissues mitochondria with tubular cristae are frequently observed in steroid-secreting cells such as the Leydig cells of the testis, cells of the corpus luteum, and cells of the adrenal cortex. They are exceptionally well developed in the mitochondria of the adrenal cortex illustrated here. The interior of these long mitochondria is crowded with unbranched tubular cristae of uniform diameter, extending nearly the full width of the organelle. Their tubular form is evident in several places, indicated by arrows, where they have been cut transversely and present circular profiles.

This shape of the cristae is evidently specifically related to the biochemical function of the cells, for in fetal and neonatal life the mitochondria have the usual lamellar cristae but then develop tubular cristae as the cells become active in steroidogenesis. Lamellar cristae may persist in limited areas of the mitochondrion in the mature gland. One such area is marked by an X at the lower edge of the figure.

Figure *240*.   A portion of a cell from hamster adrenal cortex
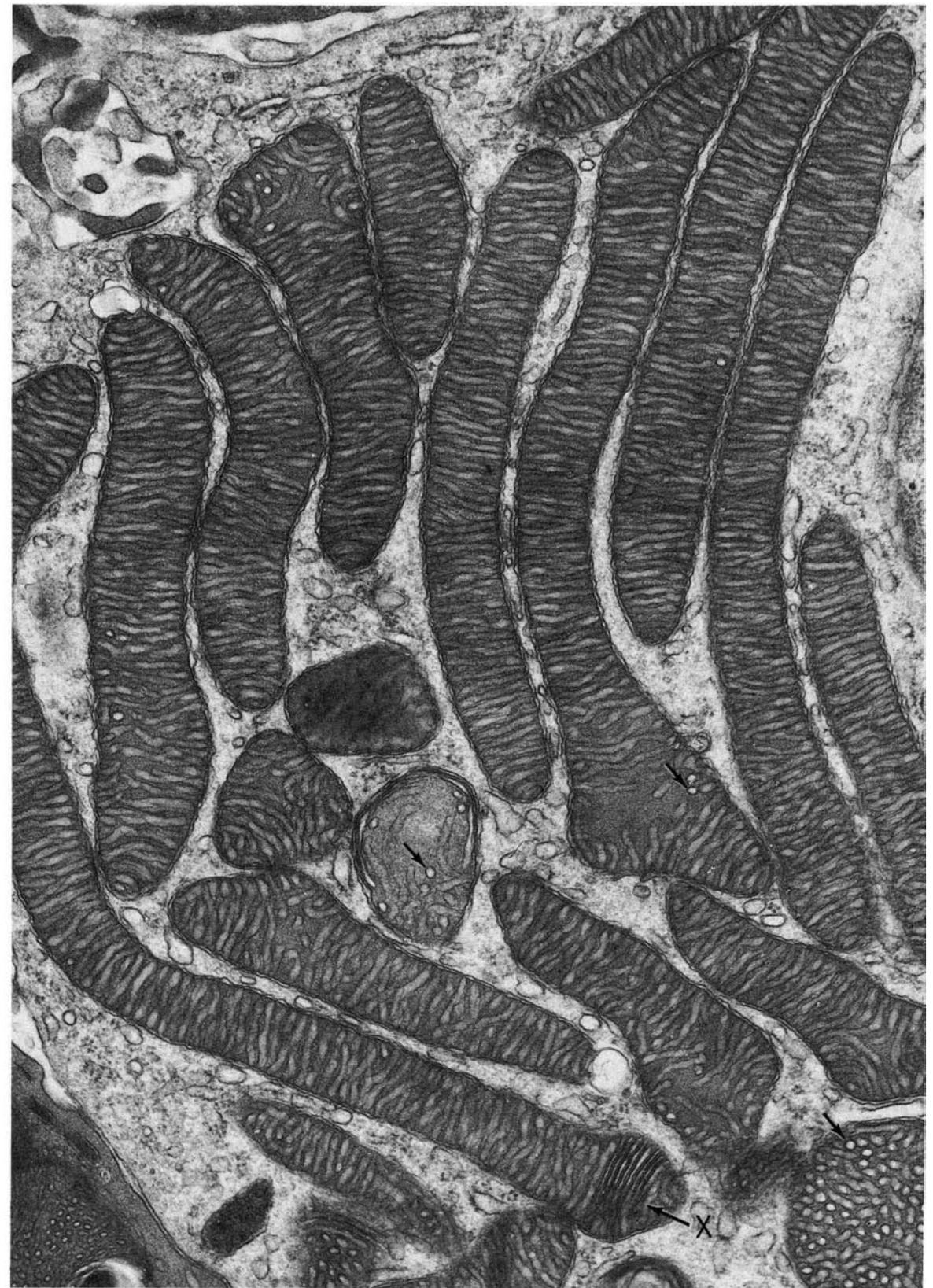
Figure *240*

Tubular cristae of protozoan mitochondria are usually fewer in number than those shown on the previous page but are otherwise very similar. Another form which they may take is illustrated by the mitochondria of the Singh amoeba on the facing page. Instead of behaving as blind-ending simple tubules, they branch and anastomose to form an irregular three-dimensional network.

Figure *241.*   Mitochondria of Singh amoeba. (Micrograph courtesy of Tom Pollard.)
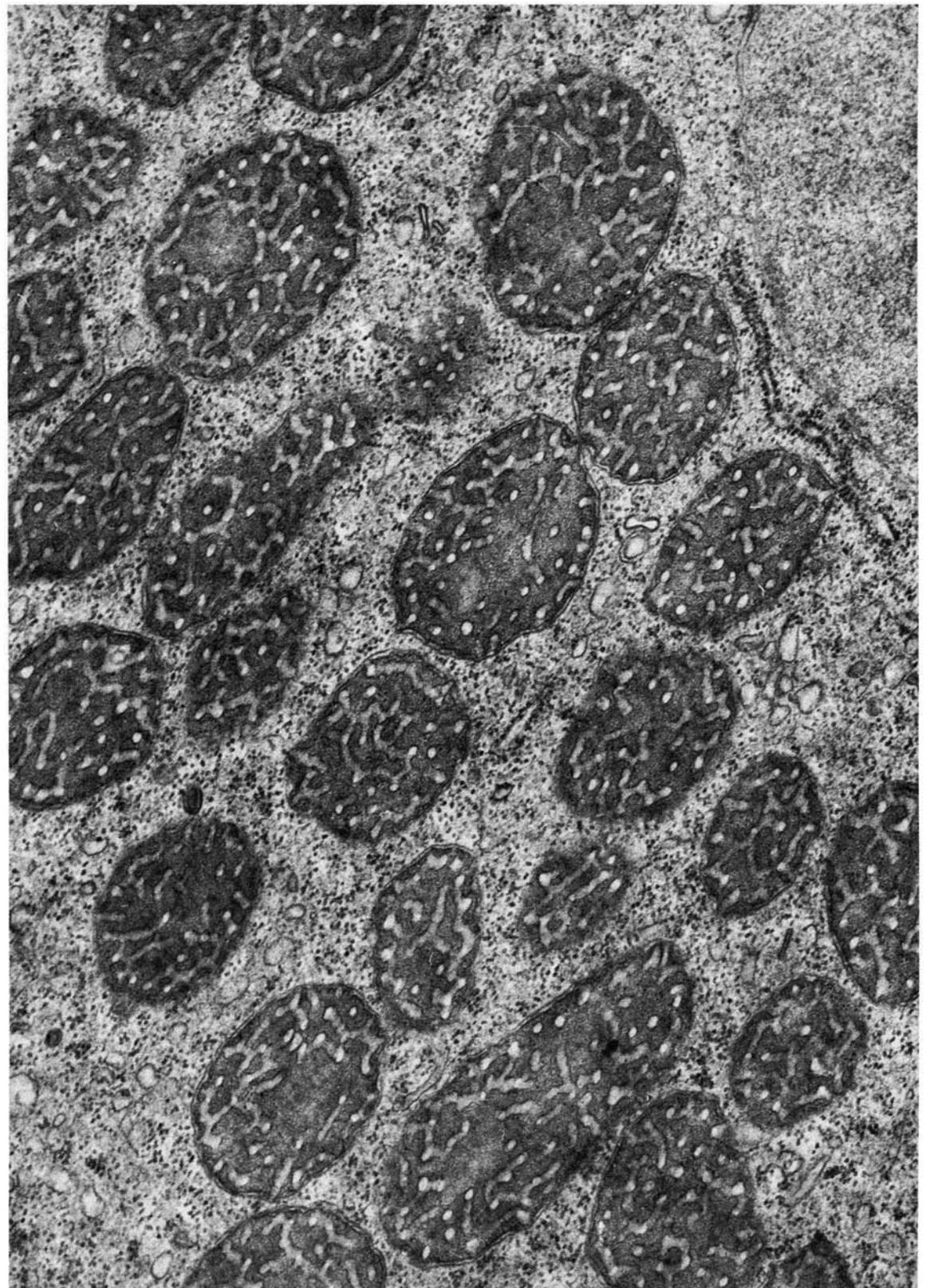
Figure *241*

Two forms of tubular cristae are presented here for comparison at higher magnification. The small granules in the dense matrix in the lower figure are believed to be mitochondrial ribosomes. This micrograph shows that they are smaller than the ribosomes in the surrounding cytoplasm.

Figure 242.   Mitochondria from the zona fasciculata of hamster adrenal cortex.

Figure 243.   Mitochondria from Singh amoeba (Courtesy of Tom Pollard.)

Figure 242, upper   Figure 243, *lower*

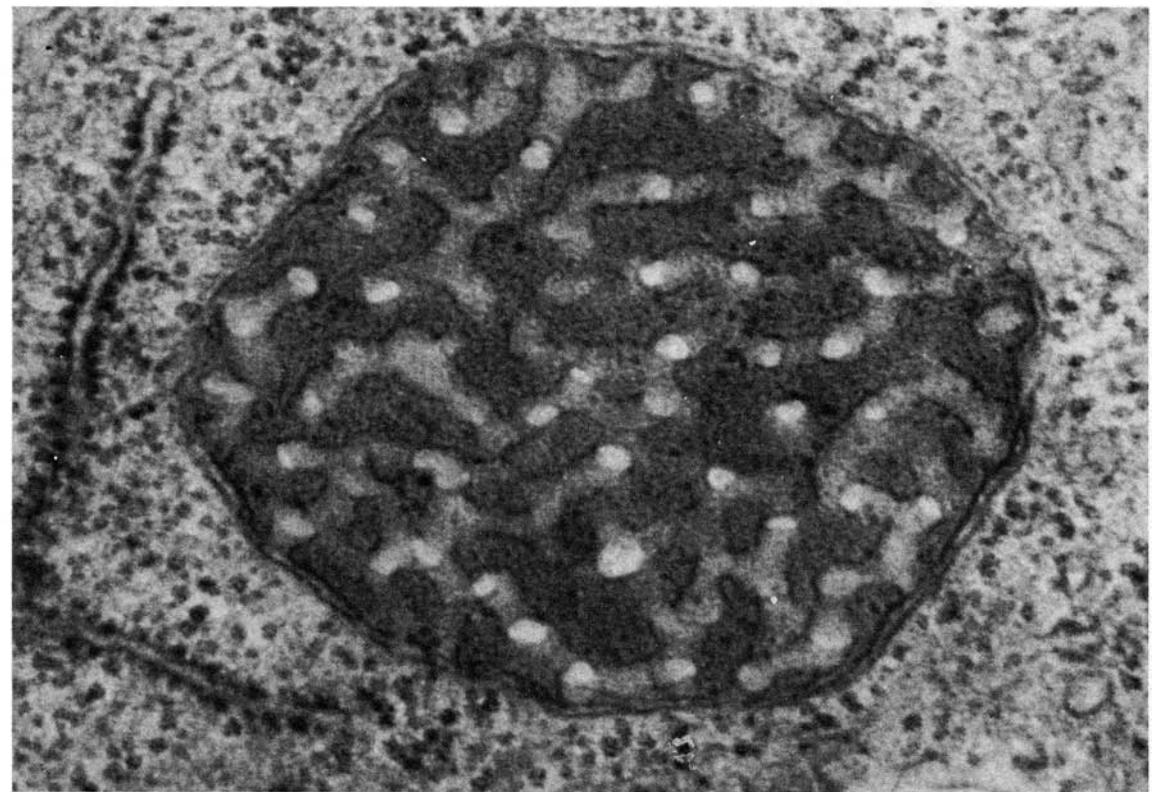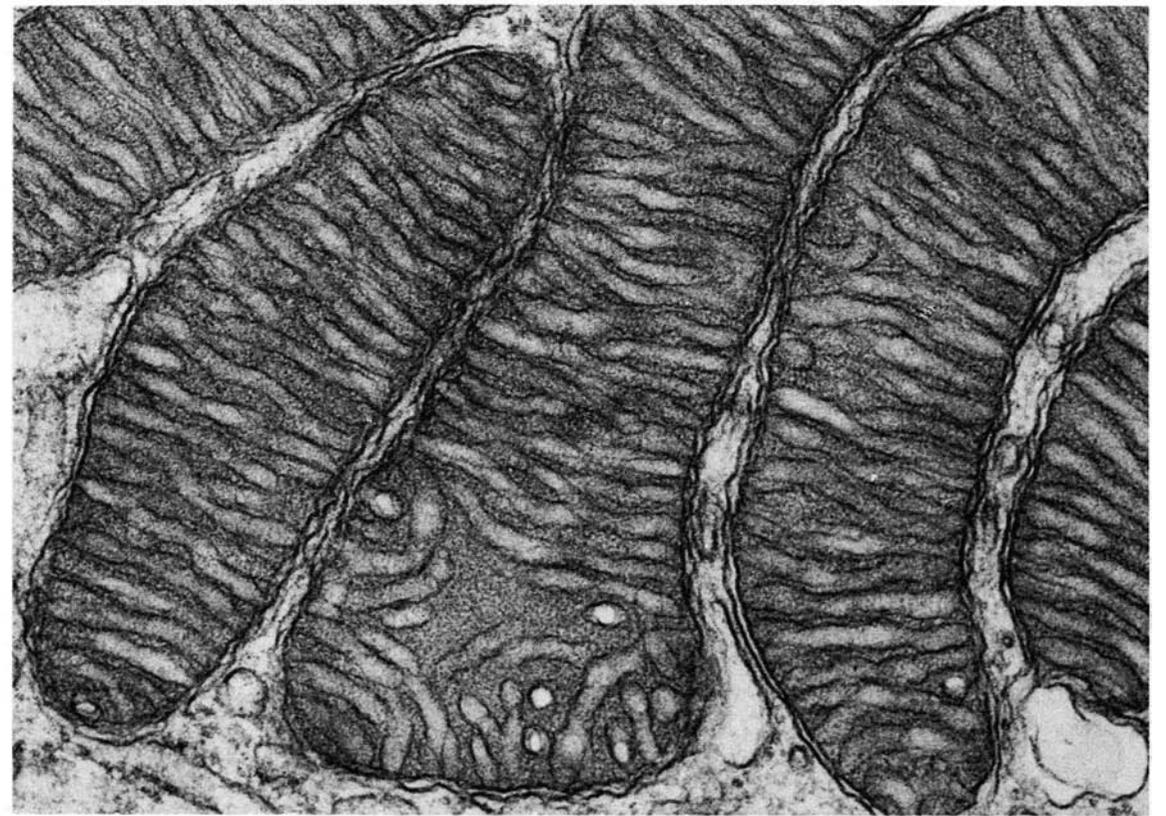In contrast to the irregular network of anastomosing tubular cristae previously illustrated, highly ordered three-dimensional lattices of interconnected tubules are regularly observed in mitochondria of *Amoeba proteus* and rarely in certain tissues of vertebrates as illustrated here.

As a rule, differences in size, external form, and internal organization are relatively slight among mitochondria of the same cell. There are, however, examples of marked changes in structure during functional differentiation of some cell types, and not all mitochondria change their form at the same rate. In the adrenal cortex of fetal or neonatal rats the mitochondria have lamellar cristae, but as the cells differentiate either in *vivo* or in organ culture the prevailing form of the cristae becomes tubular (Kahri, 1970). Similarly, immature supporting cells of *Xenopus* testis have mitochondria with conventional internal structure, but as they become mature Sertoli cells, the number of cristae increases and some of the mitochondria acquire the unusual internal structure which is depicted in the accompanying figures (Kalt, 1974). There are multiple parallel fenestrated layers of cristae that are interconnected by regularly arranged tubular elements. The resulting three-dimensional lattice presents varying patterns in different planes of section. These peculiar mitochondria occur together with others of orthodox configuration in the same cell.
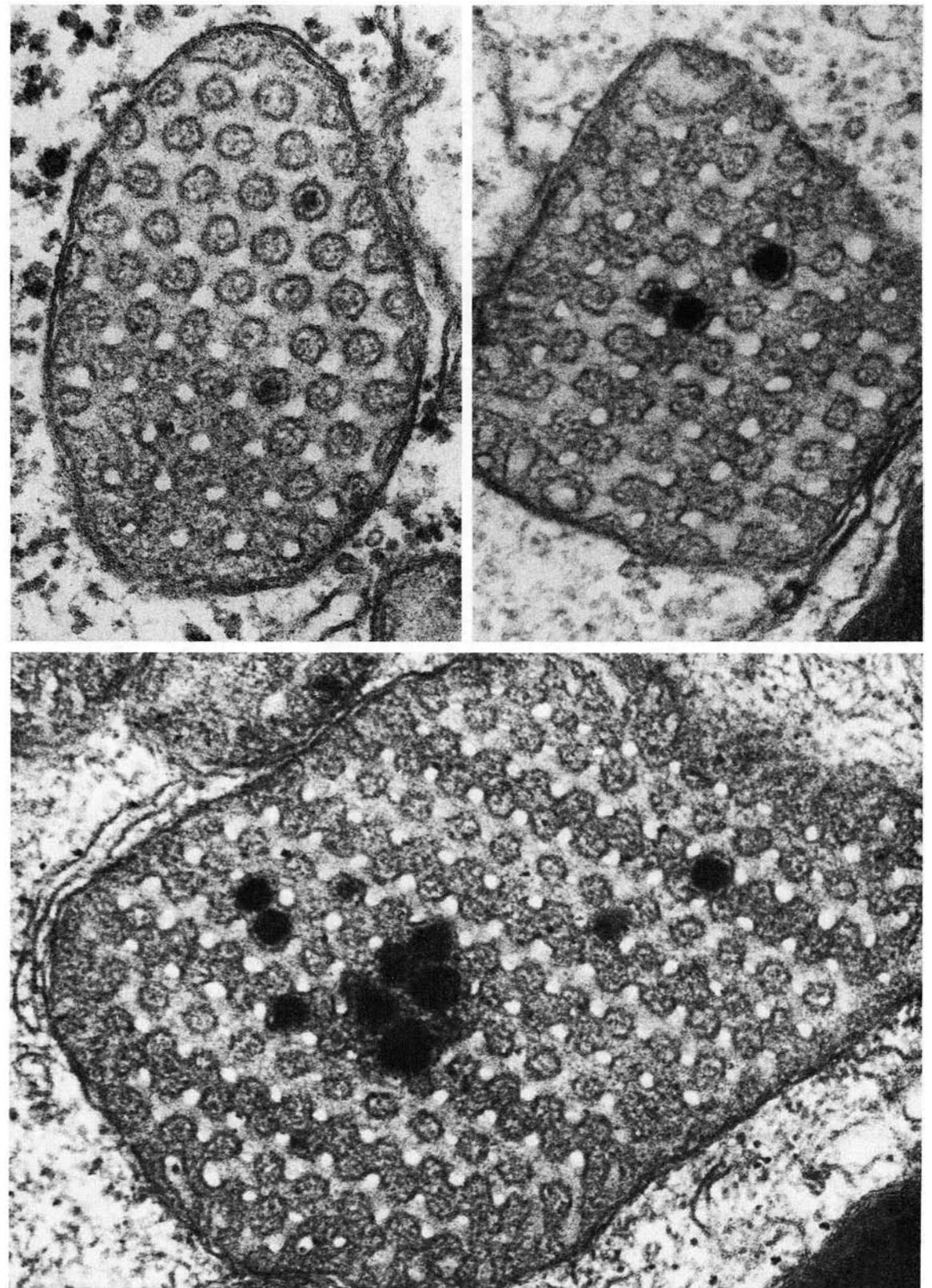

Figure *244.* Mitochondria from Sertoli cells of Xenopus in various planes of section. (Micrograph courtesy of Marvin Kalt, Anat. Rec. 182: 53, 1974.)

Figure *244*

One of the most striking and puzzling configurations of mitochondrial cristae is prismatic tubules that are invariably triangular in cross section. These were first described in muscle (Revel et al., 1963) but have since been found sporadically in many tissues and many species. They were originally interpreted as an unusual and extreme expression of the tendency of the membranes of lamellar cristae to develop angulations at regular intervals along their length. It was later pointed out that in mitochondria with prismatic cristae in the mammalian nervous system the intervening matrix shows a very regular pattern of dots with a center-to-center space of 15 nm. It was suggested therefore that the triangular shape might be imposed upon tubular cristae by hexagonal packing of filamentous elements in a paracrystalline matrix. The densities in the matrix of the mitochondrion of the facing page are seen only faintly, but the evenly spaced triangular profiles of the cristae are well shown and some at the periphery are seen to be in continuity with the inner membrane.

The large spherical mitochondria of rat adrenal cortex, shown in the lower figure, are often described as having vesicular cristae but closer examinaton reveals that they are probably tubules with alveolar branches (see at arrows). In section, both the tubules and their branches have circular profiles that have been erroneously interpreted as vesicles floating free in the matrix.
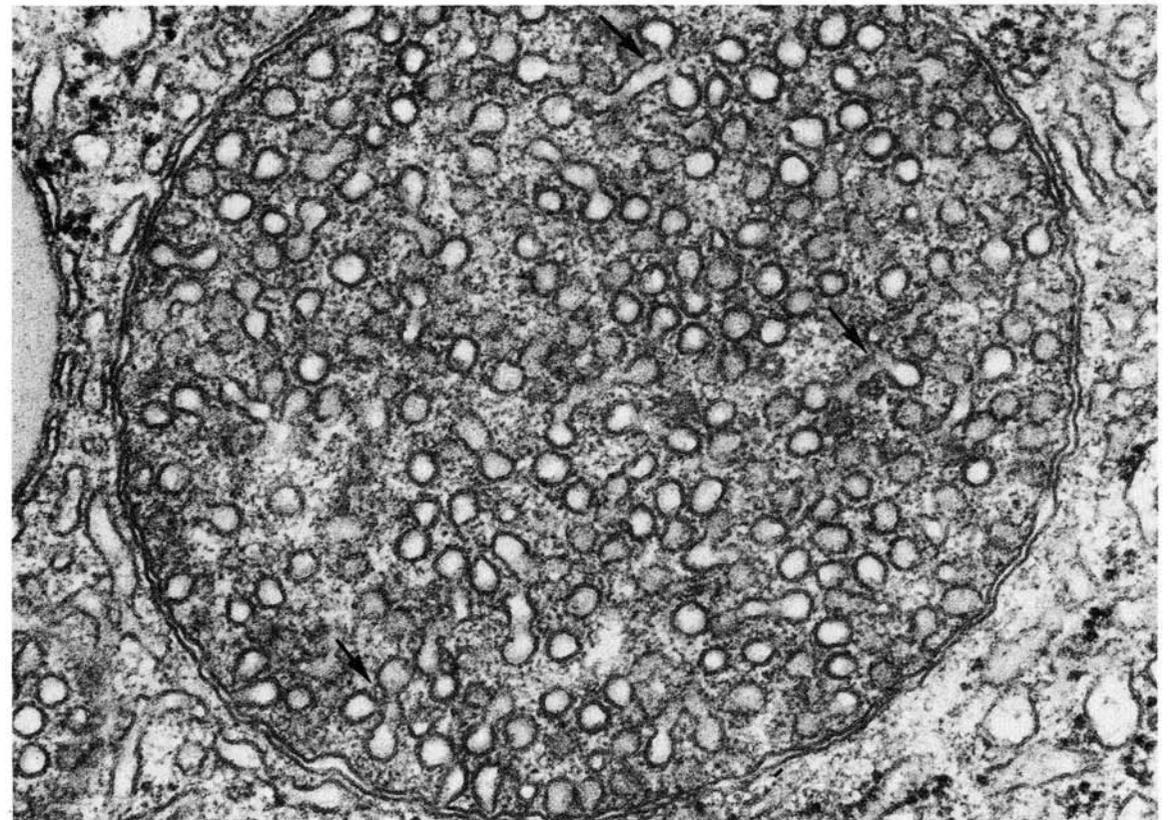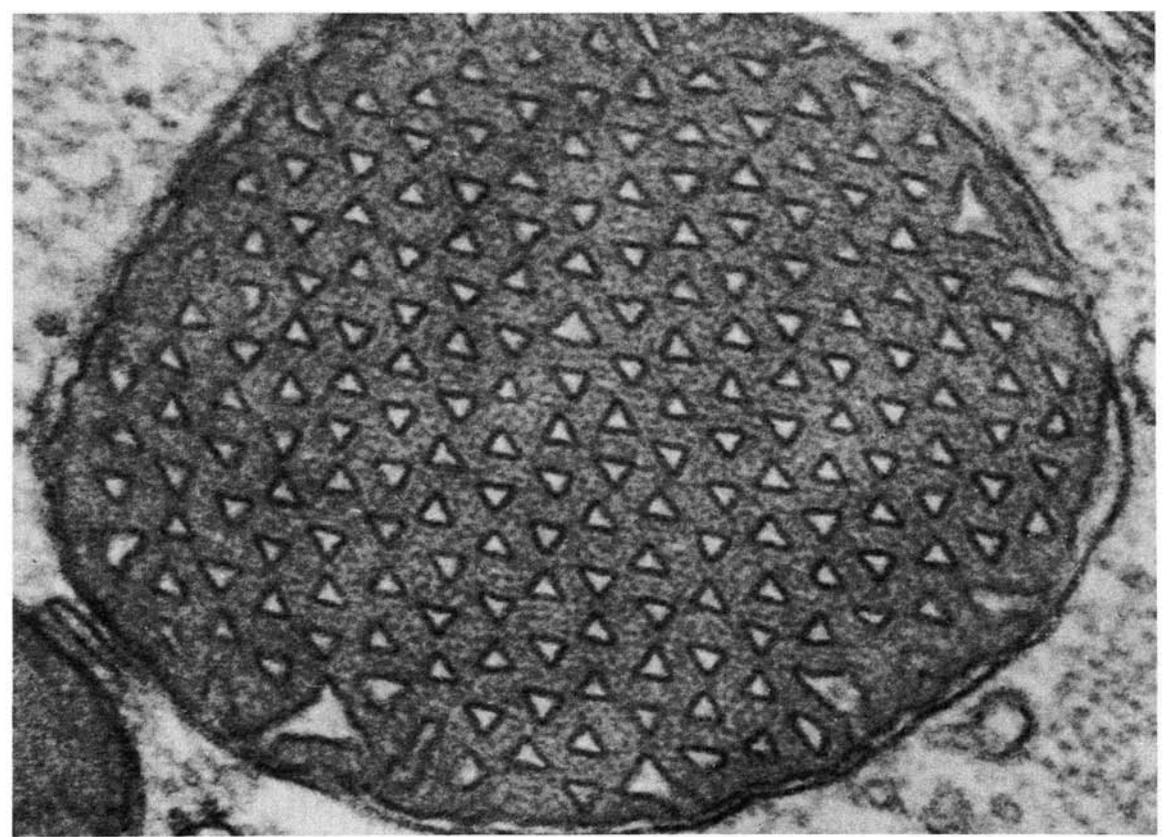

Figure *245.* Mitochondrion from an astrocyte of hamster brain. (Micrograph courtesy of K. Blinzinger, J. Cell Biol 25: 293, 1965.)

Figure *246.* Mitochondrion from rat adrenal cortex. (Micrograph courtesy of Daniel Friend.)

Figure *245, upper* **Figure** *246, lower*

In the majority of cell types the range of variation in mitochondrial size and shape is relatively narrow, but in a few cell types they exhibit an unusual degree of pleomorphism. Prominent among these are steroid-secreting cells such as those in the interstitial tissue of the testis. Reproduced here is a Leydig cell showing considerable variation in mitochondrial shape. Much more extreme examples of size difference are not infrequent, with huge spherical mitochondria 6 to 7 μm in diameter adjacent to small elongate mitochondria 0.5 μm wide.

Figure 247. Leydig cell from the testis of *Citellus lateralis*. (Micrograph courtesy of Jeffrey Pudney.)

Figure **247**

Steroid-secreting cells also exhibit unusual diversity in the form of the cristae in their mitochondria. Those shown here, for example, have small alveolar cristae along the inner membrane, longitudinally oriented lamellar cristae (single arrows), and bundles of parallel tubules coursing in various directions in the matrix (double arrows).
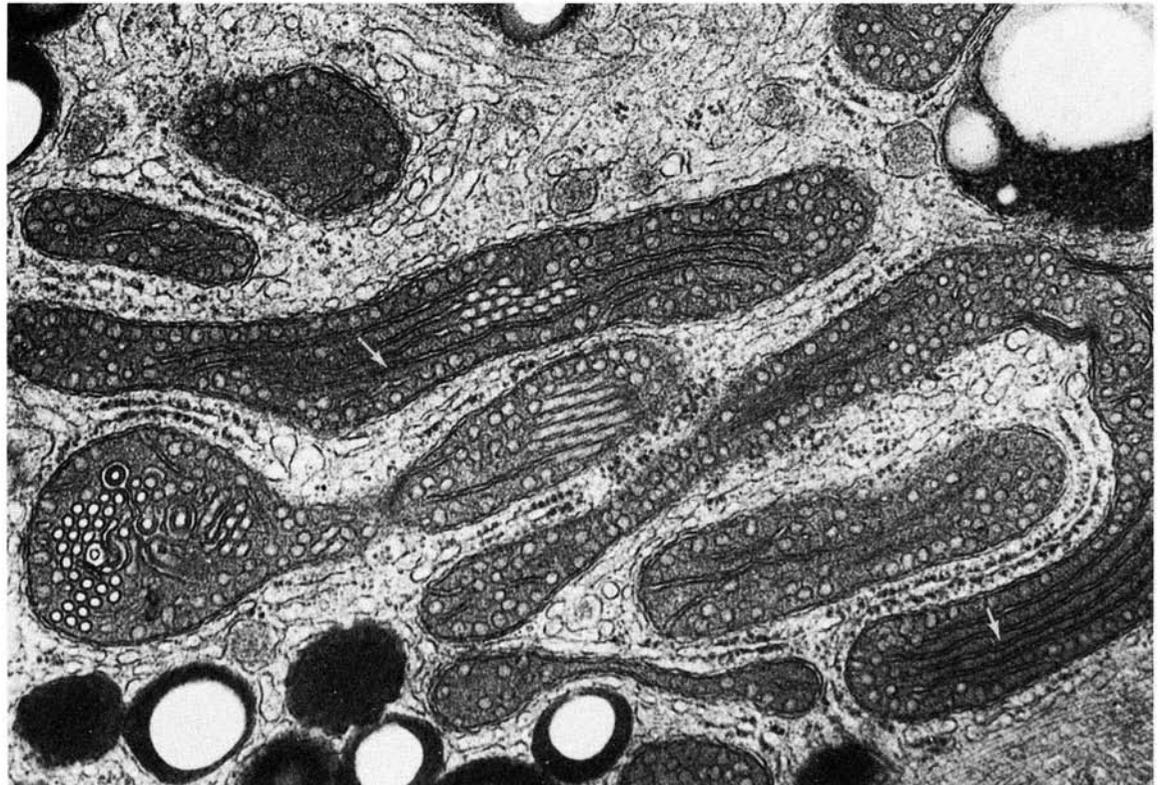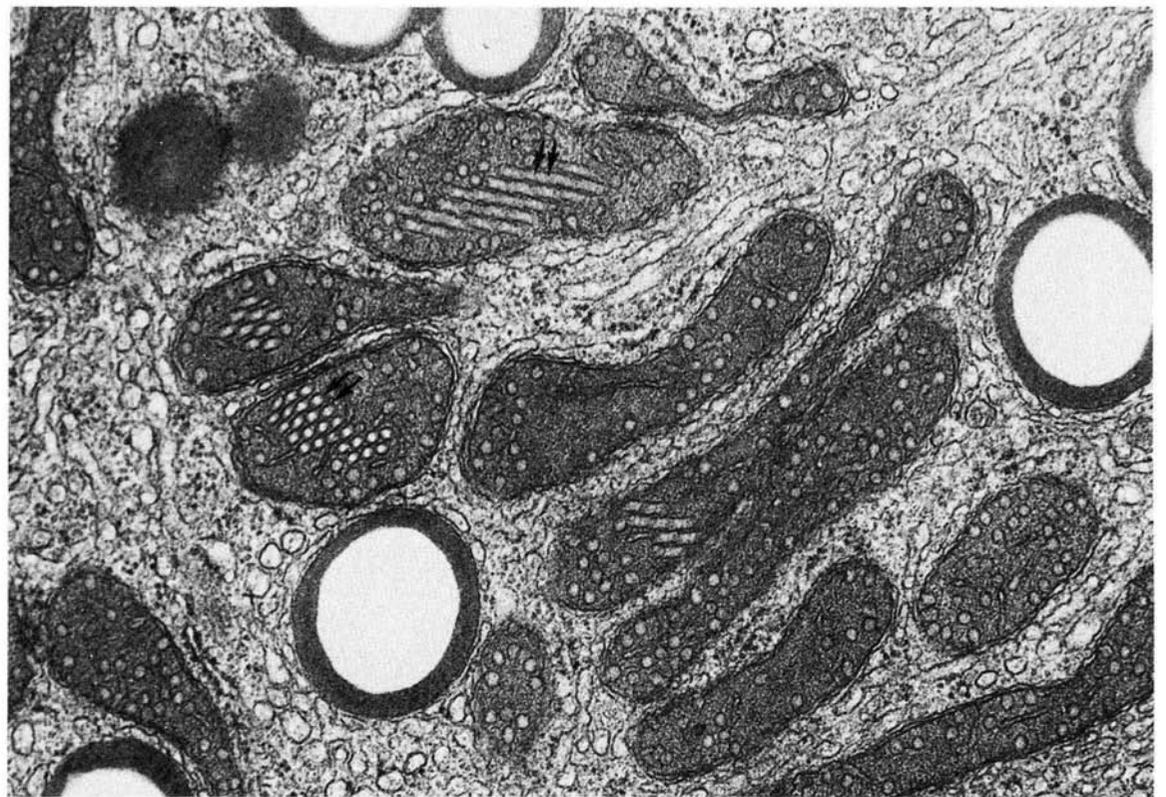
Figure *248.*   Mitochondria from testicular Leydig cells of the ground squirrel, *Citellus lateralis.* (Micrograph courtesy of Jeffrey Pudney.)

Figure *248,* upper *and lower*

The ability of mitochondria to change their internal structure in response to altered physiological demands is exemplified in the pseudobranch of fish. This organ is believed to have a functional relationship with the eye. In fish maintained under normal illumination or exposed to brief periods of darkness, the great majority of the mitochondria have the usual foliate cristae. However, when fish are kept in constant light for three to four weeks, a second category of tubular cristae appears in 70 to 90 per cent of the mitochondria. These are arranged in rows between the longitudinally oriented foliate cristae. When viewed in longitudinal section (see inset), they have the appearance of helically twisted tubules.
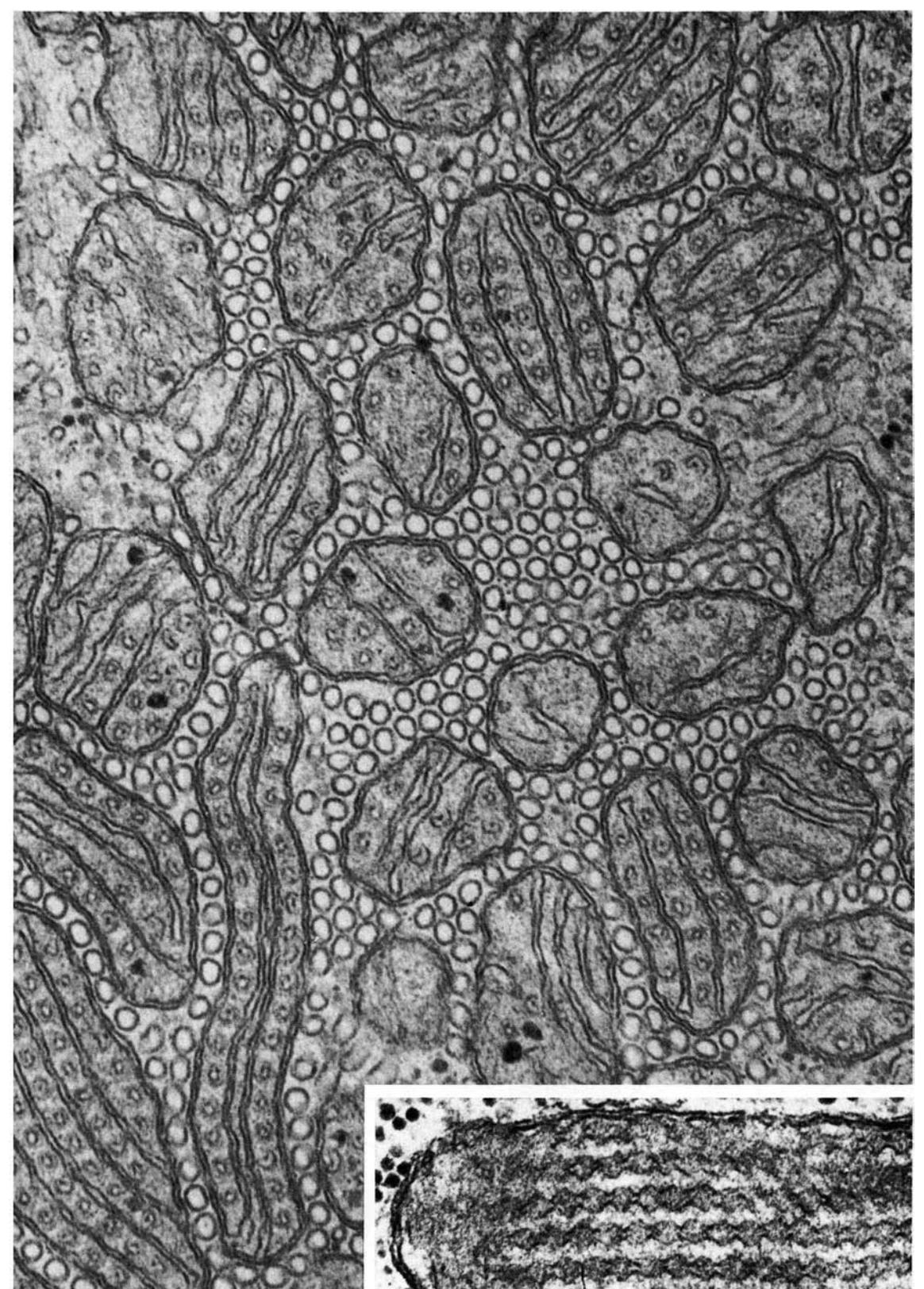

Figure 249.   Mitochondria from the pseudobranch of *Cyprinoden variegatus* exposed to constant light for four weeks. (Micrograph courtesy of Joseph Harb and Eugene Copeland.)

igure 249

MITOCHONDRIAL INCLUSIONS

In addition to the standard structural components of mitochondria, a great variety of inclusions have been described. These may be located within the matrix or within an expanded intracristal compartment. Some are of frequent occurrence and must be regarded as normal for the particular cell type, while others occur more rarely and may be manifestations of disordered mitochondrial metabolism or disease states.

Two examples of stored material accumulating in an expanded intracristal space are shown in the micrographs on the facing page. The upper figure is a mitochondrion from a frog oocyte containing a hexagonal crystal with a 7.4 nm periodicity. These intramitochondrial crystals are similar in form and lattice dimensions to yolk crystals in the oocyte cytoplasm. It is postulated that these form within mitochondria and are subsequently extruded in an outpocketing of the mitochondrial membranes which pinches off to leave an intact mitochondrion and a membrane-limited yolk crystal in the cytoplasm (Massover, 1971). These have been observed in several species of frog and are considered to be part of the normal process of vitellogenesis. To what extent, if any, the synthesis of yolk lipoprotein is directed by the mitochondrial genome is unknown.

The lower figure is a mitochondrion from a Purkinje fiber of cat heart which has accumulated a large amount of glycogen. The glycogen may either be in the form of alpha or beta particles. Such mitochondria are relatively uncommon, but various stages in glycogen accumulation can be found, from a few particles in one or two dilated cristae to large masses such as illustrated here. This is an unexpected finding in that the enzymes for glycogen production are not thought to be present in mitochondria.
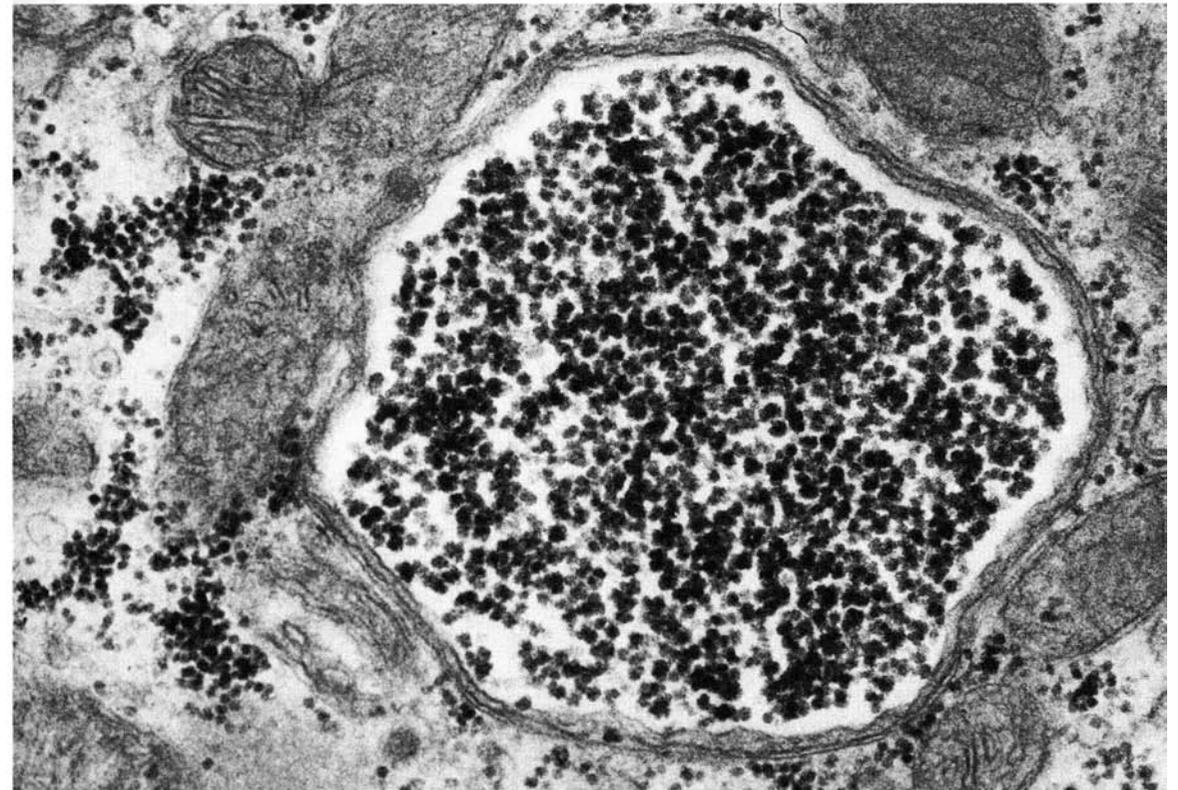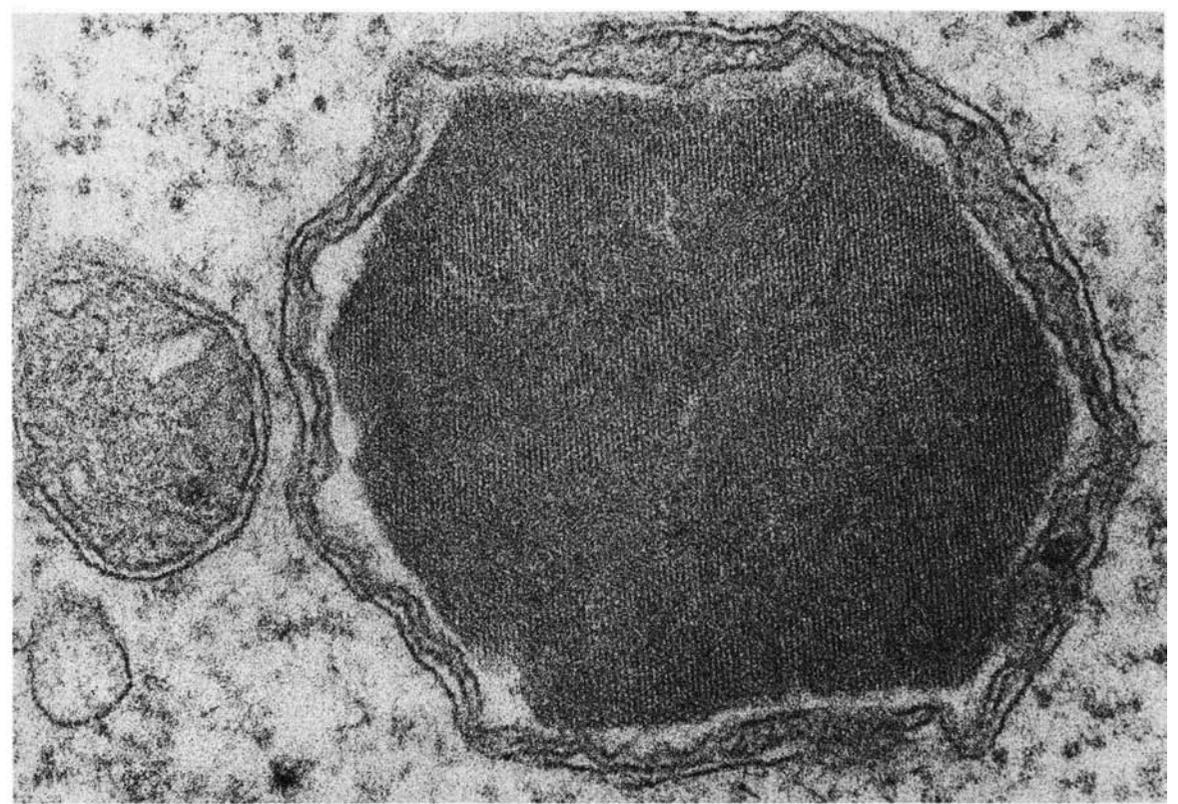
Figure **250**, *upper*   Figure **251**, *lower*

Figure **250.**   Mitochondrion from an ovarian oocyte of the bullfrog *Rana catesbiana.* (Micrograph courtesy of William Massover, J. Ultrastruct. Res. 36: 603–620, 1971.)

Figure **251.**   Mitochondria from conduction tissue of the cat myocardium.

During spermiogenesis in insects, the cristae, originally present in the individual mitochondria and later in the mitochondrial nebenkern, disappear and proteinaceous material is deposited in filaments that become closely packed in paracrystalline arrays. Crystals are a conspicuous feature of the mitochondrial derivatives of most insect sperm, but their size and shape vary greatly from species to species. In the accompanying micrograph, crystals are present in each of the two mitochondrial derivatives that flank the axoneme.
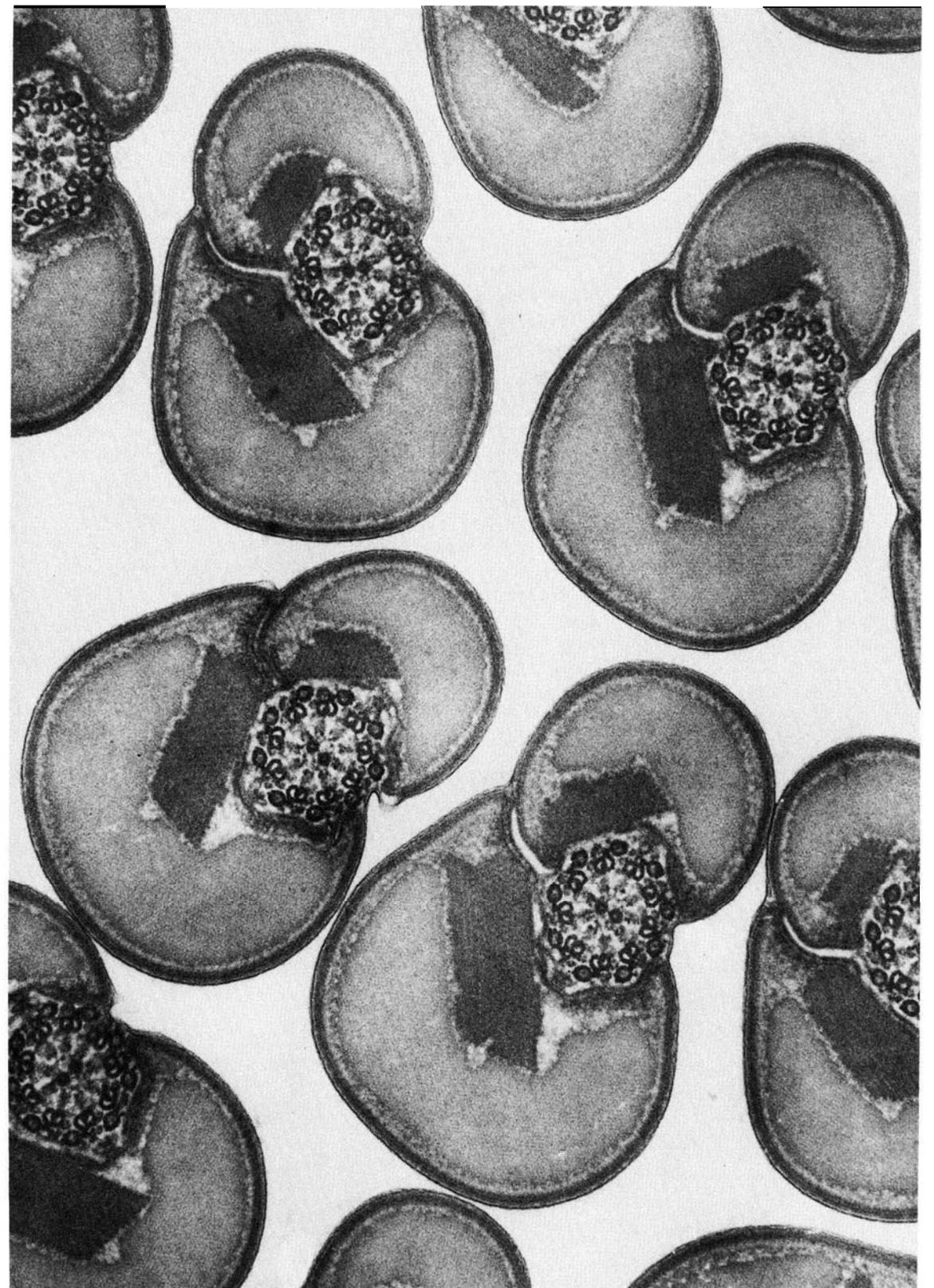

Figure 252. Mitochondrial derivatives in spermatozoa of the stilt bug, *Jalysus*. (Micrograph courtesy of David Phillips.)

Figure 252

NUMBERS AND DISTRIBUTION

The numbers of mitochondria vary over a wide range depending upon the size of the cell and its energy requirements. Reliable estimates have been made for only a few cell types. Values for the liver are **800** to **2000;** oocytes of echinoderms **150,000;** and in the giant amoeba *Chaos chaos* as many as half a million.

They may be randomly dispersed in the cytoplasm or concentrated around the cell center as in the micrograph on the facing page. They exhibit a tendency to aggregate around deposits of substrate such as lipid droplets. They are often specifically located in very close proximity to sites of high energy utilization. A number of examples of such localization will be illustrated in the pages that follow.

The transport and control mechanisms responsible for mobilizing mitochondria at particular sites in the cell are not understood, but there are studies that seem to implicate microtubules in directing their movements. In nerve axons the proximity of mitochondria to microtubules is greater than would be expected from a random distribution and small cross-links between them have been described. It was suggested that the organelles might be moved along the tubules by a sequential making and breaking of such linkages (Smith, Jarlfors and Cameron, **1977).** In studies on several cell types in tissue culture by immunofluorescence techniques, mitochondria were shown to be arranged along bundles of microtubules. Disruption of microtubules with colchimid caused a redistribution of mitochondria (Heggeness et al., **1978).** These observations are suggestive of direct or indirect involvement of microtubules in mitochondria1 movements but shed little light upon the mechanism.
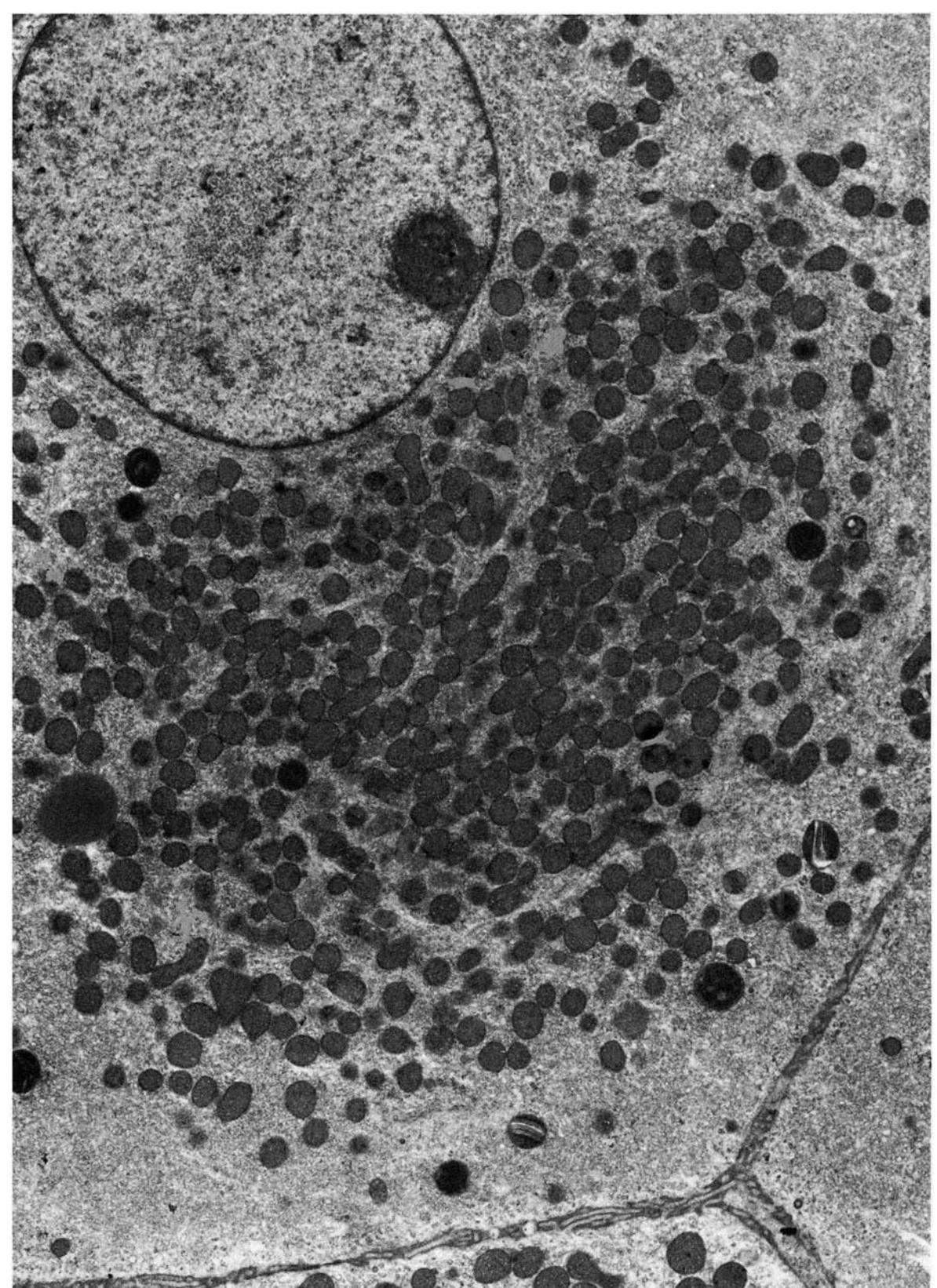

Figure 253.   Prominent clustering of mitochondria in the Golgi region of a Leydig cell from the domestic boar, *Sus scrofa*.

Figure 253

An example of localization of mitochondria in close proximity to a source of substrate is seen in the relationship of mitochondria to lipid droplets. They may partially surround small lipid droplets and when the droplets are very large, as in this liver cell from a fasted hamster, several mitochondria may be closely applied to its surface. The fatty acid oxidases of the mitochondria are involved in the metabolism of triglycerides, and this intimate relationship no doubt facilitates transport of substrate to the enzymes.

Figure 254.   Portion of a liver cell from a hamster fasted for four days.

Figure 254

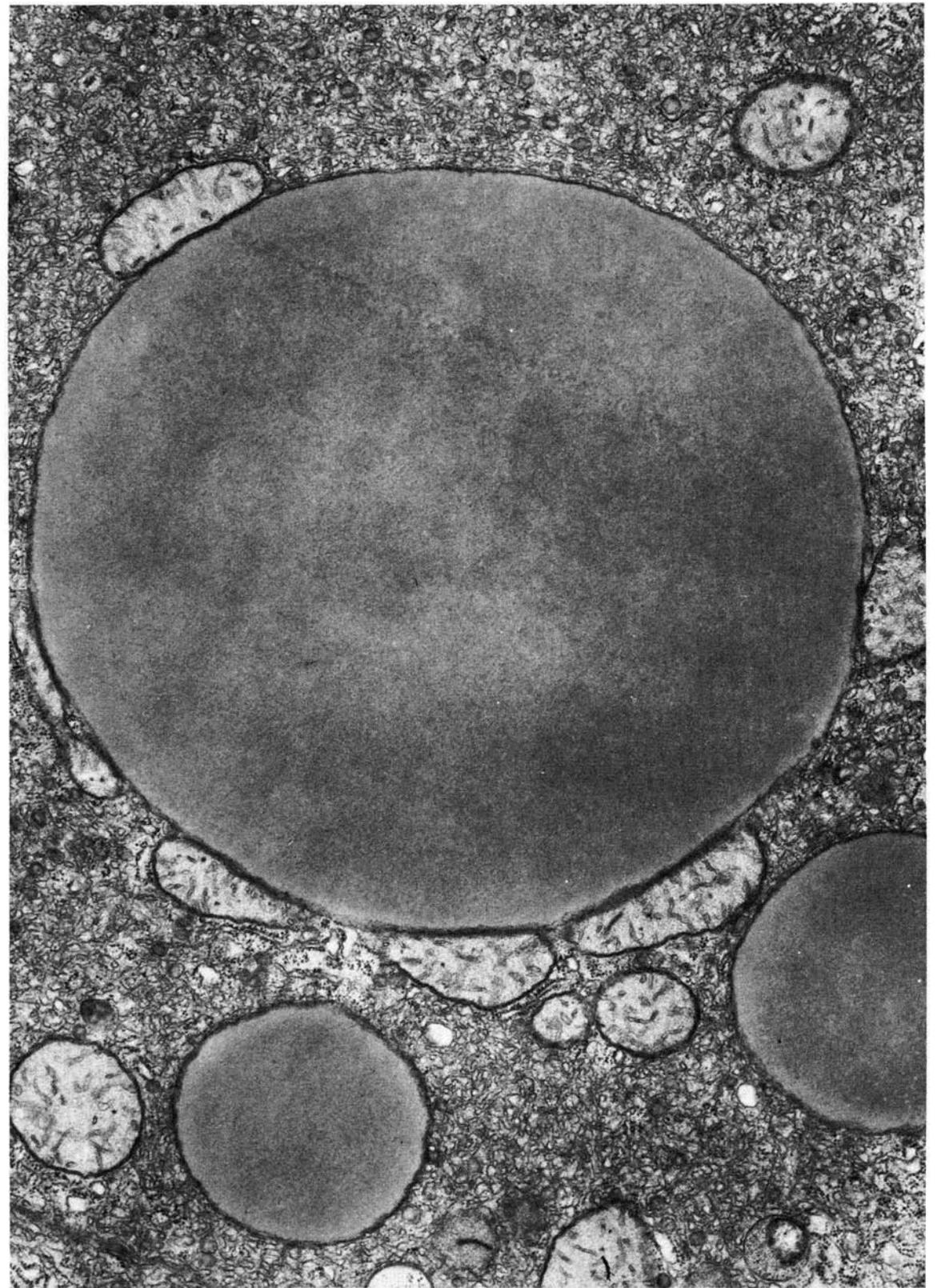Energy for muscle contraction is derived mainly from metabolism of carbohydrate, but under certain conditions lipid also serves as an energy source. In cardiac muscle, lipid droplets are often partially enveloped by mitochondria in the interstices between myofibrils.
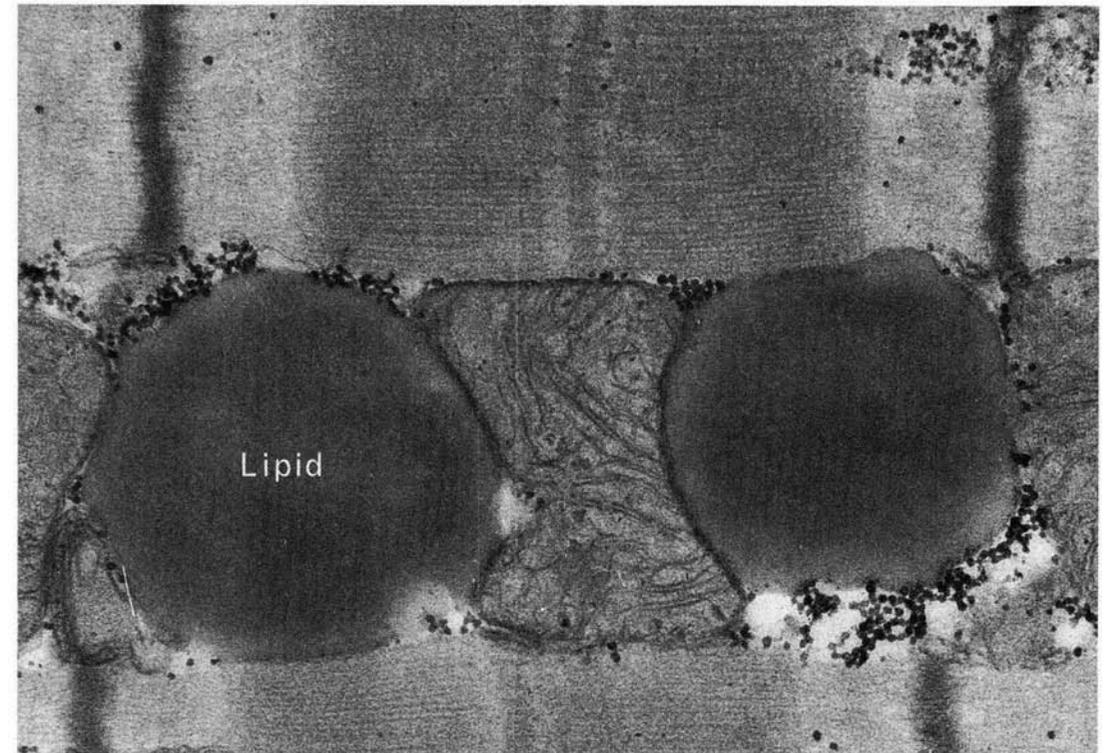

Figures 255 and 256. Mitochondria-lipid relationships in papillary muscle of the cat heart.
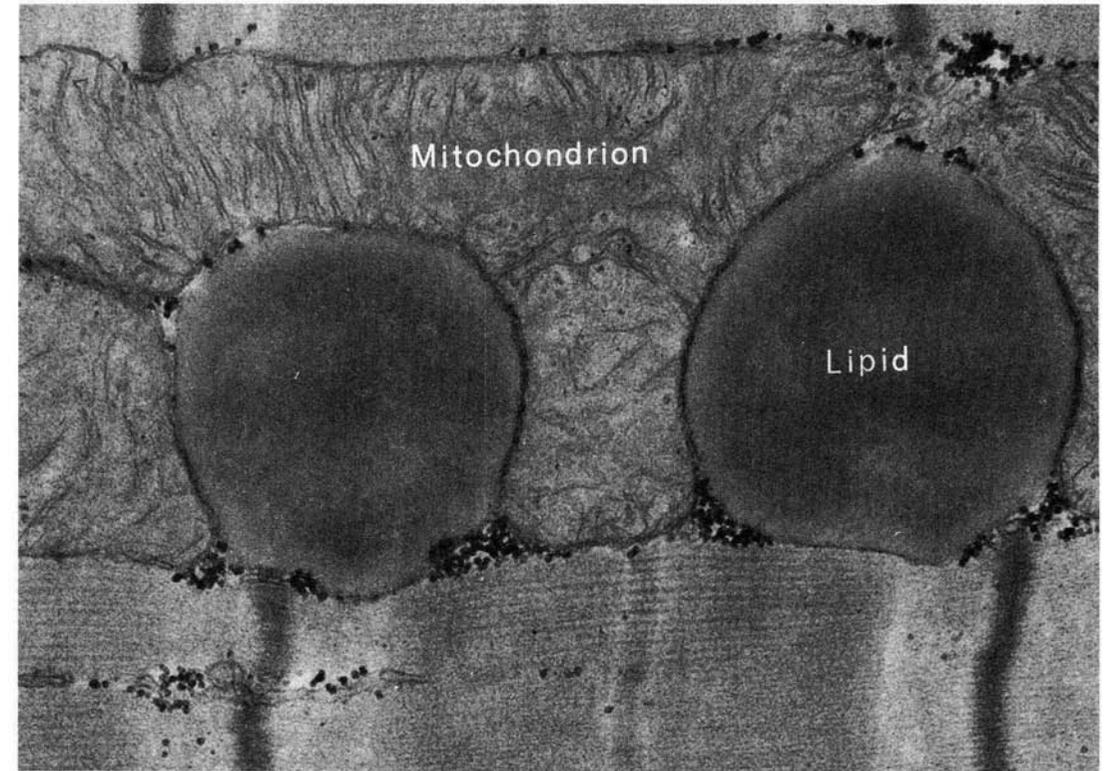
Figure 255, upper   Figure 256, *lower*

One of the most striking local accumulations of mitochondria in the body is in the inner segment of the rods and cones of the retina. The closely packed mitochondria are very long and have a complex pattern of curved cristae. The accompanying micrograph shows the inner segment of a cone, flanked by the inner segments of two rods. The mitochondria are several micrometers in length and those of the cone appear to have a denser matrix. The molecular events related to energy transduction and amplification in the photoreceptors are still poorly understood, but the remarkable concentration of mitochondria near the photosensitive region of the cell indicates that the process has high energy requirements.

Figure 257. Electron micrograph of photoreceptors in human retina. (Micrograph courtesy of Toichiro Kuwabara.)
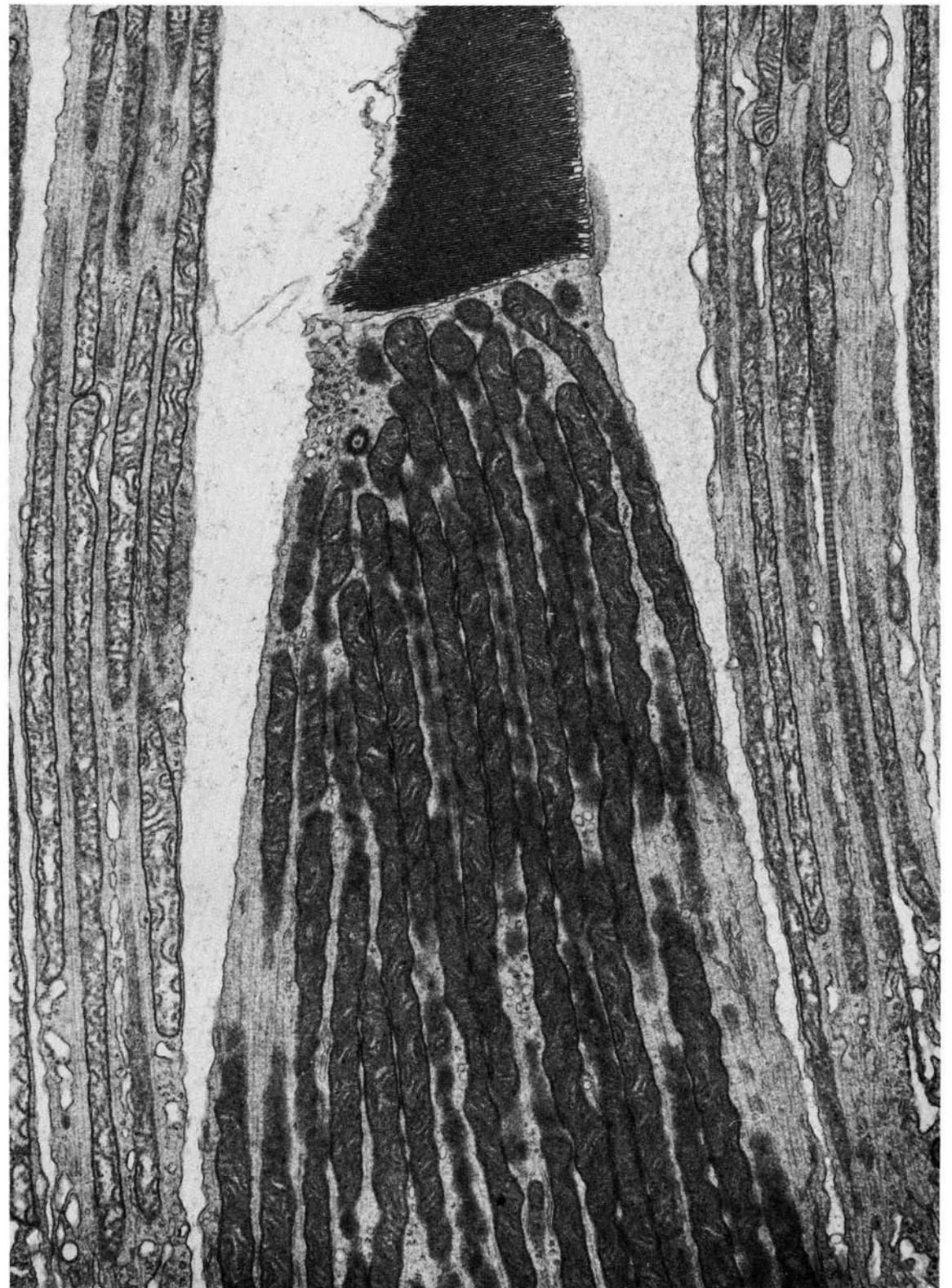
Figure 257

In epithelia engaged in active transport of ions, numerous mitochondria at the cell base are lodged in narrow compartments or interdigitating processes of neighboring cells in such a way that the mitochondria are brought into very close relationship to a large area of the cell surface. Details of the process are not well understood, but it is assumed that this arrangement provides a favorable juxtaposition of the energy source to the elaborately convoluted cell surface in which the active transport mechanism resides.

The micrograph on the facing page includes a vertical section of the base of the epithelium of the distal convoluted tubule in the kidney and a section of the subjacent peritubular capillary.
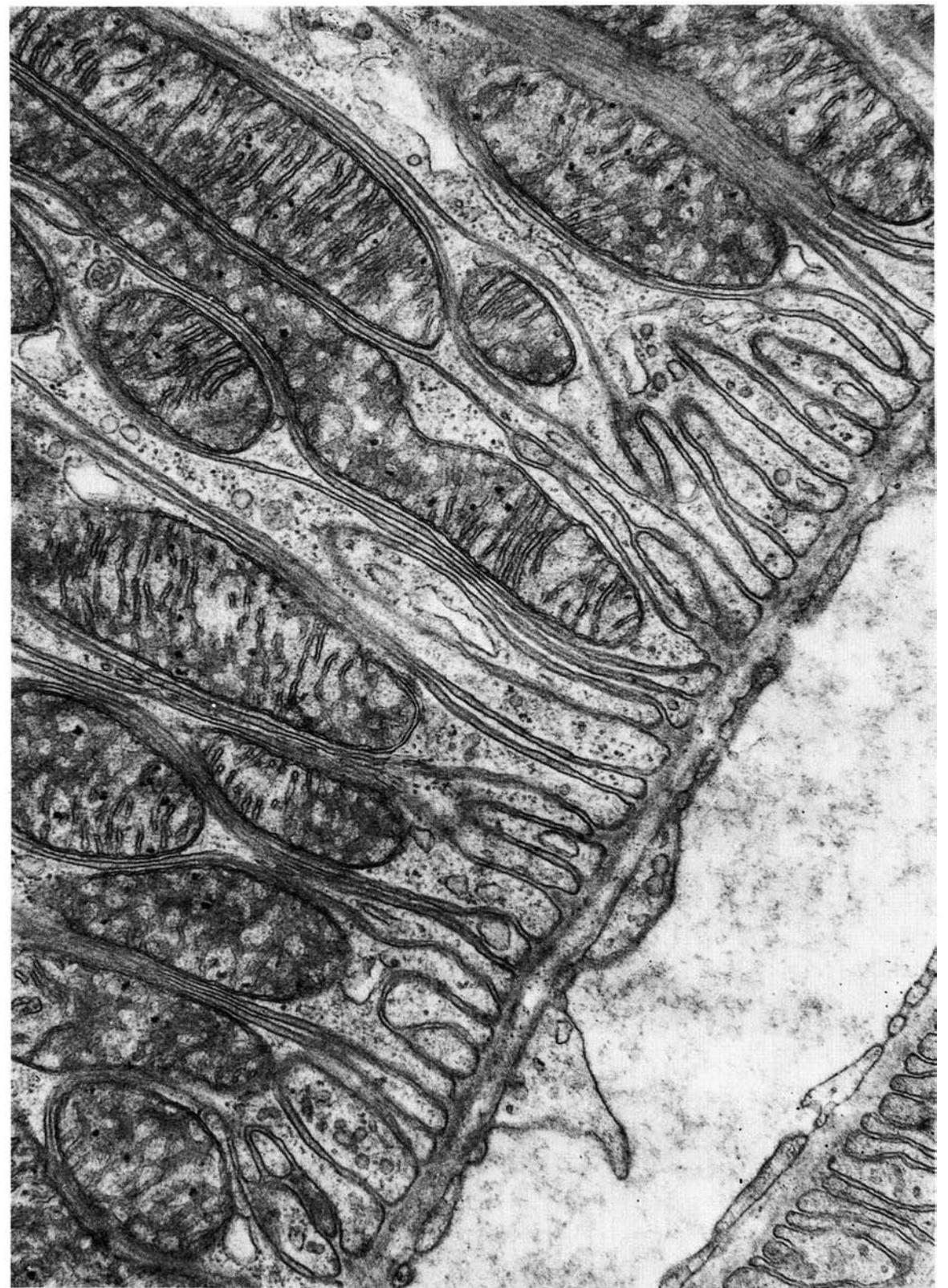
Figure 258. Distal convoluted tubules of guinea pig kidney. (Micrograph courtesy of Atsushi Ichikawa.)

Figure 258

The intimacy of the association of mitochondria with the contractile elements of muscle is well illustrated in transverse sections such as that in the micrograph on the facing page. The boundaries of myofibrils are ill defined in cardiac muscle. The mitochondria are uniformly distributed in a more-or-less continuous field of myofilaments. The diffusion distance for ATP is thus minimized.

Figure 259.   Right ventricular papillary muscle of cat heart.

Figure 259

Insect flight muscle with a beat frequency of 50 per second exhibits discrete myofibrils of uniform size surrounded by large mitochondria (sarcosomes), which often assume bizarre shapes. In this species a very dense mitochondrial matrix obscures the numerous closely packed cristae.
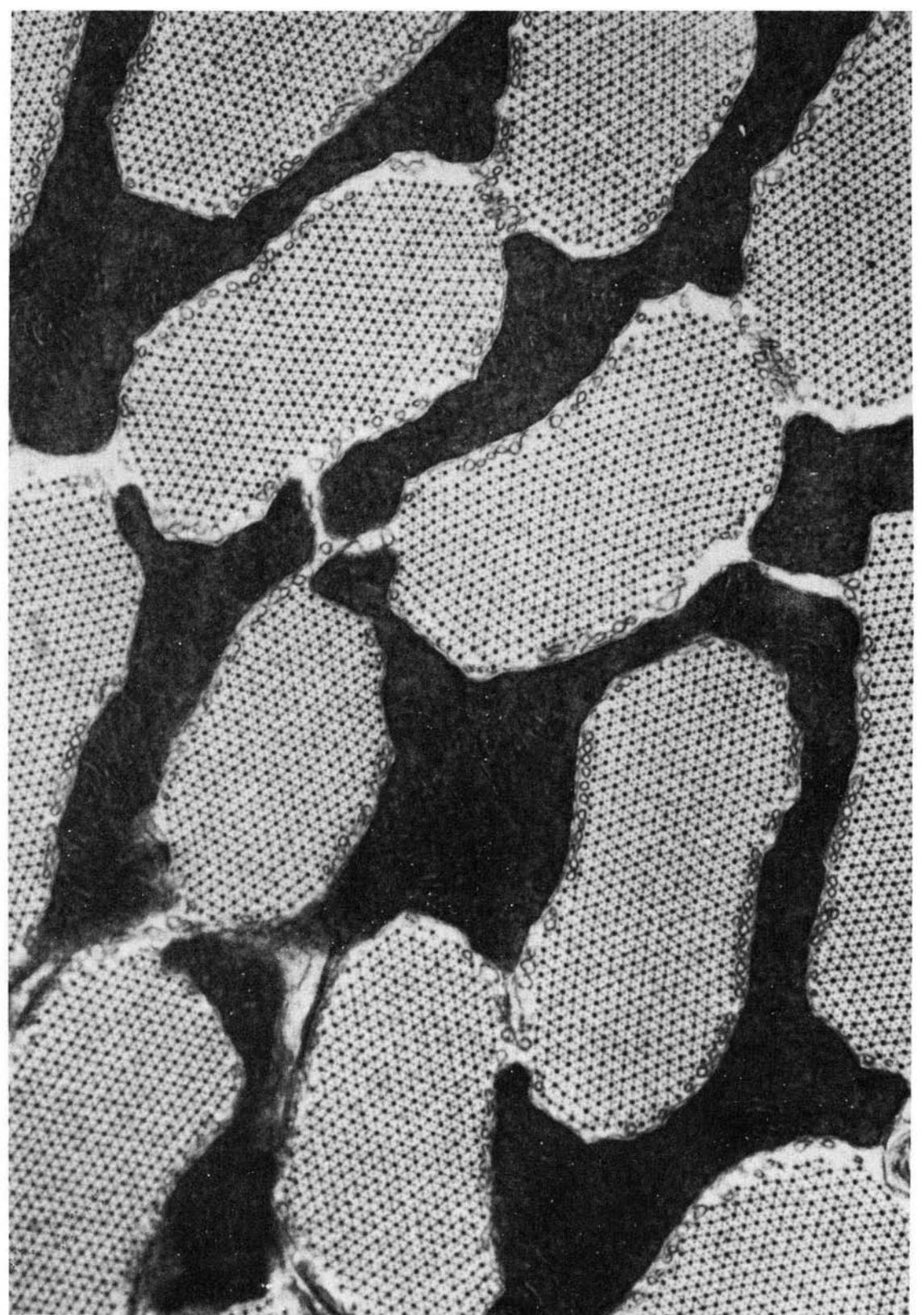
Figure 260

Figure 260. Flight muscle of the moth, *Phytometra gamma*. (Micrograph courtesy of J. Auber, C. R. Acad. Sci. (Paris) *264: 621, 1967.*)

In the midpiece of the spermatozoon of nearly all vertebrates the axoneme is enclosed in a sheath of mitochondria. In mammals, such as the dormouse illustrated here, the elongate mitochondria are arranged end to end in a helix. Thus in longitudinal sections of the sperm tail the mitochondria are seen in cross-section. Their intimate relationship to the outer dense fibers and axoneme of the sperm tail facilitates diffusion of ATP, which is essential for motility.

Figure 261. Midpiece of spermatozoa from the dormouse, *Glis glis,* in longitudinal section. (Micrograph courtesy of David Phillips.)

Figure 261

REFERENCES

Mitochondria

Andre, J. Quelques donnees recentes sur la structure et la physiologie des mitochondries: glycogen, particules élémentaires, acides nucléiques. Archives de Biologie 76:277–304, **1965.**

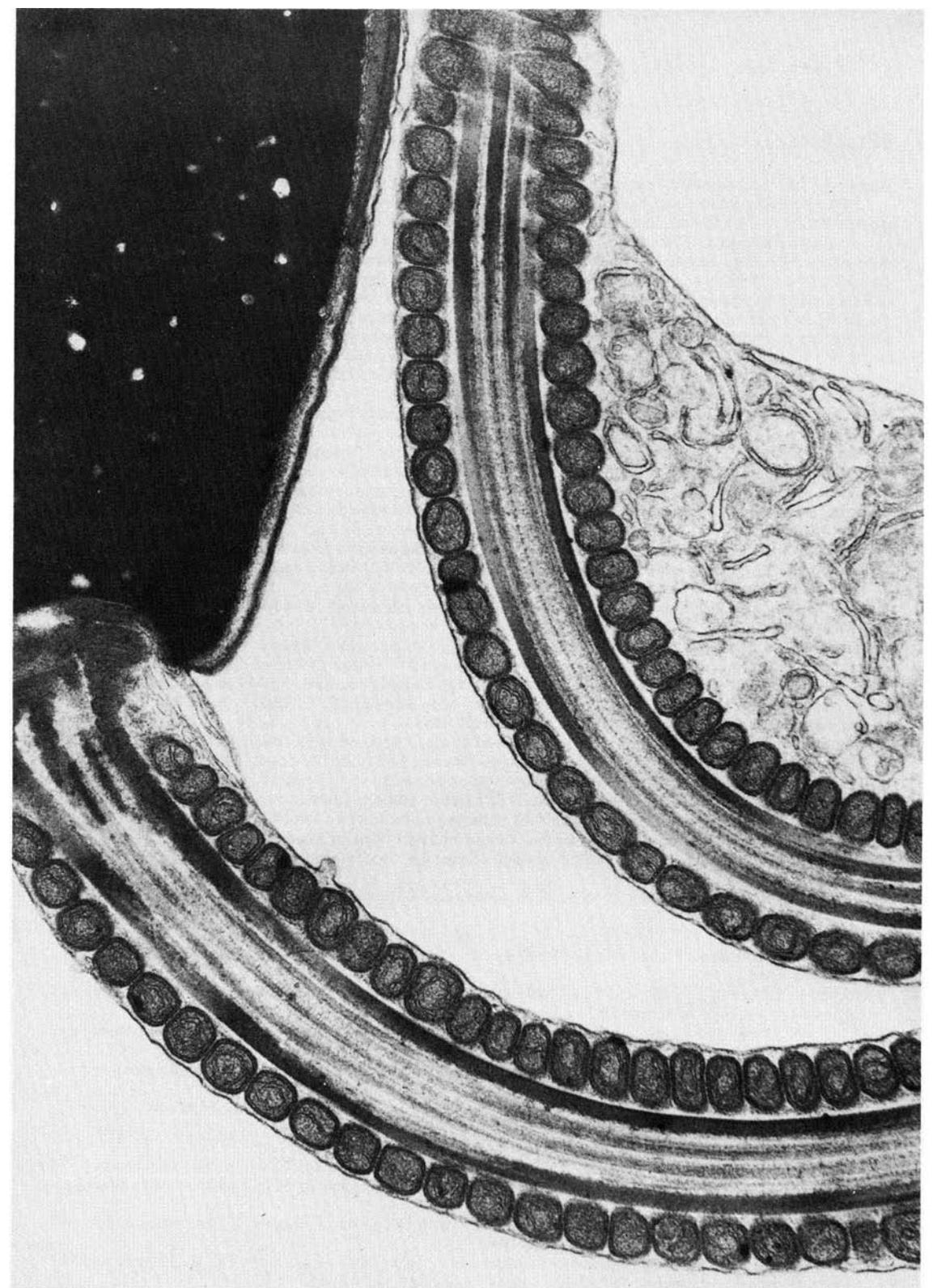Andre, J. and V. Marinozzi. Presence, dans les mitochondries, de particules resemblant aux ribosomes. J. Microscopie 4:615–626, **1965.**

Altmann, R. Die Elementarorganismen und ihre Beziehungen zu den Zellen. Viet Co., Leipzig, **1890.**

Barnard, T. and J. Ruusa. Mitochondrial matrix granules in soft tissues I. Elemental composition by x-ray microanalysis. Exp. Cell Res. 124:339–347, **1979.**

Benda, C. Weitere Mitteilungen über die Mitochondria. Verh. Otsch. Physiol. Ges., pp. **376–383, 1899.**

Bensley, R. R. and N. Hoerr. Preparation and properties of mitochondria. Anat. Rec. 60:449–455, **1934.**

Borst, P., E. F. J. Van Bruggen and G. J. C. M. Ruttenberg. Size and structure of mitochondrial DNA. In Biochemical Aspects of Biogenesis of Mitochondria (E. C. Slater, J. M. Tager, S. Papa and E. Quagliarello, eds.), pp. **51–59,** Adriatica Editrice, Bari, **1968.**

Brdiczka, D. and T. Barnard. Mitochondrial matrix granules in soft tissues. II Isolation and initial characterization of a calcium precipitable, soluble lipoprotein subfraction from brown fat and liver mitochondria. Exp. Cell Res. 126:127–136, **1980.**

Claude, A. The morphology and significance of dumbbell-shaped mitochondria in early stages of regenerating liver. J. Cell Biol. 27:146A, **1965.**

Cowdry, E. V. The mitochondrial constituents of protoplasm. Carnegie Contribution to Embryology 8:29, **1918.** (Review)

Dawid, I. B. and D. R. Wolstenholme. The structure of frog oocyte mitochondrial DNA, pp. **83–89.** In Biochemical Aspects of Biogenesis of Mitochondria. (E. C. Slater, J. M. Tager, S. Papa and E. Quagliarello, eds.), Adriatica Editrice, Ban, **1968.**

Fawcett, D. W. Observations on the cytology and electron microscopy of hepatic cells. J. Nat. Cancer Inst. 15:1475, **1955.**

Fernandez-Moran, H. Subunit organization of mitochondrial membranes. Science 140:381, **1963.**

Gibor, A. and S. Granick. Plastids and mitochondria: inheritable systems. Science 145:890–897, **1964.**

Gross, N. J., G. S. Getz and M. Rabinowitz. Turnover of mitochondrial DNA and phospholipid in rat tissues. In Biochemical Aspects of Biogenesis of Mitochondria (E. C. Slater, J. M. Tager, S. Papa and E. Quagliarello, eds.), Adriatica Editrice, Bari, **1968.**

Hackenbrock, C. R. Ultrastructural bases for metabolically linked mechanical activity in mitochondria. I. Reversible ultrastructural changes with changes in metabolic state in isolated liver mitochondria. J. Cell Biol. 30:269–297, **1966.**

Harris, R. A., C. H. Williams, M. Caldwell, D. E. Green and E. Valdivia. Energized configurations of heart mitochondria in situ. Science 165:700, **1969.**

Harris, R. A., M. A. Asbell and D. E. Green. Conformational basis of energy transduction in membrane systems. II. Measurement of configurational change by light scattering. Arch. Biochem. Biophys. 132:545–560, **1969.**

Hawley, E. S. and R. P. Wagner. Synchronous mitochondrial division in *Neurospora crassa.* J. Cell Biol. 35:489–499, **1967.**

Hogeboom, G. H., W. C. Schneider and G. H. Palade. Isolation of intact mitochondria from rat liver; some biochemical properties of mitochondria and submicroscopic particulate material. J. Biol. Chem. 172:619, **1948.**

Kahri, A. I. Effects of actinomycin D and puromycin on the ACTH-induced transformation of mitochondria of cortical cells of rat adrenals in tissue culture. J. Cell Biol. 36:181–195, **1968.**

Kalf, G. F. Deoxyribonucleic acid in mitochondria and its role in protein synthesis. Biochemistry 3:1702–1706, **1964.**

Kalf, G. F. and M. V. Simpson. The incorporation of valine-1-C^{14} into the protein of submitochondrial fractions. J. Biol. Chem. 234:2943–2947, **1959.**

Kroon, A. M. Inhibitors of mitochondrial protein synthesis. Biochim. Biophys. Acta 76:165–167, **1963.**

Lehninger, A. L. The Mitochondrion. W. A. Benjamin, Inc., New York, **1964.**

Larsen, W. J. Genesis of mitochondria in insect fat body. J. Cell Biol. 47:373–383, **1970.**

Luck, D. J. L. DNA in mitochondria of *Neurospora crassa.* Proc. Nat. Acad. Sci. 52:931–938, **1964.**

McLean, J. R. et al. Incorporation of labeled amino acids into the protein of muscle and liver mitochondria. J. Biol. Chem. 233:657, **1958.**

Nass, M. M. K. and S. Nass. Intramitochondrial fibers with DNA characteristics. I. Fixation and electron staining reactions. J. Cell Biol. 19:593–612, 1963.

Nass, M. M. K. and S. Nass. Intramitochondrial fibers with DNA characteristics. II. Enzymatic and other hydrolytic treatments. J. Cell Biol. 19:613–629, **1963.**

Novikoff, A. B. Mitochondria. In The Cell (J. Brachet and A. E. Mirsky, eds.), Vol. II, Academic Press, New York, **1961.** (Review)

Palade, G. An electron microscope study of mitochondrial structure. J. Histochem. Cytochem. 1:188–211, **1953.**

Parsons, D. F. Mitochondrial structure: two types of subunits on negatively stained mitochondrial membranes. Science 140:985, **1963.**

Parsons, D. F. Recent advances correlating structure and function in mitochondria. Int. Rev. Exp. Pathol. **4:1–54, 1965.** (Review)

Parsons, J. A. Mitochrondrial incorporation of tritiated thymidine in *Tetrahymena pyriformis.* J. Cell Biol. 25:641–646, **1965.**

Peachey, L. D. Electron microscopic observations on the accumulation of divalent cations in intramitochondrial granules. J. Cell Biol. 20:95–111, **1964.**

Rabinowitz, M., J. Sinclair, L. DeSalle, R. Haselhorn and H. H. Swift. Isolation of deoxyribonucleic acid from mitochondria of chick embryo heart and liver. Proc. Nat. Acad. Sci. 53:1126–1133, **1964.**

Reich, E. and D. J. L. Luck. Replication and inheritance of mitochondrial DNA. Proc. Nat. Acad. Sci. **55:1600, 1966.**

Schuster, F. L. A deoxyribose nucleic acid component in mitochondria of *Didymium nigripes,* a slime mold. Exp. Cell Res. 39:329–345, **1965.**

Sheridan, M. Fine structural changes seen in the livers of scorbutic and partially starved guinea pigs. Anat. Rec. 136:276, **1960.**

Sinclair, J. H. and B. V. Stevens. Circular DNA filaments from mouse mitochondria. Proc. Nat. Acad. Sci. 56:508–514, **1966.**

Smith, D. S. The structure of flight muscle sarcosomes in the blowfly, *Calliphora erythrocephala.* J. Cell Biol. 19:115, **1963.**

Steinert, M. Mitochondria associated with the kinetonucleus of *Trypanosoma mega.* J. Cell Biol. 8:542, **1960.**

Stoeckenius, W. Some observations on negatively stained mitochondria. J. Cell Biol. 17:443–454, **1963.**

Stone, G. E. and O. L. Miller. Incorporation of tritiated thymidine into deoxyribonuclease sensitive material in mitochondria of tetrahymena. J. Cell Biol. 23:89A, **1964.**

Sutfior, L. V., M. E. Holtrop and R. Ogilvie. Microanalysis of individual mitochondrial granules with diameters less than **1000** angstroms. Science 174:947, **1971.**

Swift, H., N. Kislev and L. Bogorad. Evidence for DNA and RNA in mitochondria and chloronlasts. J. Cell Biol. 23:91A, **1964.**

Swift, H., M. Rabinowitz and G. Getz. Cytochemical studies on mitochondrial nucleic acids. In Biochemical Aspects of Biogenesis of Mitochondria (E. C. Slater, J. M. Tager, S. Papa and E. Quagliarello, eds.), pp. **3–36.** Adriatica Editrice, Ban, **1968.**

Tandler, B. and C. L. Hoppel. Division of giant mitochondria during recovery from cuprizone intoxication. J. Cell Biol. 56:266–272, **1973.**

Van Bruggen, E. F. J., P. Borst, G. J. C. M. Ruttenberg, M. Gruber and A. M. Kroon. Circular mitochondrial DNA. Biochim. Biophys. Acta 119:437, **1966.**

Vogel, F. S. Structural and functional characteristics of DNA rich mitochondria of the common meadow mushroom *Agaricus campestris.* I. Intracellular environment. Lab. Invest. **14:1849, 1965.**

Vogel, F. S. and L. Kemper. Structural and functional characteristics of DNA-rich mitochondria of the common meadow mushroom, *Agaricus campestris.* II. Extracellular cultures. Lab. Invest. 14:1868, 1965.