CYTOCHONDRIA OF NORMAL CELLS, OF TUMOR CELLS, AND OF CELLS WITH VARIOUS INJURIES*

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PLATES 4 AND 5

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The cytoplasm of liver cells consists in large part of vesicular bodies just within the range of microscopic visibility (1). Pathological changes in liver cells and in tumor cells derived from them may bring about such enlargement of these bodies that their structure and relation to one another become readily recognizable. They show a rim which under some circumstances is acidophile and perhaps in large part protein, and under other circumstances is basophile because it contains ribonucleic acid (2, 3), in part at least as nucleoprotein. The greater portion which is within the rim stains faintly, is chiefly lipoid, and consists largely of phospholipids.

All of the bodies with definable rim and clear central space have been designated for convenience, *cytochondria* (cell grains (1)). Certain of them give the reactions that have served to identify mitochondria. These latter are characterized in fixed tissues by their reaction with potassium bichromate which acts as a mordant for several dyes and by their resistance to decolorization when overstained by aniline–acid fuchsin.

It would be unprofitable to review early opinions concerning the granular, fibrillar, or foam-like stucture of cytoplasm. Furthermore, it has not seemed desirable to review the extensive controversial literature concerning the relation of mitochondria to secretion, to deposition of fat and glycogen, and to other changes in normal and pathological cells.

Methods

After fixation in Zenker's fluid and staining by the Giemsa method or by methylene blue and Azur II preceded by phloxin, in accordance with the method of Mallory, the vesicle-like bodies of the cytoplasm are well shown. The usual methods for the demonstration of mitochondria in fixed tissues have been used, and prolonged fixation in Regaud's solution of potassium chromate has been followed by staining with aniline–acid fuchsin or iron hematoxylin. With acid fuchsin alcohol–soluble nigrosin in 1 per cent solution has been a useful counterstain, because it defines cytochondria that do not stain as mitochondria. Staining with iron hematoxylin has been followed by phloxin as a counterstain.

For the purposes of the present study, fixation in a mixture of equal parts of a fifth molar solution of lanthanum acetate (6.85 gm. in 100 cc. water) and 20 per cent formalin has been found to be useful, because it fixes well the ribonucleic acid of the cytoplasm which in many cells of the liver is localized about cytochondria. It has the further advantage that it acts

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promptly as a mordant for the substance that gives to mitochondria a distinctive stain and causes them to be stained by methylene blue used in combination with Azur II. Rose bengal has been substitured for phloxin used by Mallory, because it stains more deeply. Mitochondria identical in size and shape with those demonstrable by other procedures are stained deep blue. Cytochondria that do not stain as mitochondria have a pink color.

Cytochondria become swollen when tissues are subjected to the action of distilled water or of hypotonic solutions and are so much enlarged that they are readily observable. They assume a spherical form and increase in diameter two- or threefold or more. Hypotonic solutions of sodium chloride have a similar effect. It is noteworthy that osmotic swelling of mitochondria has been repeatedly observed (Fauré-Fremiet (4), Anitschkow (5). Lewis and Lewis (6), Lazarow (7)).

Fresh tissue immediately after removal from an animal killed by bleeding from the vessels of the neck has been cut in thin blocks approximately 0.5 mm. in thickness and immersed in distilled water, in hypotonic solutions of sodium chloride, or in solutions of some other substances during periods varying from 5 minutes to 24 hours. The tissue has then been fixed in formalin and lanthanum acetate, Zenker's fluid, absolute alcohol, or Regaud's fluid and embedded in paraffin. For comparison, tissue has been fixed in Regaud's fluid and stained for mitochondria by aniline–acid fuchsin, which stains mitochondria alone, or by iron hematoxylin, which stains both mitochondria and nucleic acid.

Enlargement of Liver Cytochondria in Hypotonic Solutions

The effect of water immersion on the cells and cytochondria of the cytoplasm for a time as short as 5 minutes is seen in a narrow zone about the edges of the block of tissue and in the parenchyma surrounding the larger veins which give free access of the fluid to the immediately adjacent tissue. After 1 or 2 hours of immersion, the tissue is changed throughout.

When water enters the liver cells (Fig. 1), they become much swollen and their outlines are accentuated as sharply stained lines. Cytochondria become swollen and spherical and much increased in size. They are seen as isoated acidophile bodies with deeply stained rims and more palely stained or unstained centers. With continued swelling of the cytochondria they are crowded against one another, so that the cytoplasm assumes a vacuolated or foam-like appearance (Fig. 2). The clear centers of the swollen cytochondria seem to form the meshes of a network outlined by the stained rims, now in contact with one another. In liver kept in water from 20 to 24 hours, this appearance may be almost uniform throughout.

Hypotonic solutions have an effect upon the nucleus similar to that upon the cytoplasm. It becomes swollen and in large part loses its nuclear stain. Within the nucleus, round or oval bodies resembling the cytochondria of the cytoplasm but smaller and less sharply defined are recognizable. They have a well stained rim and clearer center and are usually in close contact with one another, but in much swollen nuclei, they may be separated, apparently as discrete bodies.

Osmotic Enlargement of the Cytochondria of the Kidney

With the Giemsa stain, after fixation in Zenker's fluid, the cytoplasm of the proximal convoluted tubules stains predominantly with the acid dye, and round, oval, or elongated bodies stained pink form lines perpendicular to the base of the cell. The rim of these bodies is usually more deeply stained than the center and about some of them is a delicate rim of basophile material.

With appropriate stains mitochondria are found crowded together in such abundance that they evidently occupy the greater part of the cytoplasm of the cells of the proximal convoluted tubules and constitute most, at least, of the less well defined bodies seen with the Giemsa stain. They are often much elongated at right angles to the base of the cell and may have a beaded appearance.

When kidney tissue is immersed in distilled water, changes occur more promptly than in the liver (Fig. 3). Mitochondria lose their ability to take the stains that characterize them. The cytoplasm of the swollen cells within 15 minutes may be occupied by bodies with circular outline and vesicular appearance with a diameter much greater than that of the mitochondria. Some of them retain the mitochondrial stain at their periphery. They may be separated from one another as discrete bodies in the swollen cells, but with increased swelling they are crowded together so that the cytoplasm assumes the uniformly vacuolated or foam-like appearance (Fig. 4) seen under similar conditions in the cells of the liver. After immersion in water during 20 hours, some cells with foam-like cytoplasm remain well defined, but in the greater part of the tissue all evidence of structure has disappeared.

Osmotic Enlargement of Cytochondria of the Pancreas

In the pancreas secretion granules appear as conspicuous round bodies at the apices of the acinar cells, and the basal part of those cells (Zenker, Giemsa, or methylene blue) is occupied by deeply stained basophile material now recognized as ribonucleic acid. Within this basophile material seen in thin sections are a few round or elongated spaces about the size of mitochondria. With mitochondrial stains (Regaud's fluid, aniline–acid fuchsin, or iron hematoxylin) sparsely scattered mitochondria are found in the basal part of the cell. It is noteworthy that the basophile material fills the interstices between the secretion granules in the apices of the cells. After immersion of the tissue in water from 15 to 30 minutes (Fig. 5), two changes occur: basophile substance diminishes or disappears and the vesicular bodies (cytochondria) of the cytoplasm become swollen and finally spherical. In the basal part of the cell, round bodies are closely approximated and much more numerous than the mitochondria demonstrable in this part of the cell by appropriate stains. The secretion granules retain for a time their distinctive stain and resist the penetration of water, but after prolonged immersion (during 20 hours), vacuoles appear uniformly throughout the cell, those replacing the secretion granules being no longer distinguishable from the others.

The cytoplasm of the cells of the pancreatic islets contain minute oval and round mitochondria in great number together with minute bodies of about the same size and shape with clear center and rim unstained by the mitochondrial stain but defined by the counterstain. They seem to be more resistant to penetration of water than cells of the secreting acini. After immersion in water during 20 hours, mitochondria are stained but in large part vesicular. Cytochondria in the space between them have further increased in size and in places are separated so that they are recognizable as discrete bodies.

Osmotic Enlargement of the Cytochondria of Gland Cells of the Stomach

The cytoplasm at the apices of the cells forming glands of the stomach contains zymogen granules, but in the basal parts of these cells, it stains deeply with nuclear dyes (Zenker, Giemsa). In this part of the cell in thin sections clear round or oval spaces are surrounded by the basophile material. Here appropriate stains (aniline–acid fuchsin, or iron hematoxylin after fixation in Regaud's fluid) demonstrate the presence of sparsely scattered coarse mitochondria and between them are cytochondria shown by a counterstain but with no mitochondrial stain. When particles of stomach mucosa are immersed in water during 1 or 2 hours, basophile material disappears and mitochondria lose their characteristic stain. Throughout the cytoplasm cytochondria, including mitochondria and zymogen granules, are swollen to form larger spherical bodies recognizable as discrete structures which, when crowded together, give a foam-like appearance to the cytoplasm. Those representing zymogen granules are no longer distinctive and when water imbibition is advanced, are represented by almost uniformly distributed vacuoles.

The cytoplasm of the parietal cells of the gastric mucosa stains by the Giemsa method only with the acid dye, and coarse round or oval mitochondria are demonstrable by the usual methods. In the interstices between mitochondria are bodies of similar form which fail to take the distinctive stain but are defined by the counterstain. When stomach tissue is immersed in water, mitochondria do not lose their peculiar staining character after 1 or 2 hours and do not become swollen, but after immersion during 20 hours, they become vesicular and moderately enlarged.

The cytoplasm of the cells forming the ducts of the liver and pancreas unlike the parenchymatous cells of the these organs, contains few and small mitochondria. In the space between these mitochondria are minute bodies of similar size which stain only with the counterstain and have stained rims and clear centers. With immersion in water these cells become swollen and cytochondria are seen as discrete swollen bodies; with greater enlargement they form almost uniformly distributed vacuoles separated by their stained rims. Similar changes are seen in the cells forming the necks of the gastric glands and in those that constitute the collecting tubules of the kidney.

Osmotic Swelling of Tumor Cells

Osmotic swelling of the cells of hepatomas following the long continued administration of butter yellow causes changes in the cytoplasm of tumor cells identical with those seen in normal liver cells (Fig. 6). At the edges of pieces of tumor tissue immersed in water changes occur within a few minutes, and after 1 hour these changes may be almost uniform everywhere. Cells are swollen, basophile substance has almost completely disappeared, and distinctive mitochondrial stain is almost wholly lost. Within the swollen cells conspicuous spherical bodies, often well separated from one another and hence recognizable as discrete bodies, occupy the cytoplasm. With increasing swelling of these bodies, all stages are seen in the transition to the foam-like appearance that has been described. Identical changes as the result of water imbibition are seen in a hepatoma caused by the administration of acetylaminoflourene and in sarcomas caused by the introduction of 3,4-benzpyrene in paraffin into the subcutaneous tissue of rats.

Hydropic Swelling of Cytochondria Caused by Dimethylaminoazobenzene

Following the administration of butter yellow to rats, conspicuous depletion of the basophile substance (ribonucleic acid) of the cytoplasm of liver cells occurs (2) and is often associated with accumulation of fat. The cytochondria in considerable part lose their ability to take up the basic stain and are colored by the acid dye (pink with the Giemsa stain). In sections appropriately stained mitochondria are in general more numerous in cells next to the portal spaces, but cytochondria with no mitochondrial stain are much more abundant than in cells of the normal liver. These are stained by the counterstain and usually occur in groups. As the central vein is approached, mitochondria diminish further and in some cells may be wholly absent, the cytoplasm being occupied in large part by cytochondria that stain only with the counterstain. In places these bodies are enlarged, spherical, and conspicuously vesicular. With greater enlargement, what appear to be vacuoles are formed, and when these are abundant, the cytoplasm has a foam-like appearance. Uniformly vacuolated cells often resemble liver cells with water imbibition, but these vacuoles are often less uniform in size than in tissue exposed to the action of hypotonic solutions. They are recognizable in sections of tissue fixed in lanthanum acetate and formalin, frozen, and stained for fat and evidently contain none.

Hydropic Swelling of Cytochondria Caused by Chloroform

When the parenchyma about the central veins of the liver is injured by the administration of chloroform (0.25 to 0.3 cc. per 100 gm. of body weight injected subcutaneously with twice its volume of olive oil), necrosis may occur in contact with the vein; it is characterized by loss of nuclei and acidophilia of the cytoplasm. In these cells the cytochondria are in great part swollen and spherical and have sharply defined rims deeply stained by the acid dye. Just outside this area of necrosis, or perhaps in contact with the central vein when necrosis has not occurred, the much swollen, rounded cells have a granular translucent appearance, because their cytopaslm is occupied by spherical evidently swollen cytochondria which stain faintly with the basic dye. Enlargement of cytochondria may give the cytoplasm a vacuolated or even foam-like appearance, and comparison with frozen sections stained with Sudan IV shows that these vacuoles do not contain fat. Nevertheless, in a broad zone surrounding the swollen granular cells, liver cells contain fat droplets in abundance.

When sections of the liver of rats that have received chloroform as described above are stained for mitochondria (Regaud's fluid, iron hematoxylin, or aniline–acid fuchsin) changes are found in these bodies throughout a large part of the portal unit. Only the liver cells about the portal spaces are like those of the normal liver; they contain well stained mitochondria in great abundance, and bodies of similar size, unstained by the mitochondrial stain, are not readily found; but in the midpart of the radius from portal space to central vein, a large part of the cytoplasm is occupied by bodies that do not take the mitochondrial stain and are stained by the counterstain. In this part of the parenchyma, mitochondria may be rod-shaped or round, and some of the latter shape are apparently losing their ability to take up the mitochondrial stain, so that they stain only at the rim and have a vesicular form. In the swollen cells about the central veins mitochondria are much less abundant and appear as small, scattered, round bodies (Fig. 7). Changes in cytochondria that do not take the mitochondrial stain are best seen when tissues fixed in Regaud's fluid are stained for mitochondria with aniline–acid fuchsin and counterstained with alcoholic nigrosin (Fig. 8). The swollen spherical bodies stained by nigrosin are in some cells well defined as discrete structures and in other cells give a foam-like appearance to the cytoplasm. The changes are identical with those caused by immersion of fresh liver tissue in water.

Accumulation of Fat in the Cytochondria of Liver Cells

When the cytochondria of liver cells are enlarged by intake of water, their relation to fat deposited in visible stainable droplets becomes evident. To study this relation, accumulation of visible fat in the liver, that is, fatty degeneration, has been produced in the rat by several kinds of injury. Chloroform poisoning causes accumulation of fat chiefly in cells about the central veins or in a zone surrounding necrosis in the same position (8). Withdrawal of food causes the appearance of fat in liver cells (9) about the portal spaces. Removal of one kidney of the rat has been followed in the present study by accumulation of fat in liver cells; and in guinea pigs, fatty degeneration of the liver in the present experiments has followed the administration of diphtheria toxin.

The lesion usually designated as fatty degeneration is an intracellular accumulation of droplets of visible fat following various injuries to parenchymatous organs. Normal fat metabolism in cells of an organ such as the liver takes place with no deposition of fat droplets within the cells, but injury of cells may bring about accumulation of visible fat. The chemical and physical changes determining the appearance of recognizable droplets of stainable fat have not been defined satisfactorily, but it may be assumed that the deposited fat has failed to undergo the usual transformation into phospholipids and other components of the cytoplasm.

Small pieces of liver, the site of intracellular accumulation of fat, caused by each of the procedures mentioned above, have been immersed in water for 1 hour and after fixation in lanthanum acetate and formalin have been frozen, cut in thin sections, and stained with Sudan IV and hematoxylin (Fig. 9). The cytochondria take up water and become swollen to form spherical bodies of at least twice their usual diameter and especially about the portal spaces their rims are marked by material stained by the basic dye. When fat accumulation is in moderate quantity the contents of scattered cytochondria defined by their basophile rims have taken a deep orange color; whereas those about them may be unstained or stained in lighter shades of yellow. The contents of cytochondria in the same cell may stain with intensity varying from a pale yellow just perceptible to deep orange-yellow. This variation may be explained by assuming that stainable fat gradually accumulates within cytochondria at the expense perhaps of an invisible lipoid content. Cells at the margin of areas of fatty change may contain only a few cytochondria with faint shades of yellow. In the early stages of this fat accumulation, almost all of the fat droplets visible in the cell approximate in size the swollen cytochondria (Fig. 9) and appear as spherical bodies surrounded by a delicate rim of basophile substance; but the larger droplets of more advanced fat change may have no recognizable basophile rim and those of considerable size may very well be formed by the coalescence of smaller droplets.

RECAPITULATION AND DISCUSSION

The purpose of this publication is to show that the cytoplasm of the cells that have been studied, including those of several varieties of neoplasm, is in great part constituted by minute bodies which vary much in their chemical composition but have surface properties that make them permeable to water and to other substances in the medium about them. These bodies, which for convenience may be designated cytochondria, in most normal cells are just within the range of microscopic visibility and appear in appropriately stained sections as rounded particles with definable rim and clear central space. They are so much increased in size in certain pathological lesions that further details of their structure become evident (1). When subjected to the action of distilled water or of solutions of certain substances, they enlarge so that they are readily visible and their relation to identifiable components of the cytoplasm becomes perceptible. Their relation to ribonucleic acid, to water, and to fat indicates that they are concerned with important metabolic changes within the cells and observations that have been recorded here and elsewhere (1) show that they are concerned with a variety of pathological lesions.

Ribonucleic acid is present in or upon the surface of a large part of the cytochondria of the normal liver and is increased when normal liver cells have been transformed into tumor cells (2). Removal of this substance by ribonuclease or other means leaves a body which stains with acid dyes more deeply at the periphery than in the center. The bodies that undergo these changes are in part mitochondria, as characterized by their well known reactions to mordants and to stains. In the parenchymatous cells of the liver the cytoplasm is in great part occupied by bodies with the reactions of mitochondria, but with appropriate methods (fixation in lanthanum acetate and formalin and staining with polychrome methylene blue and rose bengal or fixation in Regaud's fluid and staining with aniline–acid fuchsin and counterstaining with alcohol-soluble nigrosin), bodies of similar form, but unstained by methods for the demonstration of mitochondria, can be found in normal liver cells. When hepatomas are formed from liver cells rendered neoplastic as the result of prolonged feeding with butter yellow, the mitochondria are small and sparsely scattered in the cytoplasm of the tumor cells, while bodies of similar shape giving no mitochondrial reaction but demonstrable by counterstains occupy the greater part of the cytoplasm (1). With a variety of injuries to the cytoplasm of liver cells, well illustrated by poisoning with chloroform, the mitochondria lose in considerable part their peculiar reaction to stains. For convenience all of the bodies that are characterized by a stained rim and clear central space have been designated *cytochondria* (1), the term mitochondria being limited to those with the well known staining reactions. Further its noteworthy that all mitochondria lose their characteristic reactions when fresh tissue is immersed in distilled water but persist as vesicular bodies indistinguishable from other cytochondria.

It has seemed desirable for the purpose of the present study to determine the effect of distilled water and of hypotonic solutions on several tissues including liver, kidney, pancreas, stomach, and some tumors. The results have been nearly uniform. Fluid enters the cell and causes swelling of it. At the same time the fluid causes the cytochondria, including mitochondria, to swell and assume a spherical form. Fluid apparently enters the cell more rapidly than it penetrates into cytochondria so that these are recognizable as discrete, much enlarged, spherical vesicular bodies with stained rim and faintly stained or unstained centers. With further swelling of these bodies, they occupy all of the space within the cell and, crowded against one another, their clear centers seem to form the meshes of a network. The cytoplasm may be said to have a foam-like appearance. The cells of the acini of the pancreas and of the gastric glands with secretion granules next to the lumina undergo changes similar to those of the liver, and their cytoplasm after 1 or 2 hours of immersion in water appears vacuolated almost uniformly. The swollen bodies that represent the secretion granules are not distinguishable from cytochondria elsewhere in the cytoplasm.

Hydropic swelling of cells with swelling of their cytochondria has been observed in association with butter yellow administration and is well illustrated by the changes that occur in liver cells adjacent to the zones of necrosis about central veins caused by chloroform. The changes are similar to those produced artifically by immersion of fresh tissues in water and reproduce the lesion that has been designated hydropic or vacuolar degeneration. What has long been known as cloudy swelling or granular degeneration is not definable exactly but is represented in part at least by the changes produced by chloroform poisoning. The uncertainty that has existed concerning the relation of the granules of "parenchymatous degeneration" to mitochondria may be in part referable to the associated loss of mitochondrial stain following injury caused by agents such as chloroform or butter yellow. Anitschkow (5) and others have attributed parenchymatous degeneration to swelling of mitochondria.

Some of the changes that occur in cytochondria produce well recognized lesions of which the pathogenesis has been obscure. In hepatomas produced by butter yellow, cytochondria may become swollen and deeply acidophile and, surrounded by a rim of basophile material, may form cellular inclusions resembling those associated with diseases caused by ultramicroscopic viruses (1).

The properties of cytochondria that have been noted serve to define their relation to the fat droplets that accumulate within parenchymatous cells in the presence of injury (fatty degeneration). When liver tissue which has undergone this change, is exposed to the action of distilled water, fat is recognizable within swollen cytochondria marked by rims of basophile material.

CONCLUSIONS

Bodies that may be designated *cytochondria* occupy the greater part of the cytoplasm of the normal and tumor cells that have been studied. They are characterized (*a*) by their behavior as discrete particles with surface properties that cause osmotic changes in the presence of water; (*b*) by reactions to stains which show that they have a rim surrounding a clearer (lipoid) center; (*c*) by their varying relation to the basophile substance (ribonucleic acid) of the cytoplasm.

Mitochondria which have characteristic reactions to stains promptly lose their distinctive reactions in the presence of solvents or as the result of pathological changes, becoming apparently indistinguishable from other cytochondria.

Changes that occur in cytochondria give insight into the pathogenesis of a variety of pathological lesions. Hydropic swelling of cytochondria caused by chloroform, butter yellow, and other agents, representing one variety of parenchymatous degeneration or cloudy swelling, results in changes similar to those following the immersion of fresh tissues in water.

When parenchymatous cells undergo fatty degeneration as the result of injury fat accumulates within cytochondria.

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EXPLANATION OF PLATES

These photographs were made by Mr. Joseph B. Haulenbeek.

PLATE 4

FIG. 1. To show swelling of cytochondria of cells of liver with formation of discrete spherical vesicular bodies following immersion of the tissue in distilled water during 2 hours. The cytoplasm of one cell (*A*) has assumed a "foam-like" appearance. Fixation in Regaud's fluid and staining with aniline-acid fuchsin and alcohol-soluble nigrosin. × 1000.

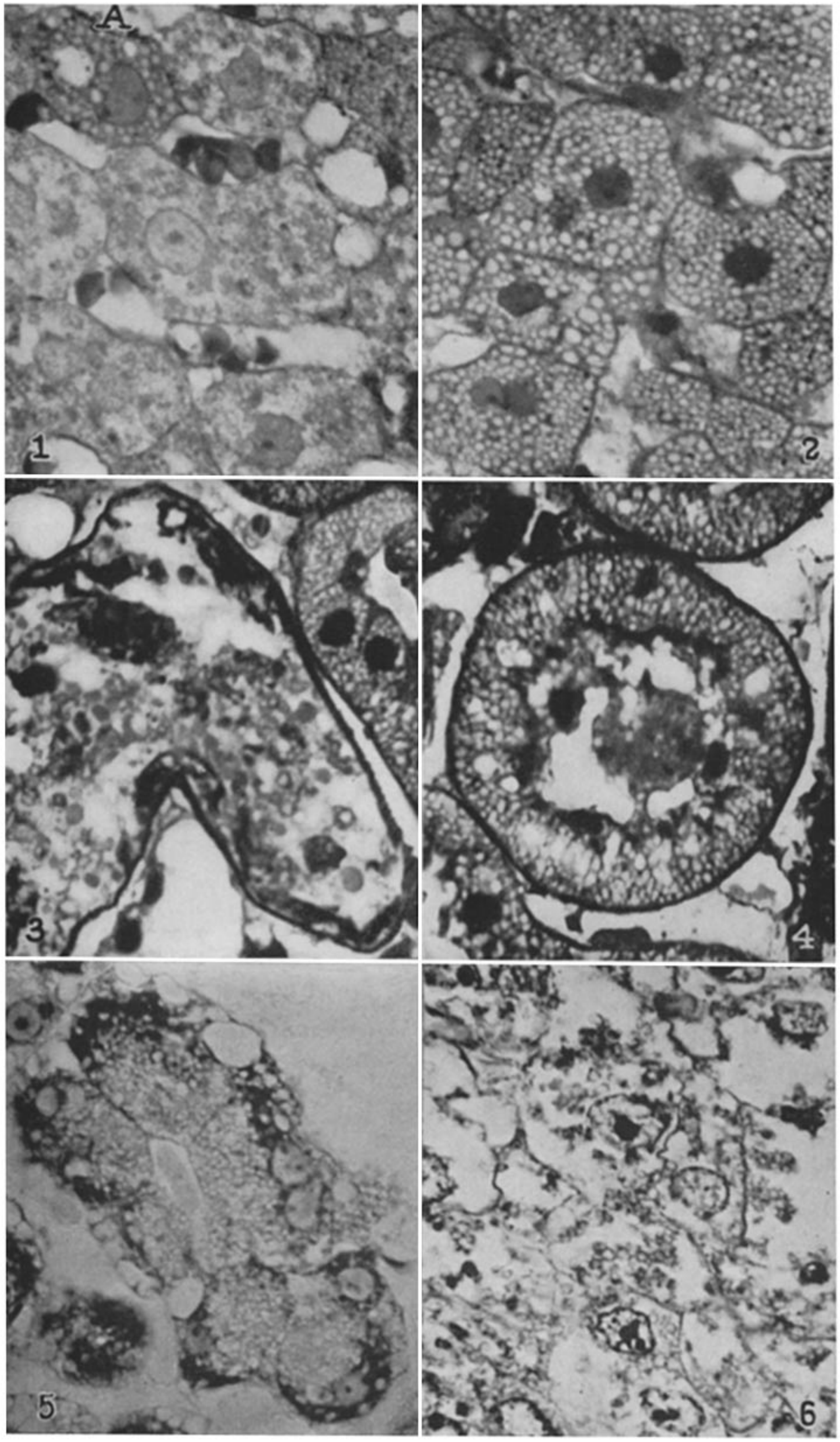
FIG. 2. To show advanced swelling of the cytochondria of hepatic cells with "foam-like" appearance of cytoplasm, after immersion in distilled water during 2 hours. Regaud; aniline–acid fuchsin and nigrosin. × 1000.

FIG. 3. To show swelling of the cytochondria of cells of the convoluted tubules of kidney with formation of discrete vesicular bodies; immersion in distilled water during 1½ hours. Regaud; iron hematoxylin and phloxin. × 1000.

FIG. 4. To show advanced swelling of cytochondria of the cells of the convoluted tubules of kidney with "foam-like" appearance of cytoplasm. The cytochondrial vacuoles form lines at right angles to the bases of the cells; immersion in distilled water during 1½ hours. Regaud; iron hematoxylin and phloxin. × 1000.

FIG. 5. To show swelling of cytochondria of the cells of a secreting acinus of the pancreas; immersion in distilled water for one-half hour. There is a "foam-like" appearance of the cytoplasm and some basophile substance remains at the bases of the cells and between vesicular bodies elsewhere. Zenker; Giemsa. × 1000.

FIG. 6. To show swelling of the cytochondria of cells of a hepatoma produced by administration of butter yellow. The cytochondria can be seen in the much swollen cells as discrete vesicular bodies. Fresh tumor tissue was immersed in tenth molar solution of lanthanum acetate during 6 hours and fixed in formalin. Stained with methylene blue and rose bengal. × 1000.



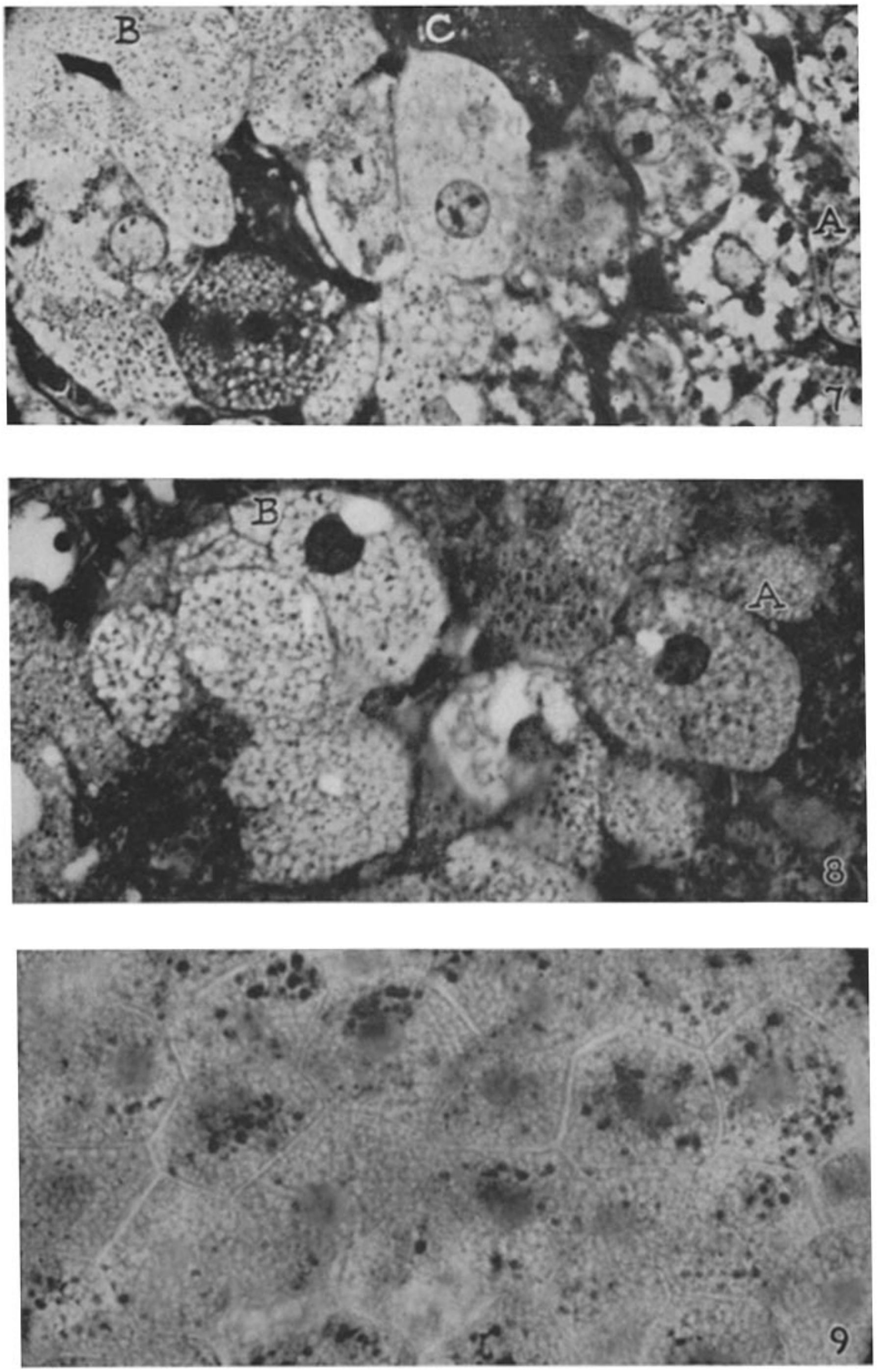
(Opie: Cytochondria of cells)

Plate 5

Fig. 7. To show swollen cells with mitochondria diminished in number and size in the liver of a rat that had received chloroform (0.25 cc. per 100 gm. of body weight) 24 hours before death by bleeding. Clumps of black material represent the mitochondria in relatively normal liver cells (*A*). In some cells the mitochondria are small and scattered (*B*) and in other cells (*C*) they have almost completely disappeared, but when this is the case, bodies of similar size, not well defined in the photograph because they do not take the mitochondrial stain, replace them. Regaud; iron hematoxylin and phloxin. $\times$ 1000.

Fig. 8. To show the swollen cells of a rat that received chloroform (0.3 cc. per 100 gm. of body weight) and was killed after 22 hours by bleeding. The sparsely scattered mitochondria are black. Swollen and vesicular cytochondria stained only with the counterstain can be seen in the cells marked (*A*) and others swollen to form vacuoles that give a "foam-like" appearance to the cytoplasm in the cells marked (*B*). Regaud; aniline–acid fuchsin and nigrosin. $\times$ 1000.

Fig. 9. To show fat and cytochondria in liver cells swollen as the result of immersion of the fresh tissue in distilled water during 1 hour. The animal received no food during 48 hours before death and fat accumulated as droplets in cells about the portal spaces. The tissue after immersion in water was fixed in lanthanum acetate and formalin, sectioned after freezing, and stained with Sudan IV and hematoxylin. The fat appears black. Its inclusion within the basophile rims that define the cytochondria is not visible in the photograph. $\times$ 1000.



(Opie: Cytochondria of cells)