AN EXPERIMENTAL STUDY OF MITOCHONDRIAL CHANGES IN THE THYROID GLAND.

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Plates 9 to 11.

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That the thyroid plays an important, though little understood, part in growth and in the coordination and regulation of vital phenomena has been demonstrated repeatedly—with unusual clearness in the case of amphibia[1]—and it is fully recognized by physiologists. We are also familiar with some of the more conspicuous constitutional disturbances which follow derangements of the normal activity of the gland. We suspect that the latter is associated in some way with the development of important racial characteristics.[2] And only recently evidence has been presented that resistance to certain infections is likewise in some obscure way linked up with the degree of development (or activity) of the thyroid and of other endocrines. This information has come through the systematic study of experimental syphilis and tumor metastases in rabbits by Brown and Pearce[3] carried out upon a sufficiently large scale to minimize individual variations. The authors mentioned found that changes occurred in the size and in the gross and microscopic appearance of the thyroid gland coincidently with definite stages in disease reactions. Unfortunately, any explanation of the basic phenomena is prevented at the outset by our lack of comprehension of the mechanism of thyroid secretion. The gland is constructed upon a peculiar and totally different plan from any others.

[1] Gudernatsch, J. F., *Arch. Entwcklngsmechn. Organ.*, 1912–13, xxxv, 457.
Swingle, W. W., *J. Exp. Zool.*, 1922, xxxvi, 397.
Uhlenhuth, E., *Proc. Soc. Exp. Biol. and Med.*, 1922–23, xx, 494.

[2] Keith, A., *Lancet*, 1919, ii, 553.

[3] Brown, W. H., and Pearce, L., *Proc. Soc. Exp. Biol. and Med.*, 1922–23, xx, 472, 476.

Recognizing the fact that investigators have advanced about as far as can reasonably be expected with data regarding the size and arrangement of the cells and their nuclei and the appearance of the intrafollicular colloid, Goetsch[4] sought for a more delicate criterion of thyroid activity, and made an investigation of the mitochondria in pathologic human thyroids. He was rewarded by considerable success in correlating mitochondrial changes with clinical symptoms—a result which is fully in accord with the large mass of information at hand bearing upon the probable physiological significance of these granulations. In the hope that an analysis of the experimental conditions which lead to definite alterations in the shape and number of mitochondria in the comparatively easily controlled thyroid glands of guinea pigs, might contribute a little to the general problem, the following experiments were undertaken.

EXPERIMENTAL.

Normal Control.

Since the thyroid is notorious for its variability in apparently normal animals, it was first essential to determine the usual appearance of its mitochondria. This necessitated considerable experimentation in technique and the observation of a selected group of guinea pigs held under slightly different physiologic conditions within the limits of normality.

After tests with many fixatives, it was discovered that the shrinkage of the intrafollicular colloid and the resultant distortion of form relations could be avoided by fixation in a mixture devised by Pianese and composed of the following ingredients.

1 per cent aqueous solution chloride of platinum and sodium	15 cc.
$\frac{1}{4}$ " " " " chromic acid	5 "
2 " " " " osmic acid	5 "
Formic acid C. P.	1 drop.

This fixative was, moreover, found to be an excellent preservative for mitochondria, which, after its use, may be stained easily by treatment with fuchsin and methyl green which gives them a bright crimson color and makes them stand out sharply against a light green background. Because of the tendency of some fluids to

[4] Goetsch, E., *Bull. Johns Hopkins Hosp.*, 1916, xxvii, 129.

cause a partial fragmentation of mitochondria so that they are rendered somewhat more granular than in the living gland, the results were in several instances checked by the Janus green staining of mitochondria in living cells and found to be substantially correct. Other preparations made by the methods of Bensley and Regaud were employed for controls. In estimating the number of mitochondria, care was taken throughout the experiments to secure a uniform degree of differentiation by washing the preparations in water and counterstaining with methyl green to a uniform extent. The mixture of Pianese has the advantage over the fixatives of Altmann and Flemming in that it penetrates easily and rapidly, so that an even preservation of mitochondria throughout the fragment of tissue can be obtained. Short serial sections were usually made in a plane parallel with the long axis of the gland and they were cut at a uniform thickness of 4 microns. The same routine was always followed in fixation and dehydration, embedding, and staining, so the results are strictly comparable. In all cases involving ligation of blood vessels, the control and experimental tissues were fixed in the same bottle, embedded in the same block of paraffin, placed on the same slide, stained together, and mounted under the same cover-glass; but in other experiments this precaution was not considered necessary.

Unfortunately, the guinea pigs were purchased from several dealers and accordingly came from a mixed stock so that their antecedent history and diet were not known. In some cases they were given a short time to adjust themselves to laboratory conditions. Failure to do this always does not vitiate the results for the reason that the control findings remained remarkably constant while changes in the mitochondria did not occur spontaneously but always in response to experimental influences. The animals were kept in individual cages, under similar conditions, and upon a practically constant diet. In consideration of the profound influence of the nervous system upon the thyroid, they were treated with extreme gentleness and unusual care was taken in other ways not to excite them. To procure tissues for examination the animals were killed with chloroform or ether. Many rats and rabbits were employed also for vitamine and insulin experiments, and the normal was carefully standardized in both cases (see Figs. 26 and 37).

A systematic examination was made by this technique of tissues from 13 adults and 11 young normal guinea pigs in various stages of development. The appearance of the mitochondria in the thyroids of normal young adult guinea pigs is represented in Fig. 4. The mitochondria seemed to be identical in the epithelium of large and small follicles, but follicles of about the same size were selected for comparison to make it more direct. The findings remained constant from day to day throughout the period of experimentation (October to June). No mitochondrial differences were detected between the sexes except during pregnancy. Although the mitochondria were relatively more numerous in embryonic thyroids (Fig. 1) and most abundant after birth (Figs. 2 and 3) their uniformity was remarkable in young adults of about 325 gm. such as were chosen for experimentation (Fig. 4). Old animals, unless very senile and almost moribund, showed no distinctive changes in the mitochondrial content of the thyroid (Fig. 5).

Interference with Blood Supply.

In surgical practice, one of the simplest and most direct methods of reducing thyroid activity is by ligation of the large arteries supplying the gland. To ascertain how the mitochondria respond to this treatment, the larger vessels to one gland were ligated in a series of animals, the gland on the other side being left untouched. Both glands were removed and examined cytologically at the following intervals after operation: 2 animals after 1 day; 3 after 2 days; 2 after 3 days; 3 after 4 and 5 days, respectively; 2 after 6 and 7 days, respectively; 3 after 8 days; 2 after 9 days; and 1 after 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, and 60 days.

No alterations were noted after 1 and 2 days of reduced blood supply but after 3 days, the mitochondrial filaments had become shorter and more granular (Fig. 6). This change gradually became more and and more pronounced each succeeding day up to the 10th day, as illustrated in Figs. 7, 8, 9, and 10, when all the mitochondria had rounded up into spherules. That the response was a very delicate one is shown by the fact that no parallel modifications could be detected in the shape of the follicles, in the appearance of the nuclei, or in the condition of the colloid substance. It was only after the 10th day that changes took place which would be noticeable in ordinary histological preparations fixed, for example, in Zenker's fluid and stained with hematoxylin and eosin. It is interesting to note that while the mitochondria retained their rounded form and occurred in about the same number from the 10th to the 25th day (Figs. 10 to 13), the nuclei commenced to exhibit unusual polymorphism which steadily increased up to the 45th day, as is shown in Figs. 12 to 15. During this later period, the mitochondria rapidly decreased in number

(Figs. 14 and 15) and continued to disappear to the 60th day, when the cells were distinctly swollen and the nuclear chromatin unusually granular (Fig. 16).

Approximately similar changes have been described in anemic necrosis of the kidney by Israel.[5] The negative results obtained by McCann[6] are perhaps to be explained by the peculiar and unusual resistance of mitochondria in nervous tissues to injurious influences and by the slow rate of autolysis as compared with glands like the thyroid and kidney.

Partial Extirpation.

For purposes of contrast and in order to investigate the effect upon the mitochondria of increased functional demand for thyroid secretion, the whole of one gland and half of that on the opposite side were removed in a series of five guinea pigs. They were sacrificed after 5, 10, 15, 20, and 25 days, respectively. It was found that while the mitochondria in the remaining fragment of tissue retained their usual form, they increased considerably in number. This may be seen by reference to Figs. 17 and 18.

These mitochondrial changes in experimental compensatory hypertrophy of the thyroid gland apparently differ from the alterations observed in exophthalmic goiter by Goetsch[4] in the failure of the individual mitochondrial filaments to increase in length, although in both conditions there is a marked increase in number. A corresponding increase in the number of granulations, probably of mitochondrial nature, has been observed in compensatory hypertrophy of the kidney by Enderlen,[7] Hirsch,[8] and De Giacomo.[9] It seems to be of rather common occurrence in regenerating tissues,[10] despite the fact that Oliver[11] has reported a decrease in the regenerating kidney cells of uranium nephritis.

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[5] Israel, O., *Virchows Arch. path. Anat.*, 1891, cxxiii, 310.

[6] McCann, G. F., *J. Exp. Med.*, 1918, xxvii, 31.

[7] Enderlen, E., *Deutsch. Z. Chir.*, 1895, xli, 208.

[8] Hirsch, C., *Anat. Hefte*, 1910, xli, 125.

[9] De Giacomo, A., *Internat. Monatschr. Anat. u. Physiol.*, 1911, xxviii, 208.

[10] Romeis, B., *Anat. Anz.*, 1913, xlv, 1.

Torraca, L., *Arch. Zellforsch.*, 1914, xii, 539; *Anat. Anz.*, 1914, xlv, 459; *Arch. ital. anat. e embriol.*, 1916–17, xv, 326.

[11] Oliver, J., *J. Exp. Med.*, 1916, xxiii, 301.

Effect of Thyroid Feeding, Fasting, and a Water-Soluble B Vitamine-Free Diet.

The mitochondria reacted quickly to the administration of whole desiccated thyroid gland substance.[12] After feeding it at frequent intervals during 1 day, they showed a tendency to round up into spherules (Fig. 19), which was more marked after 2 days. After 4 days, no filamentous forms remained and the total number had become greatly reduced (Fig. 20). The animals at the same time rapidly lost weight. It is evident, from a comparison of Figs. 20 and 12, that an end-result of the same general character as far only as the mitochondria are concerned, may be obtained by vessel ligation and thyroid feeding. But this does not mean that there is necessarily any fundamental parallelism in the mechanism of cellular response to these therapeutic measures, because, as we shall see, fragmentation and diminution of mitochondria are reactions exhibited under conditions of the most diverse kind. For example, after the daily subcutaneous injection of 1 cc. of 1 per cent potassium iodide solution in water, the mitochondria again lost their filamentous form and became spherical, though in this instance the animals gained in weight. The same result was obtained whether they were kept under the influence of potassium iodide for 10 days (Fig. 21) or 25 days (Fig. 22).

Quite the opposite was the effect of fasting. After 3 days the mitochondria lost their filamentous form (Fig. 23); after the 4th day there was a definite diminution in number coincident with the appearance in the cytoplasm of large round bodies which blackened with osmic acid (Fig. 24); while at the end of 6 days of fasting the mitochondria completely disappeared and the droplets blackening with osmic acid exhibited a further increase in number (Fig. 25). These results are in general accord with observations which have been made by several investigators[13] on the influence of inanition upon the mito-

[12] Prepared by Burroughs Wellcome Company, London, England.

[13] Russo, A., *Arch. Zellforsch.*, 1910, v, 173.
Azzi, A., *Arch. sc. med.*, 1916, xl, 22.
Ono, S. I., *China Med. J.*, 1920, xxxiv, Anat. suppl., 23.
Ma, W. C., *Anat. Rec.*, 1923, xxv, 157.
Okuneff, N., *Arch. mikr. Anat.*, 1923, xcvii, 187.

chondria in other tissues, although there is some difference of opinion as to whether the mitochondria completely disappear before death.

Eight rats fed upon a water-soluble B vitamine-free diet[14] showed in all cases a diminution in the number of the mitochondria of the thyroid gland and a rounding up into spherules, in confirmation of results previously obtained by Miller[15] and Wakefield[16] in the gastrointestinal epithelium and in the pancreas. Compare Fig. 27 with the normal for the rat as represented in Fig. 26. In this connection we may profitably bear in mind that the literature contains many references to rapid modifications in the shape and the number of mitochondria in simple aseptic autolysis, notwithstanding the fact that the nuclei may show no evident deviation from the normal.[17] An instance in point is shown in Fig. 28.

Effect of the Inhalation of Gases.

The mitochondria were also found to respond directly and with astonishing rapidity to the inhalation by the animal of various gases. Four guinea pigs were allowed to breathe almost pure oxygen in a glass chamber of about 2 liters capacity, with occasional return to air, for $1\frac{1}{2}$, 5, $6\frac{1}{2}$, and 9 hours, respectively. The mitochondria in each rounded up into spherules to about the same degree, irrespective of the duration of inhalation (Figs. 29 and 30).

In the administration of carbon dioxide, the animals had to be periodically revived by permitting them to breathe air at frequent intervals. Changes in the mitochondria were noted that were like those after oxygen as far as could be ascertained, and were also independent of whether the treatment was continued for $2\frac{1}{2}$, 4, 6, or $10\frac{1}{2}$ hours; but the alterations were not quite so pronounced, since a few rod-like mitochondria could still be found, as reference to Figs. 31 and 32 will show.

[14] Obtained through the kindness of Miss Muhlfeld.

[15] Miller, S. P., *Anat. Rec.*, 1922, xxiii, 205.

[16] Wakefield, H. W., *Anat. Rec.*, 1923, xxv, 158.

[17] Dannehl, P., *Virchows Arch. path. Anat.*, 1892, cxxviii, 485.
Takaki, K., *Arch. mikr. Anat.*, 1907, lxx, 245.
Biondi, G., *Virchows Arch., path. Anat.*, 1915, ccxx, 232.

A parallel, but perhaps less striking granular metamorphosis was discovered after the inhalation of hydrogen under the same conditions for interrupted periods amounting to $2\frac{1}{2}$, 5, and 7 hours (Figs. 33 and 34).

As a control measure, animals were allowed to breath just as little air as would suffice to maintain life for $2\frac{1}{2}$, 4, and $6\frac{1}{2}$ hours. Upon making preparations, the mitochondria were found to be unaltered, except that they evinced a tendency to be clumped about the nuclei (Figs. 35 and 36). Miller[15] also failed to detect mitochondrial changes resulting from asphyxiation. The alterations described by Champy[18] in tissue cultures, and attributed by him to asphyxiation, are difficult of interpretation.

Effect of Chemical Agents.

The following substances were deliberately selected as likely to modify cellular activity radically and in different ways: insulin, atropine sulfate, pilocarpine hydrochloride, adrenalin chloride, morphine sulfate, potassium arsenite, hydrogen cyanide, and phosphorus. All, except the insulin which was given to rabbits, were administered subcutaneously.

For experimentation with insulin, Dr. P. A. Levene very kindly placed at my disposal a series of eighteen rabbits which had received injections of about 20 mg. of insulin per kilo of body weight directly into the heart. They usually showed convulsions from 1 to 2 hours later lasting for 3 or 4 hours. Tissues were taken $\frac{1}{3}$, $1\frac{3}{4}$, 3, $3\frac{1}{4}$, $3\frac{1}{2}$, 4, $4\frac{1}{4}$, $4\frac{1}{2}$, $5\frac{1}{4}$, $5\frac{1}{2}$, $5\frac{3}{4}$, and 6 hours after injection. None of the specimens (Fig. 38) showed any noticeable departure from the normal (Fig. 37), which is interesting—à propos of theories to the effect that mitochondria are concerned in cellular respiration,[19]—in the light of the fact that insulin treatment causes a tremendous increase in the rate of oxidation. A similar result was obtained with three guinea pigs examined 6, 51, and 52 hours after repeated small subcutaneous injections of insulin. Compare Figs. 39 and 40.

[18] Champy, C., *Arch. zool. exp. et gén.*, 1914, liv, 307.

[19] Kingsbury, B. F., *Anat. Rec.*, 1912, vi, 39.

Mayer, A., Rathery, F., and Schaeffer, G., *J. physiol. path. gén.*, 1914, xvi, 607, and others.

Atropine sulfate was administered to two animals in doses of 0.001 gr. twice in 24 hours, followed by 0.02 gr. which killed them within an hour's time. Tissues were taken immediately and a change of some mitochondria into granular forms was noted (Fig. 41). Two other animals were given 0.01 gr. every day for 10 days which caused a reduction in body weight of about 10 per cent with a change in the mitochondria to the granular form (Fig. 42). Pilocarpine hydrochloride was given to two guinea pigs in 0.05 gr. doses, increased by 0.05 gr. each hour for $4\frac{1}{2}$ hours, which likewise brought about a partial rounding up of the mitochondria (Fig. 43). Two others received 0.0625 gr. doses, increased by 0.0625 gr. each day, for 3 days and 5 hours, which proved fatal. In this instance the mitochondria were all changed to granular forms and their number greatly depleted (Fig. 44). Atropine and pilocarpine, then, substances which produce distinctly different physiologic responses in glandular tissues caused in the thyroid a similar rounding up of filamentous mitochondria into spherules.

1 cc. of 1:1,000 adrenalin chloride solution injected in the neighborhood of the thyroid gland killed an animal in 2 hours; and a close study of the tissues revealed an increase in size and a rounding up of mitochondria (Fig. 45). The experiment was twice repeated with similar results. When 1 cc. was injected subcutaneously, remote from the gland, in two guinea pigs daily for 3 days and 10 days, respectively, the same result was again obtained (Fig. 46).

Two animals were given 0.125 gr. of morphine sulfate daily for 10 days without noticeable effect upon the mitochondria (Fig. 47). Two others received 0.125 gr., increased by a like amount each day for 25 days, which caused a loss of 10 per cent in weight, some rounding up of mitochondria and a diminution in their number (Fig. 48).

Potassium arsenite, administered in 0.5 cc. of 1 per cent solution doses to two animals, brought about death in 5 hours with no detectable change in the mitochondria (Fig. 49). Two others received 1 cc. of a 0.1 per cent solution daily for 11 days which caused a loss of weight of 9 per cent and a barely noticeable alteration in the mitochondria in the direction of spherule formation (Fig. 50).

Hydrogen cyanide given in a dose amounting to 0.5 cc. of U.S.P. No. 9 to two animals resulted fatally in 15 minutes, without producing any alteration in the mitochondria (Fig. 51). Another animal received 1 drop of HCN and a second drop at the end of 26 hours, and was sacrificed. Preparations of the thyroid again revealed no change in the mitochondria. Two others were given 1 drop, increased by 1 drop twice daily for 6 days, which killed them and produced a profound modification in the cell with almost complete disappearance of mitochondria (Fig. 52). Here again the time factor was evidently of importance.

Lastly, phosphorus was employed. A guinea pig given subcutaneously 0.5 cc. of a saturated solution in olive oil died in 21 hours. Preparations of the thyroid showed a granulation and swelling of the mitochondria and the appearance of the familiar droplets of fatty degeneration, which blackened with osmic acid (Fig. 53). Two others received doses of 1 cc. of the same solution, diluted with four parts of olive oil, daily for 5 days which killed them. There was a complete disappearance of mitochondria and a further increase in the masses that blackened with osmic acid (Fig. 54). With a view to further prolonging the time of treatment 0.5 cc. of the diluted solution was injected daily for 6 days which also resulted fatally, with loss of mitochondria and a tendency on the part of the blackened bodies to disappear also. It will be recalled that Scott[20] observed a similar fragmentation of mitochondria with the appearance of droplets of fat in the acinus cells of the pancreas of mice likewise poisoned with phosphorus. In addition, he discovered that the fat was formed through the clumping together, fusion, and metamorphosis of the mitochondria. The decrease in number of mitochondria had been noted several years earlier by Lubarsch,[21] who referred to them as Altmann's granules.

RESULTS.

The foregoing experiments have demonstrated the following constant qualitative and quantitative changes in the mitochondria in the thyroid glands of guinea pigs.

1. Fragmentation of Filaments into Granules.—This was the most common reaction encountered and was produced by many influences:

[20] Scott, W. J. M., *Am. J. Anat.*, 1916, xx, 237.

[21] Lubarsch, O., *Ergebn. allg. Path. u. path. Anat.*, 1897, iii, 631.

reduction of blood supply, feeding of thyroid substance, fasting and vitamine deficiency, the inhalations of oxygen, hydrogen, and carbon dioxide, and by the administration of atropine, pilocarpine, adrenalin, morphine, phosphorus, potassium arsenite, and hydrogen cyanide. It occurred with the greatest rapidity after breathing oxygen and not so readily after strong poisons like arsenic and cyanide. It was associated with both an increase and a decrease of body weight. The general similarity in response to influences of such divergent character is compatible with the hypothesis that the filamentous state of mitochondrial substance is dependent upon the maintenance of conditions which may be extremely easily disturbed in a variety of ways. It is very remarkable that no definite changes were observed as a result of the injection of insulin, which causes an acceleration of oxidative processes.

2. *Reduction in the Number of Mitochondria.*—This reaction was usually observed coincidently with the preceding, and in common with it, and may be often illusory resulting from faulty technique alone. The change was, however, noted with great constancy after reduction of the blood supply, the feeding of thyroid substance, on vitamine deficiency, and when pilocarpine, morphine, and hydrogen cyanide had been administered. It was also observed after phosphorus poisoning and fasting, but in these instances was concurrent with the appearance of fatty droplets within the cells. Such an association of mitochondrial loss with the appearance or non-appearance of visible fat indicates forcibly that a similar mitochondrial response may occur in opposed functional states.

3. *Increase in the Number of Mitochondria.*—Increase in the number was noted only in association with compensatory hypertrophy after removal of some three-quarters of the thyroid tissue. The shape of the mitochondria remained unaltered. In no case has it yet been possible to bring about experimentally an increase in the length of the mitochondrial filaments— that is to say, to produce a change such as Goetsch[4] discovered in exophthalmic goiters. In order so to do it would probably be necessary to experiment extensively along somewhat different lines.

EXPLANATION OF PLATES.

The drawings were made with the aid of a camera lucida, a Zeiss compensating ocular 8 and an apochromatic objective of 2 mm. They have been reduced one-third in reproduction so that they now present a magnification of 1,000 diameters.

PLATE 9.

FIG. 1. Specimen from an embryo shortly before birth, which showed mitochondria of normal morphology but slightly more abundant than in young adults. (*Cf.* Fig. 4).

FIG. 2. The many mitochondria observed 1½ hours after birth.

FIG. 3. 4 days after birth; mitochondria still more numerous than they are in young adults.

FIG. 4. Mitochondria in a young, healthy adult guinea pig of about 325 gm. taken as the normal.

FIG. 5. Tissue from an old guinea pig, showing mitochondria which were slightly shorter and less abundant than those of Fig. 4.

FIGS. 6 to 10. 3, 5, 7, 9, and 10 days after ligation of large blood vessels to gland, showed a progressive tendency on the part of the mitochondria to become shorter and more granular in form.

FIGS. 11 to 13. Specimens of the ligation series, procured after 15, 20, and 25 days, showed continuance of rounding up of mitochondrial filaments.

FIGS. 14 to 16. 35, 45, and 60 day stages, illustrative not only of a rounding up of the mitochondria into granules, but of the gradual lessening in their number.

PLATE 10.

FIGS. 17 and 18. The increase in number of mitochondria observed in the thyroid 10 and 25 days, respectively, after the removal of one gland and of half the gland on the opposite side.

FIGS. 19 and 20. The rounding up of filamentous mitochondria into spherules and decrease in number occurring after 1 and 4 days feeding of thyroid gland substance.

FIGS. 21 and 22. To show that the daily subcutaneous injection of potassium iodide in dilute solution for 10 and 25 days, respectively, brought about a loss of the normal filamentous shape and a decrease in size of mitochondria.

FIGS. 23 to 25. 3, 4, and 6 days of complete fasting caused a rounding up of mitochondria, a pronounced decrease in number, and the appearance in the cytoplasm of many large droplets which blackened with osmic acid.

FIGS. 26 and 27. Illustrating the normal appearance of mitochondria in the thyroid of a white rat and the decrease in their number which resulted from holding white rats upon a vitamine B-free diet.

FIG. 28. For comparison, the gland from an animal which had been dead for 7 hours, showing complete autolysis of mitochondria.

FIGS. 29 and 30. Effect of breathing pure oxygen alternating with air for 1½ and 9 hours. It caused a transformation of filamentous mitochondria into spherules.

FIGS. 31 and 32. Breathing carbon dioxide and air alternately for 2½ and 10½ hours produced comparable but less marked changes.

FIGS. 33 and 34. Breathing hydrogen under the same conditions for 2½ and 7 hours likewise caused a rounding up of mitochondria.

FIGS. 35 and 36. Animals allowed as control to breathe only sufficient air to keep them alive for 2½ and 6 hours exhibited no change in the shape or number of the mitochondria, but showed a tendency to clump about the nucleus.

PLATE 11.

FIGS. 37 and 38. To illustrate the appearance of mitochondria in the thyroid of a normal rabbit and the lack of response on their part to an injection of insulin directly into the heart 6 hours previously.

FIGS. 39 and 40. The mitochondria in the thyroids of guinea pigs were equally unresponsive showing no changes 6 hours after the subcutaneous injection of insulin or 52 hours after the repeated injection of small doses.

FIGS. 41 and 42. No alterations in the mitochondria were to be noted 1 day after the administration of a heavy dose of atropine, but the repeated injection of small doses over a period of 10 days caused all to become rounded into spherules with a slight lessening in their number.

FIGS. 43 and 44. After a dose of pilocarpine, the majority of the mitochondria became spherical in 4½ hours. Repeated doses over a period of 3 days and 5 hours caused all the mitochondria to become spherical while their number underwent a great reduction.

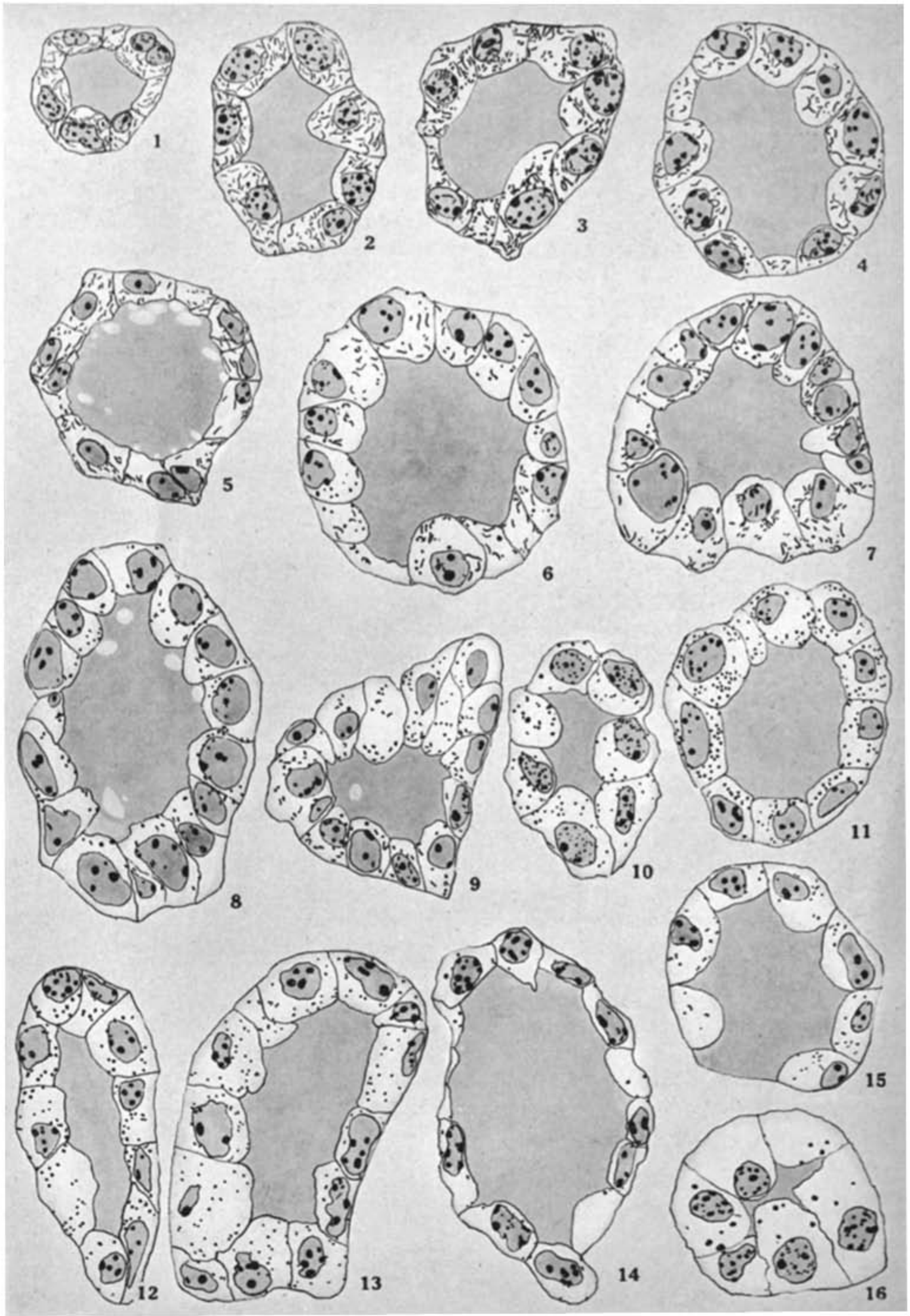
FIGS. 45 and 46. The mitochondria had rounded up and increased in size 2 hours after the injection of adrenalin as well as when smaller quantities had been injected daily for 10 days.

FIGS. 47 and 48. Daily dosage with morphine for 10 days produced no alterations in the mitochondria, but after the treatment had been continued for 25 days, they were distinctly more granular and slightly reduced in number.

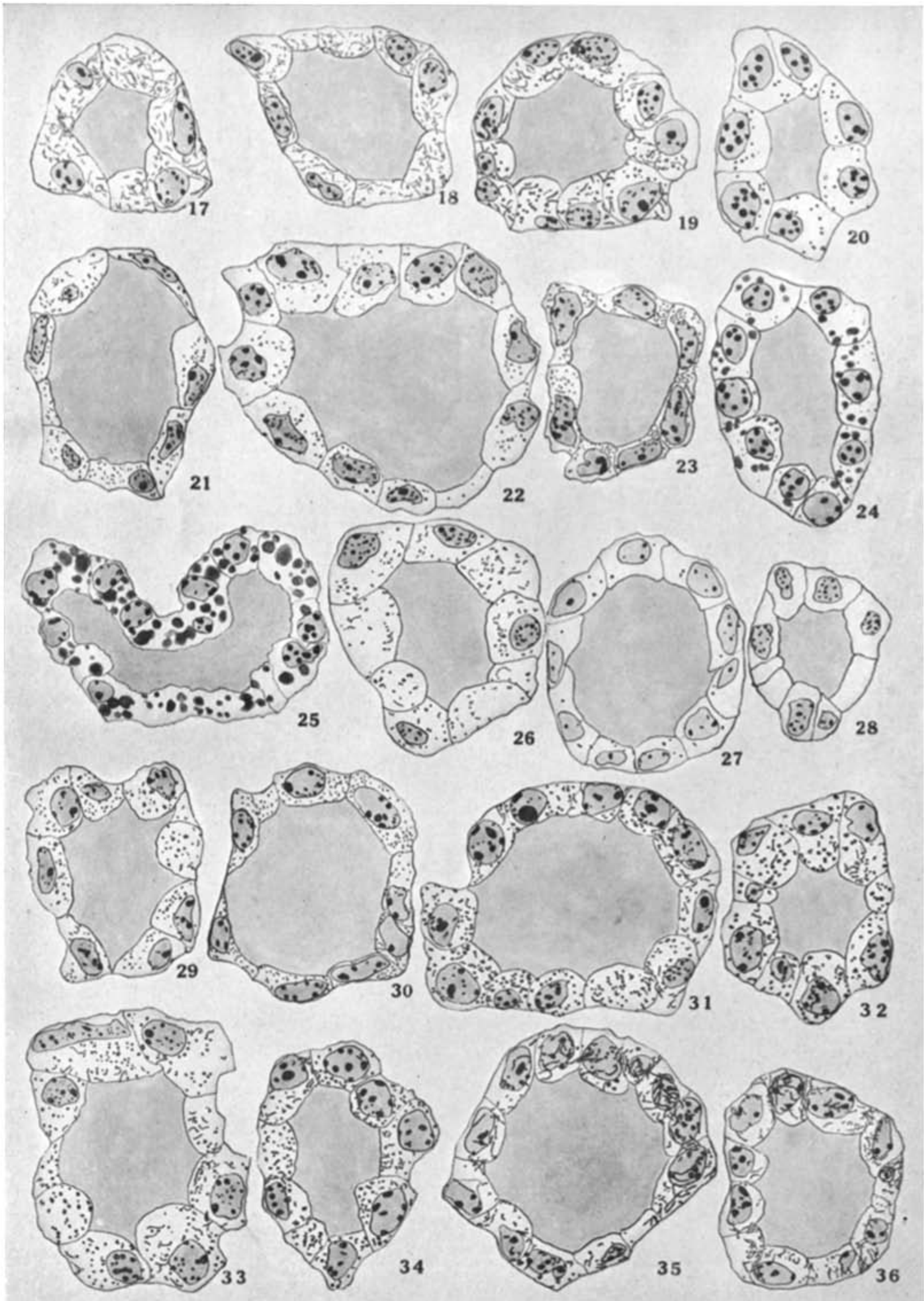
FIGS. 49 and 50. With arsenic, no changes could be detected after 5 hours, but when smaller amounts had been given for 11 days, the mitochondria became spherical.

FIGS. 51 and 52. Similarly in the case of hydrogen cyanide, a lethal dose left the mitochondria normal whereas when the administration was distributed over 6 days a considerable depletion in number was brought about.

FIGS. 53 and 54. The injection, subcutaneously, of a solution of phosphorus in olive oil produced a rounding up and disappearance of mitochondria and the formation of numerous large droplets blackening with osmic acid.



(Nicholson: Mitochondrial changes in thyroid gland.)



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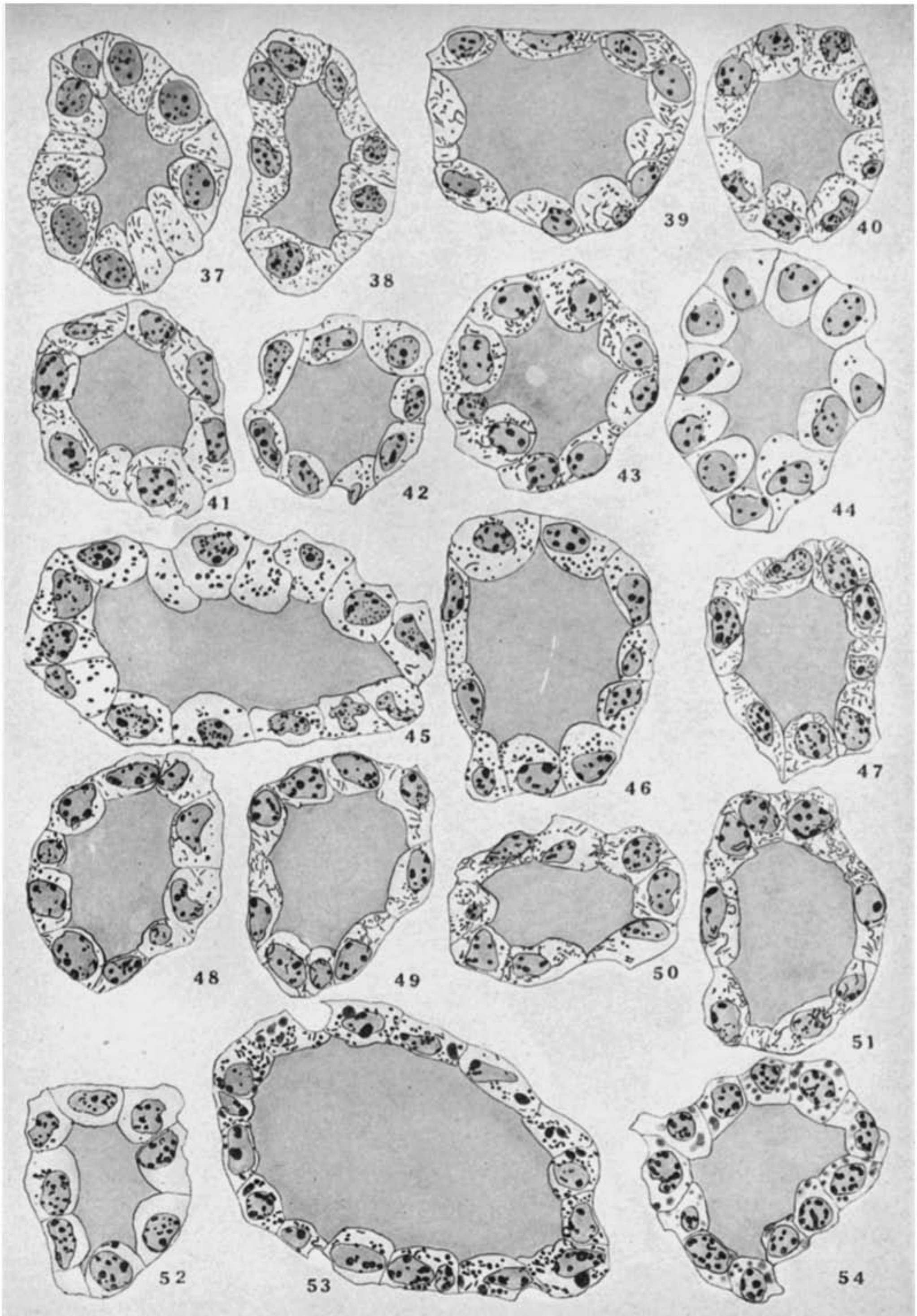

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