

Resumen por G. Carl Huber, por el autor, Wilder G. Penfield.

El aparato de Golgi y su relación con el trofospongio de Holmgren en las células nerviosas. Comparación durante retispersión.

El autor revisa brevemente las investigaciones sobre el aparato de Golgi y el sistema de canales del trofospongio de Holmgren, prestando particular consideración a la idea, mantenida por ciertos autores, de que el aparato de Golgi y el trofospongio de Holmgren son la misma estructura en las células nerviosas, que se manifiestan de modo distinto según los métodos de fijación y teñido, que dan imágenes positivas y negativas de una misma estructura. Usando la misma célula y mediante métodos sucesivos de teñido, el autor ha observado que no se encuentran canales que correspondan con el aparato de Golgi demostrado previamente, y que durante la retispersión, el retículo de Golgi se desplaza hacia la periferia celular, mientras que la posición intracelular de los canales de Holmgren no se altera. Ambas estructuras pueden demostrarse sucesiva o simultáneamente en el citoplasma de una misma neurona.

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THE GOLGI APPARATUS AND ITS RELATIONSHIP TO HOLMGREN'S TROPHOSPONGIUM IN NERVE CELLS. COMPARISON DURING RETISPERSION¹

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SEVEN FIGURES

INTRODUCTION

The intracellular relationship of the Golgi apparatus and Holmgren's trophospongium has been a subject of considerable dispute. Observations made by the writer while studying nerve cells in conditions of varying functional activity may throw some light on this problem. A preliminary brief review of the literature has been added, inasmuch as the work done on the comparison of the two structures has not been summarized in English and interesting contributions have been made since the excellent general reviews in German and Spanish by Duesberg and Cajal.

A reticular apparatus was first described by Camillo Golgi in 1898, in the cytoplasm of neurones in the central nervous system (21). In the same year Verati (44) described a similar structure in sympathetic ganglia. Outside of the nervous system, Pensa (40) was the first, demonstrating in the following year an internal reticulum in the cell cytoplasm of the suprarenals. Negri (37), also a pupil of Golgi, noted it in other ductless glands. After these pioneer observations, a large number of cytologists have observed the structure in cells of many types.

There has been considerable confusion as to the nomenclature of the structure in question. It has been variously called the internal reticular apparatus of Golgi (21), the endocellular reticular apparatus, the Binnennetz of Kopsch (33), the reticular

¹ From the Laboratory of Physiology, Oxford University. This communication formed part of a thesis accepted for the degree of B.Sc.

apparatus of Golgi-Holmgren (Cajal, 7), the canalicular apparatus (Bensley, 3), Golgi apparatus, etc.

Beginning in 1899, Holmgren (24) described an intracellular system of canals ramifying in the cytoplasm of neurones and certain other cells. He at once urged the identification of this canalicular structure, which he named 'trophospongium,' with the Golgi apparatus. Since this time the question of the identity of the two structures has been a subject of much discussion.

LITERATURE

Distribution of the Golgi apparatus in nature

A voluminous literature describing the apparatus in various types of cell has accumulated since Golgi made his original observations on spinal ganglion cells. The form of the apparatus varies somewhat from one type of cell to another, but common staining and developmental characteristics are exhibited throughout.

Cajal, in 1903, was the first² to demonstrate the Golgi apparatus in the nerve cells of invertebrates. His observations have been greatly extended for nervous and other tissue cells in all types of invertebrates by many workers, especially in the school of Nusbaum (38). Gatenby (19) states that "every sort of metazoan cell carefully examined, has been found to possess the typical apparatus" of Golgi.

Cajal (8) states that the reticular apparatus is well differentiated between the thirty-second and thirty-fourth hour of incubation in the germinal cells of the primitive streak (chick embryo). During ontological development, the reticulum and attraction sphere occupy the pole of the cell directed toward the external world, that is, the direction of cell migration or growth. According to Marcora (35), it is not until the tenth or twelfth day that the Golgi apparatus first appears in *adult* form *about* the cell nuclei of the more highly differentiated tissue. In cell division, Gatenby (20) has observed the Golgi apparatus (which is in the form of little rods lying on the archoplasm) to divide

² See Duesberg (16), p. 13.

itself equally between the two daughter cells. He concludes that "every mitosis or karyokinesis is also, as well, a dictyokinesis or nearly equal sorting out of Golgi rods between the daughter cells."

Bensley (3) described a typical intracellular reticulum in plant cells, staining with osmic acid, and watched it intra vitam break down from a living 'canalicular apparatus' to the 'multiply vacuolated' condition usually seen in the dead plant cell. This would seem to indicate that for plants, too, it may be possible, with improved technique, to demonstrate a reticular or canalicular cytoplasmic structure.

Thus the Golgi apparatus has been described as a cytoplasmic constituent of all types of cells in the animal kingdom, has been observed developing in the early stages of the embryo, and has been seen to grow and divide with cell multiplication.

Nature of the Golgi apparatus

The appearance of the structure varies somewhat from one type of cell to another, but in general it always occupies the cytoplasm of the cell and never encroaches upon the nucleus nor the cell periphery. In neurones it enters some distance into the dendrites. Its form is, in general, that of an irregular network.³ In nerve cells the meshwork is typically studded with lacunae, which sometimes appear full of impregnatable material and at other times empty, the boundaries *only* being outlined by the deposited silver or osmium.

With regard to the consistency of the apparatus, Cajal (8) has carried out some experiments which are of interest. In order to determine whether the 'materia argentofilia' was a liquid (and easily diffusible) substance or a semisolid substance not easily dislocated, he tore and crushed areas of spinal cord and brain. Upon subsequent microscopic examination, he found that at a tenth of a millimeter from the injured surface the Golgi apparatus

³ Workers in the Institute of Nusbaum have shown (38) that in the ganglion cells of cephalopods the Golgi apparatus takes on the form of threads which rarely join or cross. Likewise, in gastropods, Crustacea, and some insects the apparatus departs somewhat from its usual reticular arrangement.

within the cells was quite normal. On the very borders of the lesion or at a short distance from it were cells whose internal reticulum was broken, but replaced by tiny globules distributed throughout the cell, quite in accordance with the usual structure of the reticulum. There was no seepage nor fusion into large drops of the component material of the reticulum. His conclusion was that the material of the Golgi apparatus was viscous or semisolid and incapable of diffusing irregularly through the interstices of the neurofibrillar skeleton.

Concerning the structure of the apparatus, Cajal's theory, which resembles that of Holmgren in some respects, is briefly as follows: As seen in its most highly developed state in nerve cells, but also in other cells of the body, it is made up essentially of two different factors, a stable system of communicating spaces, and "a finely granular, vacuolated substance composed of complex material, endowed with great chemical and physiological activity—a mixture of lipoids (substances reduced by osmic acid) and of cytoproteids (with special affinity for silver)." The quantity and chemical composition of this mixture vary within wide limits according to the functional activity of the cell. This complex chemical is organized, during life, into granules or microscopic 'protomeras' endowed with the capacity to grow and, in multiplying, to bring about or assist at the chemical synthesis indispensable to cellular life.

This theory is broad enough to explain the varying morphology of the Golgi apparatus at least in normal conditions, as demonstrated by the variety of methods in use.

Functional activity of the Golgi apparatus

That the apparatus plays some essential rôle in cell life can hardly be questioned, inasmuch as it has been found generally in the cells of all types of living organisms. Moreover, there exists a proportional relationship between the normal cellular activity and the size and complexity of the Golgi apparatus. For example, the most complete and complicated reticulum is found in adult nerve and muscle cells. The function of these two classes of cells is, of course, associated with a great discharge of energy.

In 1909, Golgi (23) observed in the mucous glands of the stomach of a frog the displacement and change in form of the reticular apparatus corresponding to the varying functional activity proper to the cells. Kolster (32) similarly noted dislocation and metamorphosis in the goblet cells of the stomach accompanying the formation of secretion, and Tello (43), in studying sebaceous and mammary glands, described two phases through which the Golgi apparatus passed in active cells: hypertrophy and dispersion into fragments or droplets.

Cajal (8), in the goblet cells of the intestine, showed that in the resting stage the Golgi apparatus was small. With the beginning of the formation of secretory products, the apparatus becomes much hypertrophied. Before discharge of the cell contents, the apparatus itself becomes fragmented and partially passes out with the secretion. He showed that in odontoblasts, and osteoblasts as well, there is hypertrophy of the reticulum with the onset of cell activity.

Basile (2) has made interesting observations on the renal epithelium of the remaining kidney in animals after unilateral nephrectomy. The apparatus, which normally is found between the nucleus and lumen of the tubule, becomes hypertrophied, passes down about the nucleus, and at the end of the twelfth day is found at the base of the cell. Addison (1) noted hypertrophy of the reticulum of the basophilic cells of the anterior lobe of the pituitary after castration.

Weigl (45), after considering the changes occurring in the Golgi apparatus of gland cells, concludes that no light is thrown upon the actual function of the structure in those cells. The changes, he supposes, are due to mechanical displacement. But Duesberg (15) has reported changes in staining reaction of the Golgi apparatus during cell secretion and absorption which, in his opinion, indicated a qualitative as well as a quantitative change.

As to the actual function of the Golgi apparatus in the cell, Nusbaum (38), in reviewing the work of Weigl and the other investigators in his institute, concludes that the nature of the activity of the apparatus is nutritive (also Cajal, 8) and that it shows, typically, phases of increase and decrease associated with

this activity. Duesberg (16) and Sjövall (42) are unable to reach any conclusions as to function, and agree with Golgi (23) that "Relativement à la signification de l'organe reticulaire en question, . . . il s'agit d'un probleme dont la solution reste encore à trouver."

Functional changes in the Golgi apparatus of *nerve cells* were reviewed in a previous communication⁴ (39), and a specific reaction of the apparatus in these cells to axone section was described. This reaction consists in a dispersion of the intact reticulum to the periphery of the cell, *retispersion*, which may or may not be followed by *retisolution*. The question arises as to whether this added phenomenon throws any light on the function of the Golgi apparatus.

Retispersion after axone section is usually associated with chromatolysis. The nucleus may therefore be found at the cell periphery as well as the reticulum. If the conclusion of Cajal is correct, that the Golgi apparatus is of a semisolid consistency, then in retispersion associated with chromatolysis the two cell constituents which are displaced to the periphery are of more or less rigid form. This might argue that they were passively displaced from the center of the cell, *without* necessarily signifying any alteration in the function of either structure.

From the form of the apparatus in nerve cells (a system of lacunae joined by canaliculi), its variations (sometimes appearing hollow and at others full of impregnatable material), its resistance to alteration in abnormal conditions (such as tetanus and pathological states generally, or separation from its connecting neurones by decerebration or cord section), it may well be an endocellular storehouse or circulatory structure. That is, a structure of more or less rigid form, the lipoid and proteid content of which varies during normal cellular activity. Its function is yet obscure.

⁴ Since this was written I have had the opportunity of seeing a very interesting demonstration of changes in the Golgi apparatus of nerve cells in the spinal cords of rats following exposure to cold. Demonstration—Congrès de Physiol., Paris, July 16-20, 1920—by C. Da Fano. It is learned from this investigator that a preliminary note on the subject will appear shortly.

Methods of demonstration of the Golgi apparatus

The methods by which the internal apparatus of Golgi can be demonstrated in nerve cells have become numerous. Briefly they are:

1. Modern method of Golgi (22). Fixation in formol, alcohol, and arsenious acid, followed by impregnation with 10 per cent silver nitrate. Reduction in any developer. The sections are toned with gold chloride.

2. The Cajal method (8, 10). Fixation in formol and uranium nitrate, impregnation with silver nitrate, followed by reduction in a solution of formaldehyde, hydroquinone, and sodium sulphite. Da Fano (13, 14) has modified this procedure by the substitution of cobalt nitrate for the uranium nitrate in the fixative with good results.

3. Kopsch method (33). Immersion of fresh tissue in 2 per cent osmic acid for eight days.

4. Besta (5) stained the apparatus with thionin after fixation in formol and acetic aldehyde, and followed by mordantage in ammonium molybdate.

5. Sjövall fixes tissue in formalin followed by immersion in osmic acid.

In addition to the above well-known methods, the apparatus is occasionally stained by many of the more general methods or may be seen unstained against a darker background (12). It may be stained *intra vitam* with neutral red or janus green (18). It has been demonstrated to me by J. B. Gatenby in fresh unstained cells of the ovotestis of *Helix aspersa* when they were teased out in the tissue juice.

A description of the methods employed in this study appears below.

Mitochondria (chondriosomes) and Golgi apparatus

Most recent writers are agreed that mitochondria and Golgi apparatus are separate structures, although possibly having some genetic relationship (Duesberg, 17, Cajal, 8; Cowdry, 12; Gatenby, 18; Nusbaum, 38). On the other hand, Monti (36) has recently

maintained that in adult nerve cells the mitochondria and Golgi apparatus are one and that the apparent difference is due to the different technic employed. It is true that in procedures designed to demonstrate each structure, the other may occasionally appear as well, but this apparently is only an indication that the two structures have some common chemical constituents.

HOLMGREN'S TROPHOSPONGIUM

In 1899 Holmgren (24) drew attention to processes from outside entering the cytoplasm of the spinal ganglion cells of *Lophius piscatorius* and anastomosing there. In a postscript to this publication he cited Golgi's description of 'un fine apparato fibrillare' in the cytoplasm of the ganglion cells of mammals, and expressed the belief that the structure demonstrated by Golgi was only an intracellular part of the network of 'Saftkanälchen' which really communicated with the exterior.

In 1900 Holmgren (25) extended his observations to the spinal centers and ganglia of cats, rabbits, dogs, etc., using Carnoy's fluid principally for fixative, and iron hematoxylin or toluidin-erythrosin for stains. He showed that the 'Saftkanälchen,' or system of canaliculi, opened into the pericellular tissue spaces ('perizellulären Lymphspalten').

After these first descriptions he elaborated a somewhat complicated theory (26, 31) to explain variations in the appearance of the structure under consideration, which is briefly as follows: There is a network in the cytoplasm of certain cells to which he gave the name of 'trophospongium.' During cell activity, drops of liquid may be formed in the substance of the trophospongium thread. These drops coalesce, thus forming a canal within the thread, and this procedure may go far enough to dissolve the whole trophospongium, replacing it by trophospongium canals, the above-mentioned 'Saftkanälchen.'

In spinal ganglion cells he showed continuity between trophospongium and the enveloping subcapsular cells. In fact, according to Holmgren's view, the branching outgrowths of these enveloping cells or trophocytes, after entering the cytoplasm of the nerve cell, constitute the trophospongium. These ingrowths

possess the power of ameboid movements and their function is to take part in the nutrition of the neurone.

He proposed dividing all cells into two classes (26); *a*) a more highly differentiated class of cells all of which contain a trophospongium-canal system, and *b*) supporting cells, whose cytoplasm contains no trophospongium, but whose outgrowths, entering the cytoplasm of the cells of the first class, form a trophospongium there. Examples of this second class are subcapsular cells and neuroglia.

To sum up in Holmgren's own words (31, p. 135): "Summa summarum: ich glaube noch jetzt gültig Gründe zu haben . . . dass ein gewisser Inhalt des Netzes durch Umsetzungen in mehr oder weniger hohem Grade verflüssigt werden kann, wodurch Kanälchen entstehen, die durch azidophile Konturen, die den Resten der eigentlichen Fädchen entsprechen, abgegrenzt werden. An den Stellen des Netzes, wo die Dissolution zustande kommt, entstehen Kanälchen, die weiter sind als die übrigen Netzteile."

Numerous authors (Bethe, Cajal, Cowdry, et al.) have verified the existence of canals, *at least* within the cell.⁵

The question of the identity of the Golgi apparatus and Holmgren's trophospongium

In substantiation of his claim that trophospongium may be identified with the Golgi apparatus, at least in spinal ganglion cells, Holmgren presents in a recent article (31) excellent microphotographs of the Golgi apparatus blackened by the Kopsch method and, for comparison, iron-hematoxylin preparations in which the trophospongium canals stand out unstained in the ground cytoplasm. The general distribution of the two structures is the same and their form, although incompletely shown, is similar. Such appearances have led many authors to state that one structure is simply the negative picture of the other.

⁵ For an excellent summary of the work done on this point, see Duesberg (16), p. 47 et seq.

Such arguments are open to criticism, however. The two structures are being studied in cells of the same category, but *not in the same cell*. Also, even though they are frequently found occupying the same general location in the cell, their identity is not thereby established. Legendre (34) demonstrated similarity of general location for Golgi apparatus and Nissl granulations. But he did not thereby succeed in establishing that the two substances were identical.

Most workers have not been able to demonstrate extracellular connections for the trophospongium. In fact, Holmgren himself admits that all observers who have used only the osmic-acid or silver-impregnation methods, with the exception of Retzius and Smirnow, have concluded that the intracellular reticulum does not reach the surface of nerve cells. Sjövall (41), in ganglion cells, showed that it approached the subcapsular cells, but he could not establish true continuity.

In the opinion of Duesberg (16, 17), Nusbaum and his pupils, and also of Cajal (8), Holmgren, in maintaining the communication of the trophospongium with the exterior, has been confused by the appearance of exogenous processes which penetrate into (Duesberg, Nusbaum, Weigl), or make facets on (Cajal) the cell, without becoming continuous with the reticular apparatus.

Bensley holds that Holmgren's figures of intracellular nets in continuity with subcapsular cells stained by fuchsin do not prove continuity of structure, but more probably only a common affinity for the dye.

In a number of previous publications and in an admirable monograph ('15), summarizing the previous work, Cajal (8) has agreed that the trophospongium and Golgi apparatus in nerve cells were different representations of the same structure. The system of canals demonstrated by Holmgren, by Sjövall (41), and recently by Bensley (3), Cowdry (11, 12), et al., are produced, according to Cajal, not by a liquidation of the trophospongium, but by the fixatives used, partly or completely dissolving the reticulum. The occurrence of the canals would indicate failure of fixation of the reticulum or failure to stain it. The impregnation of the Golgi apparatus implies a fixation of the substance of

the reticulum and the deposition upon it of a metallic precipitate from the osmic acid or silver-nitrate solution.

Duesberg (16, p. 60), after a careful summary of the literature, concludes that the trophospongium, at least in nerve cells, cylindrical epithelium, and young oöcytes is identical with the Golgi apparatus in those cells. His conclusion was based on similarity of form and the changes undergone by the two structures at various ages of the animal. It should be noted here, however, that the development in the canalicular system has not been worked out as it has for the Golgi reticulum. Whether the structure appears as a set of canals or a compact network depends, in his opinion also, as in that of von Bergen, Bensley, Cowdry, et al., on the action of the reagents used.

The view taken by von Bergen (4) is somewhat different. He maintained that canals within the cytoplasm of ganglion cells are of two kinds. Type 1 resembles the Golgi apparatus in form and distribution and is really the negative picture of that structures. Canals of type 2 connect with the periphery of the cells, have no relation to the Golgi apparatus, frequently open into canals made by the microtome knife, and are in truth no more than artifacts. Canals of this type can never be stained, according to von Bergen. This theory has found few adherents. Holmgren's illustrations frequently show structures well stained which correspond to what von Bergen described as belonging to type 2.

Golgi and his school, on the other hand, refuse to consider the two structures as in any sense identical, although the former observed that some of the forms described by Holmgren are in some manner allied to the reticular apparatus. That the trophospongium and Golgi reticulum cannot be the same structure is, in the opinion of the Italian school, self-evident from the great difference in the form of the two structures. In agreement with the Spanish workers, they consider the reticular apparatus to be completely impregnated by the silver methods. The extra-cellular connections, therefore, as demonstrated by Holmgren, appear to the Italian school as additional evidence of the independence of the two structures.

Bethe (6) and Kopsch (33) likewise denied that the trophospongium is the same as the Golgi reticulum. The latter, using his entirely new but very simple osmic-acid technique, demonstrated an intracellular structure or 'Binnennetz' which corresponds very evidently with the Golgi reticulum and which, according to him, has no relation to trophospongium and does not show extracellular connections.

In considering the pros and cons of this much-debated question, it appears that the first group maintains that the Golgi apparatus and trophospongium-canal system are the same because of *similarity* of form and intracellular location. The larger part of this group deny the existence of extracellular prolongations of the canalicular structure (Cajal, Duesberg, Weigl, Bensley, Cowdry, et al.). The second group (Golgi and his pupils, Kopsch, et al.) deny that the two structures are the same because of *dissimilarity* of form. Much of the confusion arises from the variety of methods used, but also from the fact that Golgi apparatus and trophospongium have not been studied in the same cell carefully and that there has been no easily recognized alteration of one of these structures which could be evoked at will.

It is the object of this communication to draw the attention of cytologists to a method of successive as well as simultaneous demonstration of the Golgi apparatus and Holmgren trophospongium in the same cell, and to make a brief comparison of the two structures in neurone cytoplasm during retispersion subsequent to axone section.

METHOD

For this study the spinal cord and ganglia of cats were used, the Golgi apparatus being demonstrated by Cajal's formol-uranium-silver method, with certain minor modifications (39). After being stuck to the slide, the sections were counterstained by immersion in a dilute solution of Unna's polychrome-methylene-blue for from one to four hours. This was followed by passage through graded alcohols and differentiation in absolute alcohol. The sections were then mounted in Canada balsam,

and the result was a clear demonstration of Golgi apparatus and Nissl bodies, the latter appearing either green or blue, according to the intensity of silver impregnation in the cells. By this method the trophospongium canals may be demonstrated also. In some cells they will be found to stand out with great distinctness depending on the degree of differentiation. But I have found that these canals may be demonstrated more consistently and completely in the following way:

Drawings are made of the Golgi apparatus in certain selected cells (Cajal preparation). The cover-slip is then removed by immersion in xylol and the slide passed through downward graded alcohols to a 5 per cent solution of iron alum (Heidenhain), where it remains from twelve to twenty-four hours. This removes all silver from the cells as well as counterstain, and at the same time mordants the tissues for further staining. The sections are then treated as is usual in Heidenhain's iron-hematoxylin method. Great care is required to secure the proper amount of differentiation of the particular cells already drawn. At times it is necessary to mount the section in xylol and examine with high-power oil immersion, in order to be sure of the optimum differentiation of the Holmgren canals in the cell to be studied.

With the aid of the camera-lucida drawing previously made, it is easy to locate the same cell and to compare the two structures carefully. It is well to keep the nucleolus in focus during both examinations. Passing the sections up and down through the alcohol series must not be too abrupt, as otherwise a pericellular space may appear, especially in the spinal-cord sections. If a drawing was made of the canals demonstrated by the polychrome-methylene-blue in the original preparation, they will be found to coincide with those demonstrated now by iron hematoxylin in the same cell. Hematoxylin shows the canalicular structure usually in greater detail. It is evident that this staining is not influenced by the previous impregnation with silver, as there is no difference in the appearance of the peripheral as compared with the central cells. In the Cajal method the silver impregnation penetrates only a short distance below the surface of the block of tissue. No silver will be found in the central cells if the block is large.

If the section be left in the iron-alum solution for shorter periods of time, it is possible to mordant the tissue without removing the silver from the Golgi apparatus. The two structures then appear simultaneously in the same cell after staining with hematoxylin.

GOLGI APPARATUS AND HOLMGREN TROPHOSPONGIUM COMPARED DURING RETISPERSION

As mentioned above, after interruption of the axone there is a specific alteration in the distribution of the Golgi apparatus within the corresponding nerve cell, *retispersion* (Penfield, 39). As the name indicates, this alteration is a displacement of the net away from the center of the cell to the periphery. The axone base is also left clear of the apparatus (fig. 1). After this change there may or may not succeed a stage of dissolution of the Golgi apparatus, *retisolution* (fig. 2).

That the canals demonstrated by iron hematoxylin after removal of the silver as described above are really the same structure as that demonstrated by Holmgren, under the name of trophosphonguim, is apparent after comparison of his figures⁶ with those presented here (e.g., 25, p. 86, pls. I and II; 27, p. 324, pls. XXV and XXVI; 29, p. 380, fig. 2).

We may now examine the Golgi apparatus and trophosphongium in the same cell under conditions which we know produce an alteration in the normal location of the former. The question arises whether or not in retispersion the Holmgren canals will likewise assume a peripheral distribution within the cell. If the two branching structures are identical, as certain authors maintain, one would expect to find both displaced to the cell periphery.

Such, however, is not the case. In figure 1 the Golgi apparatus (in black) was drawn with a camera lucida in a spinal ganglion cell impregnated with silver and without counterstain. Reti-

⁶ Whether or not these canals have any association with the neuroglial fibers which a number of authors have described penetrating the neurone body need not be discussed here further. It is also beyond the scope of this paper to discuss the possible relationship between the trophosphongium and the so-called trophocyte. The attention will be confined to the cytoplasm of the nerve cells.

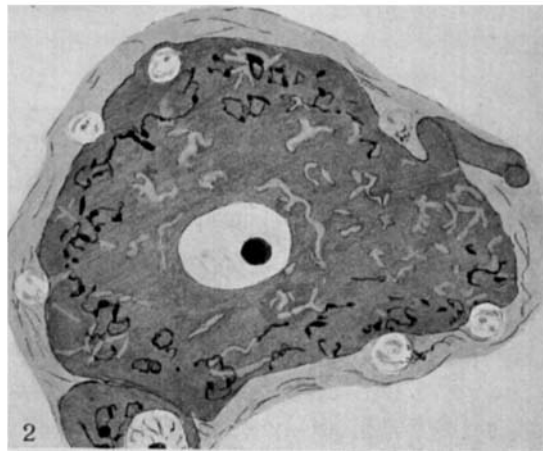
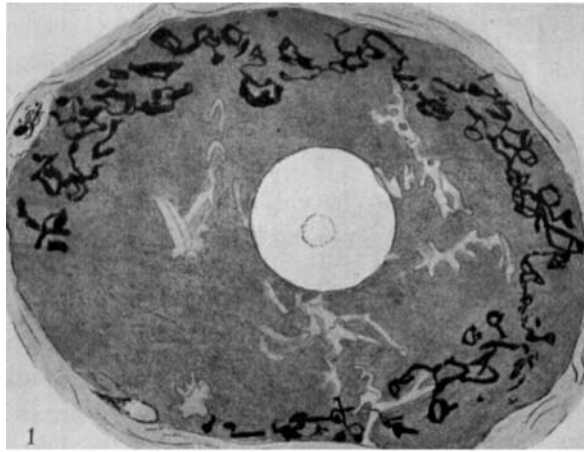


Fig. 1 Spinal ganglion cell of cat four days after section of the corresponding sciatic nerve. Retispersion evident. Successive staining method used (the Golgi apparatus and cell drawn from a Cajal silver preparation, the Holmgren canals are added after subsequent staining of the same cell with iron hematoxylin). Drawings made with Abbé camera lucida, Zeiss. Objective homog. immers. 1.5 mm., eye-piece no. 6 comp.

Fig. 2 Spinal ganglion cell of cat seven days after section of the corresponding sciatic nerve. Retispersion and retiresolution evident. Successive staining method as in figure 1.

spersion had begun throughout the ganglion, the sciatic nerve of that side having been cut four days previously. After removal of the silver, followed by staining with iron hematoxylin, the image of the same cell was superimposed upon the previous drawing by means of the camera lucida and the Holmgren canals drawn. It is apparent that the canals have not been forced to the cell periphery, as was the Golgi apparatus, under the influence of axone section, but have maintained the position occupied by them in normal cells (see also fig. 2).

If the canal system were the negative image of the Golgi apparatus, one would expect that drawings of a cell undergoing retispersion, treated in turn by the two methods described above, would show the same network, black in one drawing and unstained in the other, provided the focus of the microscope was the same in each case. On the contrary, the general distribution of the two structures appears to be quite different (compare fig. 3 with fig. 4). In figure 5, a photomicrograph, it is apparent that the Holmgren canals are not influenced by axone section, although the Golgi apparatus has undergone retispersion. The form of the Holmgren canal system differs from that of the Golgi apparatus. It is branching, often arranged concentrically. The canals are less tortuous, of greater diameter, and have a much more regular outline. The larger lacunae of the Golgi apparatus, joined by smaller tortuous threads, are never seen in the trophospongium system. The canals at times communicate with the pericellular space, but the Golgi apparatus never.

Therefore the form and distribution of the two structures in the same neurone are frequently entirely different. Also, they do not react to axone section in the same manner. The conclusion is evident that the Golgi apparatus and Holmgren trophospongium are not identical.

But what is the relationship of the two systems? This may be more easily studied in cells showing both simultaneously. Figure 6 is from a spinal ganglion of a cat seven days after section of the sciatic on that side. The Golgi apparatus, which appears to be undergoing retispersion and also retisolution, has been impregnated with silver by the Cajal method and the Holmgren canals

are simultaneously demonstrated by polychrome-methylene-blue. Nissl bodies are almost entirely absent from the cell except just about the nucleus. When the drawing was made the nucleolus was kept constantly in focus. The section of the cell drawn has therefore no greater thickness than that of the nucleolus. The

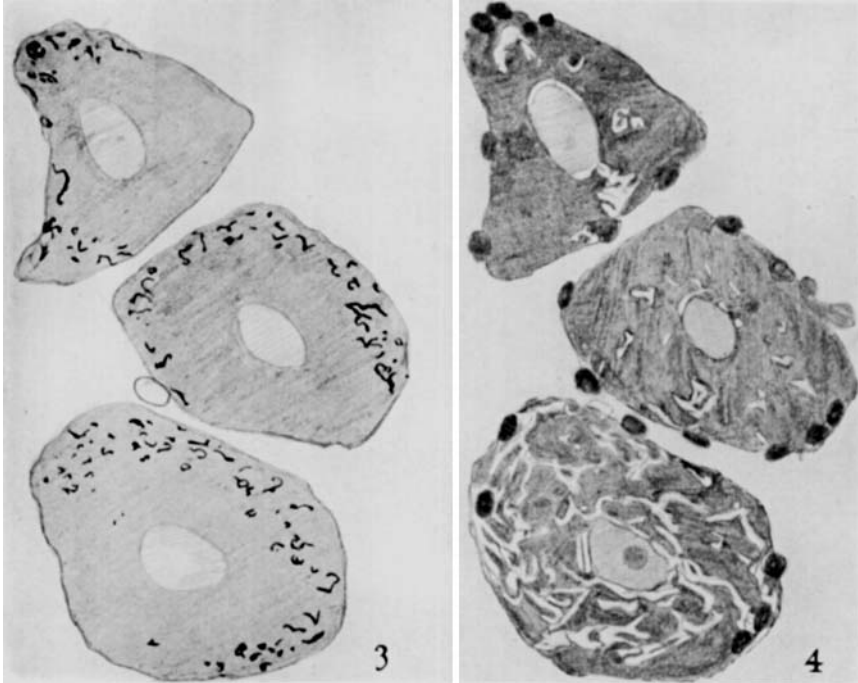
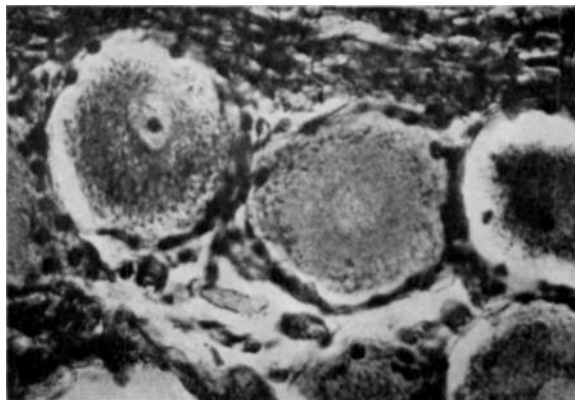


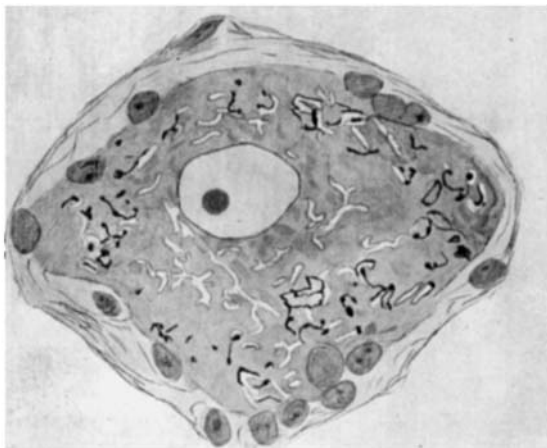
Fig. 3 Group of three cells from spinal ganglion of cat seventeen days after section of the posterior nerve roots. Retispersion and retisolution evident. Cajal silver preparation. Object. homog. immers. 1.5 mm. eye-piece no. 1.

Fig. 4 Same cells and magnification as in figure 3. The silver has been removed and followed by iron hematoxylin. In this drawing, as in figure 3 also, the nucleus at its greatest width was kept in focus while each cell was being drawn.

same contrast in the general location and form of the two structures as was noted above is evident. But in some areas a part of the Golgi apparatus is seen to lie against the wall of a Holmgren canal and to follow its contour. Subsequent staining of the same cell with iron hematoxylin showed the same canals. It is, no



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Fig. 5 Photomicrograph. Magnification of 900, Cajal silver impregnation with polychrome-methylene-blue counterstain. Cells of spinal ganglion of cat subsequent to axonection. Golgi apparatus dark. Holmgren canals unstained. In the less darkly shaded cell the Golgi apparatus is at the periphery in evident retispersion, while in the center of the cell may be seen Holmgren canals, faintly outlined in two concentric groups. The chromophil substance has largely disappeared from this cell. In the darker cell the presence of the blue-stained chromophil substance partially obscures the black Golgi apparatus at the periphery of the cell, but demonstrates the Holmgren canals both in the center and at the periphery with greater distinctness.

Fig. 6 Cell from spinal ganglion (fig. 2). Simultaneous staining method. Same magnification as figure 1.

doubt, such appearances which have led Holmgren to speak of canalization of the trophospongium. The part impregnated with silver in this section would, I presume, according to his interpretation, be that portion of the trophospongium wall not liquefied. Figure 7, a photomicrograph of normal cells, shows these two systems clearly and it is apparent that the Golgi apparatus frequently lies beside the unstained canals.

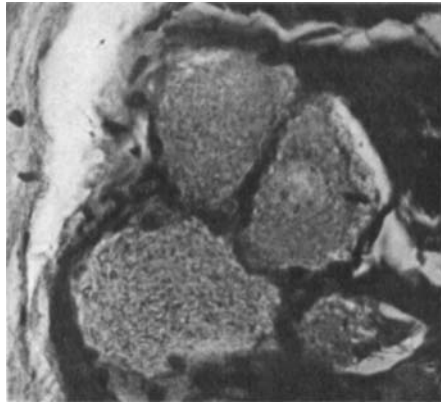


Fig. 7 Photomicrograph. Magnification of 900. Treated as in the successive staining method without, or with very little, removal of the silver from the Golgi apparatus. Cells of normal spinal ganglion of cat. In the lower large cell the relation of the Golgi apparatus, in black, to the Holmgren canals, unstained, is illustrated.

If one were to adopt the theory of von Bergen, on the other hand, that these canals are really artifacts or cracks in the cytoplasm, it would be sufficient to say that the crack tends to occur along the outline of the reticulum in certain cases. Subsequent staining of this cell with iron hematoxylin showed the same canals. In view of the excellent work of Holmgren and also the regular consistent appearance of the canals when the tissue is fixed without the slightest trace of shrinkage or distortion, it would seem extremely unlikely that these Holmgren canals are artifacts in any sense.

The above facts show that the Holmgren canals are not simply the negative picture of the Golgi apparatus. Fixation has been

in formol-uranium-nitrate solution which does not dissolve the Golgi apparatus. It is conceivable that if Carnoy's fluid, for example, were used for fixative, that the Golgi apparatus, after being dissolved or changed in some fashion, would then leave its negative image in the form of unstained canals. It does not seem likely, however, that these negatives would bear much resemblance to the canals of Holmgren. All reason for the negative-picture hypothesis disappears when the two structures are demonstrated simultaneously in the same normal cell (fig. 7) and are shown to react differently to abnormal conditions.

Occasionally, as noted above, I have seen canals and Golgi threads lying side by side. This may indicate, perhaps, a close association of function, but, inasmuch as we do not understand the function of either structure, the nature of their interrelation can only be conjectured.

SUMMARY

A short review has been made of the work which has been done, chiefly in continental laboratories, on the Golgi apparatus. It has been described as a cytoplasmic constituent of the cells of all animal organisms. It has the power of growth and has been followed through cell development and division. Its form is generally that of a network, but in certain of the lower invertebrates it appears as a thread or rod.

The trophospongium-canal system described in the cytoplasm of certain cells by Holmgren has long been urged as identical with the Golgi apparatus. Both systems find their most complex development in the cytoplasm of nerve cells.

Cajal, Duesberg, Bensley, Cowdry, Nusbaum, Weigl, and others have refused to accept the extracellular connections described by Holmgren. They have maintained or implied that the Golgi apparatus and Holmgren trophospongium were the same structure in nerve cells; that this structure appeared as a network which may be impregnated with silver or with osmic acid only after adequate fixation, and that, in case the fixative was inadequate or contained certain solvents, the structure appeared as an unstained canalicular system.

Golgi and his pupils, Kopsch, Bethe, and others, have never accepted the identity of the Golgi apparatus and Holmgren trophospongium, because of the difference in their form and also the extracellular connections of the canals described by Holmgren. On the contrary, those just mentioned above, who take a similar view with Cajal, find the form of the two structures similar and deny extracellular connections. Their general location within the normal cell is admitted to be the same.

The confusion seems to be due partly to the variety of methods of study, but also to the fact that comparison has been made in cells of the same type, but not the same cell, and there has been no means of inducing a specific change in either structure. It has been shown above that after the Golgi apparatus, as demonstrated by the Cajal method, has been studied in a certain cell, the silver may be removed and the trophospongium demonstrated in the same cell by iron hematoxylin; also that the two structures may be demonstrated simultaneously, as illustrated in the drawings and photomicrographs.

These methods provide a simple means of direct study and comparison. In the successive staining method no canals are found which correspond to the Golgi apparatus previously demonstrated. During retispersion (a specific alteration of the Golgi apparatus described in a previous publication) (39), the Golgi net is displaced to the cell periphery, while the intracellular location of the Holmgren canals is not altered.

CONCLUSIONS

That the Golgi apparatus is a universal cytoplasmic constituent has been established by a large number of investigators. In neurones the apparatus reacts to axone section in a specific manner. There is no similar response on the part of Holmgren's trophospongium. The two structures may be demonstrated independently in the cytoplasm of the same neurone, either successively or simultaneously.

The conclusion is evident. There cannot be a positive and a negative picture of the same structure, as has been widely maintained. They are separate structures demonstrable simultaneously.

Occasionally there appears a close anatomical relationship between parts of the Golgi apparatus and Holmgren's trophospongium which may indicate an intimate association of function. The methods described above provide a simple means of comparative study.

Further work upon the Holmgren canals is required to clearly demonstrate the developmental stages and, in fact, their existence antemortem.

In conclusion it is a pleasure to thank Professor Sherrington for his interest and suggestions. To Prof. Arthur Thomson I am indebted for helpful criticism, and to Mr. Chesterman, of the Anatomical Department, for the photomicrographs.

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