ON CELLULAR ACTIVITY AND CELLULAR STRUCTURE AS STUDIED IN THE THYROID GLAND.

BY W. CRAMER AND R. J. LUDFORD.

(*From the Laboratories of the Imperial Cancer Research Fund.*)

THE following observations were made as an attempt to establish a correlation between changes in the functional activity and in the structure of the cytoplasm as indicated by alterations in the mitochondria and the Golgi apparatus. Both these structures can be seen in the living cell and are not, therefore, artefacts produced by special methods of fixation. The mitochondria have, moreover, been seen to undergo spontaneous changes in size and shape in living cells (1). We have used the cells of the thyroid gland as the most suitable test object for the following reasons. They are cells of a secreting gland, but unlike most gland cells they do not contain during rest their specific secretory product within themselves to any great extent. Most cells of a secreting gland are filled with their specific secretion—we may give as instances the acinar cells of the pancreas, the medullary cells of the adrenal, and the cells of the salivary glands, while the liver cells contain glycogen as their specific secretion. It is obvious that in such cells changes in the internal structure may result for purely mechanical reasons, owing to the accumulation, and subsequent rapid discharge of secretion in the course of functional activity. In the resting thyroid gland the specific secretion accumulates as the "colloid" outside the cells, within the lumen of an alveolus.

Another reason for selecting the thyroid gland was that previous observations by one of us (2) had determined a number of conditions which are associated with rest and activity respectively of the gland. Thus, exposure to cold and the injection of β-tetrahydronaphthylamin produce an intense activity of the gland, while a resting condition is induced by exposure to a hot environment, and by thyroid feeding. The increased activity of the gland on exposure to cold and after injection of tetrahydronaphthylamin manifests itself by an intense congestion of the interalveolar and the intra-alveolar capillaries and by a disappearance of the colloid from the alveoli. A figure illustrating this condition has been published in a previous paper (3). The congestion of the intra-alveolar capillaries may be so intense, especially after tetrahydronaphthylamin, that hæmorrhages into the centre of the alveoli may occur. Sometimes the cells lining the alveoli get detached and are pushed into the central lumen of the alveoli. Conversely heat and thyroid feeding induce inactivity of the gland. We have then an accumulation of colloid in the alveoli which appear distended with colloid. The interalveolar capillaries close up altogether so that the lining cells appear to be resting directly upon the basement membrane. The thyroid gland offers, in fact, a very striking illustration of the opening and closing of capillaries in the different stages of functional activity of an organ. The staining reaction of the colloid also changes. In material fixed in Formol bichromate solution and treated subsequently with osmic acid (Schridde's method) the colloid of the resting thyroid gland, that is to say, the gland of a rat or mouse kept in a hot environment or fed on thyroid gland, stains deeply with Heidenhain's hæmatoxylin and retains the stain tenaciously on differentiation with iron-alum. In the active gland the first change is the loss of this affinity for hæmatoxylin, so that the colloid appears colourless on differentiation. The response of the thyroid to heat which was first described by one of us in 1916, has since been confirmed by Mills (4).

In a previous publication (3) has been discussed the significance which these observations have in demonstrating that the thyroid forms together with the adrenal a humoral mechanism for the heat regulation of the body. In the present paper, where we are dealing with changes in the activity of the thyroid induced by different agencies we may point out the importance of controlling the thermal environment if one wishes to get comparable results. It is probable that the neglect of this factor is responsible for many of the contradictory or inconclusive results recorded in the literature concerning changes in the appearance of the thyroid gland.

As an illustration of the rapidity with which the thyroid responds to changes in the thermal environment, Figs. i, ii and iii are given here. Fig. i represents the thyroid of a mouse kept at ordinary room temperature. Figs. ii and iii give the appearance of the thyroids of mice which for a week had been subjected to the following routine. They were kept in glass jars which were placed in an incubator kept at 37° C. at 10 o'clock in the morning and removed at 5 o'clock in the evening. From 5 p.m. till 10 a.m. of the following day they were kept in a room with an open window during cool weather in March. After having been subjected to this regular routine for several days one mouse was killed in the afternoon after having been kept for 6 hours in the incubator. The

Fig. i. Room temperature.

Fig. ii. Immediate effect of cold, following heat.

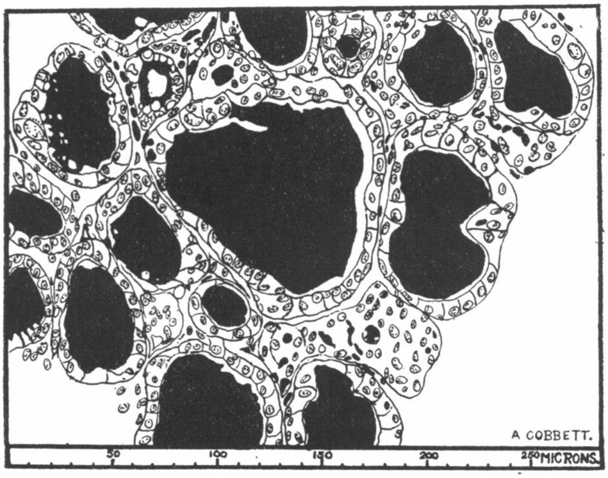


Fig. iii. Immediate effect of heat, following cold.

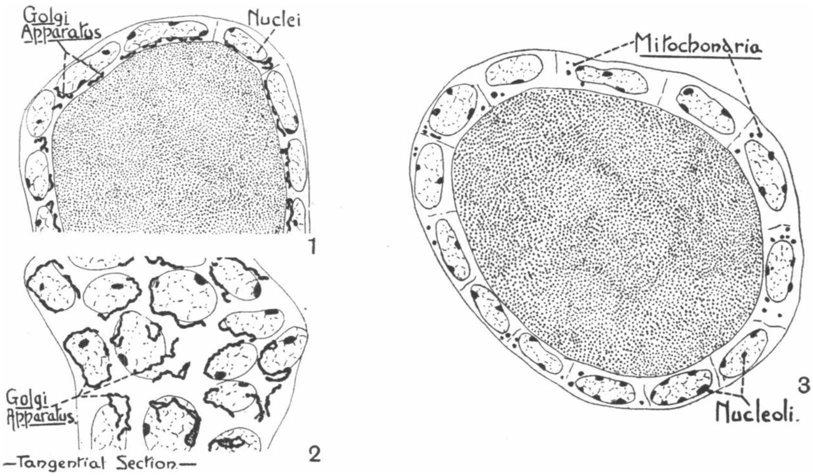
Figs. i–iii. Thyroid of mouse. × 300.

appearance of its thyroid which represents the immediate effect of a hot environment is given in Fig. iii. The other mouse had been removed from the incubator at room temperature and kept in the cool room for 4 hours, when it was killed. It shows the immediate effect on the thyroid gland of a cool environment as seen in Fig. ii. This experiment has been repeated frequently with different variations, such as one single prolonged exposure to heat, with the uniform result that exposure to heat leads to an accumulation of deeply staining colloid, which on exposure even to a moderately cool environment rapidly disappears.

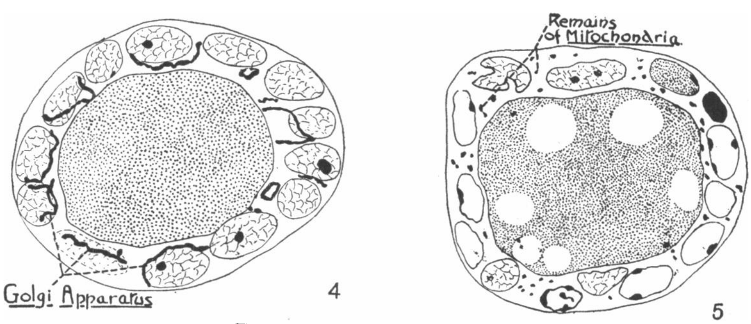
Having established a number of conditions which induce rapidly and with certainty rest and activity of the thyroid gland in such animals as the mouse and the rat, it was possible to study changes in cellular structure in these different conditions as manifested by changes in the mitochondria and in the Golgi apparatus in order to see whether cellular activity in the cells of the thyroid is correlated with definite changes in these cytoplasmic elements. So far little definite knowledge is available concerning the function of the mitochondria, although this problem has been the subject of a good deal of speculation. The mitochondria of the thyroid gland of guinea-pigs subjected to a number of different conditions have been studied by Nicholson(5). His findings have, however, not given any very clear or conclusive results. Goetsch(6) observed an increase in the number and size of the mitochondria in toxic adenomata of the thyroid associated with symptoms of hyperactivity of the gland. He pointed out the importance of finding a criterion for determining whether individual thyroid cells are functionally hyperactive or not, since many clinical cases of hyperthyroidism can frequently not be correlated with the ordinary histological appearance. He suggested that a study of the mitochondria might furnish such information. Quite recently Seecoff(7) has published observations showing an increase in the mitochondria in the thyroid gland of guinea-pigs and rats in which hyperplasia had been induced by fat feeding and a diminution of the mitochondria when involution of these hyperplasic glands was induced by iodine. Here again we are dealing with chronic pathological conditions rather than a physiological response of the gland. No figures are presented recording these changes, which detracts from the value of these observations.

The Golgi apparatus has been studied by various authors in cells of externally secreting glands in different stages of secretory activity. In the resting cells it assumes a contracted and relatively simple form and is then closely applied to the nucleus with a definite orientation. During functional activity of the cells it has been found to undergo enlargement extending from the nucleus in the direction of the lumen. In many glands the secretion arises in intimate relationship with the apparatus, which later becomes broken up into fragments.

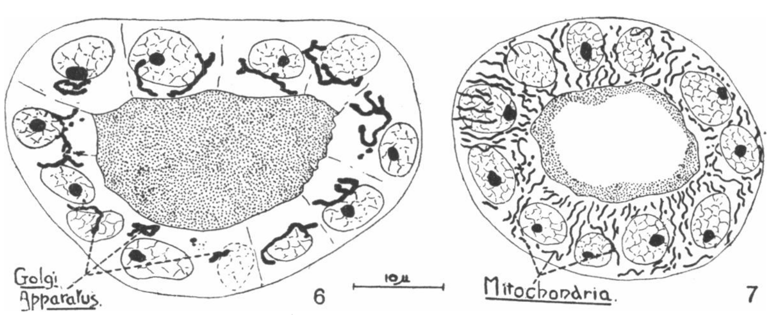
The methods employed by us for the demonstration of these two cytoplasmic structures were as follows. For mitochondria Schridde's fixation (Formol bichromate solution with subsequent treatment by



Figs. 1–3. Thyroid fed.

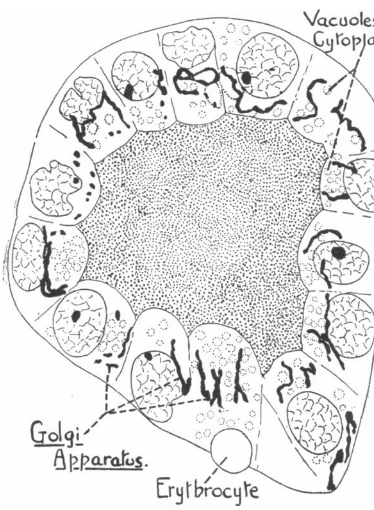


Figs. 4, 5. Exposure to heat.

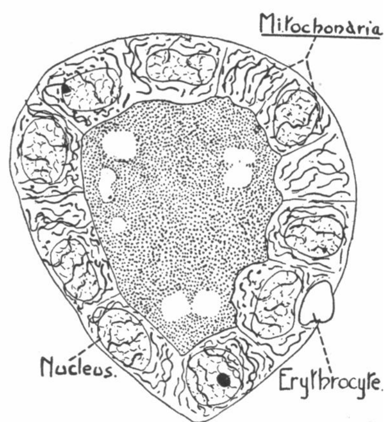


Figs. 6, 7. Fasting.

osmic acid) and staining with Heidenhain's iron-alum hæmatoxylin. For the Golgi apparatus Ludford's(8) modification of the Mann-Kopsch method (fixation in osmic acid and sublimate and subsequent treatment with warm osmic acid). The actual appearances are given in Figs. 1–11, to which the following details may be added. Figs. 1–3 from a rat fed on 0·1 grm. thyroid daily for 30 days. The animal was then in a good state of nutrition and the post-mortem examination showed no obvious changes from the normal. Fig. 4 from a rat kept at 37° C. for 3 hours. Fig. 5 from a rat kept at 37° C. for 36 hours. Fig. 6 and Fig. 7 from a rat kept without food for 20 hours in the animal room which was kept at a fairly even temperature of about 16–20° C. This may be taken as representing a condition of normal activity of the thyroid gland in the rat. Fig. 8 from a rat which had been epilated and kept in the open air on a warm autumn day for 3 hours. Fig. 9 from a rat not epilated and



8

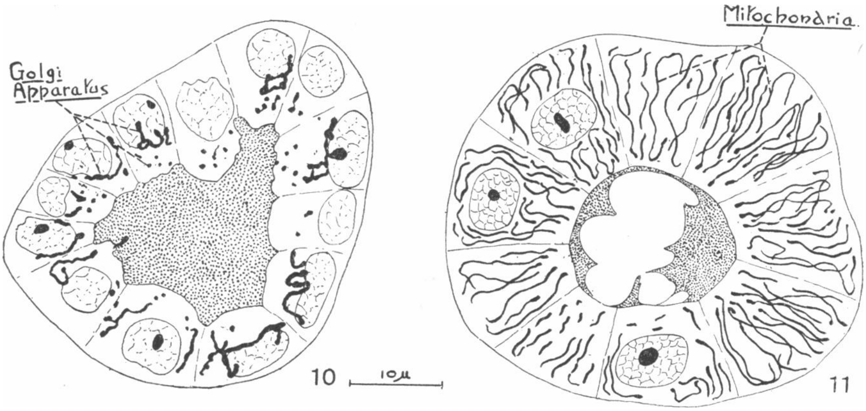


9

Figs. 8, 9. Exposure to cold.

kept for 1 hour in the open in a glass jar on a cold winter day. Both these rats had previously lived in the warm animal room. Fig. 10 from a rat killed 30 minutes after the injection of 15 mgrm. of β-tetrahydronaphthylamin (= T.H.N. in figures). Fig. 11 from another rat 30 minutes after the injection of 10 mgrm. of the same drug. All the animals were killed with coal gas and the thyroids fixed at once. The experiments have been carried out repeatedly and the figures give typical appearances for each condition.

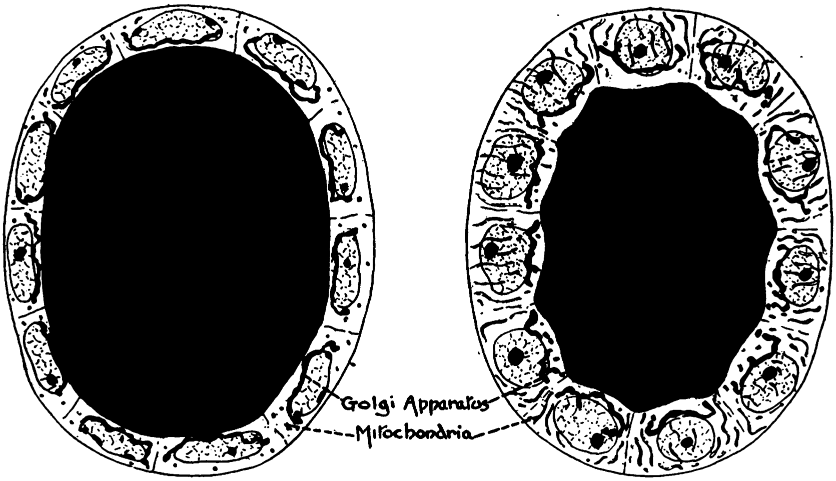
The differences are particularly striking when one compares cells of glands showing the two extreme stages, such as complete rest after exposure to heat and intense activity after injection of tetrahydronaph-



Figs. 10, 11. Action of T.H.N.

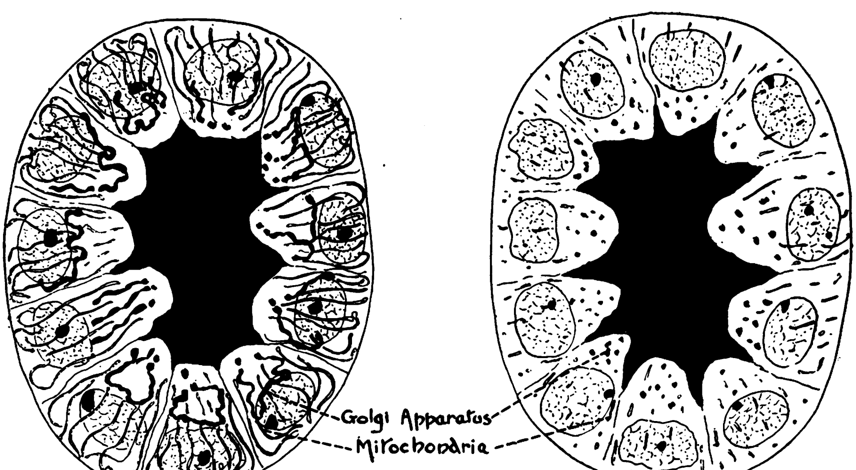
thylamin. In the cells of the thyroid of a normal animal all stages of activity may be seen in different alveoli, while the cells of each alveolus present a uniform appearance. This is due to the fact that the cells of the thyroid function in relays, as do, in fact, the cells of all secreting glands. In such glands as the pancreas or the adrenal medulla, where the specific secretion can be demonstrated within the cells, one finds that while the cells of a resting gland are fairly uniformly charged, they do not participate equally in the process of secretion when the gland is stimulated to activity. In the active pancreas for instance one may find groups of acini having discharged completely their zymogen granules, while others are still filled with them. Similarly, in the active adrenal medulla secretion begins always in the cells lying around the central vein and its immediate tributaries. These cells will show profound changes, while in parts of the gland remote from this region the cells may still present an appearance of rest. In the thyroid we find similarly a fairly homogeneous appearance in the cells of the completely resting gland while in the active gland different alveoli may present different stages of activity. The figures represent the predominant appearance under the conditions indicated.

The figures are self-explanatory, so that a detailed description is unnecessary. A pictographic summary showing the changes in the mitochondria and the Golgi apparatus in a semi-diagrammatic form is given in Fig. 12. The results may be summarised as showing a definite correlation between cell activity and cytoplasmic structure: with increasing activity the mitochondria become more and more differentiated from the cytoplasm, they enlarge and become filamentous, while with diminishing



Resting. Thyroid fed. Exposure to heat.

Moderate degree of activity. Fasting.



Extreme degree of activity. Exposure to cold. Action of β-tetrahydronaphthylamin.

After extreme degree of activity. Following exposure to cold, or action of β-tetrahydronaphthylamin.

Fig. 12. Pictographic summary.

activity their differentiation from the cytoplasm becomes less distinct so that only a few granules remain visible. After prolonged increased activity of the gland the large thread-like forms break up into shorter rods.

The Golgi apparatus shows the same changes which have been observed in cells of externally secreting glands in different stages of secretory activity; it exhibits a simple contracted form in the resting cell; during activity it enlarges, becomes convoluted, and after prolonged activity it breaks up. In the thyroid we find similarly the simple contracted form after thyroid feeding and after exposure to heat, while after exposure to cold and after injection of tetrahydronaphthylamin it is enlarged and convoluted and begins to disintegrate. This is itself confirmatory evidence that the various conditions examined by us induced rest and activity of the thyroid.

Cowdry (9) has observed changes in the position of the Golgi apparatus in the thyroid gland of the guinea-pig and has suggested that this position might vary with the direction in which the specific hormone is secreted. He found that in some alveoli the Golgi apparatus had the usual orientation, lying on the side of the nucleus directed towards the alveolar lumen, while in others it was lying on the other side of the nucleus. He makes the interesting suggestion that in the resting gland, when the specific hormone is secreted into the central alveolar lumen, the Golgi apparatus lies on the side of the nucleus directed towards the centre of the alveolus. He assumes that during activity the thyroid hormone is secreted directly into the blood vessels or lymphatics and that the Golgi apparatus wanders round the nucleus with the change in the direction of secretion and comes to lie on the opposite side. This suggestion is of importance, because, if correct it would enable us to use the position of the Golgi apparatus in the thyroid cell as an indicator of its activity. There is, however, no evidence in Cowdry's observations of an increased activity of the gland, where he found that the Golgi apparatus had reversed its position. In our observations where the state of functional activity of the gland was known, we have found no evidence to support the suggestion that the Golgi apparatus exhibits a consistent change of polarity varying with activity of the gland, although occasionally it has been seen to lie on the side of the nucleus away from the centre of the alveolus. An example of this appearance is seen in one cell of Fig. 8. Since in our preparations the position of the Golgi apparatus showed no consistent change in the conditions of extreme activity, it appears to us doubtful, in the absence of further evidence, whether Cowdry's interesting interpretation can be accepted.

In addition to the changes in the cytoplasm there are also nuclear changes. The most obvious change is a diminution of the chromatin content as evidenced by a loss of staining power of the nucleus in conditions of intense secretory activity.

The findings recorded in this paper have a general interest from the point of view of cell mechanics. In discussing the part played in the cell by surface energy due to structural surfaces account has been taken hitherto only of the so-called cell membrane which is really the most peripheral part of the cytoplasm, and the nuclear membrane. The cytoplasm has been described erroneously as homogeneous and without visible structure by authors dealing with cell mechanics, Bayliss (10) and Leathes (11) for instance, though conclusive evidence to the contrary has been furnished by cytologists, as detailed in Cowdry's book. In the mitochondrial apparatus the cytoplasm has a mechanism by which it can alter surface energy by creating within itself an enormous surface, or reducing the surface to a minimum. In the thyroid cells the former alternative is associated with intense activity, the latter with a condition of rest. We have obtained evidence of a similar relationship in the adrenal medulla and the liver. Now the concentration of lipoids in the cell membrane can be explained on physico-chemical grounds as being due to the fact that substances which lower surface tension tend to accumulate at the surface. The same will occur therefore within the cytoplasm when a very extensive surface is created by the enlargement of the mitochondria. The cytoplasmic lipoids will accumulate around the mitochondria. It is interesting to note that on the basis of their staining reactions the mitochondria have been described as being composed of a core of protein surrounded by a sheath of lipoids and further that the formation of fat globules in cells has been associated with mitochondrial activity. As the mitochondrial surface diminishes the lipoids will return to the cytoplasm. This ebb and flow of the lipoids from the cytoplasm to the mitochondrial surface and back which accompanies changes in the mitochondrial surface, must necessarily affect the concentration of the lipoids in the cytoplasm and therefore also in the cell membrane. With increased activity of the cell and increasing mitochondrial surface the lipoids will be withdrawn from the cell membrane to the mitochondria. During rest the change will be in the opposite direction. Such a process would account for alterations in the permeability of the cell.

SUMMARY.

Conditions were established which induce intense activity and com plete inactivity respectively of the thyroid glands. A very clear relationship could be demonstrated between the functional state of the gland and the mitochondria. In the resting glands the mitochondria are barely visible. In the active gland they show an enormous enlargement. The condition of the mitochondria presents therefore a criterion for the functional state of the gland, and may, as Goetsch has suggested, be useful in the study of pathological conditions of the thyroid. The Golgi apparatus shows the same changes which have been observed in the other gland cells in rest and activity: a simple, contracted form in the resting cell, an enlarged convoluted form during activity, followed by a disintegration into granules.

The significance of the mitochondrial changes from the point of view of cell mechanics is discussed. The mitochondria represent a mechanism by which the cell can produce great variations in surface energy within the cytoplasm. On the basis of accepted views such variations must be accompanied by changes in the distribution of the lipoids within the cytoplasm and the cell membrane. These changes, in turn, would affect the permeability of the cell.

REFERENCES.

1. Cowdry. General Cytology. University of Chicago Press, p. 311. 1924.
2. Cramer. Proc. Physiol. Soc. July 15, 1916; This Journ. 50. p. xxxviii. 1916.
3. Cramer. 6th Scientific Report of the Imperial Cancer Research Fund. London: Taylor and Francis. 1919.
4. Mills. Amer. Journ. Physiol. 46. p. 329. 1918.
5. Nicholson. Journ. Exp. Med. 39. p. 63. 1924.
6. Goetsch. Johns Hopkins Hosp. Bull. 27. p. 129. 1916.
7. Seecoff. Amer. Journ. Path. 1. p. 295. 1925.
8. Ludford. Journ. R.M.S. 1926. (In press.)
9. Cowdry. Loc. cit. p. 336.
10. Bayliss. Principles of General Physiology. London: Longman, Green and Co. 1924.
11. Leathes. The Lancet, Pt. 1. p. 1019. 1925.