OBSERVATIONS ON MITOCHONDRIA OF TUMORS.*

ERNEST WILLIAM GOODPASTURE, M.D.

(*From the Pathological Laboratories of the Medical School and Cancer Commission of Harvard University.*)

It is now possible to define fairly distinctly the cytoplasmic granulations known as mitochondria. The pioneer work of Altmann left these minute bodies in the same category with many different cytoplasmic structures, including secretion granules, which were preserved and stained by his special technic. Differences in solubility, chemical reaction, and morphology finally established their distinction from other cytoplasmic constituents; and a confusing array of subdivisions based on slight morphological differences was described. The single name "mitochondria," however, is now widely used to designate all the different forms of this class, and with more assurance, since the Lewises observed rapid and varied changes in form and position of single mitochondria within the living cell.

Cowdry provisionally defines mitochondria as "substances which occur in the form of granules, rods, and filaments in almost all living cells, which react positively to janus green, and which, by their solubilities and staining reactions, resemble phospholipins, and to a lesser extent, albumins."

The almost universal occurrence of mitochondria within the cytoplasm of living cells in both animals and plants, and their prevalence in embryonic and regenerating tissue naturally would lead one to predict their presence in all tumors, and their especial prominence in actively growing neoplasms. It was, therefore, a matter for surprise when Beckton in 1909 made the statement that granules, such as could be stained in the cells of many normal tissues and benign tumors by a modification of Altmann's method, tended to disappear or were entirely absent from the cells of malignant tumors; and in a later communication confirmed this

* Received for publication March 2, 1918.

conclusion, suggesting this difference as a means of distinction between malignant and non-malignant cells.

The unexpected nature of this assertion, and its fundamental importance, if true, did not permit it to go long unnoticed. In 1910 a paper from Bensley appeared in which he announced his failure to confirm Beckton's findings, stating that in all of several carcinomata examined by him, including three of the mamma, one of the parotid gland, and one of the rectum, he had demonstrated numerous mitochondria. In this paper Bensley took occasion to distinguish mitochondria from other cytoplasmic granulations, including secretion granules which were included among those described by Beckton and demonstrable by a modification of Altmann's technic. He also made the observation that in general in the tumors studied by him, the mitochondrial "granules as well as the rod and filament forms which were abundant, were much smaller, though more numerous, than in the normal epithelium of the same location. In the cylindrical-cell carcinoma of the rectum this was not the case, the mitochondria being slightly less abundant than, but otherwise indistinguishable from, those of the normal epithelium of the glands." He thus showed that in these tumors there were important modifications of the mitochondria depending on the type of growth.

Although failing to confirm Beckton's assumption that "Altmann's granules" tend to disappear or are absent from the cells of malignant tumors, Bensley recognizes that the observations upon which these conclusions were based indicate a qualitative difference between these granules in benign and malignant neoplasms which deserve further investigation.

The technical methods employed by Beckton and by Bensley differed especially in the fixing reagents used. Mitochondria are dissolved by most of the well-known fixing reagents; they disintegrate rapidly after death, and are destroyed by acetic acid. Beckton made use of a formol-Mueller solution (1 in 49), fixing small thin pieces of fresh tissue; and stained the paraffin sections with Altmann's

anilin acid fuchsin after fixation in formol-bichromate. Bensley had not obtained uniformly satisfactory results in staining mitochondria with anilin acid fuchsin after fixation in formol-bichromate; and this especially led him to question Beckton's negative results. He himself employed his acetic-osmic-bichromate solution as a fixative, staining paraffin sections in anilin acid fuchsin and differentiating with methyl green; a method given in detail in his studies on the pancreas.

An aspect of a great deal of importance in the study of mitochondria in tumors is their possible relation to specific functional activity. In a recent paper Goetsch has considered this matter, and has advanced the hypothesis that the abnormally large number of mitochondria in the cells of adenomata of the thyroid gland associated with toxic symptoms indicates an increased specific functional activity on the part of these cells. This phase of the subject will be considered in the discussion of my own observations.

Although various anatomists have claimed for mitochondria a participation in the formation of specific cellular products — even a direct transformation into such products — no observations have come to my attention in which a similar relation has been assumed in the activity of tumor cells. Observations upon this point are of importance inasmuch as there seems to be considerable evidence to show that mitochondria may give rise to myofibrils in striated muscle fibers (Duesberg), and to connective tissue fibrillæ (Meves). Meves even came to the conclusion that fibrils of epithelium, connective tissue, smooth and striated muscle, nerve cells, and neuroglia are formed from mitochondria. In 1911 Von Fieandt, in his study of the pathological new growth of neuroglia fibrils in dogs, describes their formation from minute bodies (gliosomen) resembling mitochondria, and he draws an analogy between their mode of origin and that of connective tissue fibrils from mitochondria described by Meves. Among the tumors which I have studied were four gliomata, and in their description later I

shall have occasion to mention my own observations upon mitochondria and neuroglia fibrils in human tissue.

With the abundant material available from the Surgical Clinic of the Peter Bent Brigham Hospital, the following study was undertaken with the intention of repeating Bensley's observation on the mitochondria of tumors, benign and malignant, noting any ways in which they differed from those of normal cells, and of observing their possible relations to specific functional activity, and to the formation of cytoplasmic structures of various types. The tumors studied include the following:

BENIGN TUMORS.

Adenoma of breast	4
Struma of hypophysis	6
Adenoma of thyroid	3
Papilloma of bladder	1

MALIGNANT TUMORS.

Carcinoma of testis	2
Hypernephroma	1
Lymphoblastoma of intestine	1
Carcinoma of breast	6
Carcinoma of sigmoid	1
Carcinoma of cecum	1
Carcinoma of stomach	2
Carcinoma of urinary bladder	2
Glioma of brain	4
Endothelioma of dura	1

Technic. — The method of fixation employed was practically that advocated by Cowdry, consisting of a twenty per cent solution of neutral formalin (neutralized with an excess of calcium carbonate) in two and one-half per cent solution of potassium bichromate. Small pieces of tissue from one to two millimeters in thickness were placed in the freshly prepared fixative within five minutes after removal from the body by surgical operation. They were fixed for twenty-four hours, then mordanted three days in two and one-half per cent potassium bichromate; washed in running water twenty-four hours; dehydrated in graded alcohols, and embedded in paraffin. Sections were cut four microns in

thickness and stained with anilin acid fuchsin and methyl green, according to the method of Bensley. As a rule, the fuchsin was applied directly without previous treatment of the section with potassium permanganate and oxalic acid. A difference in physical composition of mitochondria in normal and certain tumor cells was indicated by the use of a weak (three months old) solution of anilin acid fuchsin, which stained these bodies in lymphocytes and endothelial cells of lymph glands, but would not stain them in a carcinoma of the testis, whereas a fresh solution demonstrated their presence in great numbers.

Following is a brief description of the various types of tumors studied and the character of their mitochondrial content:

Hypernephroma: Slowly growing tumor composed of very large polygonal cells with well-defined cellular membranes. Most cells highly vacuolated. Mitochondria present in enormous numbers, often densely packing the cytoplasm. They are more numerous in non-vacuolated cells, and are fewer in number proportionately to vacuolation. In vacuolated cells they appear pushed to a zone about the periphery, but are separated from the cell membrane by a clear zone. In compact cells they are closely gathered about the nucleus. Most of them are granular and very small. In some cells, especially those which are filled with small vacuoles, thread-like forms lie in cytoplasmic strands between vacuoles. There are a few thick, rod-like forms. Some cells contain larger granules with vacuolated centers (degenerating forms). Mitochondria appear to constitute the major portion of cytoplasmic mass, aside from the substance removed from the vacuoles (fat and glycogen) (Fig. 2).

Lymphoblastoma of intestine: Diffuse tumor invading wall of small intestine. Cells of lymphoblastic type, closely packed, having no definite outlines, except where they are present in less numbers in loose tissue. Nuclei fairly large, round, or oblong. No well-defined nucleolus, and little cytoplasm. Mitochondria are not conspicuous. They are usually

very minute, granular, and usually arranged immediately about the nuclear membrane in a thin zone. The size of the mitochondria varies a good deal in single cells, some granules being much larger than others, but all quite small. There seems to be less mitochondrial substance in this tumor than in most others. In my experience cells of the proliferative centers of lymph follicles contain less mitochondrial substance than the mature lymphocytes. This tumor corresponds in appearance to the cells of these centers.

Adeno-carcinoma of cecum: The tumor cells tend to form glandular acini. Mitochondria are present in much fewer numbers and greatly less regularity than in the normal glandular tissue. A single acinus in the tumor may contain a few cells filled with them, the others having few or none. They occur in the form of granules and short rods. In the metastases in neighboring lymph glands there is still a tendency to the formation of acini. Here, too, there are relatively few mitochondria and great irregularity in distribution. Within the cell the mitochondria are arranged in a central core.

Carcinoma of stomach: Tumor cells tend to form glandular acini. Mitochondria are very numerous in the cells of many acini; in those of others they are few in number, or do not appear at all. As a rule, they appear more numerous in the normal glandular cells than in the tumor. They are present in the form of granules and filaments, the former predominating in tumor cells, the latter in normal tissue. Mitochondria are more numerous in the portion of the cell near the lumen, and in differentiated cells. Plasma cells and lymphocytes are numerous and contain numerous well-preserved mitochondria. Those in the normal epithelial cells seem better preserved than in tumor cells. Perhaps the granular forms in tumor cells represent early stage of disintegration, indicating less stability. Mitochondria appear to be less numerous in metastases in a lymph gland than in the original tumor.

Adeno-carcinoma of sigmoid: Mesenteric lymph gland metastases: Tumor cells tend to form glandular acini. Much necrosis in center of cellular groups. Mitochondria very numerous, occurring as granules and short rods. In greater numbers in columnar cells lining acini and in the portion of cell next to lumen. Apparently not so numerous as in normal epithelium of this region and less thread-like forms. Few or no mitochondria seen in many tumor cells.

Carcinoma of testis: Sections uniformly cellular. The individual cells are undifferentiated and are about of an equal size. Nuclei are round or oblong, containing usually a single large distinct nucleolus; there may be more than one. There is no distinct cellular outline, the cytoplasm of adjacent cells appearing to join to form a cytoplasmic meshwork between the nuclei. Cytoplasm is highly vacuolated. Mitochondria are quite numerous, usually granular in form and small; occasionally rods and threads are seen. Often the mitochondria are grouped immediately about the nucleus, but they may be strewn irregularly along the cytoplasmic strands. They are present in dividing cells. No areas of differentiated cells are found in this tumor (Fig. 3).

Carcinoma of testis: Metastases to retroperitoneal lymph gland: Rapidly growing tumor, composed of undifferentiated polygonal cells with ill-defined outline. Numerous mitotic figures. Mitochondria are very scarce as regards their general distribution, although they are very numerous in certain cells. They are extremely minute, usually granular, sometimes filamentous. In most of the cells in areas where mitosis is most active they are not found. The cytoplasm of these cells is rather scanty in amount and is homogeneous and compact, staining light green. The cytoplasm of the cells in which mitochondria are present is faintly pink, and more abundant. These cells seem more quiescent as regards growth, and they are sometimes in the neighborhood of areas of necrosis. Mitochondria are present in abundance in certain cells undergoing irregular mitosis.

Adenoma of breast: Intracanalicular arrangement. Mitochondria numerous, small, and filamentous. In greater numbers nearer the lumen. Ducts differ considerably in quantity of mitochondrial substance in their lining cells.

Senile hyperplasia of breast: Acinar and duct epithelium contain numerous small filamentous mitochondria, possibly not so many as in adenomata and carcinoma. Cuboidal epithelium lining cysts contains a few mitochondria which are coarser and usually granular.

Simple adenoma of breast: Mitochondria very numerous, quite small, and for the most part filamentous in form. They are more numerous in the cells bordering the lumina and in that portion of the cytoplasm immediately adjacent to the lumen. Very little if any difference in appearance of mitochondria in the cells of this tumor and in those of carcinoma, but more numerous and smaller than in intracanalicular adeno-fibroma. More easily stained than in carcinoma.

Intracanalicular adeno-fibroma: Mitochondria of the duct epithelium appear in general less numerous and larger than in the carcinoma of the same breast. For the greater part they have the form of rather coarse rods and granules. They are more numerous in the central portion of a duct occluded by cells than in the cells immediately lining the duct; and where there are more than a single layer of lining epithelium they are more numerous in those nearest to the lumen. They become less numerous and smaller in ducts that are undergoing atrophy. They are more easily stained than in cancerous cells.

Carcinoma of breast (1): Alveolar arrangement of cells. Considerable central necroses of alveoli. Cells relatively large and polygonal. Mitochondria as a rule are extremely small, numerous, and filamentous in form. Degenerating cells contain granules which are larger, coarser, and more

brilliantly stained. Alveoli differ considerably in the quantity and size of mitochondria. In some the cells contain large brilliant rods and granules, so that there is no uniformity.

Carcinoma of breast (2): Mitochondria very numerous, quite small, filiform, and finely granular in shape.

Carcinoma of breast with axillary gland metastasis (3): Medullary type of growth. No tendency to the formation of glandular structures. Numerous mitotic figures. Mitochondria are numerous and unusually small. They are practically all in the form of minute threads, difficult to stain. As a rule the mitochondria in cells undergoing mitosis are larger, more granular, more prominent, and more easily stained than in the surrounding cells. The cells immediately about areas of necrosis and degeneration appear to contain more numerous and more deeply staining mitochondria than others, although the form is the same (Fig. 4).

Tumors of hypophysis (struma): Mitochondria are very numerous, occurring in form of granules and short rods. In the tumors which contain many cells filled with secretion granules the cytoplasm is more abundant and the mitochondria larger than in those which contain few or no cells with secretion granules. In deeply stained preparations the secretion granules give a diffuse purplish tint to the cytoplasm and in this bright red mitochondria are very evident. The secretion granules may be stained sharply with acid polychrome methylene blue eosin, which leaves the mitochondria unstained.

Thyroid adenomata: In two adenomata of the thyroid mitochondria were found in abundance in their epithelial cells, and were in excess of those in normal epithelium. This is in harmony with the findings of Beckton and of Goetsch. In one adenoma the cells were filled with fuchsinophile granules larger than mitochondria and not dissolved by acetic acid (Zenker fixation). These cells correspond in

appearance to the acidophilic granular cells described by Bensley in the thyroid of exophthalmic goiter.

Gliomata of brain: Four examples of this tumor were studied. Two of these showed rapidly growing areas with few glial fibrils, the other two contained an abundance of fibrils. Mitochondria were found in inverse proportion to fibrils. In the rapidly growing areas they were very abundant, situated in greatest numbers about the nuclei and about the centrosphere, a few being scattered along the anastomosing cytoplasmic bridges. Those about the nucleus were small and granular, those lying along cellular processes were often filamentous. Indeed, in certain places there were quite long, wavy filaments, extending through a single cell or from one cell to another along protoplasmic strands. These filaments have the staining reaction of mitochondria, and are of about an equal diameter with these structures. Such appearances can be easily interpreted as representing a stage in the formation of glial fibrils. The glial fibrils, however, do not stain like mitochondria; consequently, if these long filaments have to do with the formation of neuroglia fibrils, they represent an early intermediate stage.

In areas which are differentiating the mitochondria are much more generally distributed throughout the cytoplasmic processes. In older areas, containing masses of neuroglia fibrils, there are few or no visible mitochondria.

SUMMARY. — The presence of mitochondria in cells of malignant as well as benign tumors is not unexpected in view of the established, almost universal presence of these bodies within the cytoplasm of living cells, and especially because of their demonstration in great numbers by Bensley in cells of some types of malignant growths.

As Beckton's work indicated, however, there is a difference in staining reaction of mitochondria in certain malignant tumors, compared with normal cells. This is shown by the fact that by using an old (three months) solution of Altmann's anilin acid fuchsin, consequently relatively weak,

I could stain mitochondria sharply in certain normal cells (lymphocytes, endothelial cells, and those of an adenoma of the breast), but the mitochondria of a carcinoma of the testis remained unstained, but were easily demonstrable by using a freshly prepared stain. This to my mind does not mean an essential chemical difference in the mitochondria, but rather a physical difference, that is to say, a greater concentration or density of the mitochondrial substance of normal tissues, so that the mordant or stain is more firmly fixed within it. The same differences are to be found in the cells of a single tumor — the mitochondria of certain cells may not stain so readily as in others.

The number and, to a certain extent, the character of mitochondria of tumors may vary somewhat from the normal tissue in which they arise, but the difference is not sufficient to warrant a distinction between malignant and benign cells, nor is the difference in staining constant or uniform.

Conclusions concerning the specific functional activity of cells, especially those of tumors, based upon such evidence as an increased amount or difference in character of mitochondrial substance do not seem to me to be justifiable. Mitochondria may be present in great numbers in cells which obviously are not concerned with specific function, *i.e.*, those undergoing mitosis, and in undifferentiated malignant tumors.

In the cerebral gliomata studied in this series of tumors there are pictures which may be interpreted as a participation of mitochondrial substance in the formation of neuroglia fibrils, but such evidence must be considered with caution.

Finally, one is led to the conclusion that mitochondria are composed of material which is utilized by the cell in its general metabolic processes, and in this way may be indirectly involved in the formation of secretion and other specific structures. Their presence in the cytoplasm at any one time represents the reserve of this substance at that time, which varies, depending upon the relative rate of its use and formation by cellular activity.

In harmony with this view is the almost universal presence of mitochondria in cells of every description, and the great variation met with in individual cells of a single tumor. The difference in the ease with which mitochondria may be stained seems to be explainable on the assumption of variations in density or concentration of this substance. In rapidly growing tumors mitochondrial material is probably being used and formed relatively rapidly and therefore does not in most cells become concentrated, as it may during the periods of rest in normal or slowly active tissues.

REFERENCES.

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

Lewis, M. R. and W. H. Amer. Jour. Anat., 1915, xvii, 339.

Cowdry, E. V. Amer. Jour. Anat., 1916, xix, 423.

Beckton, H. Arch. Middlesex Hospital, 1909, xv, 182; Brit. Med. Jour., 1909, No. 2543, 859.

Bensley, R. R. Trans. Chicago Path. Soc., 1910, viii; Amer. Jour. Anat., 1911, xii, 297.

Goetsch, E. Johns Hopkins Hosp. Bull., 1916.

Duesberg, J. Verh. d. anat. Ges. in Giessen, 1909.

Meves, F. Arch. f. Mikr. Anat., 1910, lxxv, 149.

Von Fieandt, H. Beiträge z. pathologischen anat. u. Path., 1911, li, 247.

PLATE X.

FIG. 1. — Tumor of hypophysis (struma). Cell *S* contains acidophilic secretion (?) granules. Mitochondria numerous.

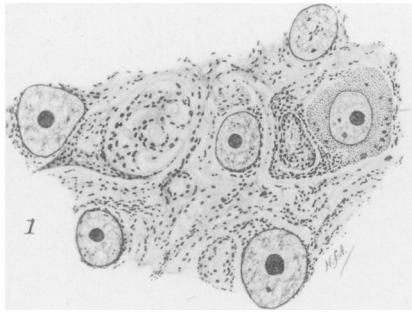
FIG. 2. — Hypernephroma. Mitochondrial granules and rods.

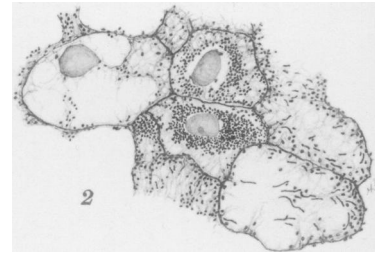
FIG. 3. — Carcinoma of testis. Mitochondrial granules.

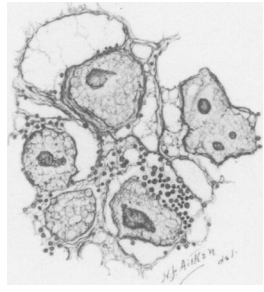
FIG. 4. — Carcinoma of breast. Mitochondria of irregular size and shape.

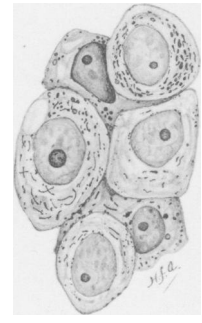
FIG. 5. — Glioma of brain. Numerous granular mitochondria arranged especially about nuclei.

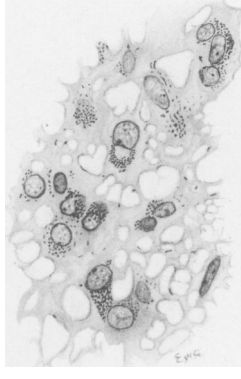
FIG. 6. — Glioma of brain. Granular mitochondria and intracellular filaments.

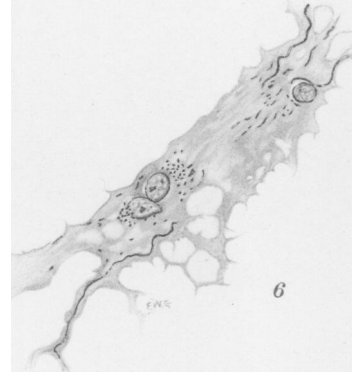

1


2


3


4


5


6