
By

Robert H. Bowen,
Department of Zoology, Columbia University.

With 3 Text-figures.

In the first of this series of 'Studies' I suggested that the conclusive demonstration of the secretory theory of the Golgi apparatus must depend upon further and more critical evidence bearing on three principal points. These are: (1) a demonstration that the general topographical relationship of Golgi apparatus and secretory granules is always such as to allow a possibility of causal connexion between them; (2) a more intimate knowledge of the finer structural features of the Golgi apparatus in general; and (3) a critical demonstration of the relation of an individual secretory granule to the material of the Golgi complex. In the preceding papers of this series (Bowen, 1926a, b, and c) I have described the objective results of an extended examination of glandular tissue, particularly in their bearing on the three points just mentioned. In this paper I propose to analyse my results with the hope of arriving at some evaluation of the theory which Dr. Nassonov (1923 and 1924a) first definitely suggested, and which I have recently (1924) tried to extend into a more general conception of the secretory function of the Golgi apparatus. My observations have been focused particularly upon the Golgi apparatus, and little or no attention has been paid to other components of the cell. This was done deliberately, in the belief that these
other components had been examined with sufficient thoroughness to indicate their probable value to a general theory of secretion. In another place I shall deal more critically with these matters, but here I purposely omit everything but the briefest reference to the secretory possibilities of cellular constituents other than the Golgi apparatus. In my opinion the older theories of secretory synthesis have broken down, and the field is now clear for a thorough examination of the Golgi apparatus as a synthetic intermediary in the process of secretion.¹

**The Topographical Relations of Golgi Apparatus and Secretory Products.**

Considering the Golgi apparatus from the standpoint of its general behavior in gland-cells, perhaps the most striking feature which it constantly presents is the increase in its amount during secretory activity. This hypertrophy of the Golgi apparatus occurs in every type of gland-cell which I have examined, and has been commented upon by other workers (see, for example, Cajal, 1914).² It is, however, important to note that this hypertrophy is not relative to the size of the cell as a whole, but to the amount of active cellular constituents exclusive of the accumulated secretory granules. In other words the Golgi material increases in amount so that its size

¹ Attention should again be called to the fact that I use the term secretion to refer either to the synthetic act of production or to the materials produced; while the term excretion is used to identify the act of extruding the secretory materials from the cell. This latter usage is not the one commonly employed by physiologists, but for the cytologist it is almost a logical necessity. The recognition of this mistake in terminology goes back at least to Ranvier (1887), who clearly brought out the difficulties of nomenclature which an uncritical use of words is still constantly emphasizing.

² So far as I know the only contradictory result is that of Maeronghi (1903), who states that (in certain glands in the skin of Ammocoetes) the Golgi material is reduced simultaneously with the transformation of protoplasm into secretory product. But his figures clearly indicate that the reduced condition of the Golgi apparatus is actually an early stage in the secretory cycle, and not an active synthetic stage at all.
relative to the whole cell is maintained (or even exceeded), its hypertrophy relative to the other components of the cell being clearly manifested only when we exclude the secretory granules. The meaning of these facts may not be evident at first, until we consider (1) that during this active period the general cytoplasmic content of the cell as a rule is not increased, and, indeed, becomes finally so attenuated in some glands as to be demonstrated with difficulty; (2) that the nucleus is rarely subject to striking changes in volume, and exhibits little or no activity so far as may be judged from its morphology—seems, indeed, actually to undergo regressive changes in the end stages of the secretory cycle; (3) while the mitochondria, according to some, diminish in number and at any rate undergo no notable changes. All these things seem to me to point in the same direction, viz. to the Golgi apparatus as the centre of secretory synthesis. It is reasonable to suppose that the elements involved in secretory activity would show changes in the direction of increased activity as expressed in size increase, change in morphology, &c. This the Golgi apparatus does, while the other elements of the cell appear to fall more and more into the background.

It has been argued, for example by Hoven (1912), that the mitochondria decrease during secretion, and must therefore have been transformed into secretory granules. Waiving the fact that this decrease has not been universally accepted as a fact, it still remains difficult to see why the mitochondria as the source of secretory synthesis should not be decidedly on the increase rather than the reverse, at least up to an advanced stage in the secretory cycle. Furthermore, even if secretory granules originate from mitochondria, the problems of why they can grow and mature without further relation to the mitochondria, or, indeed, how their growth is effected at all, remain entirely unsolved. It does not seem reasonable that the origin and growth of the granules are separate processes, but rather that growth is an evidence merely of a continuance of the processes by which the granules first came into being. These objections seem to me practically fatal to the mito-
chondrial theory of secretory origins. They are equally effective in eliminating the nucleus as the immediate source of secretory products, for while their initial formation might be ascribed to invisible intranuclear sources, still the fact of growth far removed from the nucleus is undoubted. We can perhaps retain the nucleus as a source of secretory materials in a chemical sense, but from the morphological viewpoint the 'evidence' for this view must frankly be admitted to be without any certain foundation (but see below). Arguing therefore from the general behaviour of the cellular components in gland-cells, it seems to me that such evidence as there is points indubitably to the Golgi apparatus as the element immediately involved in secretory origins. And the evidence points in this direction because, of all the cellular constituents, the Golgi apparatus alone shows throughout the secretory cycle unmistakable morphological evidences of co-ordination with the synthetic activity. We should expect a manufacturing concern to expand its plant to facilitate an increased output, and this is exactly what takes place in the Golgi apparatus during a secretory cycle. We reach thus a first and very important conclusion—that the Golgi apparatus presents morphological changes which cannot be denied and which can be synchronized with the secretory activity of the cell which contains it.

We may turn now to a more detailed analysis of the topographical inter-relations of secretory granules and Golgi apparatus as brought out in recent papers (particularly Nassonov 1923 and 1924, and Bowen 1924, 1925, 1926 a, b, and c). Consideration of the conditions, so far as known at the present time, indicates that the secretory granules may be topographically related to the Golgi apparatus in two different ways. (1) The granules may be associated with the apparatus only during their earlier growth stages, becoming subsequently detached and completing their growth in distant parts of the cell; or (2) the granules may remain in association with the Golgi apparatus until they are mature, after which time they may likewise be transported to some distant collection point usually adjacent to the glandular lumen.
The first of these two types of association seems now to be of uncommon occurrence, and unfortunately so, for it would offer unusual opportunity for a critical demonstration of the whole question. It seems to occur in the pelvic gland and probably in the pancreas of some salamanders (Nassonov, 1923, and Bowen, 1924), though in cases where the granules are very small the conditions are not favourable for analysis. In these cells the detached secretory granules are each accompanied by a cap or girdle of material impregnated like, and probably derived from, the Golgi apparatus itself. If granules actually removed from association with the Golgi apparatus are to grow (except by fusion with other granules) they must have some accompanying portion of Golgi material with them—provided always that the Golgi apparatus is the causal agent of growth. The fact that apparently detached and growing granules are provided with bits of material presumably related to the Golgi substance is topographical evidence of the highest importance—for if detached granules were found in process of growth in which this arrangement did not exist, the theory of the Golgi apparatus as a synthetic centre for secretory granules would be seriously shaken. In my estimation, however, and judging largely from my own study, the evidence for this particular type of association between Golgi apparatus and secretory granules is not yet above suspicion, and perhaps should not be stressed too much pending a more thorough inquiry into the whole phenomenon.

The second general topographical relation existing between Golgi apparatus and secretory granules is the one which commonly occurs. Consideration of the known cases indicates two general methods of developing this semi-permanent type of association: (1) the apparatus may remain as a compact network within which the granules appear and grow to maturity, subsequently migrating into a region more or less outside the Golgi confines; or (2) the apparatus may undergo an extension throughout the regions of the cell in which secretory granules occur, the association of the two being thus assured during the period of growth and maturing of the granules.
The first of these conditions is perhaps best illustrated by the mucous, so-called goblet, cells of the intestinal epithelium. The same relations are developed, usually in slightly less diagrammatic form, in the lachrymal glands (tear and some Harderian glands), in still more obscure form in the glands of the male reproductive tract (mammals), and again in salivary gland-cells of the mucous type. It is generally characteristic of this type of relation between Golgi apparatus and secretory granules that the Golgi material undergoes hypertrophy during the earlier stages of the secretory cycle, developing some characteristic shape such as that of a tube, a basket, &c., within which the secretory granules appear, are usually rapidly completed, and seem then to be gradually shifted toward the lumen by the following granules. An interesting variation is presented by the mucous salivary cells in Limax (Bowen, 1926 a). Here the Golgi apparatus is composed of many discrete pieces which are gradually pushed toward the periphery of the cell by the accumulating secretory granules. Thus, in this case, the Golgi material is moved instead of the granules, but the result is essentially the same as in the mucous cells of vertebrates.

The second condition, viz. the extension of the apparatus throughout the mass of secretory granules, may be developed in either of two ways: (1) the apparatus may remain as a more or less complete net, or (2) it may be distributed throughout the mass as distinct pieces. The first arrangement seems to be characteristic of the parotid gland of the cat, the demilune (serous) cells of the cat’s submaxillary and perhaps the mammalian pancreas. The second is found in the oil-gland of birds, and in all probability is of frequent occurrence in invertebrates as indicated in the serous cells of the salivary gland of the slug. The inguinal gland of the rabbit and the Meibomian glands of the cat exhibit an interesting intermediate condition in which one arrangement is followed during the early stages of the cycle, becoming subsequently transformed into the other by the simple process of fragmentation. The most astonishing result from this fragmentation process is that which seems to
occur in the oil-gland of the fowl (Bowen, 1926 b), and which, if I have correctly interpreted my preparations, furnishes a critical demonstration of the inter-relation of Golgi apparatus and secretory granules. If the Golgi apparatus is not intimately involved in the elaboration of the secretory granules this extended condition in certain types of cells is inexplicable. If on the contrary there is a causal connexion involving the association of the two, then we might expect to find just this wide-flung distribution of the Golgi material, especially in cells where the granules are relatively large or require a long period for their maturation.

Further development of this aspect of the problem seems unnecessary, and I can see no escape from the conclusion that in gland-cells of all kinds there exists a constant and close topographical association between Golgi apparatus and secretory granules. The reverse statement of the case is equally striking, for in parts of the cell where the Golgi apparatus is not present secretory granules do not occur, except of course as they are forced there by mechanical crowding within the cell. These facts have been emphasized again and again in the preceding 'Studies', but one aspect deserves further particular notice. This is the fact that in the early stages of a secretory cycle the secretory granules first appear only within the general area of the Golgi apparatus. Nassonov's (1923) results on the pancreas of a salamander furnish very striking demonstration of this relation, and it is a fact the importance of which it seems to me cannot be over-estimated.

Thus, in secretory cells of various kinds every possible disposition of the Golgi material is found, but always the topographical relation to the secretory products remains fundamentally unchanged. It is, indeed, true that the more the Golgi apparatus in gland-cells changes the more does it remain the same thing. As a result of the observations here summarized I conclude that the evidence, so far as it goes, points directly to the Golgi apparatus as the synthetic centre in which the secretory granules are fabricated. How their manufacture may be visualized from the standpoint of the
cytologist I will try further to outline in the succeeding sections.

Before concluding this section I wish to call attention to certain relations which I have found to obtain between the type of secretion and the disposition of the Golgi material. It seems to be a rather customary procedure, especially in more elementary discussions, to consider glands as of either serous or mucous type, with a third kind sometimes mentioned—the skin-glands. These last-named glands are quite generally characterized by the production of a secretion which has more or less marked lipoidal properties. But when one takes into account any considerable number of the remaining glands the impossibility of classifying them strictly as of the mucous or serous type becomes at once very apparent. Thus, the tear-gland has all the appearance of a mucous gland, but it produces very little mucin indeed. It is even less appropriately placed with the serous glands. Thus, the tear-gland and many others fail to fit into the usual scheme, and the classification of glands in general becomes chaotic. This lack of apparent basis for classifying glands seems to me to depend for the most part on a mistaken conception of glandular products. To me it would appear that the old mucous-serous classification is without any chemical foundation, for the simple reason that almost every gland has a more or less specific type of secretion. Sometimes this is obviously mucous in character, sometimes serous or lipoidal, but in many cases it has qualities whose peculiarities permit its inclusion in none of these groups. I have, therefore, been much interested in the discovery that the Golgi apparatus in gland-cells can be employed in a rather rough way as a basis of classification, depending upon its general topographical features in relation to the secretory cycle.

The best marked of the gland-cell types, from this standpoint, is that in which the Golgi apparatus, originally a compact, polarized structure, becomes gradually scattered throughout

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1 This relation has already been briefly developed in a preliminary note (Bowen, 1925).
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the cell as discrete fragments.\(^1\) Thus, the polarity of the cell, as expressed by the position of the Golgi apparatus, may be completely lost. But the important and constant feature is not the apparent loss of polarity but the fragmentation of the Golgi material. Such a behaviour is characteristic of the skin-glands—those in which the lipoidal nature of the secretory droplets is usually more or less emphasized. To this type belong the inguinal gland of the rabbit, the oil-glands of birds, the Meibomian glands of the eyelids, probably the mammary glands, and many others.

A second type of arrangement of the Golgi apparatus is developed typically in the goblet cells of the intestine. In these cells the Golgi material forms a more or less tubular basket-work within which the secretory granules appear. But they are subsequently transported to another part of the cell, propelled apparently by the new secretory granules which are constantly being synthesized. In these glands the secretory granules seem to be completed rapidly, and their transportation to another region of the cell is merely for storage purposes and to provide space for the production of more granules. This relation between the Golgi apparatus and the secretory granules is characteristic of all the mucous cells which I have examined, holding in a modified way even for the mucous salivary cells of _Limax_ where the apparatus from the beginning consists of discrete Golgi bodies. It is characteristic of these glands that the granules do not stain specifically after the osmic technique. These same features recur in glands which produce little or no mucin, as for instance the lachrymal glands. There is thus a community of gland-cells whose synthetic activities are carried on along broadly similar lines, as denoted by the behaviour of their Golgi apparatus. Their type is the true mucous gland-cell. We may then, it seems to me, conveniently classify these glands together, even though the specificity of

\(^1\) This behaviour should not be confused with the condition in invertebrate gland-cells, the scattered Golgi bodies of which have a very different significance.
their secretions hardly permits their grouping merely on the basis of mucin production.

Finally, there exists a third, less well-marked type of behaviour of the Golgi apparatus, well illustrated by the parotid (serous) gland of the cat (Bowen, 1926 a). In this gland the full complement of secretory granules seems to be produced early in the secretory cycle, and then a period of growth and maturing is entered upon. In this period occur the changes of staining capacity so characteristic of the parotid secretory granules. But associated with this difference in mode of producing the granules is the tendency of the Golgi apparatus to extend through the mass of secretory products, being thus brought close to, or in contact with, all the granules. Examination indicates that this behaviour is characteristic of the true serous gland-cell. It is found in the demilune cells of the cat’s submaxillary, in the serous salivary cells of Limax, and possibly in the mammalian pancreas. Here, again, we find a type of relation between Golgi apparatus and secretory synthesis which can be used as a basis of grouping gland-cells, quite apart from specific chemical similarities in their secretory products.

There appears, therefore, to be a real basis for classifying gland-cells as mucous, serous, and lipoidal—a basis which is to be found partly in the method of establishing relations between the secretory granules and the Golgi apparatus, and partly in the mere topographical history of the Golgi material. If these old terms be not employed with too rigid chemical connotations they would therefore appear to express a simple system of standard morphological types of gland-cells, having at least the recommendation of convenience.

THE STRUCTURE OF THE GOLGI APPARATUS.

It is a curious fact that among the hundreds of papers which have appeared on the subject of the Golgi apparatus very few have considered the matter of its structure. Most authors, indeed, treat this matter as though there were no problem here at all, and apparently they think of the Golgi material merely
as a network of homogeneous cords essentially lipoidal in character. It may be that this rough statement is in some cases a close approach to the truth, but in other cases it is certainly in error. I should like, therefore, to review the whole question of the structure of the Golgi apparatus, with a view to formulating a tentative conception of its morphology rather than presenting critical evidence—of which unfortunately we have at present very little. I believe, however, that the accumulated evidence is sufficient to allow a reasonable interpretation of the conditions in gland-cells.

It is, I take it, unnecessary to pay any attention to those few critics who still seem to doubt the real existence of the Golgi apparatus. No one who has seen any adequate Golgi preparations can for a moment doubt its reality. The fact that it can occasionally be seen in living cells should be sufficient evidence to silence those critics who find a stumbling-block in the fact that physical conditions render it invisible in the majority of cases. As to its general texture there is a rather wide range of opinion, some considering it as almost fluid, others as relatively solid. In all probability there is considerable variation in its physical properties in different kinds of cells, but the weight of evidence seems to indicate that it possesses an individualized identity not easily harmonized with the fluid qualities of a liquid. In some cases, the oil-gland of birds for instance (Bowen, 1926b), the Golgi pieces have a very definite form which is retained outside of the cell and is certainly indicative of the reality and definitely organized character of the Golgi material. The ground is thus cleared for a consideration of the structure itself of the Golgi apparatus.

Golgi and his pupils, who did much of the early work on the apparatus, avoided considerations which were not directly

1 I think it also useless here to discuss the question of the origin de novo of the Golgi apparatus in gland-cells. This has recently been claimed by Brambell (1925), but his observations are at variance with well-established facts in other secretory cells. In any event the matter is of too much importance to be decided without the most thorough and critical evidence. This Brambell's paper certainly does not provide.
involved in their observations, and I cannot find that this school ever developed any very definite conception of the Golgi substance. The earliest moves in this direction were made by Holmgren in the development of his well-known trophospongium theory. According to this view the cytoplasm of most kinds of somatic cells was supposed to be penetrated by processes of other cells of a trophic character, the inter-relation thus set up being of great importance in metabolic operations. At times the protoplasm of these trophic processes underwent a liquefaction, in which state the trophospongium took on the aspect of canals rather than solid cords of protoplasm. There has been a general tendency toward homologizing the trophospongium with the Golgi apparatus, and thus the structure of the former becomes at once of interest in connexion with the structure of the latter. It is, indeed, certain that, at least in most cases, the trophospongium is nothing more or less than the Golgi apparatus, but it is equally certain that in most cases these structures have nothing whatever to do with trophic processes from other cells and must be considered as a proper part of the cell itself.

These ideas of Holmgren were reflected in a variety of views according to which the Golgi apparatus was to be considered as essentially a canalicular system. An interesting development of this attitude has been offered by Bensley (1910). But in the main this conception of the Golgi apparatus as a system of canals has received little recent support and is now largely of historical interest. Cajal (1914) alone seems to have made a real effort to harmonize the actual results of silver and osmic impregnations with the canalicular theories, and thereby offered one of the earliest detailed analyses of the structure of the Golgi apparatus. He believed that the material blackened by silver methods was contained in canal-like passageways of more or less determinate location. These canals gave to the ‘apparatus’ its general topography, the visible details of which might, however, be altered according to the distribution and amount of the argentophile material at the moment of fixation. The argentophile material could be increased by processes of
growth which resulted from the multiplication of its basic, but ultramicroscopic, units of structure or 'protomeres'. The details of this analysis have not received any general acceptance, and the scattered condition of the Golgi material existing especially in invertebrate cells seems sufficient to disprove it. In addition the whole picture in gland-cells suggests no likeness to a system of canals. Nevertheless, the idea—suggested by Cajal's conception—of a lipoidal material enclosed in a definite limiting membrane might be susceptible of interesting development; although at present the existence of such a structure has no basis in actual observation.¹

However, the ideas developed by Hirschler (1913, 1914, 1916, and particularly 1918) are not far removed from this arrangement. Hirschler's papers seem to have missed the attention they deserve in the disturbances of the war period during which they were published. His preparations of material embracing Protozoa, Porifera, Mollusca, and Tunicata, and including both somatic and germ-cells, led him to view the Golgi apparatus, especially the scattered type, as essentially of a lamellar construction. Various possible curvatures imposed upon a thin plate of Golgi material would result thus in the production of spheres, half-spheres, &c. The result is to produce, in optical sections, a heavy black thread corresponding to surfaces of curvature, this thread being accompanied by a more diffuse, transparent blackening—the result of viewing the lamellar surfaces in plane view. It is the lipoidal material, the Golgi substance properly speaking, which makes up these lamellae and is responsible for the visible morphology of the Golgi apparatus. But in cases where the lamellar surface forms a sphere it is clear that the non-lipoidal contents must be something different from the surrounding cytoplasm. To this substance Hirschler gave the tentative name of 'Apparatinhalt', and his studies led him to the conclusion that it was a distinct, differentiated part of the Golgi system and not a mere result of the action of fixatives. In cases where the lamellae form only portions of a spherical

¹ But see below concerning the views recently developed by Nassonov.
surface, the 'Apparatinhalt' would necessarily be in direct contact with the surrounding cytoplasm. Hirschler was uncertain as to whether this latter condition was actually present in the living cell, and suggested the possibility that the lipoidal membranes formed normally closed containers, spherical or tubular in shape, within which the unstained 'Apparatinhalt' was enclosed. Thus, he conceived that the lipoid membrane might isolate the 'Apparatinhalt' from the surrounding plasma and thereby contribute to the heterogeneity of the whole cellular system. So far as I can find Hirschler's 'Apparatinhalt' was always a more or less hypothetical substance, its presence having never been actually demonstrated by differential staining.

This view of the lipoidal substance forming a membrane of separation between the outer cell-plasm and an inner area of differentiation has recently been adopted by Nassonov (1924 a and b) in explanation of the secretory phenomena in the epididymis and particularly in the contractile vacuole of Protozoa. No 'apparatus content' as a part of the Golgi complex is postulated, but otherwise the conception has much in common with Hirschler's suggestions. Concerning the situation in the contractile vacuole it seems impossible to arrive at any conclusion until more is known about the Golgi apparatus in Protozoa generally. But in the case of the epididymis Nassonov's conception comes very close to my own ideas presently to be described.

This brings me to the suggestions which I made some years ago as the result of a study of the Golgi apparatus during sperm-formation (Bowen, 1920). I found that the apparatus, when present in pieces sufficiently large for analysis, had actually a duplex construction. One portion impregnated heavily with osmic acid or silver nitrate, and could be stained with Fe-haematoxylin, &c., while the remaining portion, though never impregnated by silver nitrate and stained with difficulty by the usual dyes, could be slightly blackened by osmic acid under proper conditions. I suggested, and later developed the idea more fully (Bowen, 1922), that this lightly impregnated
portion was really equivalent to the idiosomic material of Meves, which was united with the heavily impregnating element, the so-called Golgi apparatus, to form what I have since termed the Golgi complex. This complex, while of duplex chemical structure, was to be viewed as essentially a morphological unit. It is perhaps pertinent, in view of Hirschler's observations, to ask whether the idiosomic substance so-called has a real existence, or whether it is simply a reflection of the lamellar structure pointed out by Hirschler. The matter is not rendered any clearer by the observations of Gatenby (1919) on material similar to Hirschler's, according to which the Golgi bodies are visibly composed of the same two elements as in the hemipteran germ-cells. In the Hemiptera, however, the idiosomic material seems to be indubitably real, and its homologue in the spermatocytes of Amphibia and Mammalia has been so often seen that its existence seems to me beyond question. It would be possible to bring all these observations into harmony by supposing that Gatenby's results, obtained by a method different from that used by Hirschler, actually revealed the 'Apparatinhalt' postulated by the latter author. Thus, the whole series of observations on the male and female germ-cells falls into place. The idiosomic substance and the 'Apparatinhalt' are one and the same thing—a substance in some way inseparably related to the lipoidal material usually designated more narrowly as the Golgi apparatus.

The conditions thus far discussed are represented in diagrammatic form in Text-fig. 1, A. It will be noted that in these cases the Golgi material itself consists of scattered pieces, each of which has the duplex character just described. This scattered condition is primarily characteristic of the invertebrates in both somatic and germ-cells. In Text-fig. 1, B, a type of Golgi complex is developed characteristic of the spermatocytes of Molluscs, Amphibia, and Mammalia, in which a process of fusion, confined primarily to the idiosomic constituent, has resulted in a polarized Golgi complex of very simple structure.

The manner in which the highly diversified networks of
vertebrate somatic cells are built up has been clearly indicated by Hirschler (1918). He followed the process whereby a simple

Diagram to illustrate the structure and disposition of the Golgi apparatus in various types of cells. A, protozoa, many arthropod cells, and the eggs of invertebrates; B, spermatocytes of Molluscs, Amphibia, and Mammals; C, somatic cells of developing molluscs (after Hirschler, 1918); D, somatic cells of vertebrates, particularly mammalian gland-cells.

network was approximated in the Mollusc (Text-fig. 1, C) by the elongation and gradual aggregation of the originally scattered Golgi bodies. In the vertebrate somatic cell (Text-
fig. 1, D) the complicated network would seem to represent merely an increase in complexity over the simple condition in the gastropod larva.

In the grosser details of morphology, therefore, the relation of the various types of Golgi apparatus to each other is now pretty well demonstrated. But in the case of networks the important question still remains as to what has become of the idiosomic component of the primitively scattered Golgi bodies. For a long time I entertained the idea that this material might accompany the lipoidal constituent in much the same way as before, leading to a structure indicated in Text-fig. 1, D. Here the heavy outline is the lipoidal Golgi material, and the idiosomic substance is represented as a stippled cloud accompanying it. Some such conception was suggested in my first paper on secretory phenomena (Bowen, 1924). But prolonged study of the Golgi apparatus in many types of somatic cells has failed to substantiate my suggestion, and I have at length abandoned the effort to find an explanation in this direction. On the other hand, it now seems very probable that the pictures obtained can be best explained by recourse to the same optical effects as those which Hirschler (1918) found in the Golgi bodies of the molluscan egg. This is almost certainly the explanation of the remarkable Golgi pieces in the serous salivary cells of Limax (Bowen, 1926 a). They are probably sheet-like plates or lamellae, curved in various ways as described by Hirschler. They possess the same heavy contour lines with more delicately blackened extensions, and with the same possibility of an accompanying content of idiosomic material ('Apparatinhalt'). But most significant is the fact, that not infrequently in fixation these delicately modelled Golgi lamellae are reduced to intensely blackened masses from which all detail and delicacy of form have vanished.

These conditions make an easy transition to the gland-cells of many vertebrates. Here, as noted again and again in the preceding 'Studies', pictures of the Golgi apparatus are obtained in which definite black contours mark the peripheral limits of the network, while in plane view the impregnation is
apparently less dense and often spread out in fenestrated sheets. In other words, in many cases the network is not made up of threads, but the Golgi material is spread out in lamellar fashion to form a fantastic platework that defies analysis. Such plateworks are remarkably developed in the epididymis of the cat, for example, but they occur in less pronounced development in many other kinds of gland-cells which I have studied. Now the interesting thing about these structures is that, as in the mollusc, the apparatus often impregnates in a heavy, coarse way, the delicacy of the real structure being completely lost. The whole collapses into a gross network which makes a fine 'demonstration' of the Golgi apparatus, but from which all possible reality has certainly departed. It is this type of impregnation which seems at times to follow the use of silver nitrate, and in my opinion this has been one reason why our insight into the Golgi apparatus has proceeded so haltingly.

My notion of the general topographical development of the Golgi apparatus (omitting for the moment any consideration of its possible duplex composition) would accordingly be somewhat as follows. I look upon the Golgi apparatus as a specialized cytoplasmic substance which may be moulded into any shape demanded by particular cellular conditions. Thus the material may be scattered in separate pieces or integrated into a complex unit. In the usual concentrated form it may assume a more or less net-like pattern, but the 'strands' of the net are often flattened into broad lamellae. The patterns thus derived may be endlessly complicated, but they are all readily interpreted merely as diverse ways of spreading out the Golgi material in broad contact with the cytoplasmic background of the cell. In one sense this material is of a homogeneous nature, and does not contain any internal system of cavities, at least not as a fundamental part of its own structure.

We thus arrive at a conception of the Golgi material somewhat more elastic than that usually contained in the description of the apparatus as a mere network. But the problem raised by the visible presence of the idiosomic substance in the
germ-cells remains still unsolved. I know of no observations on adult (vertebrate) somatic tissues which help us to an answer. Indeed, the fact seems to be that most authors do not seem to consider that there is any problem involved, and entirely ignore the matter. But the nature of the idiosomic substance is of great importance to any real understanding of the Golgi problem and must sooner or later receive more adequate attention. Concerning its possible explanation the following suggestions will indicate the direction of my own ideas.

The occurrence of a duplex structure in cellular constituents is not restricted to the Golgi apparatus. The same differentiation is well known in mitochondria, where it is best developed in the chromophobe and chromophilic substances of the spermatocyte and spermatid chondriosomes. In many cases where the spermatocyte chondriosomes are favourably shaped the chromophobe material is easily distinguished, while in other cases—thread-like mitochondria for example—the distinction is simply technically impossible. And yet in the succeeding spermatids the two substances are nevertheless present. It would appear then that in attenuated forms of mitochondria the distribution of the two materials cannot be visibly made out, although their differentiation is reasonably probable from the conditions in granular forms. This differentiation of the mitochondrial substance into two definite materials has received little real attention, but I believe that it is essentially analogous to the conditions in the Golgi apparatus. We are helped still further to an understanding of the possible situation by a consideration of the structure of the chromatin. This material seems to appear in two different phases, oxychromatin and basichromatin. These are presumably interchangeable and in different phases of nuclear activity the balance may go now in one direction and now in the other.

These considerations offer, it seems to me, a reasonable basis for interpreting the conditions in the Golgi apparatus. The idiosomic substance is a derivative of the Golgi material proper. In certain conditions it becomes very clearly individualized as in the male germ-cells, while in other cases it may be latent.
or perhaps so intimately related to the lipoidal constituent as to be lost in the impregnation of the latter. But even in the spermatid where the differentiation is most striking, cases are known (Lepidoptera and grasshoppers) where the idiosomic substance is demonstrable with difficulty or not at all—a matter of considerable comparative interest when dealing with somatic cells where the differentiation is usually not to be made out. This conception not only accounts for the observed facts, but it also permits the possibility that in secretory cells the idiosomic or chromophobic material may be more or less extensively differentiated within the Golgi area, but invisible with the technique employed. I am inclined to think, however, that such a development does not exist. But in any event this conception of the finer details of the Golgi material, coupled with what has already been concluded concerning its gross morphology, gives us an understanding of the whole situation which it seems to me is indispensable to a critical study of secretory activity per se.

These considerations clear the ground for an examination of the possible relation existing between the Golgi apparatus and individual secretory granules.

**THE RELATION OF A SECRETORY GRANULE TO THE GOLGI COMPLEX.**

It is one thing to note certain obvious relations existing in a general way between the Golgi apparatus and a mass of secretory granules and to conclude therefrom that the Golgi apparatus produces the granules; it is quite another thing to reduce this relation to the critical case of a single granule, and thus to arrive at some real conception of how secretory products are actually related to the Golgi substance. By way of approach to this problem, I suggested in a previous paper (Bowen, 1924) that the origin of secretory granules might be better understood by comparison with the acrosome of the animal sperm, which apparently develops in a similar way but on a larger scale and free from the difficulties which con-
fuse the picture in a secretory cell. I wish here to extend these earlier suggestions in a somewhat more detailed manner.

For purposes of comparison I have drawn in Text-fig. 2 a series of diagrams illustrating the formation of the acrosome in an insect and a mammal—cases which are both very well known. It will be seen that in both cases the acrosome makes its first appearance in association with the idiosomic constituent of the Golgi complex. In Text-fig. 2, A, the acrosome is just appearing as a small clear vesicle which gradually grows to its final size always in close contact with, in fact more or less embedded in, the idiosomic substance (Text-fig. 2, B). Meanwhile there has appeared within the acrosomal vesicle a small, darkly staining and highly characteristic acrosomal granule. Together these form the acrosome, being moulded into final shape after the Golgi complex (Golgi remnant) has been cast off—apparently a useless remnant (Text-fig. 2, C). The condi-

**Text-fig. 2.**

Diagram to illustrate the formation of the acrosome. A to C, from hemipteran spermatids (after Bowen, 1920); D to F, from mammalian spermatids (after Lenhossek, 1898; Meves, 1899; and Gatenby and Woodger, 1921). The nucleus is partially indicated in outline; the idiosomic substances is represented in stipple, A, acrosome; a, acroblast (= Golgi apparatus); G, Golgi remnant.
tion in mammals (Text-figs. 2, D to F) is essentially similar with
the very interesting difference that the acrosome originates by
the fusion of many small acrosomal rudiments which form
within the idiosomic mass. This fact makes it very difficult
to connect their origin with the nucleus, or indeed with any-
thing but the Golgi complex, more specifically the idiosomic
portion. Further, each of these rudimentary acrosomal
vesicles develops within itself a smaller acrosomal granule.
The basis of the definitive acrosome is produced by a fusion
process, the vesicles merging to form a single, large, acrosomal
vesicle, and the granules to form a single, large, acrosomal
granule—the end result being as in the case of the Hemiptera.

Comparing these conditions in the spermatid with the active
gland-cell, we may note first a curious structural similarity
between the acrosome and the individual secretory granules of
many kinds of glands. With the technique employed in these
'Studies', and by other methods in case of the mucous type of
glands, it is possible to demonstrate that secretory granules
quite generally possess the same differentiation into a clear
vesicular substance within which is a darkly staining granule
(cf. Text-figs. 2 and 3). This is very clearly shown in the
serous salivary cells where the inner granule stains intensely
with acid fuchsin or aurantia according to the maturity of the
granule. In lipoidal gland-cells the same type of structure was
long ago noted and discussed by Altmann (1894), and various
authors have figured it in mucous cells fixed by fluids not
containing osmic acid. This may, it is true, be a merely super-
ficial resemblance, but in comparing the acrosome to a secretory
granule, or vice versa, it is decidedly of more than passing
interest. Curiously enough, there seem in addition to be types
of secretory granules in which the vesicular envelope is not
present or else extremely difficult of demonstration, as in the
pancreas, and in the pelvic gland of salamanders; and similarly
there are acrosomal types, as in the grasshopper and mollusc,
in which the corresponding material is also apparently absent.

Having thus established a probability in favour of the
essential homology between secretory granules and the acro-
some, we may return to comparisons of the relation of these bodies to the Golgi apparatus. There is now at hand abundant evidence to show that there is a constant association of secretory granules with the Golgi apparatus during the period of growth. Whether this association must persist until the actual maturity of the granule is perhaps doubtful, since in the developing spermatid the acrosome is capable of remarkable changes subsequent to its separation from the Golgi apparatus. But pushing farther the analogy with the acrosome it should be possible to arrive at a very intimate picture of the origin of the secretory granules. They should be found in their earliest stages attached to, or embedded in, the Golgi material, just as in the case of the acrosome (Text-fig. 3). Nassonov has suggested that the Golgi material forms a surface of separation between granule and surrounding plasma, which is responsible
for the differentiation of the granule. The fact, however, that
the acrosome does not necessarily occupy a position within
the Golgi material leads me to a different view. I would suggest
that the surface membrane is really provided by the wall of the
clear vesicle within which the secretory granule proper is
differentiated. Thus the materials for secretory granules would
be elaborated primarily in the Golgi apparatus and thence
transferred to the granules, the Golgi material itself being
not directly transformed as some supposed to be the case in the
origin of secretory granules from mitochondria. The condition
is perhaps comparable to the gastric vacuole formed in intra-
cellular digestion, the wall of which apparently serves as an
interface between the surrounding cytoplasm and the contents
to be digested. Finally, the relative conditions of idiosomic
and lipoidal constituents in the Golgi complex of the spermatid
help us perhaps to a better understanding of the apparent
lack of idiosomic substance in the secretory cells.

The critical proof of the whole matter is thus reduced to
the possibility of demonstrating the smallest visible secretory
granules in actual contact with Golgi material. And here,
unfortunately, my results are not yet conclusive. In an earlier
paper (Bowen, 1924) I figured a few cases which I thought
might illustrate this attachment of granule to apparatus. In
the case of the intestinal cells, however, I am now definitely
inclined to consider the appearance as an artifact. But the
appearances in the pancreas there described, and the cases
noted in these 'Studies', leave me still in doubt as to their
correct interpretation. The remarkable vesiculated or mesh-
like development of the apparatus sometimes noted, the
structural details observed in excellent impregnations of the
cat's epididymis, the remarkable development of the apparatus
in the rabbit's vas deferens, and above all the extraordinary
relations which seem to obtain in the oil-gland of the fowl—
all these seem only explicable on the assumption that they
represent pictures of the secretory activity, somewhat altered
no doubt, but still essentially real. But if my own studies are
thus somewhat equivocal, the results of Nassonov (1928 and
1924 a) on the pancreas seem more nearly decisive, particularly in the case of the salamander where the very minute granules can be differentially stained and their association with the Golgi area demonstrated. His results on the epididymis are even more suggestive, although, as I have indicated in another place (Bowen, 1926 c), my own preparations leave me in doubt as to the interpretation of this particular case. But obviously our conceptions of the general relationship existing between granules and Golgi material are essentially identical. We have both discarded the older topographical views of the Golgi apparatus, and find in it a plastic substance which assumes endlessly varied dispositions, many of which are obviously adapted for intimate relationship with vesicles to be developed in contact with, or embedded in, the Golgi material.

But, finally, there is no valid reason for anticipating that we shall be able to make out the finer details of the process in any but the most favourable cases. Even in the spermatid it is often impossible to effect a demonstration of the relation between Golgi apparatus and acrosome, particularly when the latter is of the multiple type. So in the case of the vastly more difficult secretory cell one really specific demonstration is practically sufficient, and the occurrence of numerous cases where complete demonstration fails must not be a source of undue scepticism. This is particularly true when we recall that in many gland-cells it is practically impossible at present to demonstrate secretory granules until they have attained nearly or quite their full size. Making, therefore, due allowance for possible errors in interpretation, there yet seems to remain a residue of evidence which indicates that the individual secretory granules are morphologically related to the Golgi material in a manner essentially comparable to that existing between the Golgi complex and the developing acrosome. And at this point the possibilities of cytological analysis of the problem come to an end.
The Role of Other Cellular Constituents in Secretion.

It is not my purpose here to enter into any discussion of the older theories of secretory formation, but merely to point out briefly how other cellular constituents may be conceived in the light of the ideas suggested in the preceding sections. It must be clear in the first place that the Golgi apparatus is not an autonomous structure, but a part of the cellular system as a whole. As such I conceive it merely as the immediate focus of secretory synthesis, an activity to which other parts of the cell must contribute in one way or another. Thus the materials for the synthetic operations must come from the surrounding protoplasm and in their production the mitochondria, the general cytoplasmic background, and the nucleus may all be more or less involved. It is in this direction that I would be inclined to explain the wide distribution of mitochondria in gland-cells, where they often have close topographical relations with secretory granules but are quite lacking in any of those peculiarities which are so emphasized in the morphological types assumed by the Golgi apparatus.

The only feature of this general cellular relation to secretion which perhaps calls for more particular comment is that of the role of the nucleus in secretory phenomena. One of the most widespread and deep-seated beliefs in zoology seems to be that the nucleus is the source of enzymes and secretions generally. The basis for this belief is astonishingly tenuous, and it is only with some difficulty that I have found any actual evidence which falls within the realm of judgement of the cytologist. And even here, from the morphological standpoint, so much doubt has been raised by competent observers that almost nothing remains of an objective nature. The old indices of nuclear size, shape, position, and staining capacity were long ago shown by Heidenhain (1907) to be fallacious, and even the retrogressive phases sometimes undergone during the close of a secretory cycle are just as well or better explained as part of the general debility which seems to overtake all parts of the
secretory cell except the Golgi apparatus. Certainly it cannot be safely used as a criterion of nuclear wearing out due to unusual synthetic activity, for in some cells no such change occurs. Variations of the chromidial hypothesis are still put forward as evidence of the nuclear origin of secretions, but the studies of Nassonov and myself lend to such views not the remotest semblance of support. The one case in which clean-cut changes occur that seem possibly related to the synthetic activity of the cell is that of cells in which the nucleus assumes a remarkably branching form, as described by Korschelt (1889) and others. But even this seems only an indication that the nucleus is interested in secretion merely as a part of the whole system to which it belongs. That this can often be the only way in which the nucleus enters into the secretory process is indicated by the fact that secretory granules may make their first appearance far removed from the nucleus and then grow larger in the cytoplasm. Since increase in size does not necessarily involve any change in the composition of the granules, it does not appear why the nucleus must be invoked to produce granules which can then grow far removed from its immediate influence. Indeed, this same feature of secretory production seems also to cast much doubt on the view that the nucleus is the direct seat, chemically speaking, of the materials which make the granules.

In the absence of morphological evidence in favour of the direct intervention of the nucleus in secretion, the cytologist is powerless to push the matter farther. And unfortunately so, for just here the results of experimental work on the cell are brought in to support the discredited outcome of early morphological studies. The general purport of this work has been to show that a non-nucleated bit of protoplasm is able to carry on the synthetic activities of a cell for but a brief space of time, while the nucleated piece regenerates and appears none the

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1 Here also may be mentioned the remarkable size attained by the auxocyte nucleus, which in the egg has usually been taken to mean some nucleocytoplasmic interaction connected with the production of yolk. But the exact nature of the connexion is still problematical.
worse for the operation. Hence it is concluded that the nucleus is the centre of synthetic operations and particularly of the formation of those intracellular enzymes upon which living activity is now supposed to depend. But it is at least equally possible that the nucleated piece alone continues capable of constructive metabolism because it possesses the complete cell system, while in the non-nucleated piece the system is disrupted. One might guess that if the Golgi apparatus, for example, could be similarly eliminated, the cell would show disturbances equally fatal to its continued life.

I would suggest, therefore, that secretion is an activity in which the cell system as a whole is very probably involved, and over which the nucleus conceivably exercises some controlling influence; although of how such an influence could be exercised we are quite ignorant, unless we find it possible to accept the accounts of nuclear extrusions into the cytoplasm, which have aroused so much enthusiasm in some quarters and in others a scepticism equally lively. But the actual synthetic centre for the differentiation of secretory granules is the Golgi apparatus.

It would be a most engaging hypothesis to extend this synthetic activity of the Golgi apparatus to the production of the intracellular enzymes whose presence can be proved chemically but which have thus far escaped morphological inquiry; that, in other words, the source of the visible, extracellular enzymes is likewise the source of the invisible, intracellular enzymes. That such a common origin is possible cannot at present be doubted, but our scanty knowledge of these things makes any hypothesis whatever almost pure speculation. I believe, therefore, that the nuclear theories of the origin of secretory products must be abandoned, except as they deal with the problem in a purely hypothetical and indirect manner. The immediate source of secretory synthesis lies in the area presided over by the Golgi apparatus, and in that sense the Golgi apparatus may be said literally to produce the secretory granules.
Summary.

As a result of recent studies on secretory synthesis, the following conclusions have been reached:

1. Many gland-cells run through a regular 'secretory cycle', beginning with a small cell devoid of secretory granules, progressing through a period in which large numbers of granules are produced and terminating in an act of extrusion of the granules. The cycle may or may not be repeated according to the nature of the cell.

2. The Golgi apparatus is from the beginning present in all kinds of secretory cells, and during the secretory cycle becomes very greatly hypertrophied, establishing a volume in rough relation to that of the secretory products.

3. The topography and behaviour of the apparatus is different in different kinds of glands, but is roughly divisible into three general types characteristic of cells which produce serous, mucous, and lipoidal secretions.

4. The secretory granules make their first appearance only within the area delimited by the Golgi apparatus.

5. In a few cases relations have been made out which indicate that the secretory granules arise in close connexion with the Golgi material.

6. It is concluded that secretory granules are differentiated by the Golgi material, but that no direct transformation of the one into the other occurs such as was claimed by some authors in the case of the mitochondria.

7. It is suggested that the Golgi material is structurally homologous throughout the range of animal cells, and that the so-called idiosomic substance, sometimes associated with it, is to be looked upon as one phase of a duplex system in which the relative development of lipoidal and idiosomic substances may undergo considerable variation.

8. It is suggested that the relation between the Golgi apparatus and secretory granules is homologous to that existing between the Golgi apparatus and the developing acrosome of the animal sperm, and that our rather complete under-
standing of the latter phenomenon can thus be used as a basis for interpreting the much more obscure phenomena in the gland-cell.

9. No cytological evidence of the origin of secretory products from the nucleus receives any general acceptance at the present time. The nucleus can be considered as the source of secretions only in the indirect sense that it may possibly exercise some control over the process as a whole or may collaborate with other parts of the cell system in preparing materials for the actual synthetic operations of the Golgi apparatus.

10. The establishment of the views here developed must depend finally upon further critical evidence bearing upon the exact relation which exists between individual secretory granules and the Golgi complex.

**Literature Cited.**


— (1926 b).—"Studies. II", ibid., vol. 70.

— (1926 c).—"Studies. III", ibid., vol. 70.


Hirschler, J. (1913).—‘Über die Plasmastrukturen in den Geschlechts-
— (1914).—‘Über Plasmastrukturen in den Tunicaten-, Spongien- und 
— (1916).—‘Über die Plasmakomponenten der weiblichen Geschlechts-
zellen (zytologische Untersuchungen am Aseidien-Ovarium)’, ‘Arch. 
f. mikr. Anat.’, vol. 89.
— (1918).—‘Über den Golgischen Apparat embryonaler Zellen’, 
ibid., vol. 91.
Hoven, H. (1912).—‘Contribution à l’étude du fonctionnement des 
cellules glandulaires. Du rôle du chondriome dans la sécrétion’, ‘Arch. 
f. Zellforsch.’, vol. 8.
Korschelt, E. (1889).—‘Beiträge zur Morphologie und Physiologie des 
v. Lenhossek, M. (1898).—‘Untersuchungen über Spermatogenese’, 
Meves, F. (1899).—‘Über Struktur und Histogenese der Samenfäden des 
Meerschweinchens’, ibid., vol. 54.
Nassonov, D. (1923).—‘Das Golgische Binnennetz und seine Beziehungen 
— (1924 a).—‘Fortsetzung. Untersuchungen an einigen Säugetier-
— (1924 b).—‘Der Exkretionsapparat der Protozoa als Homologon des 
Golgischen Apparats der Metazoazellen’, ibid., vol. 103.
Ranvier, L. (1887).—‘Le mécanisme de la sécrétion’, ‘Journ. de Micro-
graphie’, vol. 11.