The Morphology of the Golgi Bodies with reference to Secretion in the Liver-cells of the Slug, Anadenus altivagus, as seen under the phase-contrast Microscope

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With one plate (fig. 1)

SUMMARY

Golgi bodies in the living liver-cells of the slug, Anadenus altivagus, exist in two forms: (a) homogeneous granules or spheres of dark contrast, and (b) spheres showing a duplex structure with a light greyish internum and a dark externum, which may be single or composite. The greyish internum of these duplex spheroids grows into the secretory granules, the dark externum disappearing in the process of growth. Mitochondria appear as fibres of light greyish contrast with a dark granule at each tip. This dark granule disassociates itself from the mitochondrion and forms the Golgi granule of dark contrast—the Golgi 'pre-substance'. The Golgi pre-substance, stainable with neutral red, forms the Golgi spheroids.

INTRODUCTION

The Golgi bodies in the secretory cells of animals have been the subject of investigation since their discovery, but almost invariably cytologists have employed techniques which involve metallic reduction of silver or osmium. But, as has been repeatedly pointed out by Nath (1944, 1956) and Baker (1950, 1953, 1954), not much reliance can be placed on such techniques, which involve long osmication or silver deposition in the tissue. These authors have also pointed out the great importance of the study of the living cell, which has been rendered much easier and more reliable by the recent advances in microscopy. A great deal of work on germ-cells and neurones has been done by phase-contrast microscopy in this laboratory and elsewhere, but living gland-cells do not seem to have attracted much attention.

Hirsch (1939), to whom we owe much of our knowledge of gland-cells, has described two phases of the Golgi bodies in the living pancreas: (1) 'pre-substance', composed of solid granules of Golgi substance showing no differentiation into external and internal regions, and (2) the 'Golgi system', which consists of a chromophil externum and a chromophobe internum. The secretion, according to this author, arises in the internum of the Golgi system.

In view of the above facts the study of the gland-cells of various animals has been undertaken by the author, largely by phase-contrast microscopy. The present studies of the living cells of the liver of the slug, Anadenus, have more or less confirmed the findings of Hirsch (1939). In this material, however, the Golgi spheroids seem to be bodily transformed into secretory...
granules, as no Golgi granules representing the separated 'externum' or the 'Golgirest' of Hirsch could be identified amongst the secretory granules. Similar observations have also been made by the author on the liver-cells of a local snail, the account of which will be published at a later stage.

**Material and Technique**

The studies of the liver-cells of the slug, *Anadenus altivagus* Theobald, were carried out at Simla, Panjab, during the months of August and September 1955. The liver was removed from the living animals and was placed in 0.7% sodium chloride solution, to 100 c.c. of which 0.2 c.c. of 10% calcium chloride solution had been added. The same physiological solution was also used as the liquid medium for the microscopical preparations.

Neutral red chloride (B.D.H.) and Janus green (Horleco, U.S.A.) were employed supravitaly at the standard concentration of 0.01% in the above physiological solution. The latter, however, did not prove successful. The bright and the dark phases of the microscope were used alternately for studying the action of the vital dyes.

The Carl Zeiss 'W' phase-contrast microscope, fitted with a photochanger, was used for the study of the living cells, and the Carl Zeiss micro-reflex camera-attachment with Contax 35 mm camera were used for photomicrography. All the photographs were taken with a K8X ocular and '1.25/100' oil-immersion objective. The photographs were further enlarged three times and are untouched.

**Observations**

The youngest cells in the liver of *Anadenus* are comparatively small, round, or oval cells generally lying in pairs. The cytoplasm at this stage reveals a number of mitochondrial fibrillae, which give light greyish contrast. To each end of the fibrilla is attached quite a prominent granule showing a very high phase-change. Some of the mitochondrial fibrillae, however, seemed to be devoid of such granules. In addition to the mitochondria a few dark, separate discrete granules of various sizes were also observed in the cytoplasm, showing a phase-change equivalent to the tip-granules of the mitochondria and staining with neutral red (fig. 1, A and B). These can be homologized to the 'Golgi pre-substance' of Hirsch (1939).

In a larger cell the nucleus becomes excentric, a position which it maintains from this stage onwards. The mitochondria, which are fibrillar and granular, are pushed into the lower region of the cell. In addition to the very small granules described above, the cell now shows a few Golgi bodies which are in

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**Fig. 1 (plate).** Photomicrographs of living liver-cells under positive phase-contrast.

A and B, early cells showing mitochondria and Golgi granules.

C, cell showing formation of the composite spheroid.

D and E, cells showing various phases of the Golgi bodies.

F, G, and H, cells showing Golgi bodies and secretory granules.

I, cell showing polarity in the distribution of the Golgi bodies and secretory granules.
the form of large granules, rings, or crescents, the latter showing a duplex structure (fig. 1, D and E). A critical study of the Golgi rings or crescents, however, reveals that they are more or less spherical bodies in the form of a greyish sphere of a greyish contrast enclosed completely or partially by a sheath of much darker contrast. It appears, therefore, that the rings and crescents are optical sections of these Golgi spheres. By changing the focus it can be seen that the dark rim of the Golgi ring travels along the surface of the grey sphere; and the Golgi sphere appears as a dark granule both in the uppermost and lowermost planes. The Golgi elements show a gradual seriation between the tiny granules of dark contrast and large Golgi spheroids of duplex structure (fig. 1, D and E). It appears that the tiny granules grow directly into the latter form.

During the process of growth the tiny dark granules first assume the shape of a much darker sphere of a very dark contrast. This sphere, in the majority of cases, seems to develop a greyish internum, which grows and forms the secretion in the older cells. The rim or externum of dark contrast thins out under the pressure of this growth till ultimately it seems to be absorbed completely in the process, leaving behind a secretory granule of large size and greyish contrast. Occasionally, however, the Golgi sphere of dark contrast seems to become crescentic in appearance and develops a greyish sphere in its concavity. With the growth of this greyish sphere, the crescentic dark substance seems to envelop it partially, thus giving the appearance of a crescent in optical sections (fig. 2).

With further growth in the size of the cell the number of the Golgi bodies and the secretory granules also increases till ultimately the cytoplasm of the cell becomes choked with these bodies, thus completely obliterating the mitochondria from view (fig. 1, F and G). In addition to the above simple form, the Golgi bodies also show a composite structure in which a large irregular sphere

![Diagram of Golgi Bodies](image-url)
of greyish contrast seems to be surrounded with a number of much darker crescentic caps on all sides (fig. 1, F and I). These composite spheroids arise by the union of individual crescentic forms of the earlier cells (fig. 2). Such a fusion has actually been observed by the author under phase-contrast (fig. 1, c). These composite spheroids, however, also form the secretion in the normal manner described above, and the dark crescentic caps seem ultimately to be consumed.

With further growth the contents of the cell show a marked polarity in their distribution (fig. 1, H). The Golgi bodies are generally crowded on one side of the excentric nucleus, and the secretory granules fill up the opposite end. The smallest Golgi granules are generally found at the extreme nuclear end of the cell, whereas the larger ones are near the secretory granules and intermingled with them. It may be pointed out here that the amount of the secretory granules in a cell appears to be inversely proportional to the amount of the Golgi bodies; this supports the direct origin of the former from the latter. In larger cells the mitochondria could not be seen.

The secretory granules, the grey internum of the Golgi spheroids, and the tiny dark granules (Golgi pre-substance) show a great affinity for neutral red; but the dark rim of the Golgi spheroids does not seem to take up the dye at all, nor do the larger, homogeneously dark spheres.

**DISCUSSION**

As was pointed out above, not much work has been done on the morphology of the Golgi bodies in the living gland-cells of animals; but the available data clearly show that cytologists hold two widely different views on the morphology of these cell inclusions.

Lacy (1954), in his extensive studies of exocrine and endocrine cells of the mammalian pancreas, believes that the Golgi apparatus in these cells 'is a system or network of canals or vacuoles, distinct from both lipoidal bodies and mitochondria'. According to this author, 'this system of canals is homologous with the Golgi apparatus of the vertebrate neurones'. Lacy also claims to have seen such canals in frozen-dried sections and living material. Similarly, Moussa (1952), Gatenby (1953), and Adamstone and Taylor (1953) describe the Golgi material as a canalicular system in living neurones.

Morgan (1953, a and b), on the other hand, believes in the spheroidal nature of the Golgi bodies in the living pancreas of the mouse.

Hirsch (1939), who has made an extensive study of the gland-cells in animals, describes two phases of the Golgi bodies in these cells: (1) 'Praesubstanz', which is in the form of separate dark spheres or granules and takes up neutral red and Janus green B, and (2) the 'Golgisystem', which is comprised of a chromophobe internum surrounded by a chromophil externum. The secretion, according to Hirsch, is differentiated in the internum of the Golgi system.

The present studies of the living liver-cells of *Anadenus altivagus* under the phase-contrast microscope have convinced the author that the Golgi
substance in these cells exists in the form of separate granules or spheres. The small granules, which have a limited range in size, exhibit a very high phase-change under phase-contrast and correspond to the Praesubstanz of Hirsch. But as the Golgi granules become active in the process of the formation of the secretion, they begin to show an area of a grey contrast in their interior which, in some cases, is stuck in the concavity on one side of the granule. With further growth in the size of this greyish sphere the dark material (corresponding to the Externum of Hirsch) spreads over the greyish sphere (corresponding to the Internum of Hirsch) and envelops the latter completely or incompletely, thus giving the picture of rings and crescents with a duplex structure in optical section. It seems from the observations of the author that the greyish spheres of the Golgi elements grow into the secretory granules. In the process of this transformation, the sheath of dark contrast seems to be completely lost. This conclusion is further strengthened by the fact that both the secretory granules and internum of the Golgi spheres are stainable with neutral red and present the same phase-change under phase-contrast. The amount of the secretory granules is found to be inversely proportional to the amount of the Golgi bodies in the growing cells of the liver of Anadenus, and this suggests their direct origin from the latter cell-inclusions.

It has also been found, and quite commonly too, that a number of incompletely invested Golgi spheroids come together and the exposed portions of their grey interna fuse with each other, thus giving rise to the composite spheroids. These composite spheroids are homologous with the 'mulberry spheroids' described by Thomas (1948) and Cain (1948) in living neurones and with the Polysystem of Hirsch (1939). These composite spheroids, however, form the secretion in the normal way.

In spite of the most diligent search, the author has not been able to find any structure in the living liver-cells of Anadenus to which the name 'canaliculi' could be attributed.

Regarding the origin of the Golgi bodies, it may be pointed out that the constituents of the youngest cells are the mitochondrial filaments possessing a dark granule at each tip, and a few dark, separate granules—the Golgi pre-substance. But with the growth of the cells the number of Golgi granules increases. In the opinion of the author the tip-granules of the mitochondrial filaments break off and form the Golgi pre-substance. This view gets support from the fact that some mitochondrial filaments do not possess these dark granules, and that both the tip-granules of the mitochondrial filaments and the Golgi pre-substance present the same phase-change under phase-contrast. The Golgi pre-substance is also stainable with neutral red.

These observations are in accord with the views of Hirsch (1939), who found that in the living pancreas the small granules are formed on the surface of the mitochondria. These granules, according to Hirsch, remain in contact with the mitochondria for some time but later become detached, move towards the Golgi field, and constitute the Golgi pre-substance.
The origin of the Golgi bodies from mitochondria has also recently been described in both the fixed (Nath and Chopra, 1955) and living (Nath and Gupta, unpublished) male germ-cells of Anadenus.

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