Studies on the Cytology of the Harderian Gland of the Rat.

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With 9 Text-figures.

In a recent study by the author (1933 a, b) of the secretory process in various gland-cells, it was shown that (a) the secretory granules originated at the base of the cell in relation to the mitochondria, and later moved into the Golgi zone where maturation occurred, and (b) that development of the granules occurred in every case in intra-cellular vacuoles, these vacuoles being also the spaces inside which the vital dyes, Janus green and neutral red, are segregated. The present short study was undertaken, since it was noted that secretion formation in the Harderian gland-cell is different from that of gland-cells included in the former studies, and that in consequence certain points raised in the former work were in this case more clearly demonstrated. The purpose of the paper is then a description of the cytological elements of the gland-cell secretory granules, mitochondria, and Golgi apparatus, and a comparison of these structures with those obtained by vital staining with neutral red and Janus green.

The Harderian gland of the rat was used, the animals being killed by coal gas. Vital staining with both neutral red and Janus green was carried out supra-vitally at 37°C, using a 1/30,000 solution in Ringer of the former dye, and a 1/15,000 of the latter. Fixations used were 10 per cent. formol, Zenker formol, Da Fano, Mann's, and Champy's fixatives. Mitochondria were demonstrated by intensive staining using iron-alum haematoxylin, following the mitochondrial technique given by Cowdry (1918), i.e. 24 hours in 5 per cent. iron alum at 35°C., and 24 hours in 1 per cent. haematoxylin and glycerol.
after Champy fixation. The Golgi apparatus was demonstrated by Da Fano's method, and by Nassanow's (1924) modification of the Kolatchev technique.

**HISTOLOGICAL APPEARANCE.**

As described by various authors, Sundwall (1916) and Buschke (1933), the gland consists of two portions, an upper smaller lobule occupying the upper portion of the eye-socket, and a lower larger and more diffuse lobule occupying the floor of the optic cavity. As there is no marked histological difference between the lobules, they are here considered together. The histological appearance is that of alveoli with a wide lumen surrounded by cells completely filled with secretory granules. These granules are of a lipoidal character, and are not present in paraffin sections following non-osmic fixation. A membrana propria lies at the base of the secretory cells, and between it and the cells is a well-marked arrangement of basket cells. The lumina of the alveoli lead directly into the ducts.

**VITAL STAINING.**

In the unstained gland the alveolar cells are seen to be filled with large numbers of highly refractile secretory granules. These granules are often extremely large, being 2.0–2.2 μ in size. Many of the cells, however, contain smaller granules, and all stages are to be seen. When stained supra-vitally with neutral red, it is at once noticed that certain cells, about 10–15 per cent. of the total number, are selectively stained, so that under the low power those cells containing red-stained granules are easily visible through the gland. The appearance of the majority of the gland-cells is shown in Text-fig. 1. Even after heavy staining only one or two neutral red-stained granules appear, and occasionally the periphery of one or two of the larger granules is also stained. Very often, however, these cells remain completely unstained. In the stained cells a remarkable condition is found. After very light staining, 2 minutes immersion in 1/80,000 neutral red solution, almost all of the formed granules are stained to some degree as in Text-fig. 2. This varies from a complete staining of the periphery as in granule A, to
a partial peripheral staining as in granule B. In addition there are numerous smaller neutral red-stained granules which show a complete staining, though it is occasionally possible to focus a colourless granule inside. With more prolonged staining, 2–3 hours at 37°C, the appearance found in Text-fig. 3 is obtained. All the stained granules here show complete staining of the periphery, the stained area around the granule in all cases being much larger than that shown in Text-fig. 2. The irregularity of staining shown by some of the granules of Text-fig. 2 is still preserved, the red area on one side of the granule being often three or four times as wide as that on the other. Fusion between the red-staining portions as at X is also common. In addition, a few unstained granules still remain, these being probably fat granules, and are not related in any way to the secretory elements.

Staining with Janus green is found to give rise to two appearances. In some of the cells staining of the mitochondria occurs. These are small granules or short rods lying all through the cell, and are similar to those pictured by Sundwall (1916), and Beattie and McDonald (1933). They are shown in Text-fig. 5, which is drawn from a fixed preparation following the mitochondrial technique given. In Text-fig. 4, however, the more usual appearance is shown. As in the case of neutral red, certain of the cells show a well-marked staining of a portion of the periphery of the secretory granule, and in addition a number of smaller green-stained bodies. With Janus green, however, the complete periphery of the granule is rarely if ever stained. Partial staining, such as is usual in the more lightly neutral red-stained cells, is commoner, the stained area being in the shape of a small green crescent or batonette attached to one pole of the granule. By staining first in Janus green and then in neutral red double staining is obtained, the innermost portion of the batonette being green and the outer red.

**Golgi Apparatus.**

In the Cajal, Da Fano, and Champy techniques, all of which were used, an exactly similar type of Golgi apparatus was found. This is shown in Text-fig. 6 taken from a Da Fano
preparation, and Text-figs. 7 and 8, which are Champy preparations. The Golgi apparatus consists in almost all cases of peculiar small crescentic bodies applied to the surface of the secretory granules. In smaller granules such as are present in Text-figs. 6 and 7, it may completely surround the newly forming granules A. In all cases, even after a very short treatment with 2 per cent. osmic acid, both the secretory granules and attached Golgi bodies blackened completely, and both Text-figs. 7 and 8 are drawn from cells strongly bleached by hydrogen peroxide. Of these, Text-fig. 7 is a cell in an advanced stage of refilling, the main mass of granules next the lumen being in the process of formation. Those granules around the nucleus are in all probability granules which have not been thrown out in the last discharge of the cell. Text-fig. 8 is a still earlier stage of secretory formation shortly after discharge, the smaller granules A, completely surrounded by a ring of osmiophilic material, being in an early stage of granule formation. With increase in size the osmiophilic ring is broken, the Golgi body being reduced to a crescentic body on the periphery of the granule. In completely matured cells the osmiophilic substance is no longer visible.

It is interesting to note in this connexion the work of various authors on lipoid secretory gland-cells. Heidenhain (1890) described in the pelvic and other glands of Triton a double appearance found in the secretory glands after ordinary fixation. Each granule apparently consists of two parts, the granule itself, a spheroidal stainable body, and a dark shell-like hood, the optical cross-section of which was a crescent. In a later paper by Fleischer (1904), he describes in the lachrymal gland of the ox peculiar crescentic structures applied to the surface of the granules as a cap, the Halbmondkörperschen. Sundwall (1916), working on the lachrymal gland of the same animal, was unable to confirm these findings. Bowen (1924) suggested a direct homology between these bodies and the osmiophilic bodies described by Nassanow (1923), and Bowen (1924), in various glands of salamanders. Ludford (1925) described similar crescentic bodies around the secretion granules in the sweat glands of the mouse following osmication, and identified these structures as Golgi bodies. In a later paper by Bowen (1926),

an exactly similar appearance is shown in the cells of the oil-gland of the common fowl, and here again the crescentic structures are identified as Golgi bodies. In the Harderian glands of the cat, rabbit, and duck Bowen (1926) found the Golgi apparatus
as a network lying at the periphery of the secretion mass, which is not extraordinary in view of the fact that the morphology of these glands varies from species to species. In both the cat and mouse which were investigated by the present author it was established beyond all doubt that these crescentic structures shown in Text-figs. 6, 7, and 8 were the Golgi bodies. In cells of the lachrymal gland of both animals osmicated for five to six days, nothing but the crescentic bodies were obtainable, and these were present mainly in the more immature cells. They were also the only structures found using silver impregnation following Cajal and Da Fano’s fixatives. Identification of these crescentic bodies by any other than the osmic methods, as described by Fleischer (1904), was however difficult. At times in tissue fixed in formol it did appear that the crescentic bodies were revealed by basic stains though their small size rendered this difficult. It is thus quite probable that these osmiophilic bodies are homologous with the crescents described by Heidenhain (1890) and Fleischer (1904), though their small size in rodents renders identification by any other than the osmic or silver methods difficult.

DISCUSSION.

In drawing any conclusions from the above investigation the results following vital staining must be compared with those structures displayed in fixed preparations. In previous studies by the author (1933a, b), it has been clearly demonstrated that neutral red and Janus green stain newly forming secretory granules, since both dye substances are segregated in the intracellular vacuoles surrounding these immature granules. Using this as a basis it is then clear that the cells showing selective staining with the dyes are cells in which secretory granules are being built up. In the Harderian gland of the rat this is apparently brought about by a simultaneous ripening of all the granules. Cells in which the secretion is fully formed, as in Text-fig. 1, show practically no staining of the granules since the perigranular vacuole has disappeared. With excess staining with neutral red the appearance found in Text-fig. 3 is obtainable, the granules showing a marked perivacuolar enlargement owing
to excess deposition of the dye substance. The perigranular vacuoles of Text-fig. 3 are in every way comparable to the krinom bodies of Chlopin (1927), and to similar bodies induced by the author (1933 b) in the pancreas and salivary gland-cells. Their fusion, as at X in Text-fig. 3, would show that this may be a part origin of the large masses found in other gland-cells. The relation of these perigranular vacuoles to the Golgi bodies is of great interest. In the author’s previous studies (1933 a, b), it was demonstrated in the various cells examined that the maturation of the granule, which was accompanied by loss of its surrounding vacuole, occurred inside the Golgi network. After excess vital staining which induces krinom deposits, the krinom which is the enlarged, fixed, and stained vacuole lay usually inside the network, the osmiophil strands of this surrounding it. This has been demonstrated by Ludford (1930), and has been confirmed by the present author. Krinom deposits lying outside the Golgi zone in the pancreas are not, however, in any relationship to the Golgi apparatus. It is clear then that in the case of perigranular vacuoles in the Golgi area the Golgi strands lie outside the vacuole, and in the case of the lachrymal gland it would seem that the probable development of a secretory granule in the Harderian gland is that represented in Text-fig. 9. As will be seen it is a modification of that originally given by Bowen (1929). In stage a, the granule A is first formed inside a vacuole B, in which vital dyes may be stored, as in Text-figs. 1–4. The vacuole is in turn surrounded by an osmiophilic ring C, such as are seen in the early stages in granule formation shown
in Text-figs. 6 and 8. Enlargement of the vacuole as in Text-fig. 9b and c is accompanied by a limitation of both osmiophilic substance and vacuole to one side of the growing granule, so that both in sections have usually a crescentic appearance. In this connexion it is of interest that Heidenhain (1890), in his pictures of the development of the secretory granules of the pelvic glands of Triton, distinctly pictures a space between the crescentic bodies and the secretory granule. Owing to the small size of these bodies in the rat this is difficult to see, except in vitally stained preparations, but there is little doubt that the space is the crescentic perigranular vacuole, and Heidenhain's drawing corresponds closely to Text-fig. 9c. Later development, d, occurs through a disappearance of the vacuole as in more mature cells, the osmiophilic substance being limited to the crescentic body on one side of the now almost mature granule. It is not clear, however, what finally happens to this osmiophilic cap. Many mature cells show granules without it, and its later fate is uncertain.

Conclusions.

1. A study of secretion formation in the Harderian gland of the rat shows that the secretory granules are formed inside vacuoles, which may be selectively stained by Janus green and neutral red.

2. A comparison of the fresh and fixed preparations makes it probable that the Golgi bodies are in every case applied to the surface of the perigranular vacuole, throughout the development of the enclosed granule.

3. A scheme for granule development in this gland is advanced.

List of References.


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