Some Observations upon the Golgi Elements of the Anterior Pituitary Cells of Normal and Stilboestrol-treated Male Rats, using the Sudan Black Technique

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With one Text-figure and one Plate

INTRODUCTION

THIS investigation was undertaken in order to discover whether the Golgi elements of anterior pituitary cells possessed the duplex structure described by Baker (1944) for cells of certain vertebrates and invertebrates, after fixation in formal-calcium chloride and staining in sudan black.

Pituitaries of normal mature male rats and comparable animals implanted with tablets of stilboestrol were used. The latter were studied for comparative purposes, since it is known that both synthetic and natural oestrogens activate the glandular cells of the anterior hypophysis, causing, among other changes, hypertrophy of the Golgi elements (Severinghaus, 1937; Foster, 1942).

METHODS

Six control and nine experimental rats with an average weight of about 150 gm. were used. The animals were killed by a blow on the neck, the experimental animals being killed at varying times after the subcutaneous implantation of a 10 mgm. tablet of stilboestrol.

The technique for demonstrating the lipoids of the Golgi zone with sudan black followed that described by Baker (1944). Since, in some instances, there was a tendency for a precipitation of the dye on the surface of the frozen sections, the following method was devised to overcome this difficulty.

The sections were first washed to remove the formal-calcium-cadmium chloride solution in which they had been stored and were then dipped in a 12 per cent. solution of gelatine, kept liquid in the 37° C. incubator. The slide was then lifted out and allowed to drain for half a minute, when the gelatine on the back was wiped off. The thinnish layer of gelatine on the section side of the slide was allowed to dry off until it became tacky, and was then coagulated by holding the slide, gelatine face downwards, beneath the surface of a dish of 90 per cent. alcohol. The section was then transferred to the filtered sudan black solution. The time necessary for adequate staining was, of course, found to be rather longer by this method and varied with the
thickness of the gelatine film. In practice, the slide was periodically removed from the dye, rinsed in 50 per cent. alcohol, and examined under the microscope.

At the completion of staining, the slide was rinsed in 50 per cent. alcohol and the gelatine film was readily removed by immersion in warm water. After further washing in distilled water, the preparation was mounted in glychrogel (Cowdry, 1943).

**Results**

1. **Normal Animals**

   (a) **The Position of the Golgi Zone.** In many instances the Golgi zone was clearly identifiable in the glandular cells of the pars distalis and details of its structure could be made out. This was particularly true of the basiphil cells, Golgi elements of which, as is well known, are revealed in Nassonov-Kolatchev preparations as fairly large spheroidal nets, generally situated at a distance from the nuclear membrane (cf. Text-fig. 1, i and Pl. 1, i, iv, v; Text-fig. 1, ii–vi). The details of the Golgi zones of the acidophil cells were, generally speaking, not so clearly defined, but the position and shape of the zone were almost exactly similar to those found in Nassonov-Kolatchev preparations; the zone was like a peruque capping the nuclear membrane. (Cf. Text-fig. 1, i, and Pl. 1, iii, vii, viii; Text-fig. 1, ii, iii, iv.) The Golgi elements in the small chromophobes were more difficult to make out, but in a number of instances it was possible to see that in some it had the shape and position characteristic of the acidophil type (Text-fig. 1, iii), while in others, its shape and position were such as are to be found in the basiphil cell (Pl. 1, vi). These observations were in agreement with those of Severinghaus (1936), who observed that in the mature gland, the two distinctive types of Golgi element characteristic of the acidophil and basiphil cells were present also in the chromophobes. Finally, the large chromophobes showed the same two forms of Golgi zone as was noted in the chromophils and small chromophobes—they are in fact, as has been shown by Severinghaus (1933), chromophils in the process of granule depletion.

Although the sections were not counterstained, very little difficulty was encountered in the identification of the cells types mentioned above, particularly as the preparations were studied in conjunction with stained Nassonov-Kolatchev material. The small chromophobes were the smallest elements of the cell population and were devoid of granules. The yellowish refractile granules of the acidophils were easily recognizable, and the basiphils were recognizable by means of their larger size, their indistinct granules, and characteristically placed Golgi bodies. The large chromophobes of the acidophil type were either partially or completely devoid of granules, as were those of the basiphil type. In the latter, this was reflected in a considerably reduced intensity of cytoplasmic staining with the sudan black.

(b) **The Structure of the Golgi Zone.** The Golgi zones of both acidophil and basiphil cells possessed three features in common and, in fact, differed
Text-fig. 1.

i. Normal φ. a, peruke-like Golgi element capping the nucleus of an acidophil cell; b, spheroidal Golgi zone of a basiphil cell. Nassonov-Kolatchev. Modified Mallory stain. ×650.

ii. Control φ. a, Golgi zone of acidophil cell; b, Golgi element of basiphil cell. ×325.

iii. Control φ. a, acidophil cell with Golgi zone immediately above and capping the nucleus; b, basiphil cells with characteristic Golgi elements, in which vacuoles are faintly discernible; c, acidophil type of small chromophobe with small Golgi zone in contact with lower edge of nucleus. ×325.

iv. Control φ. a, acidophil cell with Golgi element consisting of granules of dense lipoid. ×325.

v. Control φ. b, one of two large basiphil cells showing general form of Golgi zones, and their relation to nuclei. ×650.

vi. Control φ. Curiously shaped basiphil cell in which vacuoles are faintly detectable in the Golgi zone, the central region of which is occupied by diffuse lipoid. ×650.

vii. Experimental φ. Stilboestrol 32 days. a, acidophils with hypertrophied Golgi zones in which the diffuse lipoid and dense lipoid material is readily seen; b, hypertrophic Golgi area of a basiphil showing similar features to the above. ×325.

viii. Experimental φ. As above. c, Golgi elements showing rows of enlarged vacuoles enveloped in dense lipoid material. This is a more highly magnified area from the centre of vii. ×650.
from one another only in the two respects already mentioned—(a) in shape, and (b) position in relation to the nucleus.

The three features were:

1. A zone of diffuse, less deeply staining sudanophil material, embedded in which were:

2. Small clear vacuoles, and


The position and shape of the diffuse sudanophil material were primarily responsible for determining the morphology of the Golgi zone as a whole. That is to say, the difference between the acidophil and basiphil types was directly related to differences in the size, shape, and position of the diffuse sudanophil material and not to the nature of the vacuoles and the strongly sudanophil inclusions (cf. Pl. 1, i and vii). In some cases, particularly in small cells, where the diffuse sudanophil material could not be readily made out, the distribution of the strongly sudanophil material was sufficient to characterize the type of Golgi zone (Pl. 1, iii).

The vacuoles, although normally small, were variable in size (Pl. 1, viii), and, in occasional instances, were to be seen outside the zone of diffuse sudanophil material (Pl. 1, iv). The relationship between these vacuoles and the strongly sudanophil elements was not always clear-cut. Sometimes, as in Pl. 1, iv, v, viii, some of the vacuoles appeared to be unconnected with the sudanophil elements, but this may perhaps have been due to the extreme thinness of any sudanophil film investing them.

In those instances where there was clearly a relationship between the intensely sudanophil particles and the clear vacuoles, the former appeared either as small granules of which one and sometimes two were seen to be in contact with the edge of a vacuole (Pl. 1, iv, v, vii) or else as sudanophil arcs capping single vacuoles (Pl. 1, ii, viii). There was evidence in some instances that these 'caps' were approximately crescentic in form (Pl. 1, viii), but in others they appeared to be of a uniform thickness (Pl. 1, iv, v). A few examples of vacuoles apparently completely invested with a sudanophil film were observed (Pl. 1, viii).

In some cells there was a high proportion of small, strongly sudanophil, granule-like bodies of variable size, apparently unrelated to any external or internal vacuole. Pl. 1, iii shows a rather small acidophil type cell whose Golgi zone consisted entirely of these apparently solid granules.

Thus, in these Golgi bodies, in addition to the region of diffuse sudanophil material usually present, there were observed to be:

1. Vacuoles apparently unrelated to the strongly sudanophil bodies;

2. Strongly sudanophil elements apparently unrelated to vacuoles; and

3. Vacuoles associated with caps or granules of strongly sudanophil material.
2. Experimental Animals

(a) The Position of the Golgi Zone. Any alteration in the position of the Golgi zone was clearly due to the generalized hypertrophy of the whole area. This hypertrophy in response to oestrogens is already well known from observations based upon the study of material prepared by the Nassonov-Kolatchev method (Severinghaus, 1937). When the hypertrophy was considerable, there was a tendency for the Golgi region of the basiphil cells to encroach upon the nuclear region and make contact with the nuclear membrane (Pl. 1, xi), and this resulted in a somewhat acidophil-like morphology (cf. Pl. 1, xi and vii). In neither type of cell, however, was there evidence of any fundamental shift in position, and only occasionally was difficulty encountered in deciding the type of cell under observation.

(b) The Structure of the Golgi Zone. The three features mentioned as being characteristic of the Golgi regions of normal cells were again readily seen in the experimental animals, and, generally speaking, the differences between the two were quantitative rather than qualitative, although certain qualitative differences were observed.

Even after a relatively short period of implantation with stilboestrol there was quite a marked increase in the amount of diffuse sudanophil material, relative to the cytoplasmic volume (cf. Pl. 1, v and xi; vi and x), and with more prolonged treatment the increase was unmistakable (cf. Pl. 1, vii and xii; Text-fig. 1, ii and vii). Concurrently, the intensity of the staining of the diffuse material also increased, but this may perhaps have been due to a closer packing of the sudanophil particles and hence may have been a quantitative and not a qualitative change. The Golgi elements of all cell types were similarly affected, but since one of the characteristic effects of prolonged treatment with oestrogens is a granule depletion in the chromophil cell (Severinghaus, 1937), the cells observed in those glands subjected to a more prolonged treatment were commonly extremely degranulated—they were in fact large chromophobes.

The effect of the oestrogen upon the vacuoles and strongly sudanophil elements was, after fairly prolonged treatment, quite clear-cut. First, there was an increase in both the amount of vacuolation (cf. Pl. 1, vii and xii) and the amount of strongly sudanophil material (cf. Pl. 1, v and xi), although the latter was not always so marked. As in the controls, there were vacuoles apparently unrelated to sudanophil elements and there were granular sudanophil elements unassociated with any external or resolvable internal vacuole (Pl. 1, ix, xii, xiv). Secondly, there was a qualitative change resulting from the coalescences of vacuoles. This, in its extreme form, is shown in Pl. 1, xv and Text-fig. 1, vii, where vacuolar chains of large size were produced; in other instances, the evidence for vacuole fusion was based on the occurrence of vacuoles considerably above the normal size (Pl. 1, xii, xv). These were often associated with several sudanophil granules and crescents, instead of the more usual one or two seen in the controls. The sudanophil elements were generally of the same type as in the controls, that is to say: crescents (Pl. 1, ix,
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\(x, xi\), caps of apparently uniform thickness (Pl. 1, xi) and occasionally continuous films (Pl. 1, ix). Appearances such as those shown in Pl. 1, xiv, where the sudanophil material was elliptical in shape, suggested that ring-like configurations were also present.

The results described above show that the essential structural features of the Golgi body were maintained after fairly prolonged treatment with stilboestrol. The hypertrophy produced by oestrogens was due to (1) an increase in the amount of sudanophil material of both types, and (2) an increase in the amount of vacuolation.

**DISCUSSION**

Since sudan black is known to have a high and apparently specific solubility in lipoids (Baker, 1944) it can be justifiably assumed that the sudanophil material of the Golgi elements just described consists, at all events in part, of lipoid substances. The Golgi zones of the cells of normal and experimental animals thus consist of regions containing diffuse lipoids in which are embedded three sorts of structures: (a) clear vacuoles, (b) lipoid-containing granules, and (c) clear vacuoles partially or completely invested with dense lipoid material. It is reasonable to believe, for reasons indicated by Baker (1944), that the technique preserves these structures in a form not greatly dissimilar to that obtaining in the living cell and, in any case, it seems improbable that any distortion produced would exceed that caused, for example, by the Nassonov–Kolatchev procedure. There is in fact a very close similarity between the morphology of the Golgi zone as revealed by the latter method, and that demonstrable by the use of sudan black.

Severinghaus (1932), using the Nassonov technique, showed that two sorts of Golgi element, associated respectively with acidophils and basiphils, were present in the anterior lobe of the rat pituitary. The result of the present investigation supports his observations. The fact that two distinctive forms of Golgi element regularly exist in this way in the two sorts of chromophil cell (Severinghaus, 1933, 1937) of the rat, the guinea-pig (Kirkman, 1937), and perhaps less markedly in the cat (Dawson, 1946) is of theoretical interest, since it suggests that the Golgi zone, in spite of its lability, must be linked to the 'cytoskeleton'. In the rat, the characteristic Golgi zone morphology of the two types of chromophil cell is retained after treatment with oestrogens, except where the dosage is particularly heavy; but the evidence again suggests that the pattern of the Golgi zone is related to the ultrastructure of the cytoplasm.

The Golgi material of the anterior pituitary cells of the rat, as revealed by the Nassonov–Kolatchev technique, for example, consists of a network of osmiophil threads and granules associated, according to observers such as Severinghaus (1937) and Ayers (1941), with clear vesicles or vacuoles. The latter described such vesicles as having three different relations within the cell: (1) the smallest, appearing as 'clear, tiny ovoid spaces incorporated in the strands of the Golgi net', (2) '... slightly larger vesicles eccentrically
located so that only a thin rim covers the outer side—such vesicles being up to three times the size of the previous type—and (3) vesicles present in the cytoplasm, some of which 'have a bit of osmiophil material adherent'. There can be little doubt that these vesicles are similar to the vacuoles observed in the sudan black preparations, and it seems likely that the stranded osmiophil material in which they are embedded, or with which they are associated, corresponds partly to the diffuse and partly to the denser lipoid already described. Also, cytoplasmic vacuoles associated with dense lipoid material were occasionally observed in the sudan black preparations (Pl. 1, iv).

The 'classical' reticulate appearance of the Golgi elements in pituitary cells as seen after the Nassonov and similar techniques may well be due to the shrinkage and distortion of the complex revealed by the sudan black method. Some evidence in support of this notion has been brought forward by Worley (1943 a and b, 1944), who showed (1) that structures comparable to the Golgi elements in regard to their position in the cell and their relation to other cytoplasmic parts (e.g. secretion droplets, &c.) were, in various invertebrate and vertebrate cells, stainable supravitally with methylene blue, and (2) that shrinkage induced by dessication in many instances resulted in a picture very similar to that normally produced by the customary osmium tetroxide impregnation techniques. In the living pancreas, for example, there was a progressive distortion of the stained spherical bodies from which, after a time, there extended thread-like processes whose anastomoses with one another produced a network.

Furthermore, the observations of Worley are in support of those of Baker (1944) and the writer, in that they provide evidence for a duplex structure of the Golgi element—a concept very extensively developed by the investigations of Hirsch (1939). Worley showed that the Golgi vesicles in the cells he studied regularly progressed from a small methylene-blue-stained granular stage to one where the granule had enlarged and differentiated into a clear vacuole with a chromophil pellicle of varying thickness. The vacuole was the site of production of the secretion product, liberation of which was associated with the fragmentation of the chromophil cortex. It seems probable that these two parts of the vesicle correspond to Hirsch's 'Internum' and 'Externum', and the writer believes that this correspondence may be extended to the vacuoles and the associated lipoid elements of the Golgi bodies of the rat anterior pituitary. It is possible, moreover, that the dense sudanophil structures unassociated with vacuoles are equivalent to Hirsch's 'Presubstanz', from which the corticated vacuoles of the fully developed Golgi zone are probably derived. Baker (1944) has suggested the extension of the term 'Externum' so as to include the diffuse as well as the dense lipoid material.

The hypertrophic effect of oestrogens upon the Golgi element of anterior pituitary cells has been clearly established (Severinghaus, 1937), but sudan black because of its high solubility in lipoids gives more precise information about the details of this hypertrophy. Severinghaus (loc. cit.) has described an increase in the vacuolation of the Golgi zone after oestrone injections and
the method used in this investigation showed that not only may there be an increase in the number of vacuoles, but, after prolonged treatment with stilboestrol, coalescence of these vacuoles occurred (Pl. i, xv). There appeared also to be an increase in the amount of both diffuse and dense lipid material and in some instances coalescence of the former to produce sudanophil strands. Such changes as these, considered in relation to changes known to occur in the Golgi bodies of other endocrine as well as exocrine glands (Bowen, 1929; Kirkman and Severinghaus, 1938; Foster, 1942; Bourne, 1942), seem to indicate a state of heightened secretory activity. Finally, as has been suggested before (Severinghaus, 1936; Foster, 1942a), it is thought likely that the physiological inhibition produced by oestrogens is due either to over-activation of the secretory mechanism (of which the Golgi element is almost certainly a part) or to an ultimate interference with some stage in the metabolism of the hormonal secretion product.

Summary

1. It was found possible to demonstrate the Golgi zones of the cells of the anterior pituitary (pars distalis) of the rat with Sudan black. The position and general morphology of the Golgi bodies closely resembled that revealed by the standard osmium tetroxide techniques.

2. The Golgi zone was generally found to contain three sorts of element: (a) diffuse sudanophil material in which were embedded: (b) clear vacuoles and (c) strongly sudanophil bodies. The latter were often associated with the surfaces of the vacuoles, when they were in the form of (1) crescentic caps, (2) partial or complete rims of apparently uniform thickness, or (3) small granules. Sometimes the strongly sudanophil bodies were granular and unassociated with vacuoles; there were also vacuoles with no demonstrable lipid material in association with their surfaces.

3. The effect of stilboestrol was to cause hypertrophy of the Golgi zones and this was associated with (a) an increase in volume of the region of diffuse lipoids, (b) an increase in vacuolation, and (c) a rather more variable increase in the amount of strongly sudanophil material. These changes would appear to indicate a state of heightened secretory activity.

4. The strong affinity of the Golgi area for Sudan black suggests that it consists, at any rate in part, of lipoids.

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EXPLANATION OF PLATE I.  
Figs. i-xv are from camera lucida drawings.

i. Control $\delta$. Basophil cell showing general form of Golgi zone.

ii. Control $\delta$. Acidophil cell. Upper focal plane, showing diffuse lipid and vacuoles with caps containing dense lipid.

iii. Control $\delta$. Acidophil cell with peruke-like Golgi zone consisting of dense lipid particles.

iv. Control $\delta$. Large basophil cell with vacuoles outside area of diffuse lipid, and vacuoles unassociated with dense lipid.

v. Control $\delta$. Basophil cell, showing diffuse lipid, and vacuoles associated with incomplete dense lipid rims or granules.

vi. Control $\delta$. Basophil type of small chromophobe showing Golgi zone.

vii and viii. Control $\delta$. Acidophil cells showing vacuoles of varying size with dense lipid rims and crescents, and also isolated granules containing dense lipid.

ix. Experimental $\delta$. Stilboestrol 6 days. Acidophil cell, showing vacuoles with complete investments of dense lipid.

x. Experimental $\delta$. Stilboestrol 19 days. Acidophil cell showing enlarged area of diffuse lipid and numerous vacuoles of varying size, with crescents, rims, and granules of dense lipid.

xi. Experimental $\delta$. Stilboestrol 19 days. Basophil cell showing enlarged area of diffuse lipid, vacuoles of varying size, some unassociated with dense lipid.

xii. Experimental $\delta$. Stilboestrol 19 days. Acidophil cell showing vacuoles outside zone of diffuse lipid.

xiii. Experimental $\delta$. Stilboestrol 49 days. Acidophil cell showing greatly increased zone of diffuse lipid.

xiv. Experimental $\delta$. Stilboestrol 43 days. Acidophil cell showing two markedly elliptical vacuoles with rims of dense lipid.

xv. Experimental $\delta$. Stilboestrol 56 days. Basophil cell showing hypertrophy and partial fusion of the vacuoles and coalescence of the dense lipid material to form strands.

REFERENCES

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