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THE SIGNIFICANCE OF THE INTERNAL RETICULAR APPARATUS OF GOLGI IN CELLULAR PHYSIOLOGY ¹

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IT IS natural for us to attempt to reduce physiological activities to a cellular basis. The cells of which we are composed are in a very real sense vital units. Some of them continue to survive many hours after death, and, while we live, many of them are continually dying. But we ought not to be disappointed if examination under very high powers of the microscope fails to reveal any definite structural groundwork in the cytoplasm. That it is not homogeneous, as it often appears to be, does not require proof, for chemical and physical homogeneity would be inconsistent with physiological activity. We must have some faith in things unseen: we must extend our conceptions to include the morphology of the ultramicroscopic and invisible: otherwise we fail.

Like a great factory, the cytoplasm must be spacially organized for the separation and integration of chemical reactions. Industries are co-ordinated through trade: the cells by the blood stream. But the cell is many times more efficient than any factory. Within its small compass it rapidly brings about chemical changes which are only possible outside the body at high temperature and with the aid of much complicated machinery. It is marvelously well regulated and works with wonderful harmony. The analogy is, of course, hopelessly inadequate but it is nevertheless useful for our purpose. As in a factory, certain areas of the cytoplasm are set apart to perform specific duties. I have in mind, for example, the contractile portion of the muscle cell and the secretory pole of the gland cell. The most recently recognized area and the one about which we know the least appears in many cases to be of fluid nature. It has been called the Golgi

¹ Delivered February 24, 1923.

apparatus after its discoverer, but the term "apparatus" is unfortunate because it suggests a mechanism of a rather inanimate type. With the dawn of an era of experimentation in technique, much attention is being paid to this portion of the cytoplasm. Already the literature has become so unwieldy that to save valuable time we gladly avail ourselves of carefully constructed reviews, like those of Duesberg, Cajal and Pappenheimer, to determine what has or has not been done. It is indeed a striking commentary upon the domination and apparent self-sufficiency of classical methods of cytological analysis that this cytoplasmic constituent, which frequently occupies an area as large as the nucleus, should have remained almost unnoticed for so long.

1. *Morphology.* As a result of the discovery that the apparatus is argentophile after certain fixatives (Golgi) and that it may be blackened by prolonged treatment with 2 per cent. osmic acid (Kopsch), many facts have come to us regarding its shape. In the somatic cells of mammals and the majority of vertebrates it is usually encountered in the form of a more or less dense network consisting of anastomosing strands of uneven girth but of smooth outlines. It may be closely drawn together into a rather compact mass, or dispersed throughout the cytoplasm in isolated fragments, as is often the case in nerve cells. It is never of exactly the same morphology even in neighboring cells of the same kind, which indicates great lability and has given rise to the belief that it may be changing in shape from moment to moment.

In attempting to understand changes in form, we have to be on our guard, because slight variations in technique may result in networks which are either unusually robust, or else thin and anemic looking, or in the complete disintegration of the apparatus into droplets of irregular size. But well controlled preparations do show conclusively that the shape of the apparatus is in a general way typical of different cell types. For instance, its appearance is quite characteristic in acinus cells of the pancreas and polymorphonuclear leucocytes—in the one it is a large network of coarse meshes located between the nucleus and the lumen, in the other it is a small rather dense mass to be found in the cytoplasm in the concavity of the nucleus. Furthermore, if

glands like the submaxillary and thyroid are examined in several different groups of animals the same general style of Golgi apparatus is repeatedly met with. In other words, variations in its morphology are probably very closely related to variations in cellular organization and function.

In the lower metazoa the information at hand appears to show that, generally speaking, the Golgi apparatus is rather prone to occur in disconnected fragments, instead of in the form of complicated networks. Particularly is this true in stages of oögenesis and spermatogenesis. Isolated masses are also to be detected in the protozoa. To this circumstance is due the practice of some authors in speaking of Golgi "bodies." The word "body" unavoidably carries the impression of distinctive form and to some extent of solidity as contrasted with relative fluidity.

2. *Occurrence.* Material resembling the typical Golgi apparatus, as it was first described in the nerve cells of mammals, is of very wide occurrence. If we search through the cells of the human body we find it present in each and every one except in those which are dead and dying, like non-nucleated red blood cells and desquamating epidermal cells. Not only does a Golgi apparatus occur in all embryonic cells but with growth and differentiation, it undergoes a definite sequence of changes suggesting, as pointed out by Bensley, that we have to do with a material which behaves as a unit in the developmental cycle. In the phylogenetic series we are confronted by a similar condition of affairs. It is safe to say that a Golgi apparatus or its representative has been seen in all vertebrates which have been properly examined, also in many invertebrates and in plants (Laburu and others). In establishing homologies, however, it is necessary to keep before us the fact that we are actually dealing with mixtures of unknown substances which vary in density as compared with the ground substance, so that as yet we are only permitted to outline the probable distribution of the Golgi apparatus, with due qualifications and in the most tentative way. Borderline cases are perplexing because the properties of the material unquestionably vary progressively in the life history of specialized cells so that it is difficult to say when we are dealing with a

true Golgi apparatus or with substances which may be in part its products—an uncertainty which is also encountered in dealing with mitochondria. With our present technique we may just skim the surface and recognize a few of its most general attributes. When, after further experimentation, we are able to examine it more closely, with really refined methods, it is quite possible that we shall find distinctive variations in cells of different categories. As in other biological problems, so also here a close study of morphology and behavior must precede chemical and physiological analysis.

In our analogy of the factory, it is like the discovery of the existence in all manufacturing plants the world over of rather pretentious buildings, characterized by certain distinctive architectural features and built of materials almost wholly unknown to us.

3. *Size.* Some cells are evidently fitted to perform their duties with a large Golgi apparatus (gland cells) and others with a relatively small one (muscle cells). We know also that the Golgi apparatus is usually well-developed in the active stages of cytomorphosis, that it becomes gradually smaller as the cell ages (except in plants) until it finally disappears with senility and death. There is also satisfactory evidence to the effect that this peculiar cytoplasmic area becomes enlarged in certain pathological conditions. For instance, Tello discovered a marked degree of hypertrophy in tumors of the mammary gland and his results are in harmony with an increase in the size of the apparatus which Da Fano subsequently noticed in the mammary gland during pregnancy and lactation. Other instances might be cited but we shall do well to err, if anything, upon the side of conservatism in recording experimental changes and to accept only alterations which are very pronounced because slight variations in size occasionally occur spontaneously without apparent rhyme or reason. Some of them may be due to slips in technique or to the influence of changes in light or temperature upon the silver reaction or the blackening with osmium. For the present an accurate quantitative determination of the Golgi apparatus is beset with almost insurmountable difficulties. Nobody has yet

attempted to establish a definite Golgi apparatus—cytoplasmic ratio on the basis of relative volumes. We cannot even say, with confidence, whether the material is present in relatively larger amounts in protozoa or in man.

Passing now to a consideration of the actual mechanism of changes in size we at once plead complete ignorance. There has, however, been no dearth of speculation. On cell division the networks are broken up into smaller masses (resembling perhaps the Golgi “bodies” of lower forms) which are distributed approximately equally to the two daughter cells, in which the networks are again reconstituted. Sometimes this process is characterized by great regularity; at other times it has the appearance of being rather haphazard. Attempts have not been wanting to bring the so-called “Golgi bodies” into line with other cytoplasmic components, especially the plastids of plants, which are, at least in some cases, self-perpetuating and multiply by direct division without loss of their individuality. Gatenby in particular has come out squarely with the declaration that “both mitochondria and Golgi bodies are able to assimilate, grow and divide in the cytoplasm somewhat as a protist assimilates, grows and divides in a watery medium.” By this he does not intend to imply that they are symbiotic organisms. He wishes only to indicate that they possess a marked degree of independence. He is not alone in this contention but the idea of the individuality of chemical substances, expressed in relation to the Golgi apparatus, in a system which is itself a co-ordinate whole, does not make a strong appeal to those of us who have been chiefly occupied with the somatic cells of vertebrates in which the networks frequently attain to a high degree of complexity and in which they often undergo hypertrophy without preliminary fragmentation into small aggregates. Under these circumstances, growth by some process of accretion seems more likely to prevail.

4. *Position.* Another point of interest, and we trust of immediate practical importance, is that the Golgi apparatus appears to move about in the cytoplasm in a remarkable and orderly manner. In cells of fixed secretory polarity like the acinus cells of the pancreas, which have already been mentioned,

and the cells of the salivary gland, it is always placed between the nucleus and the discharging pole. In the thyroid, on the other hand, I have found that, at least in the adult guinea-pig, it normally migrates from one end of the cell to the other. That is to say, from its usual position between the nucleus and the lumen it may approach the lumen or flow around the nucleus to the opposite end of the cell adjacent to the peripheral vascular network. This reversal may take place in entire follicles or within single cells. There seems to be a kind of ebb and flow. In order to view this phenomenon in true perspective, we may recall that the position of the Golgi apparatus is subject to great variation in the cells of the choroid plexus, studied by Biondi, and that a somewhat similar but progressive change in its position has been noted by Golgi in mucus-secreting intestinal epithelial cells.

Since we have reason to believe that the thyroid differs from other glands like the pancreas in being able, under conditions which are but little known, to pour its secretion directly into the peripheral vascular network, I made the suggestion, in January of last year (1922), that we have in these migrations a real indicator of physiologic reversals in polarity. If this theory is confirmed experimentally, preparations of this type revealing the position of the Golgi apparatus will afford accurate information at the time the tissues are taken regarding the direction of secretion. In this way it may be possible to effect a close correlation between the actual discharge of thyroid secretion and the response by the organism to its action. I also suggested that an examination of the Golgi apparatus in the parathyroids and the hypophysis, in both of which intracellular secretion antecedents remain to be discovered, might yield clues regarding the direction of discharge, but preparations were made of these tissues from adult guinea-pigs without my being able to discover any definite orientation on the part of the apparatus.

But the probability of success with the thyroid is clearly indicated by Masson's paper on the position of the centrosomes in malignant thyroid tumors published last June (1922). It has been abundantly shown that the centrosomes are often closely related in a topographic sense to the Golgi apparatus. Like the

Golgi apparatus they usually occur in thyroid cells between the nuclei and the lumen. But in tumor cells Masson found them to migrate in the direction of the peripheral blood vessels. In one of his illustrations it may be seen that though most of them are in the usual position, a few are reversed. In another, all are reversed and the colloid has disappeared from the lumen and has accumulated about the peripheral blood vessels. The follicles seem to have been turned completely inside out in respect to the direction of secretion. Successful preparations of the Golgi apparatus would probably have revealed the same phenomenon.

Last April Courrier and Reiss published a short note dealing with the position of the Golgi apparatus in the parathyroids of new-born kittens. With the apparatus as an indicator, they claim to have established the existence of a definite secretory polarity and the presence of a network of capillaries of two types. They believe that at this stage the cells are arranged in cylindrical columns, the surfaces of which are bathed by nutritive capillaries and the central areas drained by others of excretory nature, and that the position of the Golgi apparatus between the nucleus and the central capillary indicates the existence of a definite functional polarity. The authors point out that this discovery of polarity opens up a somewhat new conception of endocrine cells, it having been customary thus far to deny the presence of definite secretory polarity in all of them except the thyroid. They call attention, for example, to Van der Stricht's claim that the lutein cells may secrete from any point of their surface—*i.e.*, that they are apolar—and to the fact that Colson is of the same opinion in respect to the cortical cells of the suprarenals. Now the possibility is raised that other cells, in addition to those of the parathyroids, may be definitely polarized. This information supplied by Courrier and Reiss, if confirmed, may be useful in helping us to find true secretion antecedents in the parathyroids. When we know the pole of the cell from which the secretion is discharged we shall at least know where the antecedents are likely to be most concentrated.

In a second paper published in June, Reiss made a study of the position of the Golgi apparatus in the secretory cells of the

anterior lobe of the cat's hypophysis. What he found is indeed most striking. As a preliminary to the discussion he remarks that, from the work of Stewart and others, it seems likely that the three types of cells with which we have to deal are closely related. From being chromophobe, they become basophile and then acidophile. In the first named, the apparatus is, according to Reiss, without special orientation which corresponds nicely with the view that these cells are resting. In the basophile cells it is invariably found between the nucleus and the periphery of the cluster, and finally in the acidophiles it is located between the nucleus and the central area. Reiss claims to have observed all transitional stages in this sequence. His interpretation is that we have a mechanism by which the cells are able in one stage to pour a secretion toward the periphery and then to turn about face and discharge a second and different product into the centre of the cluster. It is questionable whether these suggested oscillations in secretory polarity could have been detected without the clue offered by the migration of a conspicuous structure like the Golgi apparatus. We naturally await confirmation of Reiss's work with some eagerness.

A significant observation to be emphasized is that reversal in the position of the Golgi apparatus may also be induced experimentally in tissues in which it does not occur normally. D'Agata discovered a change in position in epithelial cells of the newt's stomach following scarification, and Basile found that it could be reversed in the cells of the straight and convoluted tubules in one kidney through the extirpation of the other. From its normal position between the nucleus and the lumen it migrates to the opposite pole facing the peripheral blood vessels. Unhappily these observations have not been confirmed but the illustrations presented by Basile are so clear and convincing that it is difficult to imagine how he could have gone astray. But we must not be premature and hasten to the conclusion that the purpose of the Golgi apparatus is to elaborate secretion, because we find it to be equally highly developed in nerve cells and in others in which secretory activities are not pronounced. This does not mean that in gland cells it may not take some part in the formation of

secretion since so marvelous an integrating and unifying principle is manifest in all vital processes. We have to steer a middle course. What the observations which I have related do show is that we are now able to follow significant changes in the position of an important and hitherto unrecognized cytoplasmic area. From the technical point of view we are on firmer ground than in the study of variations in the shape and size of the Golgi apparatus, inasmuch as it is altogether unlikely that any error in manipulation would constantly bring about so definite a shifting in the relative position of parts of the cytoplasm.

To return again for a moment to our confessedly inadequate analogy of factory organization, it is as if the above-mentioned large and unknown buildings, presumably containing machinery vital to the industry, were found to be capable of undergoing periodic migrations to the other end of the lot.

5. *Constitution.* Thus far stress has been placed upon objective findings which are subject to verification. Unfortunately any logical and comprehensive interpretation which will fit all the facts is a thousandfold more difficult. We are not even justified at this time in hazarding speculations as to what is going on within this newly discovered region of the cell, although several investigators have not shown any reluctance to express their views on the subject. It has already been intimated that while our methods are so crude we cannot exclude the possibility that under the same heading we are grouping a variety of formations. The affinity of the Golgi apparatus for silver salts after appropriate fixation has been mentioned and the ease with which it may be blackened by prolonged immersion in osmic acid. It may occasionally be stained with iron hematoxylin and resorcin-fuchsin. Either it or one or more of its components is soluble in alcohol, for unless preparations are dehydrated very promptly no trace of it remains. It has been repeatedly asserted that the Golgi apparatus is at least partly of lipoidal nature. According to Gatenby it has in this respect much in common with mitochondria. Bowen is of the opinion that the Golgi bodies observed in insect spermatogenesis are made up of two components, one staining darkly and the other lightly—an idea which has been

elaborated by subsequent investigators, but I see no good reason to suppose that the Golgi apparatus in the somatic cells of higher vertebrates is heterogeneous in the same sense.

The chief obstacle is that the Golgi apparatus cannot be clearly seen in living unstained cells examined in approximately isotonic media—to be specific, in mammalian tissues, because several workers claim to have observed Golgi bodies in the living cells of certain invertebrates. Nor has it been possible to stain the material with vital dyes, although many have been tried. This small branch of cytology has in fact advanced about as far as did our knowledge of mitochondria before the introduction of janus green. At that time there were many “doubting Thomases” who have since been converted. We hope and expect a similar development in the case of the Golgi apparatus. But in fairness we are obliged to admit that, as revealed to us in fixed preparations, upon which we must for the moment rely, it is an artefact in the sense that it conveys an impression which does not fully or accurately represent the condition of affairs in the living cell. We suspect that the dense black outlines give rise to a false idea of relative solidity for the reason that when cells are carefully crushed under the microscope it may be seen that the mitochondria and other granules have freedom of motion and that they are not impeded by the presence of a semi-rigid network in the area which we know to be occupied by the Golgi apparatus. I have found, moreover, in the thyroid that it is by no means a simple matter to displace the Golgi apparatus by centrifugation, from which it is safe to deduce that it is, in the tissue examined, of about the same specific gravity as the remainder of the cytoplasm.

6. *Reactions to Injury.* -It was soon shown by Cajal and other investigators that the Golgi apparatus is very sensitive to autolytic influences. In nerve cells removed from the body it loses, within a very few minutes, its distinctive net-like form and breaks up into a very fine dust-like deposit. The sequence of alterations following experimental injury to nerve cells has been the subject of several papers, first by Marcora and later by Cajal and Penfield. As a result, the apparatus becomes dispersed into

the peripheral cytoplasm and finally disappears completely. In phosphorous poisoning I have noted a corresponding fragmentation but no peripheral migration. It is a singular fact that unlike the mitochondria (W. J. M. Scott) the Golgi apparatus takes little or no part in the ensuing fatty degeneration.

The time at my disposal forbids reference to further work along this line except to make the general comment that investigators in biology and medicine have not been slow to grasp the fact that the Golgi apparatus offers an entirely new criterion of cell injury, the study of which may yield surprising results of far-reaching importance. It is to be regretted that the problems involved have not always been approached in a spirit likely to bring adequate returns. In recent years cytology has become a very highly specialized science and must be treated with due respect. Nobody would launch forth upon a complicated chemical analysis without adequate training in chemistry. Disappointment would follow as surely as day follows night. The instances are exactly parallel. No matter how detailed are the instructions, a technician cannot be expected of his own initiative to follow them successfully and to arrange for suitable controls. The investigator himself must buckle right down to work, and prepare himself for some disappointments, if he is to reap the reward. In a study so delicate it is even within the bounds of possibility that trained individuals looking eagerly for some distinctive changes will all unconsciously be influenced to report some deviation from the normal, especially when the normal is not readily established as in the case of human tissues removed at operation or at autopsy. It is open to serious question whether the study of mitochondria has not also suffered grievously from the hastily planned and ill-considered observations of investigators who are masters in their own fields and have simply been attracted by the shimmer of something which is strange and new.

7. *Relation to the So-called Canalicular Apparatus.* Before concluding this address, brief mention should be made to a kind of evidence which we apprehend only dimly but which seems to shed some light upon the nature of the materials which we have under consideration. About the time that Golgi announced his

discovery (1898), Holmgren and other workers found a system of clear canals within the cytoplasm of a large variety of cells belonging to the same categories in which the presence of the Golgi apparatus was being reported. They were seen after many fixatives, but especially trichlorolactic acid, and exhibited the property of remaining uncolored when the rest of the cytoplasm was stained. Their close resemblance in form and position to the apparatus of Golgi attracted widespread attention, so much so that Cajal was led to propose the name of Golgi-Holmgren canals to include both formations. This action, however, has not passed unchallenged. Duesberg has reacted strongly against its unqualified acceptance. He is of the opinion that the two formations are identical in neurones and non-nervous cells which possess a localized trophospongium (canalicular system) but that in non-nervous cells with a diffuse trophospongium spread throughout the cytoplasm they cannot be the same because the Golgi apparatus is restricted to one pole of the nucleus.

We note also that Penfield has found distinctive changes in the morphology and position of the Golgi apparatus in nerve cells after section of the posterior nerve roots. And, further, that when the same cells were bleached and stained with iron hematoxylin he was able to observe a system of clear canals in the cytoplasm which in no way corresponded with the remnants of the Golgi apparatus. He naturally concluded that the clear canals and the blackened Golgi apparatus are two entirely different formations. These observations merit very careful consideration. A close examination of his figures shows that the clear canals which he found are not exactly the same as the canalicular apparatus in normal nerve cells. The canals are angular and to some extent suggestive of shrinkage spaces: they are abundant in the peripheral cytoplasm and in some cases appear as if they might penetrate into the cell from without; whereas in normal cells of the same kind the canalicular apparatus presents rounded contours and is usually situated in the intermediate zone of the cell, leaving a layer of cytoplasm immediately about the nucleus and just beneath the cell membrane clear. This may mean that we are dealing with a canalicular apparatus distinctively changed

by section of the nerve roots or that we are confronted by an altogether different type of tubular system.

It is, I think, significant that von Bergen discovered in certain nerve cells shreds of blackened material within clear canals—an observation which has led investigators to infer that the clear canals contain a fluid which is argentophile, osmophile, soluble in alcohol, and exhibits all the properties which we are accustomed to refer to the Golgi apparatus. Though I have been unable to repeat this observation of von Bergen, I have obtained similar but less striking information pointing in the same direction. In nerve cells the clear canals, like the blackened apparatus, may be diffuse, eccentric, or circumnuclear in position after fixation in formalin, trichlorolactic acid, and osmic acid. The same three morphologic types are seen after staining with Weigert's hematoxylin. What appear to be transitions may be detected between clear canals and aggregates stainable with resorcin-fuchsin, and also between clear canals and osmic acid blackened masses. In other words, in both instances there seems to be a progressive increase of the stained material at the expense of the clear canals. My preparations suggest positive and negative impressions of one and the same thing. In the pancreas I have bleached out the blackened networks and find in all cases a corresponding system of clear canals remaining, although the size of the cells is altered by the repeated hydration and dehydration. But it is unsafe to go as far as to claim that the clear canals and the blackened reticula occurring in all cells are visible expressions of the reactivity of one and the same cytoplasmic area.

We know that clear, chromophobe spaces in the cytoplasm are not always of the same origin. They may represent areas from which the mitochondria have been dissolved; instead of being restricted to a definite location they may be experimentally produced throughout the cytoplasmic area; in some cases pointed clefts which are apparently technical artefacts may be continuous with a canalicular apparatus in its proper location. And these are not the only possibilities that complicate the problem. Repeated attempts on my part to make clear canals artificially in mixtures of gelatin and lecithin, fixed by methods designed to

reveal the canalicular apparatus, have not been particularly fruitful. Preparations made in this way and stained with iron hematoxylin contain canals and vacuoles of many sizes. By careful selection, however, it is possible to gather together a series of canals which resemble to some degree the intracellular formations which so perplex us. That intracellular canals are not always fixation artefacts may be concluded from Bensley's observation that they may be seen in living islet cells of the pancreas. But the mere act of taking living cells from the body and of bringing them under the microscope for study may, and probably does, initiate changes in the cytoplasm which may be wholly or in part responsible for the appearances in question.

The same investigator made a parallel study of the clear canals in plant and animal cells by improved methods of technique. Through the study of both living and fixed tissues he found a very significant series of changes in growing cells of the onion tip. In the youngest cells he discovered a system of clear canals, agreeing in many details with those brought to light by the same methods in animal cells. With increase in age the canals enlarged and finally gave rise to the familiar plant cell vacuole. On the basis of these observations he suggested "that the network of canals found in so many animal cells is the physiologic and morphologic equivalent of the vacuolar system in the plant cell." This far-reaching generalization has recently (1922) received support from the botanists, Guilliermond and Mangelot. These investigators worked with barley cells and arrived at a similar conclusion by employing methods adapted to the demonstration of the Golgi apparatus in animal cells. If further work shows that this is in truth the case, interesting and new opportunities for experimental study will be opened up of a kind essentially different from those contingent upon the discovery of the nucleus.

To come back to the starting point in our discussion of what we are pleased to call "cellular organization" which is, after all, the central problem of physiology, it is as if an inhabitant of Mars observed one of our large manufacturing plants with a powerful telescope and discovered a large and conspicuous building,

and, further that he noted similar buildings in other centres the world over, capable of changes in size and shape and of migration from place to place. It would be only natural for him to try to discover what mysterious activities go on within them. This is what we are endeavoring to do with the Golgi apparatus in animal cells. At present we see through a glass darkly but we hope that this haziness is merely the rather invigorating cloud of mystery which usually surrounds a new development in science. We do not like to think that we are only hot in the pursuit of a phantom.