PRELIMINARY NOTES ON THE MITOCHONDRIA OF THE PARATHYROID OF THE WHITE RAT.—BY SINA S. SINGER, Sc. M., From the Histological Laboratory of the New York University Dental College and the Histological Laboratory of Dalhousie University, Halifax, Nova Scotia.

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This paper embraces a study of the mitochondrial granules in the parathyroid gland of the white rat.

The mitochondria, now recognized as a definite class of granules, are found in the cell protoplasm of almost all living tissues. Therefore the presence of the mitochondria must have an important bearing on the function of the given cell. Many observers express different theories and have hazy ideas about the behaviour of the mitochondria.

The real function of the mitochondria is still unknown. Perhaps, this is due to the fact that the study of mitochondria is recent, having been begun within the last two decades. The variety of names attributed to the same kind of granules also was a hindrance to the progress of the study of mitochondria. At present, the mass of literature concerning this subject is largely descriptive.

However, E. V. Cowdry (1918) has presented experimental results and careful studies of the true mitochondria granules from unicellular living cells up to the highest type of organism.

In order to give a historical review of mitochondria, only a few of the more important investigators and their contribution towards this study will be mentioned.

Flemming's (1882-1884) "fila" theory of the constitution of protoplasm proves his careful study of granules. Altman (1890) studied all types of tissues only on the vertebrata, while F. and R. Zoja (1891) focussed their attention on invertebrates. The Zojas concluded that the mitochondria play an important role in nutrition, which view is also held at present.

For an interval of ten years after this time, the study of the granules of the cytoplasm was neglected. Attention was de-

voted solely to the nucleus and its granules. The fixatives used for nuclear details contained mixtures of mercuric chloride, alcohol, chloroform, or acetic acid, because of their rapid penetration and their action on chromatin. These solutions act fatally on the mitochondria unless certain precautions are taken, for the mitochondria are dissolved by such treatment.

In 1889 Benda had introduced the term "mitochondria" for a definite class of granules. Meves (1908) worked on the granules of the cytoplasmic embryonic tissues, and found mitochondria in all embryonic tissues. From this he deduced that the mitochondria have an influence on the modification and on the differentiation of cells, in spite of the fact that at that time the origin of these cell differentiations had been more or less explained to the satisfaction of cytologists without reference to mitochondria. His theory that mitochondria play an important part in heredity attracted world-wide attention, being supported by the discovery that the mitochondria of the spermatozoon enter the egg of fertilization. Simultaneous with these facts, strong support by Morgan and others was given to the chromatin hypothesis.

Regaud (1908) studied the chemistry of mitochondria, proving them to be a compound of phospholipin and albumin, and progress since that time may be attributed to this information regarding the chemical constitution of the mitochondria. He regarded mitochondria in the same light as do the modern physiological chemists and pathologists and tended to promote their interest in phospholipins.

M. R. and W. H. Lewis (1914) devised a method by which they are able to select a certain group of mitochondria and observe their behaviour for a considerable length of time. In 1915 they found that mitochondria continually change in shape by bending in various directions, elongating, contracting, thickening, thinning, etc. The Lewis' also observed mitochondrial networks in living cells of tissue cultures. By changing the osmotic pressure of the fluid bathing the cells in their tissue cultures, they found they could modify the shape of the mitochondria. There was a marked increase

when hypotonic solutions were used, a distinct decrease with hypertonic solutions.

Goetsch (1916) reported that the mitochondria were definitely increased in the cells of pathological tissues. In 1918 he carefully studied mitochondria in twelve simple goiters and fifty exophthalmic goiters and one hundred and twenty-five adenomas. According to Key (1924) Goetsch found in this series that in adenomas with toxic symptoms, the mitochondria were present in excessive numbers; in exophthalmic goiters they were present in enormous numbers; in simple colloid goiters, without toxic symptoms, few or practically no mitochondria were present, while in hypertrophy with symptoms, they were present in moderate numbers. These results indicate that the toxicity of the thyroid varies directly with its mitochondrial content. Goetsch concludes "that a study of mitochondria was the only reliable method of determining the state of activity of certain pathologic thyroids, as in adenomas, every type of growth and degree of hyperplasis might be present and the symptoms still vary from hypothyroidism to hyperthyroidism."

From the foregoing brief historical review one can see that the actual function of the true mitochondria is still doubtful, but it is evident that they are destined to play a noteworthy part in medical research. Further work in the study of the chemistry and physiology of mitochondria will, undoubtedly, aid in understanding the behaviour and relation of these granules to the normal and pathological cells in the given organism.

Throughout this work the term "mitochondria" is used to designate a specific class of granules. An adjective such as rod-like, granular, short, and long is almost always added to the term "mitochondria" to describe or limit its meaning.

The work for this paper was done in the histological laboratory of New York University Dental College (1926-1927) and in the histological laboratory of Dalhousie University (1927-1928) under the direction of Professor Raymond J. Bean of

the Laboratory of Histology and Embryology, Dalhousie University.

Parathyroids taken from ordinary stock colony rats of various ages were examined. The 120-day-old rat is taken as the standard. The rat diet includes milk, water, corn, and bread daily and contains fish and cabbage once a week and meat and lettuce twice a week.

For all the material prepared the following technique was employed: A rat was killed by ether. By means of iridectomy scissors, the parathyroid with a piece of thyroid tissue was removed and then placed in a given fixative, dehydrated, washed, cleared, and embedded in paraffin and sectioned. The thickness of the cross sections varied from 3 to 5 microns. The ordinary egg-albumin-water method was used for mounting and various stains tried. Because the mitochondria are very sensitive, and their shape often changed by slight injury, great care should be taken in handling them. It is advisable to put the given tissue into the fixation fluid immediately, in order that it may not dry.

Three types of cells have been recognized in the parathyroid gland of the white rat (Hoskins, 1924). The chief cells predominate and are found in all parathroids. In some cases a zone of cells which resist ordinary staining methods is found about the periphery of the organ; and a third type, the chromophile may occasionally be distinguished. These chromophiles described by Dr. Hoskins lie at the margin of the gland, and are believed to be due to faulty technique in the preparation of the specimen. In addition to these types, chromophile cells have been observed lying scattered among the chief cells, which will be discussed later as a fourth type.

Regaud's (1910) iron hematoxylin method of Heidenhain: Fixation:

(1) Fix in 3 per cent potassium bichromate 80 volumes, commercial formalin 20 volumes, for 4 days, changing every day.

(2) Mordant in 3 per cent bichromate for 7 days, changing every second day.

(3) Wash in running water hours, dehydrate, clear, embed and section 4 microns, and fix to slides by albumen-water method.

Staining:

(1) Pass down through touol, absolute alcohol, 95 per cent, 70 per cent, and 50 per cent alcohol, about 30 seconds each, to aq. dest. in staining jars.

(2) Mordant in 5 per cent iron alum at 35 degree C for 24 hours. Rinse in aq. dest.

(3) Stain for 24 hours in hematoxylin made up as follows: Dissolve 1 gm. pure crystals of hematoxylin in 10cc. of absolute alcohol and add 10cc. of glycerine and 80cc. of distilled water.

(4) Differentiate in 5 per cent iron alum under microscope.

Note: The crucial point in the technique is passing from the mordant to hematoxylin. The slides must be rinsed in distilled water, otherwise, the iron alum will form a dense black precipitate in the stain. On the other hand, if they are rinsed too much, all the iron alum mordant will be removed. It is necessary to strike the happy mean in which a darkening of the hematoxylin alone occurs. It is always difficult to get good hematoxylin, and I find it best to keep on hand a ripe alcoholic solution.

Following the procedure described above, mitochondrial granules were found in every cell of the parathyroid gland. However, the distribution and morphology of these bodies varies with each cell. Most cells are polygonal in shape, though some are elongated or triangular. The part of a polygonal cell nearest the nucleus is designated as the "proximal part." In the elongate and triangular types, the respective parts may be designated by the terms 'basal" and "apical."

In the parenchyma many chief cells show an abundance of mitochondria in the proximal region, which gradually diminish in numbers towards the apex. In other cases, the dis-

tribution of mitochondria in parenchymatous cells may show a complete reversal of this order. They may be grouped in the form of a pyramid with its apex directed towards the apex of the cell, or in rare instances chief cells show at the apex scattered longitudinal rods similar in appearance to the tubercle bacillus. In contrast to mitochondria, the granules of the vesicular nuclei take the stain more readily and appear as opaque blotches.

On the concavity of the nucleus of some chief cells, an irregular line of granules was found by focusing carefully. This type of mitochondria shows a strinkingly close similarity to G. Bobeau's (1911) illustration. In other chief cells where the nucleus was at the basal membrane, the mitochondrial granules were at the side of the nucleus. It appeared, in certain instances, that the cell walls were more darkly stained at the base. This brings out the fact that in these examples the mitochondria are concentrated in the basal portion of the cell.

The second type of cell found in the parenchyma, forming the light zone at the periphery, has a paler cytoplasm with darker granules which, on focusing, appear to be mostly rod-shaped or granular. These granules are near the nucleus projecting in rays towards the apex of the cell. These cells are best demonstrated when Regaud's formalin bichromate mixture is used a fixative and in conjunction with the iron-hematoxylin staining method of Heidenhain. The mitochondrial granules show blue-black on an iron-gray background, while the thick cell membranes and intercellular connective tissues are also clearly defined.

The third type, the marginal chromophile cell, is not always present in the parenchyma of the parathyroid glands. The mitochondrial granules are spherical and evenly distributed through the cytoplasm. Considerable difficulty was experience in working out a technique satisfactory for a clear demonstration of these cells. In most cases, the cytoplasm stains so deeply that the mitochondrial granules cannot be distinguished.

The fourth type is the central chromophile cell. Its nucleus is smaller and stains darker than that of the chief cell, and the cell membrane is not clearly defined. In general, the cytoplasm is characterized by large numbers of deeply stained spherical granules, but occasionally a crescentic aggregate of granules may be clearly distinguished lying close to the nucleus in the apical portion.

Comparing the four different types of cells, it is certain that the chief cells have the largest proportional number of mitochondrial granules. Often the whole parenchyma in the parathyroid gland of the white rat is composed of these chief cells. The fact that they are common to every parenchyma and that the three other types of cells are variable in their presence also adds to the possibility that the chief cell is of greatest importance. Hoskins (1924)—"The chief cells are the only functionally impurtant type in the parathyroid of the white rat."

The second type of cell with the paler cytoplasm is in the minority, but this fact should not be interpreted to mean that these cells have no functional value. This point of view is not in accord with that of Dr. Hoskins (1924) who states—"It is possible, however, that the light zone is the result of some slight variation in the technique and is quite without functional significance."

The third type of cell in the parenchyma, in 95 per cent of the examined tissues, exists as a single marginal layer which occasionally invades the interior of the parenchyma en masse. This type of cell, is, perhaps, due to a variation in technique.

The type of cell designated as the fourth type may be the result of improper staining, because some sections that take a uniform stain show the absence of dark nuclei.

In addition to a study of the parenchyma of the parathyroid gland, an attempt was made to determine the existence of mitochondria in the connective tissue cells of the supporting framework. Of all the stains tried, Heidenhain's iron-hematoxylin and Bensley's copper chrome hematoxylin are the only

stains that bring out the connective tissues which are fibrous strands varying in thickness. Embedded in these fibers are large cell bodies filled with dark granules. The nucleus of the connective tissue cell is more resistent to this staining procedure than the cytoplasm. Often the connective tissue cells are blurred and look like dark blotches, but, wherever the cytoplasm is less deeply stained, mitochondrial granules are visible. Here again the granular-shaped type of mitochondria are found to predominate.

Summary and Conclusions.

From the work described above on mitochondrial technique, the cells in the parenchyma of the parathyroid gland of the white rat may be divided into four types.

A—Chief Cells.
B—Light Cells.
C—Marginal Chromophiles.
D—Chromophiles.

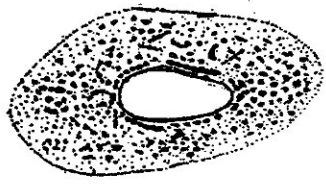
Note:—Hoskins (1924) classes C and D as one type.

A—Chief Cells.

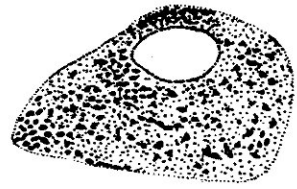
The nucleus of the chief cell is large. The mitochondria are scattered throughout the cytoplasm and are most densly grouped in the proximal portion, being less concentrated but more distinct in the rest of the cell. On one side of the nuclear wall are short filamentous mitochondria with bulb-like swellings. There is a light space between these granules and the nuclear wall. In the distal part, large spherical mitochondria are very numerous; few are found at the side of the walls of the cell.

B—Light Cells.

In the second type of cell, the distribution of the mitochondria is occasionally similar to that of the chief cells. However, the granules are fewer, more distinct and on a paler protoplasmic background. The nuclear wall is encircled by a spherical, light-stained string of mitochondria. By careful focusing, distinct, .ear-shaped granules of uniform size, somewhat darker than the protoplasm, become visible. Frequently, some



I.



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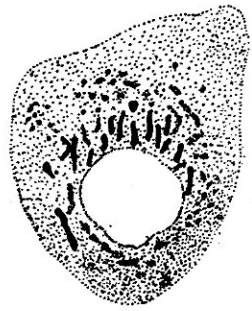
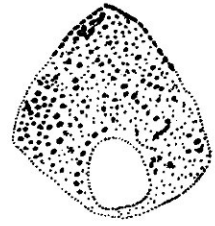


III.

IV.



V.

CHIEF CELLS SHOWING DIFFERENT TYPES AND DISTRIBUTION OF MITACHONDRIA IN PARATHYROID OF WHITE RAT.
—*Sina S. Singer.*

larger isolated, spherical granules appear. The granular-shaped mitochondria predominate in this type of cell. At the extreme distal portion are found single rod-shaped mitochondria, four to five in number, all having the same thickness. These are difficult to distinguish at first glance. Just above the nucleus, appearing to come from it, is a cord-like arrangement of mitochondria, resembling minute thumb-prints. Bunches of light-stained granular mitochondria are often present at the sides of the nucleus.

C—Marginal Chromophiles.

The third type of cell is not always present in the parenchyma. Its presence is often attributed to variations in mechanical techinque This cell appears in a single layer around the margin of the gland. Sometimes this layer invades the interior of the cell. At the margin of the parenchyma, the cells are very dark, and it is difficult to distinguish the nucleus from the cytoplasm. In the cells which invade the parenchyma, however the difference is sharply marked because the cytoplasm becomes lighter. Only spherical mitochondria are common to this type.

D—Chromophiles.

Most chromophiles are elongated cells containing a very dark nucleus and a lighter protoplasm. These cells are very few. The mitochrondria in them resemble in number, structure and location, those of the chief cell. A colorless area is almost always found between the nucleus and the protoplasm.

In the connective tissue cells, the nuclei are very dark and kidney-shaped. The cell walls are not distinguishable. On both sides of the nucleus a lighter stained protoplasm contain granular mitochondria.

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