ELECTRON MICROSCOPIC STUDY ON THE CATECHOLAMINE METABOLISM IN THE HUMAN ADRENAL MEDULLA

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In recent years a considerable number of reports have been published on the fine structures of many organs. However, no electron microscopic study has been made on the human adrenal medulla. Adrenomedullectomy has been recently employed in the treatment of peripheral occlusive vascular diseases (Durante, 1954; Paaby and Noring, 1955; Ferrand and Alger, 1957). The author has examined the ultrastructure of the human adrenal madulla at adrenomedullectomy and investigated the catecholamine metabolism in the gland by electron microscopy.

MATERIALS AND METHODS

Sixteen patients were used for this study. The cases consisted of the following: Thromboangiitis obliterans 12; Arteriosclerosis obliterans 1; Occlusion of bilateral femoral arteries 1; Gastric ulcer 1; Embryonic cancer 1.

Pieces of tissues were fixed in 1% osmic acid buffered to pH 7.4 with veronal—acetate with 0.3 M sucrose for 2 to 4 hours. After dehydration (Ethanol for 2 to 4 hours), embedding in buthyl-methylmethacrylate (9 : 1 to 8 : 2), and sectioning, the specimens were examined with Akashi 50-EI electron microscope. Some of the sections were stained with saturated uranyl acetate solution.

Three patients with thromboangiitis obliterans had been given daily oral dose of 225–300 mg nialamide (1-[2-(benzylcarbamyl)ehtyl]-2-isonicotinoylhydrazine) for 3 to 7 days before the operation. Four patients with thromboangiitis obliterans had been given daily subcutaneous dose of 2.5 mg reserpine for 7 to 10 days before the operation.

OBSERVATIONS

A. The ultrastructure of the human adrenal medulla

The human adrenomedullary parenchyma consists of light and dark cells.

1. Light cells (Fig. 1)

Light cells occupy the major part of the medulla and their form is polygonal. There are abundant osmiophilic granules, of spherical or rod-like form, in the clear or semi-opaque cytoplasm.

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The appearance of osmiophilic granules is variable; ranging in form from loose collection of component microgranules to sharply defined and intensely homogeneous osmiophilic granules, in various sizes. The spherical form is more frequently seen than the rod-like form. The size of the spherical granule varies from 200 to 300 mμ in diameter. The rod like granules have the length of 250–400 mμ and the width of 60–80 mμ (Fig. 2).

The microgranules have diameter of 100–200 Å. The granule is invested with a limiting membrane with a thickness in the order of 80–100 Å. This membranous structure is osmiophilic and appears as a single dense line. Inside the limiting membrane a peripheral space of lower electron density generally separates the membrane from a central core (Fig. 11). The homogeneous forms are more frequently noticed than the microgranular forms, and the limiting membranes are mostly obscure.

The osmiophilic granules fill the cytoplasm to such an extent as to totally obscure other cytoplasmic structures. The granular endoplasmic reticulum and round or elongated mitochondria are occasionally recognized throughout the cytoplasm. The Golgi apparatus is recognized in a paranuclear area (Fig. 3). Centrioles are very rarely seen near the nucleus, and their fine structure corresponds with that commonly observed in other types of cells (Fig. 4). The nucleus enclosed by a bilaminar nuclear membrane has homogeneous granular nucleoplasm and nucleolus, and multinuclear cells are frequently seen.

2. Dark cells (Fig. 5)

Dark cells are occasionally scattered among the light cells.

The dark cells can be distinguished by their high electron density from the light cells, and their shape is irregular. The cytoplasm is very dense and filled with such osmiophilic granules as in the light cells. In the dark cells also, the spherical granules are more frequently seen than the rod-like granules. The granular endoplasmic reticulum and mitochondria are similar to those in the light cells.

3. Interstitium

Between the parenchymal cell membrane and the endothelial lining of the blood sinusoid, there is a subendothelial space, which communicates with interparenchymal cell spaces. Unmyelinated nerve fibers are more frequently seen than myelinated nerve fibers (Fig. 6).

B. The nialamide-treated human adrenal medulla

Following nialamide administration, considerably wide changes occur in the mitochondria. In the light cells, the mitochondria are clear: the matrix is diluted and the cristae are shorter than normal. However, the outer membrances are retained without being distrupted. Then the mitochondria grow larger, the matrix lightens, and the cristae break up and finally disappear (Fig. 7). Besides the swelling, a ring-like modification of mitochondria is observed (Fig. 8).

This mitochondrial modification is ascertained also in the adrenal medulla of the rabbits after daily oral administration of 50 mg nialamide for 38 days

(Fig. 9).

In the selfsame cell, the standard-type mitochondria mingle with the swollen or ring-like modified mitochondria and the mitochondria show up eminently against the cytoplasm (Fig. 10). No significant change of other organelles is noticed.

C. The reserpine-treated human adrenal medulla

After injection of reserpine, intense changes occur in the osmiophilic granules and granular endoplasmic reticulum.

In the light cells, there is an apparent decrease in electron density of the granules (both spherical and rod-like forms), and the microgranular forms with the limiting membranes are very conspicuous (Fig. 11, 12). There is a noticeable increase in amount of the granular endoplasmic reticulum (Fig. 13). Occasionally lipochondria are crowded in some light cells (Fig. 14). No distinct change of other organelles is recognized.

Similarly to the untreated cases, the osmiophilic granules are not also observed in the subendothelial space or blood sinusoid.

There is no accumulation of reduced substances in the extragranular cytoplasm, and the morphological heterogeneity between the light and dark cells is preserved.

In the dark cells, no conspicuous cytological feature is yet obtained after nialamide or reserpine treatment.

DISCUSSION

Light and dark cells

It has been known that if the adrenal medulla is subjected to the action of chromic acid or potassium dichromate, a dark brown color appears in the medullary cells, and that the brown color represents a secretion which is formed in the cells.

Hion (1927), using formol-dichromate-fixed rat adrenals stained by the Altmann-Kull method, described on the four categories of medullary cells; a, b, c, and d: "a" and "d" cells were agranular and supposedly nonsecreting while "b" and "c" cells, because of the content of fuchsinophile granule, were actively secreting ones.

Bennett (1941) favored the use of the word "fuscogenic" instead of "chromaffin" reaction, from the fact that so-called "chromaffin" reaction depends on the oxidation of certain organic compounds (adrenaline, various aldehydes and ketones, polyphenols and polyamines etc.) in the medulla to brown polymers, and that this oxidation can be produced as well by oxidizing agents not containing any chromium at all. According to Bennett, the cells of the adrenal medulla may undergo a secretory cycle, and at any given moment a search of the gland may reveal various cells in each of the many phases of this cycle: active secretion, depletion, resynthesis and presecretion.

In recent years the adrenal medullae of different animals have been shown to contain noradrenaline in addition to adrenaline (Holtz, *et al.*, 1947; Euler

and Hamberg, 1949).

Afterwards, the existence of adrenaline and noradrenaline in the separate adrenal medullary cells has been proved by various histochemical reactions (Hillarp and Hökfelt, 1953; Eränkö, 1951 and 1955). Therefore, it has been the accepted view that the adrenal medullae of various animals have two different types of cells: one stores mainly adrenaline and the other exclusively noradenaline.

Adrenal medullae of various species have been examined with the electron microscope (in rat, Lever, 1955; in mouse, Sjöstrand and Wetzstein, 1956; in mouse, guinea-pig and cat, Wetzstein, 1957; in rabbit, De Robertis and Vaz Ferreira, 1957, in frog, Burgos, 1959).

Lever (1955) has described two sorts of parenchymal cells in the adrenal medulla of the rat: light and dark cell, not being of distinct type, but transferable to each other giving an intermediate type. According to him, it was concluded that the light cells could be considered in the rat medulla as being either in the process of active secretion or alternatively in the process of replenishing their stock of secretory material, from the observations on a proliferation of the light cells following cold subjection of the animal.

On the other hand, Wetzstein (1957) has reported that there are chief cells and accessory cells in the adrenal medulla of the mouse, and these cells are of different type from which two distinct neurohormones, adrenaline and noradrenaline, may be secreted.

Up to the present time, however, no report is available on the ultrastructure of the human adrenal medulla.

In the anthor's study, it is considered that two types of cells, light and dark, are completely different from each other in the human adrenal medulla, from the fact that the light cell is quite different from the dark cell in the points of cell form and electron density, and that the intermediate form is absent.

Catecholamine-containing granules

In the adrenal medulla, the most conspicuous component is the osmiophilic cytoplasmic granules and a very high amount of catecholamines is stored mainly in the granules (Blashko and Welch, 1953; Hillarp and Nilson, 1954; Hillarp *et al.*, 1954). Studies on the properties of the isolated amine granules from the adrenal medulla once suggested that the catecholamines might be kept within a semipermeable membrane (Hillarp and Nilson, 1954).

It has been reported that an insoluble residue, at lysis of the granule in hypotonic media, may consist of the limiting membranes and the ATPase activity may be concentrated at a main part consisting of the limiting membranes; the catecholamines may be present in the granules in an osmotically inactive form, probably in combination with an equivalent amount to adenosinetriphosphate (Hillarp, 1958 and 1959; Carlsson and Hillarp, 1958; Schümann, 1960; Vendsalu, 1960).

The presence of a limiting membrane around the granule in the adrenal medulla has been confirmed by electron microscopy (Lever, 1955; Sjöstrand

and Wetzstein, 1956). In the electron micrographs of the human adrenal medulla, many granules appear homogeneous but other granules have a microgranular inner structure, and the limiting membrane is strongly osmiophilic and appears as a single, dense line. However, the author has been unable to observe the presence of the large clear space (Höfe) surrounding most of the granules, which was indicated as a result of the dissolution of lipids (Sjöstrand and Wetzstein, 1956; Wetzstein, 1957).

There are two divergent views on the genesis of catecholamine-containing granules, whether they might be cell organelles (Sjöstrand and Wetzstein, 1956; Wetzstein, 1957) or secretory inclusions (De Robertis and Vaz Ferreira, 1957). The polarity of the adrenal medullary cells has been reported: nerve endings terminate on their capillary poles and medullary secretion is associated with an actual discharge of secretory droplets into the vein (Bennett, 1941; Blaschko and Welch, 1953).

In electron micrographs, the excretion of catechol-containing granules by a mechanism of membrane flow has been recognized: the catechol-containing granules are secretory inclusion and not cell organoids (De Robertis and Vaz Ferreira, 1957; Burack and Draskoczy, 1962). Hillarp *et al.* (1954) have observed that adrenaline secretion in the adrenal medulla is not accompanied by the discharge of the granules, but adrenaline is liberated and then rapidly transported out of the cells. According to Lever (1961) the adrenal medullary secretion may be accompanied by a dissolution and fragmentation of the granules *in situ* within the cells. On the other hand, the existence of dynamic extragranular amine pools has been discovered, which shunt the freshly formed catecholamines past the granules directly into the blood (Udenfriend and Wyngaarden, 1956; Bygdemann *et al.*, 1960; Hillarp, 1960 c). Hillarp (1960 c) has speculated that the level of AF (free amines in the cytoplasmic sap) regulates the rate of the amine synthesis by a feed-back mechanism and the nerve impulse needs only to produce a shortlasting increase in the membrane permeability.

In the human adrenal medulla, it appears that the catecholamine containing granules are not cell organelles but are formed in the Golgi region from where they presumably acquire limiting membranes and the catecholamines may be liberated from the granules *in situ* within the cells and probably rapidly transported out of the cells without accumulation of amines in the extragranular cytoplasm, from the fact that the granules are closely related to the Golgi vesicles, and that there is no dissolution of the granules and limiting membranes, no granule in the subendothelial space or blood sinusoid and no accumulation of reduced substances in the extragranular cytoplasm.

Nialamide-treated medulla

A considerable number of reports have accumulated on monoamine oxidase (MAO) and MAO inhibitors in recent years. Monoamine oxidase is an enzymic system that catalyses the oxidative deamination of many amines, and the sympathicomimetic amines may be an important substrate (Blaschko, 1952). Monoamine oxidase is mainly bound to the mitochondria (Cotzias and Dole, 1951;

Hawkins, 1952; Blaschko *et al.*, 1957), and in the adrenal gland the enzyme is present in the medulla as well as in the cortex (Langemann, 1951). Since MAO inhibitor brings about a pronounced increase of catecholamines by blocking the deamination of amines in various organs, especially in the brain most remarkably (Shore *et al.*, 1957; Carlsson *et al.*, 1958; Belford and Feinleib, 1961; Smith, 1963), it is an interesting drug in the field of psychiatry as well as in many other fields of medical scince.

Following the administration of nialamide (MAO inhibitor) interest changes occur in the mitochondria in the human adrenal medulla, as above described.

The swelling of mitochondria—an early sign of cell damage—has been known for a long time, and it is a reversible process in the early stage. This swelling, probably due to retention of water, may be studied in a number of pathological states: fasting, disturbances in cell hydration, various poisoning, neoplastic transformation, etc. (Oberling, 1959; Rouiller, 1960; Deshpande *et al.*, 1961).

According to Rechnagel and Malamed (1958), clear swelling of mitochondria would be due to osmotic disorder of the cell. Although this explanation may be correct in some cases, it cannot become a general rule. Moreover, the fact that the normal mitochondria mingle with swollen and clear mitochondria in the selfsame cell, seems to be a proof that the mitochondria may not respond so simply as an osmometer. Recent biochemical studies on isolated animal mitochondria have established these particles as the center of a variety of metabolic reactions: as cellular particles associated with enzymes of the cytochrome system, fatty acid oxidation, and oxidative phosphorylation (Blaschko, 1952; Watson and Siekevitz, 1956 a and b).

After administration of nialamide, the swelling of mitochondria may be indicative of their degeneration; a genesis of the mitochondrial degeneration may be due to retention of toxic metabolites within mitochondria by the inhibiting action of nialamide on the monoamine oxidase.

A definite explanation is not yet obtained on the ring-like modification of mitochondria, although various mitochondrial variations have been reported (Lever and Chappel, 1958; De Robertis and Sabatini, 1958; Bargmann and Knoop, 1960; Duncan and Hild, 1960). Zbinden and Studer (1958) have proved histochemically the accumulation of catecholamines in the adrenal medulla of the iproniazid (MAO inhibitor)-treated rat. However, no conspicuous change is recognized in the osmiophilic granule and cytoplasm in the nialamide-treated human adrenal medulla.

Reserpine-treated medulla

A large number of studies have been published demonstrating a pronounced loss of catecholamines from the adrenal medulla after administration of reserpine (Schümann, 1958; Zbinden and Studer, 1958; Eränkö and Hopsu, 1958 and 1961; De Schaepdryver, 1959; De Schaepdryver and Presiozi, 1959; Hillarp, 1960 a and b; Burack *et al.*, 1961; Euler and Lishajko, 1961; Leduc, 1961; Callingham and Mann, 1962; Klein, 1962).

With regard to pharmacological action of reserpine in the adrenal medulla,

it is necessary to distinguish between a direct peripheral action and indirect central effect of reserpine. In the first case, there is a liberation of catecholamines from their bound form *in situ* followed by the enzymic destruction which is subject to the interference from monoamine oxidase inhibitors. In the second case, there is a true secretion of catecholamines into the blood followed by the pharmacological effect inherent to the release of these amines. (De Schaepdryver, 1959; Leduc, 1961; Callingham and Mann, 1962). It has been reported that reserpine may not inhibit the amine synthesis, but block the process of amine storage (Bertler, 1961; Bertler *et al.*, 1961; Axelrod *et al.*, 1961; Kirshner, 1962). Leduc (1961), contrariwise, has pointed out that reserpine may directly activate monoamine oxidase and possibly affect the synthesis as wall as the storage mechanism.

Accoring to Iwase (1963), it seems probably that reserpine may inhibit the synthesis of catecholamines to some extent, from the fact that the amount of urinary excretion of adrenaline, noradrenaline and V.M.A. decreased, more or less, in the patients with thromboangiitis obliterans after reserpine administration.

From the fact that the intragranular proteins in the adrenal medulla may not be altered by reserpine administration, and reserpine may not necessarily act by disrupting the integrity of the intracytoplasmic granules (Schümann, 1958; Burack *et al.*, 1961), it is tempting to assume that the microgranular inner structure after injection of reserpine may be mainly representative of nonreleased intragranular proteins. According to Lever (1961), there are only a few secretory granules present twenty four hours after intravenous injection of 1 mg of reserpine in the rabbit, and the amounts of the granular endoplasmic reticulum and secretory granules within a cell may appear very roughly to be inversely related.

In the electron micrographs of the reserpine-treated human adrenal medulla, there is an apparent decrease in electron density of the granules, and the microgranular forms with the limiting membranes are very conspicuous. The catecholamine depletion by reserpine of above described dosage may not be due to any profound damage of the granules and limiting membranes, since there is no destruction of their structure, although the dissolution of the granules was indicated as an expression of adrenaline release in the denervated adrenal medulla of the rat (Lever, 1955).

Moreover, from the fact that there is no noticeable reduction in the number of the granules and there is no granule in the subendothelial space or blood sinusoid, the microgranular forms with the limiting membranes may be an expression of catecholamine release *in situ* within the cells, notwithstanding Lever (1955) has considered the microgranular forms as representative of granule formation. Therefore, the feature of microgranular forms with limiting membranes may be more conspicuous in the reserpine-treated human adrenal medulla than in the untreated human adrenal medulla.

Besides the granules, there is a remarkable increase in amount of the granular endoplasmic reticulum. This fact implies that the granular endoplasmic reticulum may play an important role on the synthesis of catecholamines.

Although protein synthesis may result from a cooperation between mitochondria—the sites of energy generation—, and microsome—the supposed sites of formation of new proteins (Gustafson, 1954)—, there is no distinct change of mitochondria in the reserpine-treated medulla.

After injection of reserpine there is an increase in amount of lipochondria. Lipochondria have been named by Melczer (1931) and Baker (1951) instead of the Golgi apparatus and have been considered as secretory granules (Lacy, 1954) or fat degeneration of the Golgi apparatus (Ito, 1956). For the present, however, it may be uncertain whether lipochondria are closely involved in the secretory process of the human adrenal medulla.

Adrenomedullectomy

The theoretical basis for the employment of adrenomedullectomy in the treatment of peripheral occlusive vascular diseases lies in the elimination of hyperfunction of the adrenal medulla associated with these diseases (Durante, 1954; Paaby and Noring, 1955; Ferrand and Alger, 1957), and in the reduction of hypersensitivity of the blood vessels to circulating catecholamies after sympathectomy (Freeman *et al.*, 1934; Ascroft, 1937; Cannon, 1939).

However, definite evidences which support the theoretical basis have not yet been obtained.

In the present study, there is no such a feature as indicative of hyperfunction or hypertrophy in the adrenal medulla of the patients with thromboangiitis obliterans.

SUMMARY AND CONCLUSION

An Electron microscopic study has been made on the adrenal medulla in the patients with peripheral occlusive vascular diseases before and after administration of nialamide or reserpine.

The human adrenomedullary parenchyma consists of light and dark cells: the light cells which occupy the major part of the medulla and have the clear or semi-opaque cytoplasm, and the dark cells which are occasionally scattered among the light cells and can be distinguished from the light cells by their high electron density and irregular shape.

The two cell types may be completely different from each other in both form and function.

The appearance of osmiophilic granules, of spherical or rod-like form, is variable; ranging in form from loose collections of component microgranules to sharply defined and intensely homogeneous osmiophilic bodies, in various sizes.

The swelling or ring-like modification of the mitochondria are recognized in the light cells of the nialamide-treated medulla. The swelling of mitochondria may be the expression of the mitochondrial degeneration, which may be due to any toxic metabolites produced by the inhibiting action of nialamide on the monoamine oxidase localized in the mitochondria.

Following injection of reserpine, significant changes occur in the granules and granular endoplasmic reticulum, in the light cells. There is a noticeable

reduction in electron density of the granules of both spherical and rod-like form, and the microgranular inner structure as being invested with the limiting membrane is more conspicuously visible. The microgranular form with the limiting membrane may be indicative of catecholamine release from the granule within the cells. And there is a remarkable increase in amount of the granular endoplasmic reticulum. This suggests that the granular endoplasmic reticulum may play an important role on the synthesis of catecholamines in the adrenal medulla.

Reserpine appears to release catecholamines without any pronounced damage of the granules and limiting membranes.

The catecholamine containing granules appear to form in the Golgi region and the catecholamines may be liberated from the granules *in situ* within the cells and probably rapidly transported out of the cells without accumulation of amines in the extragranular cytoplasm.

In the patients with thromboangiitis obliterans, there is no such a feature as being indicative of hyperfunction in the adrenal medulla.

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EXPLANATION OF FIGURES

All photographs are electron micrographs of the human adrenal medulla except Fig. 9.

FIG. 1. Light cells and intercellular spaces.
Abundant osmiophilic granules, of spherical or rod-like form, are seen in the semi-opaque cytoplasm. A 34 year male with thromboangiitis obliterams (T. O.) ×20,000.

FIG. 2. Catecholamine-containing granules in the light cell.
The homogeneous osmiophilic form is more frequently observed than the microgranular form. A 58 year male with arteriosclerosis obliterans. ×24,000

FIG. 3. Golgi-apparatus in the light cell.
The granules are closely related to the Golgi vesicles. A 30 year male with T. O. ×30,000.

FIG. 4. Centrioles in the light cell.
A 46 year male with T. O. ×40,000.

FIG. 5. Light and dark cells.
Morphological heterogeneity between them is very conspicuous. A 49 year male with gastric ulcer. ×12,000.

FIG. 6. The bundles of unmyelinated nerve fibers enfolded by a Schwann cell are seen between adrenal cortex and medulla. A 40 year male with T. O. Uranyl acetate stain. ×18,000.

FIG. 7. The nialamide-treated medulla (daily dose of 225 mg for 3 days).
The swelling of mitochondria is seen. A 30 year male with T. O. ×26,000.

FIG. 8. The nialamide-treated medulla (daily dose of 300 mg for 7 days).
The ring-like modification of mitochondria is recognized. The granules appear to form in the Golgi region from where they presumably acquire the limiting membranes. Uranyl acetate stain. A 40 year male with T. O. ×24,000.

FIG. 9. The adrenal medulla of the nialamide-treated rabbit (daily dose of 50 mg for 38 days). The ring-like modification of mitochondria is conspicuous. ×40,000.

FIG. 10. The nialamide-treated medulla of the same case as presented in FIG. 8. The standard-type mitochondria mingle with the swollen or ring-like modified mitochondria. Uranyl acetate stain. ×15,000.

FIG. 11. The granules in the reserpine-treated medulla (daily dose of 2.5 mg for 7 days). The microgranular inner structure with the limiting membrane is conspicuous. A 46 year male with T. O. ×30,000.

FIG. 12. The reserpine-treated medulla of the same case as presented in Fig. 11. Higher magnification of the microgranular inner structure in the catecholamine containing granules. ×70,000.

FIG. 13. The reserpine-treated medulla of the same case as presented in Fig. 11. There is a noticeable increase in amount of the granular endoplasmic reticulum. ×20,000.

FIG. 14. Lipochondria in the reserpine-treated medulla (daily dose of 2.5 mg for 10 days). A 46 year male with T. O. ×16,500.

Scale line indicates 1 micron on each figures.

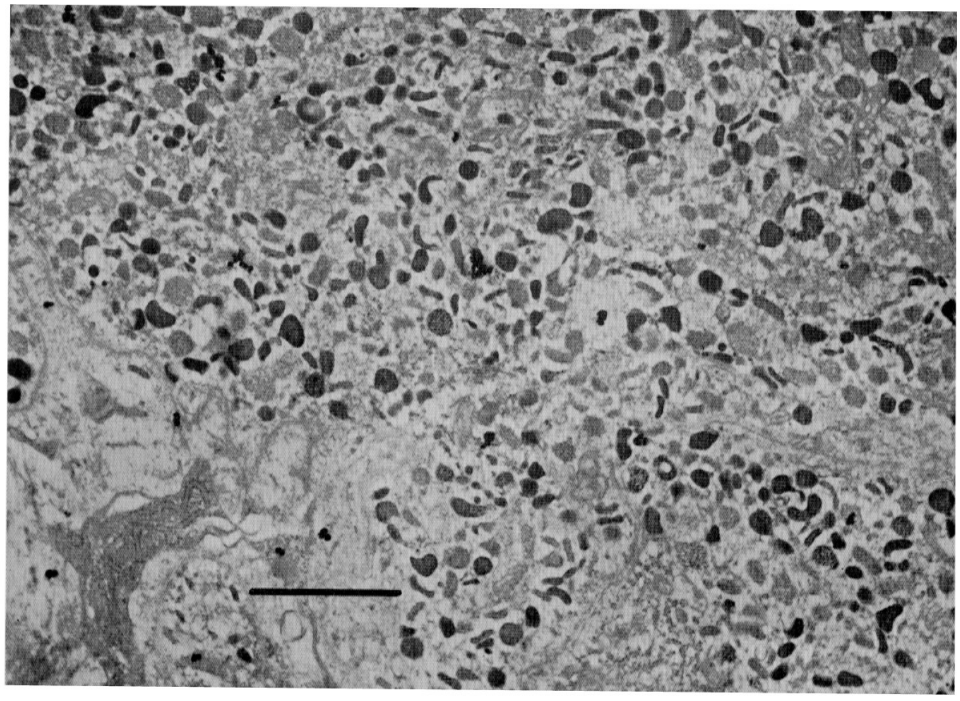

FIG. 1

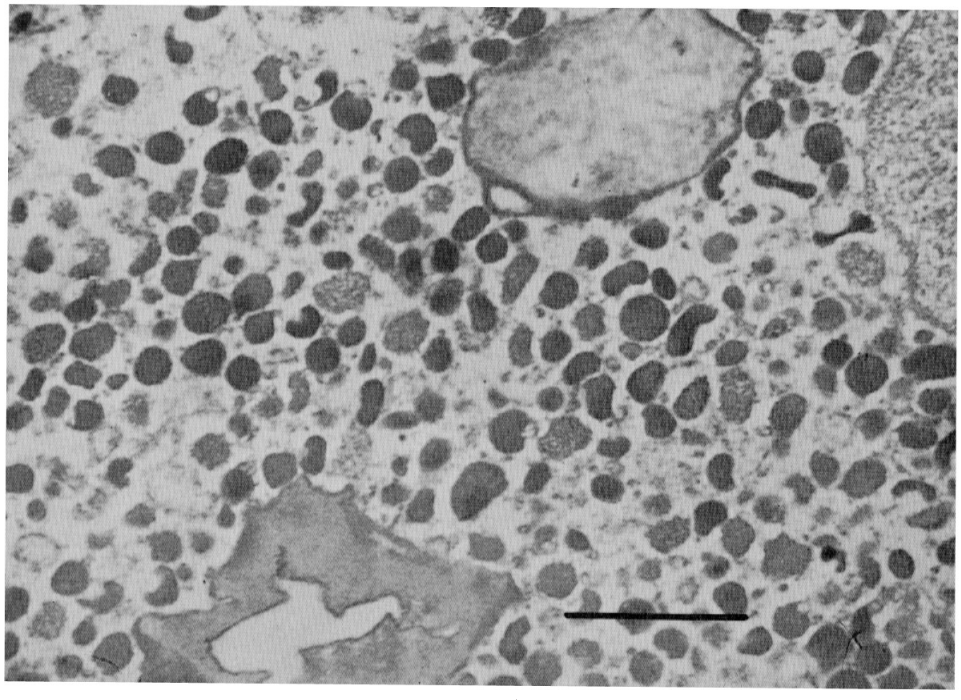

FIG. 2

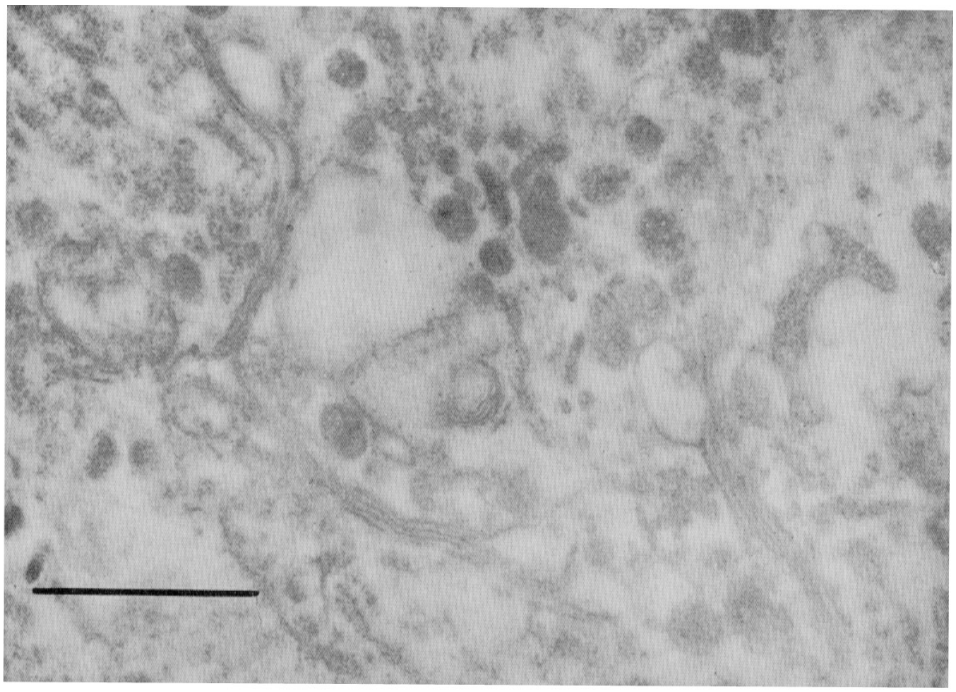

FIG. 3

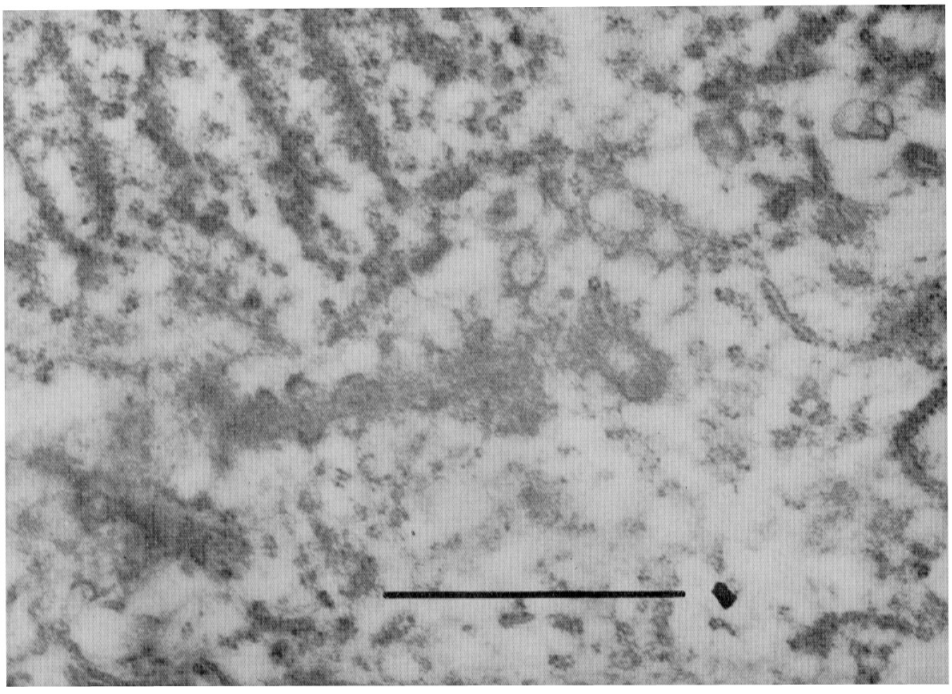

FIG. 4

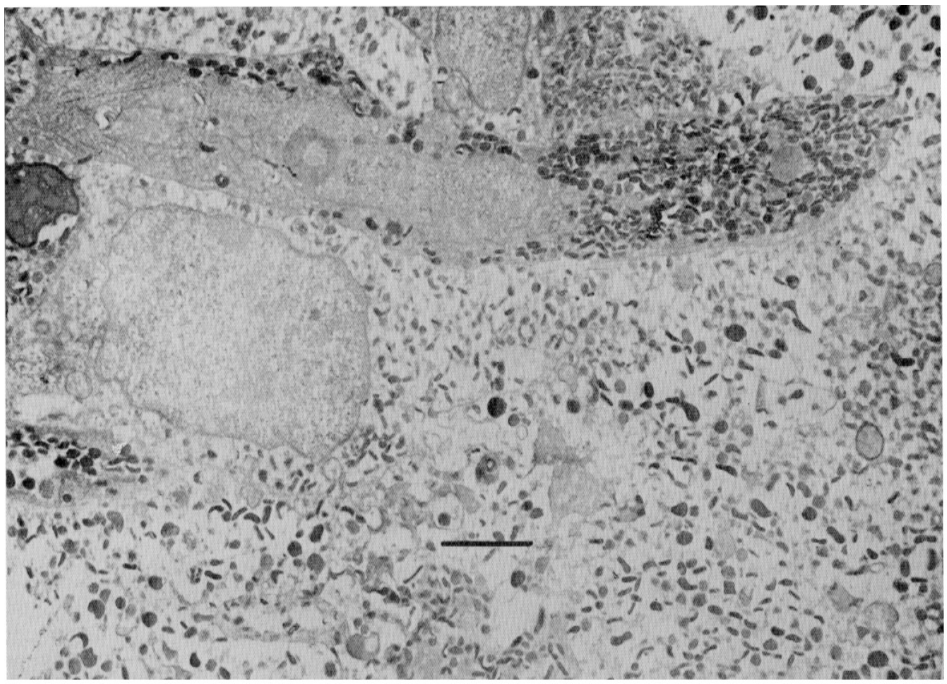

FIG. 5

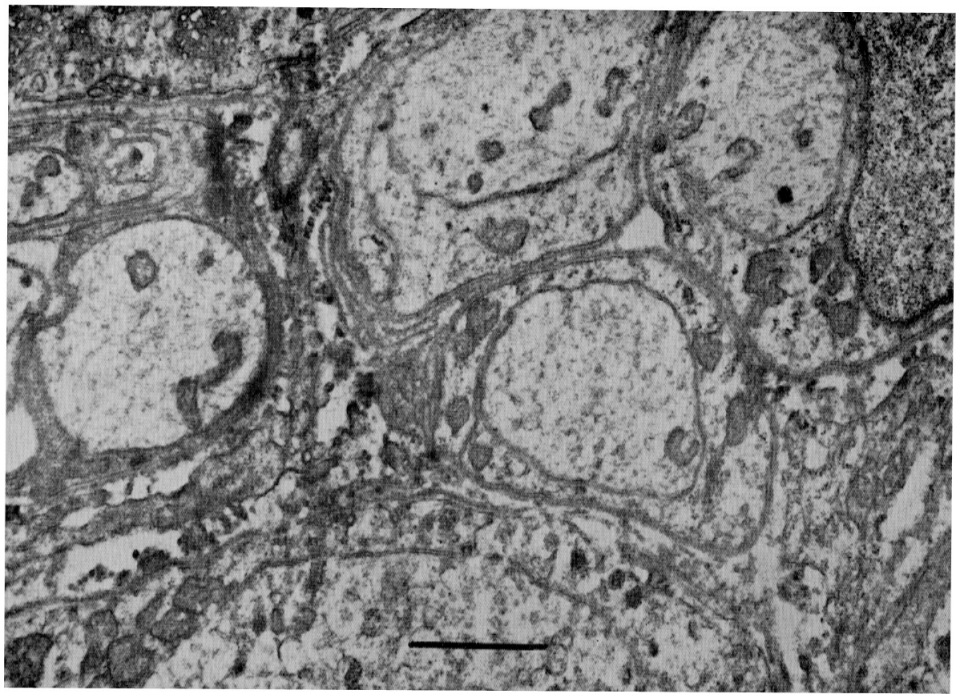

FIG. 6

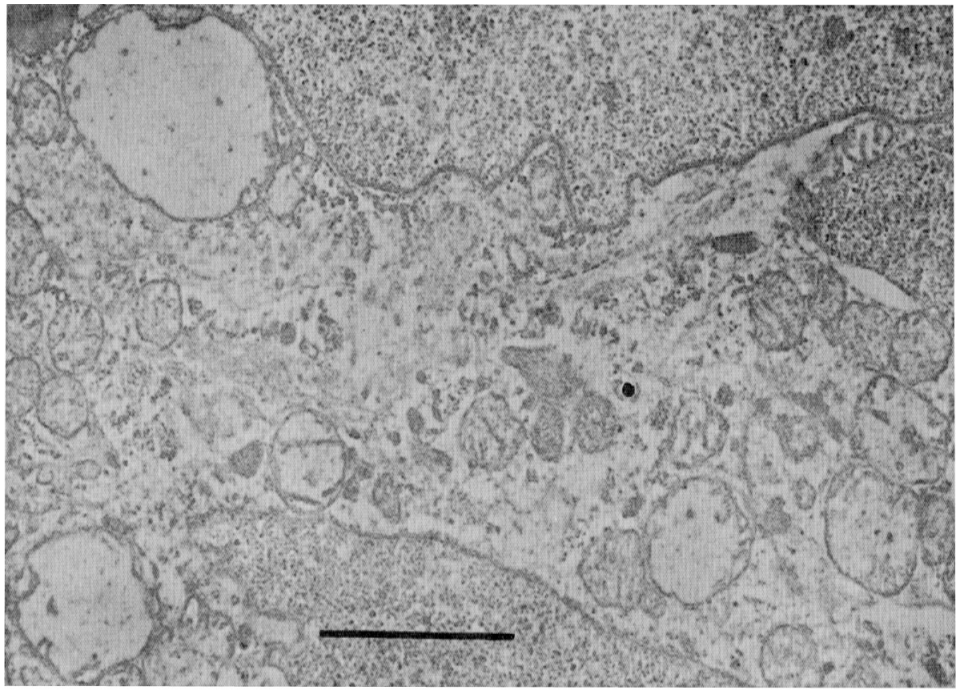

FIG. 7

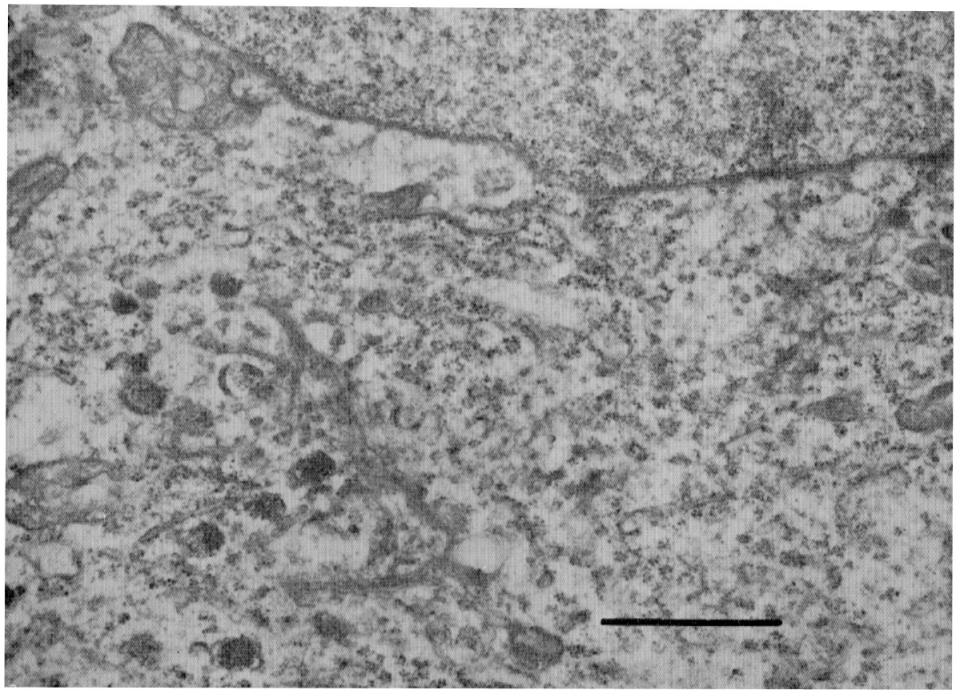

FIG. 8

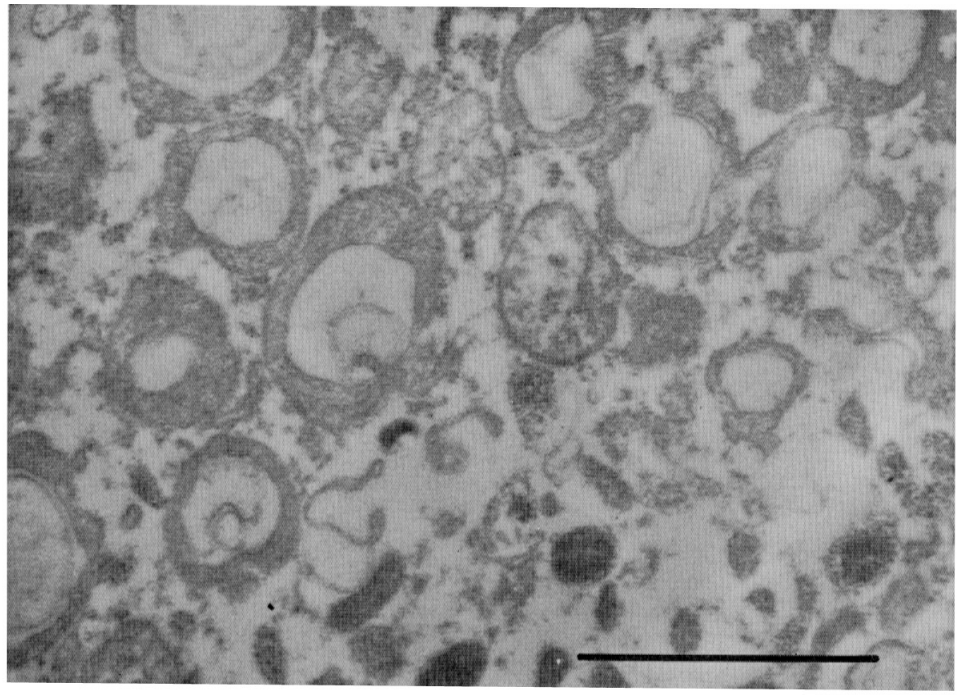

FIG. 9

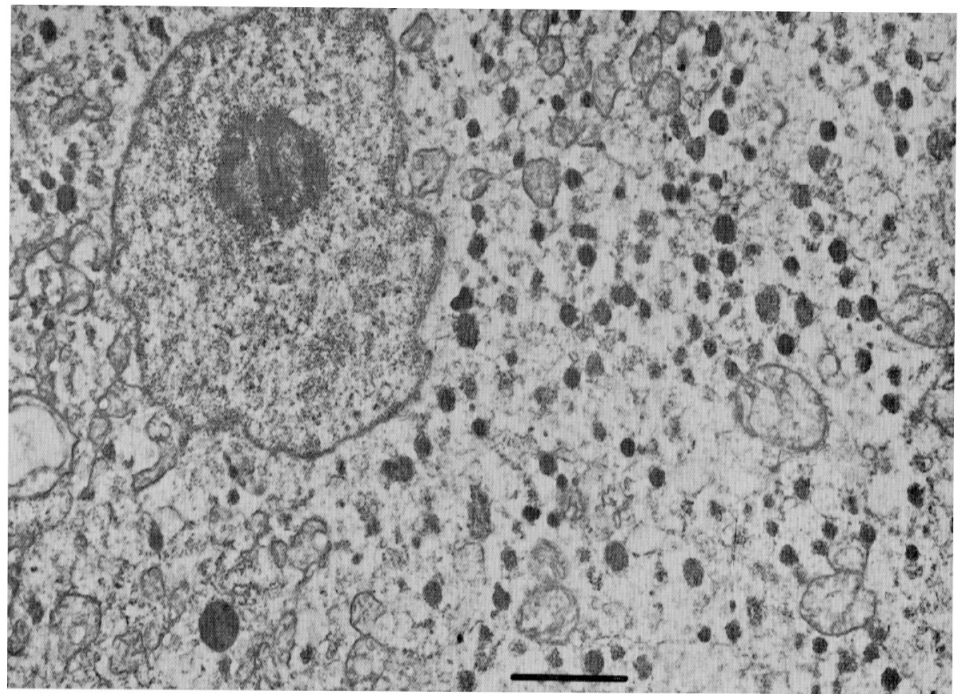

FIG. 10

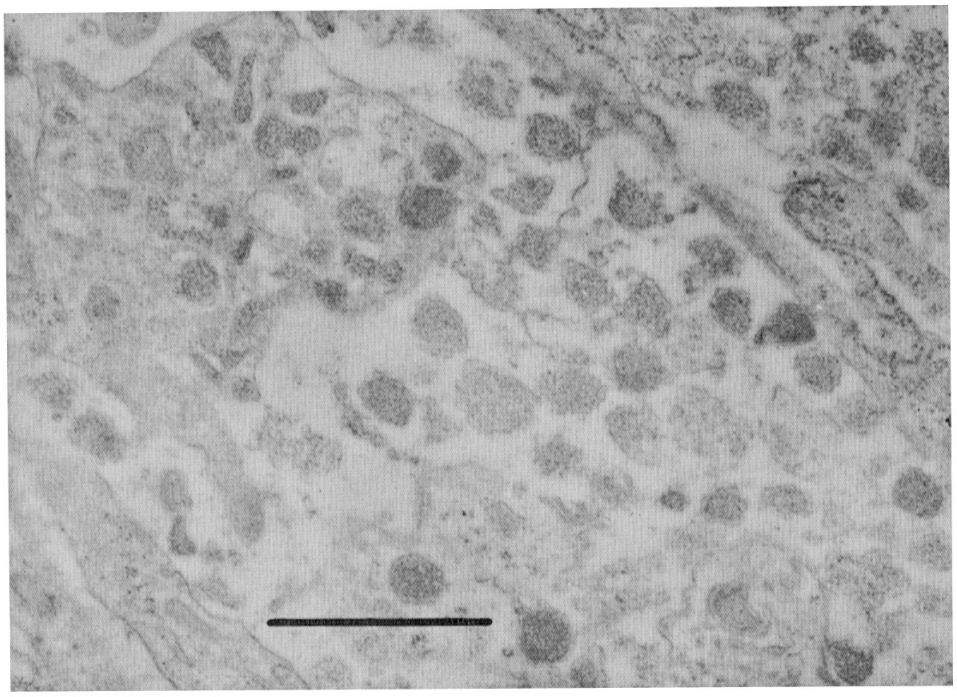

FIG. 11

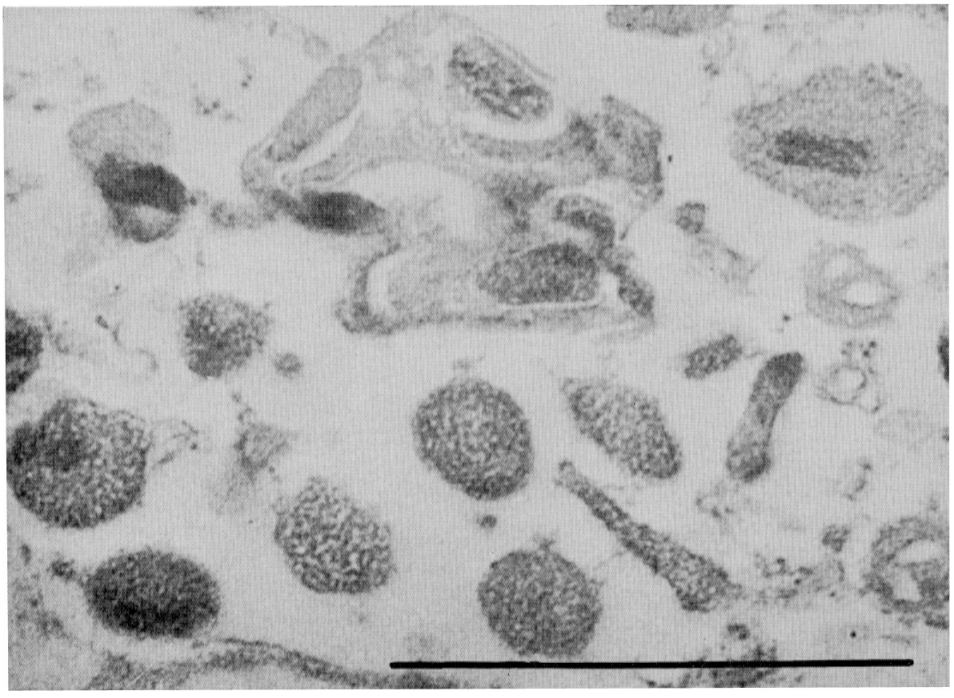

FIG. 12

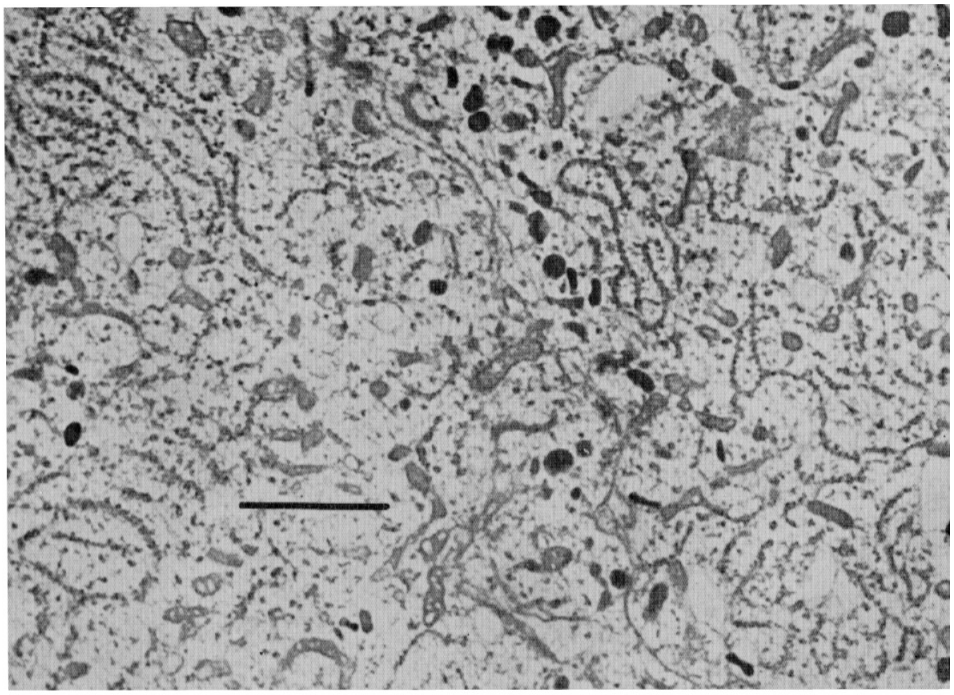

FIG. 13

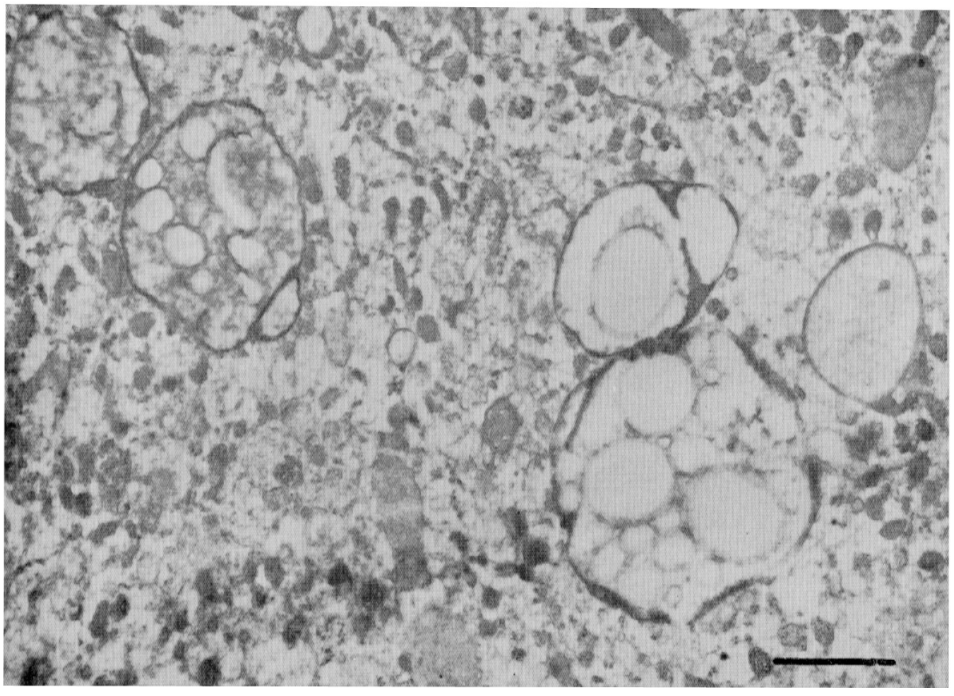

FIG. 14