The Structure of the Golgi Apparatus in the Tissues of Amphibia.

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With Plates 17–21 and 3 Text-figures.

Introduction.

Studies of the Golgi apparatus in the cells of Vertebrata greatly outnumber those dealing with that aspect of the cytology of the tissues of Invertebrata. Yet, with regard to actual details of structure of the Golgi apparatus our information appears to be more accurate in the latter group. It seems from the researches of Hirschler, Monné, Nassonova, and others that the osmiophilic substance in the cells of Invertebrata is almost invariably lamellar in form. The lamellae may constitute the boundaries of closed vesicles, or may be more or less irregularly folded structures, that do not enclose a definite region of non-osmiophilic material. Exact descriptions of the form of the osmiophilic substance in vertebrate tissues are exceptional. Most recent workers have been interested primarily in the orientation of the apparatus with respect to some cellular function, and have either ignored the question of structure or have been satisfied to speak of it as a network, a single black mass, or a number of separate black bodies. The small number of recent studies that have attempted exact description of the structure of the osmiophilic and argentophilic Golgi apparatus in vertebrate cells has indicated that in these, as in Invertebrata, the structure is lamelliform. Examination of figures in a number of other works strongly suggests that the authors demonstrated a lamelliform Golgi apparatus, though this fact is not emphasized in the text. It is especially important to determine whether this sort of morphology is generally characteristic of the Golgi apparatus of all animal cells, because the writings of Parat and
others have recently raised the question of whether the substance called the Golgi apparatus is strictly comparable in all cells. It seems to the present writer that this scepticism has been fostered especially by the vagueness of our information concerning the structure of the Golgi apparatus in a wide variety of tissue cells. The present contribution is the report of an extensive survey of the Golgi apparatus in tissue cells of one class of Vertebrata to determine whether its structure in all these cells conforms to one general type, that may be compared with that in the cells of Invertebrata.

Material and Methods.

The study has been made entirely from the tissues of Amphibia, which long have been recognized as the best vertebrate cytological material. The Golgi apparatus is most easily demonstrated in a variety of tissues by dropping intact live larvae into the fixing reagent. A large quantity of the fluid is always swallowed; the animal is well fixed throughout; and subsequent osmication blackens the Golgi apparatus in nearly every cell. Preparations have been made of larvae of Rana pipiens, Triturus torosus, and various species of Amblystoma; with such uniform results that most of the tissues could have been described from any one of these forms. The last genus has been used in most cases, because it was more frequently available and because the cells are slightly larger than in the frog or the newt. Larvae of Amblystoma, from the time they begin feeding until they have grown to 25 mm., respond excellently to technical treatment as entire animals. An extensive check has been made on the tissues of adult urodele amphibians. One or more organs of each of the following adults was prepared: Amblystoma opacum, Amblystoma punctatum, Triturus viridescens, Triturus torosus, Triturus pyrrhogaster, Salamandra salamandra, Pseudotriton ruber, Siren lacertina, Cryptobranchus alleghaniensis, Necturus maculosus, and Amphiuma tridactylum. Drawings are presented from only part of this material. It is sufficient to say
of the remainder that it shows no exceptions to the facts described below.

The methods for demonstration of the Golgi apparatus have been described and critically compared in a series of articles by Bowen (1928 a, b, c). The present investigation has employed no new methods, but has been made entirely from material prepared by four of the most widely used procedures; silver impregnation, according to Cajal or Da Fano, and osmication according to Weigl (Mann-Kopsch) or Kolatchev. The procedures have been carried through almost exactly as described by Bowen (loc. cit.). Osmication in the Kolatchev method has been in a 2-per-cent. solution at 35° C. For success with the silver methods the writer is indebted to Doctor José Nonidez for suggesting the use of Merck Reagent Grade Chemicals for all reagents. Glass-distilled water was used throughout the Cajal and Da Fano procedures, except in the final washing. The author wholly agrees with Bowen that the silver methods are definitely outmoded. They are exceedingly capricious, not highly specific, and the quality of general fixation rarely approaches that by the Kolatchev method. They have been employed in this study merely as a control, for greater certainty in identification of the Golgi apparatus; and, as a matter of fact, it now seems best to regard silver methods as useful only in that regard. Certainly it is extremely hazardous to report upon the morphology of the Golgi apparatus from material studied only by silver impregnation; for these pictures are rarely, if ever, free from artifact, either due to defective fixation or to erratic deposition of silver. The Weigl method succeeds in many instances with vertebrate tissues, but the general cell fixation is distinctly inferior to that by Champy’s fluid in the Kolatchev method, especially with respect to the preservation of chondriosomes. This last brings us to one of the chief reasons for use of Champy’s fluid wherever possible, namely, the fact that any cytoplasmic study is incomplete unless it involves parallel demonstration of both chondriosomes and Golgi apparatus—and surely the most obvious method of doing this is to fix material in Champy’s fluid and to subsequently divide it into two parts, one to be carried through the Kolatchev technique
and the other through the Kull procedure. This is the author's invariable habit, which has been followed in all the technical work incidental to this study. Accordingly, there is a comparable set of all this material, in which the chondriosomes are demonstrated. It is planned to report in detail upon this in a later work. It seems pertinent at this time, however, to state that these Champy-Kull preparations show very clearly that there is no possibility that the structures described below as the Golgi apparatus are any part of the chondriome.

For the reasons just set forth, most of the study has been made from Kolatchev material. It should be added that one must exercise a certain amount of selection from the osmicated material. Tissues that have been osmicated for 6—7 days at 35° C. are nearly always correctly prepared. Four days or less gives insufficient blackening; 9 days or over results in over-osmication. These times are not arbitrary; and there is another criterion which is the final one in determining whether one is studying tissues that are correctly blackened; namely when the Golgi apparatus is blackened to the right degree for analysis of structure one sees both black and grey areas, never a uniform black. The reason for this last is inherent in the microscopy of the Golgi apparatus as described below.

Observations.

In order to appreciate the significance of the figures drawn from osmic and silver preparations, it is important to understand the microscopic appearance given by the delicate structures impregnated. On these slides a structure seems uniformly black only when one is looking through a considerable thickness—over one-half micron. Thus only a massive body of that thickness offers a considerable expanse of uniform black. In the slides selected for this study such an appearance is never seen. The Golgi substance, as actually observed, presents two aspects, black regions and adjacent grey areas. The grey areas occupy only a shallow focal plane, the black regions may be followed through considerable focal depth; the latter in nearly every case
are narrow lines; the former are broad areas. Obviously such an appearance can only be presented by various aspects of a lamellar structure or platework, viewed by transmitted light. The black lines represent the lamella as seen on edge. Focusing shows that they extend in the vertical plane, and such vertical parts of the platework can frequently be found to become continuous at some level with a broad grey area of shallow focal depth, which is the lamella extended in the horizontal plane. Such an optical effect can never result from viewing a network of filamentous elements. In such a case the black regions must always be points (the filaments seen on end), while the filaments in the horizontal plane will be grey lines. The latter microscopical appearance is very familiar to cytologists who study lightly stained chromosomes or chondriosomes. Indeed, next to the small spherule, the filament, generally cylindrical, seems to be the most common geometrical form assumed by structural elements of the cell—a fact which makes their microscopy familiar to all. Lamellae or plateworks, on the other hand, seem to be very rare, and accordingly the microscopic appearance of such a structure is strange to most observers. There is an obvious corollary to the above conclusions regarding the microscopic appearances of lamellar structures, which it is important to keep in mind in interpreting the figures of this paper, or of others: namely, if one encounters a Golgi apparatus that gives a predominant appearance of black lines and shows no black points this can only be a platework or lamellar structure. This follows even though because of the light osmication or their thinness one cannot observe the grey areas that are the lamellae in the horizontal plane.

Briefly to summarize the microscopy of the Golgi apparatus: where one sees thin black lines of considerable focal depth and adjacent wide grey areas of shallow focal depth he is observing a lamella or platework; where there are grey lines of shallow focal depth and black points of considerable focal depth, one has a filamentous network. Text-figs. 1 and 2 diagrammatically illustrate the contrast between these two types of structure in correctly impregnated material. Text-fig. 1 is a hypothetical
structure, never present in a columnar cell (though seen in cartilage and notochordal tissue). Text-fig. 2 is the appearance encountered in nearly every type of cell of amphibian tissues.\textsuperscript{1}

\textbf{Text-fig. 1.}

A series of diagrams to show the expected microscopic appearance of an epithelial cell, with Golgi apparatus in the form of a network of threads or filaments.\textsuperscript{1}

A. Entire cell.\textsuperscript{2} B. Optical section of upper third of cell (i.e. section nearest observer).\textsuperscript{2} C. Optical section of middle third of cell.\textsuperscript{2} D. Optical section of lower third of cell (i.e. section farthest from observer).\textsuperscript{2} E. Cross-section of A, in region marked X. Whatever aspect of a filamentous Golgi apparatus is studied, one sees but two possible images: either black dots (or points), which have considerable focal depth; or grey lines of shallow focal depth. The former are parts of the filamentous network that run vertically; the latter are horizontally oriented filaments. No epithelial cell of Amphibia contains this filamentous sort of Golgi apparatus. It is characteristic only of cells of the specialized connective tissues, cartilage, bone, notochord, and odontoblasts.

\textsuperscript{1} It should now be clear why it is important to select correctly prepared material. If the tissue is under-impregnated one sees only the black lines, since in surface view the plates are invisible because of insufficient blackening. Unless studied very critically, such a slide gives the impression that the Golgi apparatus is composed of filamentous elements that constitute a network. Osmication for four days or less (Kolatchev) frequently gives this appearance. If, on the other hand, the preparation is over-impregnated the surface aspects of the plates will appear solidly black and indistinguishable from the edgewise views—and the observer receives a very definite impression that the Golgi apparatus is a massive, lumpish body. The author has many Kolatchev preparations that give just this appearance; they have resulted from allowing material to remain for nine days or more in the osmic acid.
Observations.

Epithelial Cells.

In cuboidal and columnar epithelial cells the Golgi apparatus is easily resolved into a vertical collar, or sleeve-like structure, most frequently encircling the distal end of the nucleus and extending for some distance into the cytoplasm distal to the nucleus.

Fig. 1, Pl. 17, shows one-half of a cell of the epithelial lining of the pancreatic duct. The grey band across the nucleus is the surface view of the collar. It is continuous at either side of the nucleus with a heavy black line, which is, of course, an edgewise view of the collar as it passes around the nucleus in the vertical plane. Fig. 2, Pl. 17, shows a similar structure from the lining of the gall-bladder. In mucous cells from the lower end of the oesophagus (fig. 3, Pl. 17) is the same simple type of collar, here located between nucleus and lumen of the oesophagus.

1 The term 'distal' is here used to mean towards the free surface of the cell, and 'proximal' towards its base.
section transverse to the long axis of the cell (fig. 4, Pl. 17) shows that the structure is a complete ring, which encloses droplets of secretion within its circumference. In mucous cells of the intestine of the Amblystoma larva the collar is similar but is somewhat less regular. Four focal planes of one such cell are shown in fig. 5 a–d, Pl. 17; while successive cross-sections of the same cell are seen in figs. 6 a–c, Pl. 17. In this and in the very elongate goblet-cell of the intestine of Necturus (fig. 7, Pl. 17) there appear to be perforations in the plate-like Golgi apparatus, a condition encountered in some other cells as well. In the non-mucous cells of stomach and small intestine the irregular collar-like Golgi apparatus encircles the upper part of the nucleus and extends into the distal cytoplasm (figs. 8 and 9, Pl. 17). A similar structure occurs in the columnar cell of the gastric mucosa of Amphiuma (figs. 10–13, Pl. 17). The latter two figures are from a Da Fano silver preparation, showing that essentially similar structures are demonstrated by osmium and silver techniques. The effect of alteration of the cell shape on the form of the Golgi apparatus has been studied in the superficial gastric epithelium of the larval Amblystoma (length, 17 mm.). In the empty stomach these cells appear like those illustrated in figs. 10–13, Pl. 17. When the stomach is distended by a solid mass of food (2 hours after swallowing a 3-mm. section of Tubifex) the epithelial cells are greatly compressed, and even the nucleus is flattened horizontally. The Golgi apparatus also suffers distortion, parts of the platework being actually folded back upon themselves, see right side of fig. 14, Pl. 17. Specimens fixed after gastric digestion is completed (15 hours after feeding) and food has all passed into the duodenum, again show the high columnar epithelium with the

1 The darker reticulation seen in fig. 12, Pl. 17, is often observed in silver preparations. It is projected on a uniformly grey background, the plate-work in surface view, and these two together seem the obvious equivalent of the osmiophilic substance revealed by Kolatchev preparations. The delicate network described by D'Agata, 1910, in the gastric epithelium of Triton was only the reticulation. The older observations of Golgi, 1909, on the frog's stomach agree exactly with the results of the present study, except that I have not observed such great variation in position of the apparatus with respect to the nucleus.
normal type of Golgi apparatus. Similar phenomena have been briefly studied in the epithelium of the bladder.

In most parts of the uriniferous tubule the Golgi apparatus is a collar around the distal part of the nucleus. Fig. 15 a–d, Pl. 17, are successive focal planes through one type of cell of the proximal segment of the mesonephric tubule of Necturus, showing the continuous, though very irregular, collar. Certain cells of the distal segment of the tubule have the very rare vertebrate condition of a Golgi apparatus in the form of small separate platelets around the nucleus (figs. 16–18, Pl. 17). The collar in the ciliated cell of the neck of the mesonephric tubule projects slightly from its circumnuclear position into the distal end of the cell (fig. 19, Pl. 17). Ciliated cells of the tracheal epithelium also have a Golgi apparatus much like this (fig. 20, Pl. 17).

The structure of the Golgi apparatus has not been studied in the skin, but in other stratified squamous epithelia, and in transitional epithelium it is not greatly different from that in the simple epithelia described above. In mucous cells of the upper part of the oesophagus the apparatus is an extensively perforated, collar-like structure, within which are droplets of secretion (figs. 21 and 22, Pl. 18). In the superficial non-mucous cells of the pharyngeal epithelium the apparatus is a ring encircling and extending distally to the nucleus (fig. 23, Pl. 18) (circular grey bodies are fat droplets); and much the same structure is found in cells of the second, deeper layer of the two-layered epithelium (fig. 24, Pl. 18). When compared with these two cells it appears that ameloblasts have the Golgi apparatus considerably hypertrophied (fig. 25, Pl. 18). In the lining of the gill chamber the two-layered epithelium is extremely thin. In such a flattened cell the Golgi apparatus takes a position parallel to the plane of surface of the epithelium and surrounding the nucleus (figs. 26 and 27, Pl. 18). This is a significant type of modification since, in effect, we here have flattened the cell of fig. 24, Pl. 18, until the collar-like Golgi apparatus, somewhat difficult exactly to resolve in fig. 24, Pl. 18, is spread out in a single plane, making its lamellar structure evident at a glance. In the epithelium lining the bladder, the Golgi apparatus is an
extremely irregular, collar-like structure. Parts of the platework are so narrow as to approximate the thickness of the plate. Thus parts of the Golgi apparatus are filamentous, such parts appearing like black dots, when they are seen on end (figs. 28, 29, and 30, Pl. 18).

If the simple squamous epithelia are not subjected to considerable pressure the Golgi apparatus, lamellar in structure, assumes a position between the nucleus and the adjacent cavity. This is shown in the endothelial cells of a small larval vessel in fig. 32, Pl. 18, in which the Golgi apparatus is located close to the centre of that nuclear surface adjacent to the lumen of the vessel. It has been shown that this is also the location of the centrioles (Pollister, 1933), and hence it appears that in these endothelial cells the Golgi apparatus is in close topographical relationship to the centrioles. In this respect they differ from the other epithelia so far described; and they are more strictly comparable with the cells like leucocytes, described below. In many peritoneal epithelial cells the Golgi apparatus is likewise between nucleus and lumen (fig. 33, Pl. 18). In more flattened epithelium, however, it appears as if the apparatus had slipped into the zone of less pressure at the side of the nucleus (fig. 34, Pl. 18), somewhat as one may interpret the situation in the cells lining the gill chamber.

Compared with the simple conditions described above, the structure of the Golgi apparatus is often extremely complicated in some types of glandular cells. This has already been described in the liver (Makarov, 1931, and Pollister, 1932), where the extensive development of intercellular bile canaliculi is accompanied by a distribution of the Golgi apparatus along this complicated surface of secretion. In this instance the Golgi apparatus is probably not a continuous band, but is in the form of a number of separate, more or less complicated platelets (fig. 35, Pl. 18). The same structure is observed in well-fixed Da Fano preparations (fig. 36, Pl. 18).

Another case where the Golgi apparatus is oriented, not with reference to the nucleus, but along the zone of discharge of secretion from the cell is seen in the zymogenetic cells of the cardiac glands of the stomach. These large cells are roughly
pyramidal, the tips of all the cells being in contact with the dilated terminal portion of the common duct, which is continuous with the neck of the gland. On two opposite sides of each cell is a straight canaliculus, which extends from the tip of the cell to near its proximal (basal) end. This canaliculus is shared with one adjacent cell. It is difficult to demonstrate the Golgi apparatus in these cells when they are full of secretion, a fact known since 1909, when Golgi failed to find it in the frog’s stomach, and concluded that this was one type of cell that lacked the apparatus. It is beautifully demonstrated, however, in an exhausted cell like that drawn in fig. 37 a–c, Pl. 19 (from a 14-mm. larva of Triturus torosus which had been feeding heavily shortly before it was fixed). The large grey circular masses in the basal part of the cell (left) are fat droplets, always present in many cells of a well-fed amphibian. The Golgi apparatus consists of a number of extended, more or less irregular plates, much like that already noted in the hepatic epithelium. Careful study of these three sections of one cell shows the orientation of the Golgi plates with reference to the secretory zone, which, marked by the presence of small secretory granules, is seen to extend along two sides, where the canaliculi are located, and around the tip of the cell (fig. 37 a, Pl. 19). In a and b the band of Golgi platelets is adjacent only to the distal ends of the canaliculi; in c it can be seen that the band is continuous with a group of Golgi platelets that extends out along the canaliculi and around the tip of the cell. At no point is the Golgi apparatus between nucleus and lumen. It is always lateral to the nucleus; and careful study of the course of the capillary at the base of the cell has made it clear that the orientation of the Golgi apparatus in this cell is most accurately expressed by stating that it is located along a course between the blood capillary and the secretory surface. With this in mind, it seems pertinent to remark that this is one way of describing the orientation of the Golgi apparatus in all the epithelia described above, except the simple squamous.

The pancreas cell has recently appeared as a prime favourite for studying the relation of the Golgi apparatus to secretion. There can be no doubt, however, that it is one of the most
difficult cells in which to demonstrate this cell component. The problem is of the same nature as that encountered with the zymogenic cell of the gastric gland; when the cell is full of secretory granules, which is usually the case, the Golgi apparatus is not definitely demonstrable. In the writer's slide collection is more material of the pancreas than of any other organ. In much of this there is perfect preservation and impregnation of the Golgi apparatus in endothelial cells, duct cells, leucocytes, fibroblasts—in short, in every type of cell in the organ except the exocrine glandular cells. Fixation appears good, with the minimum of shrinkage or distortion, but within the cytoplasm of the glandular cell there is no trace of structure that could be identified as the Golgi apparatus. Aside from an occasional obvious artifact, all one can see is a diffuse greyness in the region of the so-called secretogenous zone. It is very different, however, in the few cases where an exhausted pancreas has been preserved. Then one duplicates the pictures that have been so beautifully presented by Nassonov, of an extensive plate-work, in close association with which are often vesicles, which have been interpreted as a stage in the synthesis of the secretory product (figs. 38 and 39, Pl. 19). As a whole the apparatus forms a nearly complete collar.

Blood Tissue.

The structure of the Golgi apparatus of erythrocytes has not been determined. From the observations of Cajal, 1915, on birds, and of Cowdry, 1921, on mammals, it seems unlikely that it is greatly different from that of the leucocyte. In the latter it is very easily demonstrated; and in any cell the black lines and grey areas clearly indicate that here also the structure is essentially a lamellar one (figs. 40, 41, 42, and 43, Pl. 19) (all osmic preparations), and fig. 44, Pl. 19, a Da Fano preparation. The Golgi apparatus is located in the approximate centre of the main mass of cytoplasm. In fig. 42, Pl. 19, the chondriosomes are likewise shown. Fig. 45, Pl. 19, is a non-granular leucocyte that was compressed between aorta and notochord so that the cell was somewhat flattened. In comparison with the spherical cells of figs. 40–4, Pl. 19, it appears that the Golgi apparatus is
very little altered in shape, obviously much less so than the nucleus. In figs. 40–5, Pl. 19, it is difficult to determine the form of the apparatus as a whole. In fig. 41, Pl. 19, there is a central space in the apparatus, a feature which is characteristic of many of the cells, and is a clue to the interpretation of the scheme of structure of the Golgi apparatus in all leucocytes. Selection of cells in which the structure can be definitely determined shows that as a whole it is in the form of a disk with a central perforation—perhaps more clearly comparable with the Golgi apparatus of epithelial cells if one describes it as a horizontal collar. This is shown with great clarity in fig. 46, Pl. 19, a rare condition where the collar is undistorted and extended in one plane. Comparatively common are cells like those drawn in figs. 47 and 48, Pl. 19, in which the collar is more or less folded. Fig. 49, Pl. 19, shows a collar like that of fig. 48, Pl. 19, as seen from the edge. Study of figs. 47–9, Pl. 19, which have been drawn with the greatest possible regard for accuracy, will show that the individual bends of the plate-work, or collar, are roughly radial to the central perforation, and are always a right-angle or less. This latter characteristic of the distortion of the lamellar structure, so far as I can determine, is an almost invariable rule not only in the case of the leucocyte but for the Golgi apparatus of all types of amphibian cells. The single notable exception is the distortion of the Golgi apparatus under considerable pressure, as in the flattened gastric epithelial cell of fig. 14, Pl. 17. Once this collar-like structure of the Golgi apparatus has been clearly determined for certain selected leucocytes, as above, it becomes clear enough that the commoner appearances illustrated in figs. 40–5, Pl. 19, are produced by a slightly greater degree of distortion of the collar. The author was able to convince himself, by careful focusing, that the Golgi apparatus of every leucocyte studied was a distorted collar, fundamentally like that shown by analysis of figs. 46–9, Pl. 19. Only occasionally does one find the collar incomplete at one point as in fig. 47, Pl. 19.

The orientation of the Golgi apparatus with reference to the nucleus is to a certain extent variable. The nucleus is roughly the shape of a thick biscuit, frequently somewhat concavo-
convex on the broader surfaces. In many cases there is a perforation through the approximate centre of the biscuit, in which type of cell the disk-like Golgi apparatus is oriented with its horizontal dimension adjacent to and parallel with the concave surface of the nucleus, and with its central perforation nearly opposite the central hole in the nucleus. This orientation with reference to the shape of the nucleus is approximated in all the spherical leucocytes to the following extent: the collar may be somewhat oblique to the adjacent concave surface of the nucleus, but the obliquity rarely exceeds a forty-five degree angle; and in no case observed is it a right-angle. The apparent discrepancy between this statement and some of the figures is due to the fact that no drawing has been made that includes a whole cell.

This cell, with a Golgi apparatus localized in the approximate centre of the main mass of cytoplasm, has long been familiar. Its prototype is the epithelium of Descemet's membrane, in which it has been demonstrated that the Golgi apparatus surrounds the centrioles (Ballowitz, 1900). It has always been assumed that the Golgi apparatus of leucocytes was in close topographical relationship with the centrioles, but no previous demonstration of the exact relationship between the two is known to the present author. Fig. 51, Pl. 19, is a leucocyte from a preparation fixed in Helly's fluid and stained with iron haematoxylin. By this method an aster is demonstrated to occupy a large region of the centre of the main cytoplasmic mass. At the centre of this aster is a more heavily stained mass, to which the term centrosome has been applied (Pollister, 1933 a). On the surface of the centrosome are located the two minute centrioles. In lightly impregnated large leucocytes of Necturus the centrosome may be stained with thionin. It can then be seen that the centrosome, and hence the centrioles on its surface, lies in the middle of the central perforation of the collar-like Golgi apparatus (fig. 50, Pl. 19.)

The structure and orientation of the Golgi apparatus of the leucocyte have been described at length because of the fact that

1 This method of staining the centrosome was discovered by Mr. Robert L. Bowman, one of my students.
this cell, as will subsequently be shown, may be very plausibly regarded as a type of a variety of cells as great as the epithelia described above. It is profitable at this point to compare briefly the leucocyte and epithelial cell types. Fundamentally the structure of the Golgi apparatus itself is much alike in the two. In each it is a lamellar body, in most instances constituting a single mass. In the epithelial cell the mass is a cylindrical structure, a vertical collar; in the leucocyte it is a perforated disk, a horizontal collar. These are two morphological features that appear much alike. When the analogy between the two cell types is extended to include the organization of the entire cell, however, it becomes apparent that the two cell types are fundamentally very different. The vertical collar of the epithelial cell usually partially surrounds the nucleus; and in every case it is far removed from the centrioles which have been shown to be in the distal end of the cell (Pollister, 1933b). By contrast, the Golgi apparatus of the leucocyte is in the centre of the main mass of cytoplasm, not closely adjacent to the nucleus; and the centrioles are in very close topographical relationship with it. Text-fig. 3 summarizes this comparison in diagrammatic form.

Connective Tissues.

In mesenchyme cells (fig. 52, Pl. 19) and fibroblasts the lamellar Golgi apparatus is localized like that of the leucocyte. It is very likely here also in the form of a collar, since in flattened cells (fig. 54, Pl. 20), or those with a considerable volume of endoplasmic cytoplasm (fig. 53, Pl. 19), the Golgi apparatus is of this character. In the more highly differentiated connective tissue cells the Golgi apparatus becomes considerably altered, but it is clear that this is a specialized condition derived from the less complicated Golgi apparatus that is characteristic of mesenchyme cells and fibroblasts. This series is fully illustrated in the case of hyaline cartilage. A cell of a blastemal rudiment of a trunk vertebra of a 19-mm. Amblystoma larva is shown in fig. 55, Pl. 19. The Golgi apparatus is localized near the cell centre and is not greatly different from that of the fibroblast. The area of the plate-work is greatly increased in lateral extent
in a more highly differentiated cartilage cell, e.g. in the somewhat flattened outer cells of a branchial arch rudiment (figs. 56 and 57, Pl. 19). The swelling of the cell that accompanies the

**Text-fig. 3.**

The two types of topographical relationship between Golgi apparatus and centrioles. **A. Epithelial Cell Type,** Golgi apparatus (G.A.) in the form of an irregular vertical collar, that usually surrounds the distal end of the nucleus (N.) and extends into the adjacent cytoplasm. Centrioles (C.) in the extreme distal end of the cell, some distance from the Golgi apparatus. With this type are grouped all epithelial cells except simple squamous. **B. Leucocyte Type,** Golgi apparatus a somewhat bent horizontal collar located near the centre of the main mass of cytoplasm. Centrioles within the space in the centre of the collar. With this type are grouped cells of blood, connective, muscle, nerve, and germinal tissues.

development of the matrix seems to involve especially the region of the Golgi apparatus, which thus becomes extended throughout a considerable volume of the cell. This extension is not accompanied by a compensatory growth in area of the Golgi plate-work. Instead, the width of the individual regions of the
plate-work appears much narrower. Indeed, this reduction proceeds to such an extent that the width of the plate equals its thickness, the result being that the Golgi apparatus appears as a filamentous network rather than the typical plate-work. One section of a typical cartilage cell in which this type of Golgi apparatus has been developed is shown in fig. 58, Pl. 20, from the centre of a branchial arch rudiment. In this instance some plate-like portions of the Golgi apparatus still persist, but in many cartilage cells the entire apparatus is an extensive network, all the elements of which are uniformly filamentous, as in the cell of notochordal tissue (fig. 59, Pl. 20). The Golgi apparatus is likewise of this character in bone cells, and in all odontoblasts except the terminal one, where it appears to have a complex lamellar structure.

Cajal, 1914, has reported a similar series of changes in development of hyaline cartilage of the chick. Pensa, 1913, has shown that the apparatus surrounds the centrioles. The Golgi apparatus of the single-layered perichondrium of amphibian cartilage is shown in two views in figs. 60 and 61, Pl. 20. It is a small plate-work opposite the centre of one flat side of the nucleus.

Germinal Tissue.

In somewhat flattened cells of the gonadal ridge of Amblystoma the Golgi apparatus is clearly a complicated plate-work, of considerable extent (fig. 62, Pl. 20). In rounded cells of the same type the apparatus is localized in the centre of the main cytoplasmic mass, and is unquestionably lamellar (fig. 63, Pl. 20); but its exact form as a whole is very difficult to resolve. It seems most likely that this appearance is given by a structure like that in fig. 62, Pl. 20, which is here, however, spread out upon the surface of a sphere, the idiosomic mass. Essentially the same structure has been noted in the early spermatocytes of Necturus. This is a condition that is thoroughly familiar to students of spermatogenesis and oogenesis; where the Golgi apparatus is on the periphery of a spherical body, the idiozome, which is known to contain the centrioles. The arrangement is clearly but one variation of the Golgi apparatus-centriole relationship characteristic of leucocytes and kindred cells.
Muscle Tissue.

Smooth muscle is developed by modification of mesenchyme cells. Consequently one would expect the Golgi apparatus of these fibres to show resemblances to that of the parent cell; and such is the case. In the fibre of the intestinal wall of a 17-mm. Amblystoma larva (fig. 64, Pl. 20), the Golgi apparatus is the familiar collar-like body, located midway along the length of the nucleus. The centrioles are known to have this same relation to the nucleus in this cell (Pollister, 1932 and 1933); hence, it is quite possible that they are enclosed within the Golgi collar, as in the leucocyte. In adult fibres the ring or collar persists in this same region, but is extended to and beyond either end of the nucleus as a narrow ribbon (figs. 65 and 66, Pl. 20) from the uncontracted circular muscle of the intestine of Amblystoma. Study of cross-sections of the fibres, like fig. 67, Pl. 20, shows that in the inner layer of circular fibres the Golgi apparatus is invariably on the side of the fibre adjacent to the submucosa. In the longitudinal layer there appears to be no constant relationship to adjacent parts of the organ. Figs. 65-7, Pl. 20, are clearly uncontracted fibres. The adjacent longitudinal muscle-layer of this specimen was fixed in the contracted state, as indicated by the irregular nuclear contours and the increased girth of both nucleus and fibre. In these contracted fibres the ribbon-like Golgi apparatus is considerably folded in contrast to the uncontracted state (fig. 68, Pl. 20).

The Golgi apparatus of cardiac muscle is extremely complicated and very extensive. In my preparations nearly all parts of the myocardium appear, from analogy with conditions in smooth muscle, to have been fixed in the contracted state. The region occupied by the Golgi apparatus is similar to that in smooth muscle; it is along one side of the nucleus and prolonged into the cones of sarcoplasm at either end of the nucleus (figs. 69-72, Pl. 21). In cardiac muscle tissue, however, the apparatus is in the form of several parallel, more or less twisted long ribbons, that anastomose with one another. This situation makes it doubtful whether one can reasonably compare a
collar-like section of the apparatus, like that in fig. 69, Pl. 21, with the collar of smooth muscle. However, due to the nature of the myocardium of Necturus it is possible to determine quite definitely that the Golgi apparatus of the cardiac muscle is to be regarded as a modification of that of the leucocyte. There is a peripheral layer of undifferentiated tissue containing no myofibrillae and directly continuous with the more central tissue that contains the typical cross-striated myofibrillae. Adjacent to each nucleus of this non-myofibrillar outer myocardium is a collar-like Golgi apparatus, extremely like that already noted in many types of cells (fig. 73, Pl. 21). Such structures are also common in parts of the ventricular myocardium of the Amblystoma larva.

These structures of amphibian cardiac muscle are strikingly similar to those noted by MacDougald, 1936, in the chick myocardium. MacDougald studied the behaviour of the Golgi apparatus in histogenesis and concluded that in the earliest stage of differentiation it was in the form of a ring or knot. In later stages he traced the elongation of this ring towards and beyond either end of the nucleus. One intermediate stage was almost exactly like the adult condition in the fibre of Necturus. In the adult fibre of the bird, however, the differentiation proceeds to one more stage, when the apparatus disappears entirely from its original position alongside the nucleus and there remain only the parts at either end of the nucleus, a condition which agrees with that reported by Luna (1911) in the cardiac muscle of Cavia. In the turtle heart Eastlick (1937) has reported what may be an intermediate condition, where there are still some remnants beside the nucleus, in addition to the portions at either end. It is also of some interest that Rojas and Carrea, 1936, report that in the presumably less specialized tissue of the atrioventricular bundle of the adult beef heart the Golgi apparatus remains of the localized type, similar to that described by MacDougald as characteristic of young, relatively unspecialized cardiac muscle.

Tissues of the Nervous System.

Those tissues of the nervous system that are epithelial closely
resemble the other cells of that type; they contain a Golgi apparatus in the approximate form of a vertical collar around the distal end of the nucleus (figs. 74-6, Pl. 21). In the thinner epithelia the plate-work is between nucleus and lumen (fig. 77, Pl. 21). In the thin ciliated epithelium of the roof of the fourth ventricle the cell processes are restricted to one small region of the free surface of the cell. The Golgi apparatus consists of several separate platelets that are found only in the part of the cell immediately below these cilia (fig. 78, Pl. 21). The Golgi apparatus of the sheath cell is a complicated lamellar structure the exact form of which has not been determined (fig. 79, Pl. 21).

In the nerve-cell of a small mesenteric ganglion (fig. 80, Pl. 21), the Golgi apparatus takes the form of a plate-work in the part of the perikaryon closely adjacent to one side of the nucleus. Smaller cells of cranial and of dorsal spinal ganglia show a similar Golgi apparatus, though often less definitely localized (figs. 81 and 82, Pl. 21). The same complicated plate-work is in the cells of the larval spinal cord (fig. 83, Pl. 21) and the larval medulla. Large cells in the common ganglion of the ninth and tenth cranial nerves show a unique condition of having a Golgi apparatus in the form of small plates scattered throughout the whole perikaryon (fig. 84, Pl. 21). This last closely resembles the sort of Golgi apparatus that has been described in many invertebrate ganglionic cells.

In the spinal ganglia of the adult frog, Dornesco and Busnitzca, 1935, have described the Golgi apparatus in the form of separate bodies, or dictyosomes. Their figures are so much like fig. 84, Pl. 21, that they must have been studying the same sort of structure. Their interpretation, however, is that they are dealing with a black osmiophilic rodlet and an adjacent grey osmiophobic (really less osmiophilic) material, of a different sort. In view of the present demonstration of unmistakable plate-works unaccompanied by any non-osmiophilic material in a great variety of amphibian cells, it seems warranted to assume that the interpretation of Dornesco and Busnitzca was erroneous; that in the spinal ganglia of the frog the Golgi apparatus is in the form of separate osmiophilic platelets, which
appear solid black when seen on edge and grey in face view. Wholly aside from this difference in interpretation, it would appear, if Dornesco and Busnitza's results on the frog are confirmable in adult Urodela (which has not been attempted in the present study), that between the early larva and the adult the Golgi apparatus of the spinal and cranial ganglionic cells undergoes a fragmentation into a number of separate bodies. If that is the case the large cell of fig. 84, Pl. 21, is best regarded as more highly differentiated than the smaller cells of the same ganglion (figs. 81 and 82, Pl. 21).

Thickness of the Lamellae.

Allowing for differences in the magnification and for difficulty of making accurate drawings at a table-level projection of 3,050 diameters, examination of the figures of Plates I-V shows that there is a remarkable approach to uniformity in thickness of the lamellae, that is, in the width of the black lines that are views of the lamellae on edge. An effort has been made to measure this thickness in nearly all the cell types, by projection with an Abbé camera lucida to table-level (giving a magnification of 1,600, the highest at which the projected image is sharp), and comparison of this projected image with a series of measured black lines drawn with a ruling pen. For such measurements were selected only views of platelets which appeared as straight black lines that shifted neither to left nor right within a focal depth of approximately one micron (as determined by the scale on the fine adjustment of Zeiss Model LWoG). The thickness of the plates is on the very border of resolution, and such micrometry is very difficult. All determinations were made in a room completely darkened except for illumination of the card on which the black lines were inscribed, and this illumination was carefully adjusted in amount and quality to give optimum conditions for comparison with the microscopic image. Not more than ten measurements were made on any one day, since eye fatigue sets in very soon, and seems to be unrelieved by a brief rest. It is obvious from the above that the measurement is attended by considerable
difficulty, but nevertheless the uniformity of the result is a striking confirmation of the impression of similarity in thickness that one gains from study of the figures, or even more from prolonged study of the material from which the figures were drawn.

In all, measurements have been made of plates in 144 different cells, representing all the major types of tissues, and taken from six genera of Urodela and one anuran genus. Of these 124 were classified as comparing most closely with a line 0·326 mm. in width, and 20 compared most closely with a 0·396 mm. line. These groups represent a width of the actual plate of 0·0002 mm. and 0·00025 mm. respectively (0·2 micron and 0·25 micron). The theoretical limit of resolution in microscopic observation is usually stated to be in the neighbourhood of 0·2 micron, and the author's practical experience fits in with this theory. Hence, strictly speaking, the majority of these measurements only indicate that the thickness of the plates is not over 0·2 micron. The fact that 16·1 per cent. of the plates appeared slightly greater than 0·2 micron may be interpreted as an indication that the thickness is very little less than that. An unexceptionable method of describing these results in terms of microscopy is to state that the thickness of the plate is such that in a focal depth of approximately one micron it gives the appearance of a solid black image 0·2 micron wide.

From the measurements certain conclusions may be drawn concerning the nature of the technical demonstration of the Golgi apparatus. Measurements of the plates in Da Fano preparations give results identical with those on the same tissues (leucocytes, hepatic epithelium, and superficial gastric epithelium) that were osmicated by the Kolatchev method, indicating that in each case the deposition of silver or of osmium has been within the same region. Very lightly osmicated preparations (where surface views of the plates could not be detected) and the very heavily osmicated preparations show plates identical in thickness with those in an intermediate stage of blackening. Evidently longer immersion in osmic acid does not change the region of distribution of the deposit, but leads to increase in density of the deposit within the same locality—presumably
either on the surface of, or within the substance of the Golgi apparatus. ¹

It did not seem necessary to extend the micrometric study farther within the amphibian material at hand, since experience in observation and measurement of the plates at a uniform magnification had made the author so familiar with their standard thickness that any significant deviation from the normal would have been at once apparent. For the amphibian tissues studied, it can be unhesitatingly stated that the Golgi apparatus consists of a material arranged in lamellae not over 0.2 microns in thickness. Within this group of tissues, then, the Golgi apparatus is a substance that varies widely in two dimensions (although for the most part within certain morphological schemata), while the third dimension remains unvaryingly constant. This is a unique property, possessed by no other cell component, and it does not seem unduly optimistic to hope that it may prove a clue that will presently lead to determination of the approximate chemical nature of the substance of the Golgi apparatus. Beyond question, it offers a new point of departure for study of the details of the mode of functioning of the Golgi apparatus within the cell.

DISCUSSION.

The foregoing observations show that in a great variety of tissues of Amphibia the osmiophilic and argentophilic material

¹ The reaction of osmic acid with the Golgi apparatus seems to be of a distinctly different sort from that with fat droplets. In material osmicated for six days the latter never appear solid black even when in the form of spheres far larger than one micron in diameter, e.g. see figs. 37, 39, 23, Pls. 19 and 17. Partington and Huntingford, 1921, have concluded that the blackening of olein is due to a reduction of the osmium tetroxide to a hydrated form of osmium dioxide. Possibly the black reaction that demonstrates the Golgi apparatus involves further reduction. Certainly it seems unjustified to conclude, as many have done, that the substance of the Golgi apparatus is of a fatty nature, in view of the difference in appearance of fat and Golgi material on the slide, and in view of the further fact that the Golgi apparatus will reduce silver nitrate with the precipitation of metallic silver—a reaction which never occurs in the presence of substances demonstrably lipoidal in nature.
known as the Golgi apparatus is in various forms which have as a common basis the lamella, probably approximately 0.2 microns in thickness. The demonstration of a lamellar structure agrees substantially with many observations on the tissues of Invertebrata. It remains to consider to what extent this structure is characteristic of the Golgi apparatus in the tissues of other Vertebrata.

Golgi called the structure 'apparato reticolare'. To many who have written reviews of the Golgi apparatus this has apparently implied a reticulum of which the strands were filamentous. However, a reference to the French edition of Golgi's earliest description of the apparatus (1899), in nerve-cells of the owl, Strix flammea, makes it clear that his concept of the structure was not that of a predominantly filamentous network but was rather essentially as it is seen in the smaller ganglionic cells of Amphibia. For he states, p. 68, 'L'aspect caractéristique de cet appareil réticulaire interne peut provenir de la forme prédominante en ruban, des fils, du mode de se diviser, de s'anastomoser et du cours de ceux-ci (spécialement dans les grandes cellules on observe un cours nettement tortueux), de la présence dans cet appareil de minces plaquettes ou de petits disques arrondis....' Golgi's accompanying figure of a Purkinje cell clearly shows this sort of structure. It seems fairly certain that this concept of an apparatus in the form of a ribbon-like reticulum was that of all the workers of the Golgi school, since the customary method of describing the apparatus was to state that it was a network like that described in the nerve-cells. Golgi's (1909) figures of the apparatus in the gastric mucosa of the frog are fine representations of a plate-like, irregular collar, exactly like that described for the same tissue in the present paper.

If one interprets the microscopic appearances as outlined on pp. 239–41 it becomes clear, from study of the figures and in many cases from the written descriptions, that there have been many observers who have demonstrated a Golgi apparatus of lamellar structure in vertebrate tissues. Thus most of the figures in Cajal's (1914) report of his comprehensive survey of the Golgi apparatus in vertebrate embryonic and adult tissues indicate
that the elements of the Golgi apparatus are plate-like or ribbon-like, a notable exception being the filamentous network of the inner cartilaginous cells—as in the present study. In the careful studies of Nassonov, 1923, 1926, on a variety of epithelial tissues the Golgi apparatus is clearly shown to be lamellar; and the same is true of the works of Jasswion, 1925, and of Makarov, 1931. From study of a great variety of vertebrate glandular cells Bowen, 1926, concluded that in nearly every case he was observing a structure that was essentially a plate-work. Some of the plainest figures of a lamellar osmiophilic material are given in Morelle’s (1927) paper on the pancreas. In many human tissues Kopsch, 1926, has figured the Golgi apparatus in a manner that indicates it is plate-like; and with respect to the Golgi apparatus of nerve-cells of the spinal ganglion he speaks of it thus, ‘... es besteht aus rundlichen oder bandartigen ... Faden’. Severinghaus, 1933, gives an exceedingly accurate description of a lamellar Golgi apparatus of very specific form in the secretory cells of the anterior lobe of the mammalian pituitary gland. One noteworthy exception to the above is the work of von Bergen, 1904, who was one of the first to apply the Kopsch osmic reaction to demonstration of the Golgi apparatus in a wide variety of tissues. In one cell he figured a plate-work, but in most cases the apparatus was described as consisting of slender fibres of uniform diameter. Some of these tissues, e.g. nerve-cells (Esterman and Gitlitz, 1927), have since been shown to have a lamellar apparatus. In the epithelial tissues it seems very likely that his study was made from slides that were of the type considered in the present paper as under-impregnated. Such preparations will show only the black lines which might be interpreted as elements of a filamentous network (see p. 240, footnote), though careful focusing will always show that they are edgewise views of plates. As a matter of fact, demonstration of a Golgi apparatus of net-like type with filamentous elements in a few cell types in no way invalidates the view that in general its structure is lamellar (cf. the cartilage cell) unless it is also shown that the diameter of the filaments is appreciably different from the thickness of the plates.
It is of importance to realize that the evidence is predominantly in favour of the view that the Golgi apparatus in vertebrate tissues is some variation of a plate-like structure, because students of the tissues of Invertebrata have been almost unanimous in ascribing such a structure to the osmiophilic substance: e.g. Hirschler, 1918, in embryonic and larval tissues of Lymnaea; Monné, 1930, in adult tissues of Gastropoda; Nassonova, 1927, in tissues of Hirudinea; Krjukowa, 1929, and Beams and Goldsmith, 1930, in salivary glands of Chironomus; Beams and King, 1932, in nerve-cells of Orthoptera; Polusyynski, 1911, and Dornesco, 1934, in nerve-cells of Crustacea; Weiner, 1925, in germinal epithelium of Tegenaria; Dumitresco and Dornesco, 1933, in nerve-cells of spiders; Wilson and Pollister, 1937, in epithelium of sperm duct of scorpion, Centrurus; Sokolska, 1931, in various tissues of Ascidians; Schlottke, 1931, in various tissues of Hydra. In these tissues it seems the more general condition for the Golgi substance to be in the form of separate, frequently rather widely scattered Golgi bodies, often incorrectly called dictyosomes; a condition that is exceptional in amphibian cells. In many invertebrates, however, epithelial tissues have been reported that have a Golgi apparatus in the form of a single complicated plate-work, approaching the condition that is more usual in Amphibia. The separate Golgi bodies of Invertebrata are often in the form of cups or closed spheres. Hirschler has been much impressed by the ubiquity of the latter structure. He has pointed out that the closed sphere isolates a definite region from the general cytoplasm, and he has suggested, 1927, that the Golgi apparatus in all animal cells may be of this duplex structure, consisting of osmiophilic substance enclosing a special sort of non-osmiophilic material. Very recently this view seems to have been partially adopted by Hirsch, 1937, as the basis of an elaborate theory of the method of functioning of the Golgi apparatus. The tissues of Amphibia offer no evidence of the presence of a special non-osmiophilic region accompanying the osmiophilic Golgi apparatus.

On the basis of orientation of the Golgi apparatus it is possible to classify fairly logically the great majority of the tissues of
Amphibia into one or the other of two classes: A, the epithelial type, in which the Golgi apparatus is in the form of a collar usually surrounding the nucleus, and with the centrioles far distant from the Golgi apparatus—in the extreme distal end of the cell (including columnar, cuboidal, and stratified epithelia); and B, the leucocyte type, in which the Golgi apparatus is a horizontal collar, or some modification of this form, in close topographical relationship with the centrioles (including endothelium, blood-cells, connective tissue cells, muscle-fibres, nerve-cells, and germinal cells). One is led to speculate on whether there are also marked physiological differences between these two types of cells. An obvious difference is in the relationship to the blood-stream or to the tissue fluid. In type A one notes the familiar epithelial polarization; the cell in contact with the blood-stream at its proximal or basal end; the opposite end in contact with the lumen of some cavity containing a fluid different from blood or tissue fluid; and the sides of the cell in contact only with adjacent elements of the epithelium. By contrast the leucocyte is unpolarized in its relation to blood-stream or body fluid; it is bathed in the same medium on all surfaces. Clearly the unpolarized B type of physiological relationship is characteristic of connective, muscle, and germinal tissues. The endothelial cell likewise has essentially similar fluids on either surface. It is also true that the nerve-cell is essentially unpolarized with respect to the blood and tissue fluid, and accordingly we should expect evidence from its internal architecture to show that it can be placed in Group B. The present study offers no clear data on this point, apparently because it has not included study of early neuroblasts. For Cajal, 1914, figures a neuroblast of the chick embryo with what appears to be a small collar-like Golgi apparatus, which he does not hesitate to suggest is in close topographical relation with the centrioles. This type of neuroblast apparatus has likewise been observed by Alexenko, 1930. As the nerve-cell becomes differentiated from the neuroblast, the typical complicated type of Golgi apparatus appears as a result of growth of this simple ring. This is very much like the changes of the Golgi apparatus in the histogenesis of smooth and cardiac
muscle; and it gives the same sort of basis for classifying the nerve-cell in Group B, along with the other tissue cells that are physiologically unpolarized. In this connexion it is also interesting to note that the medullary cells of the mammalian adrenal gland, which are considered to be closely related genetically to the nerve-cells, have a Golgi apparatus sharply localized about the centrioles (see especially Pilat, 1912).

If cells originally in the epithelial physiological relationship were to lose this relation it would be expected that the internal structures would adopt the unpolarized orientation. Such may be assumed to be the course of events in the differentiation of the epithelioid glandular cells of the anterior lobe of the pituitary gland; and it seems certain from the observations of Zimmerman, 1898, on the centrioles, and from the recent work of Severinghaus, 1933, that the compact centralized type of Golgi apparatus within the B type of pituitary glandular cell surrounds the centrioles.

The cells of the mammalian adrenal cortex are arranged in epithelial cords around a virtual lumen. The blood capillaries are basal to the cells, and on their sides the cells are in contact with other members of the epithelium. Thus from a purely morphological aspect these cells are much like the typical exocrine glandular epithelia; but physiologically there is the all-important difference that the lumen with which the distal end of the cell is in contact is not continuous with any duct system; and, so far as can be told, no secretion is ever passed out of that end of the cell. Consideration of the observations of Pilat, 1912, and of Tschassownikow, 1929, makes it clearly evident that the sharply localized Golgi apparatus surrounds the centrioles, so that internally this cell is like those that are physiologically unpolarized. This indicates that the orientation of the Golgi apparatus and centrioles is not primarily a consequence of the cell merely assuming the shape and external morphological relations of an epithelial cell. To bring about the type A polarized condition it is furthermore necessary that the cells should have specific physiological relationships, such that one end is in contact with the blood or tissue fluid w:he
the other is in relation with a region containing a considerably different fluid.\footnote{The posthumous work of S. G. Tschassownikow, 1929, pertains to the matter of the position of the centrioles. This observer described a secretory cycle in the pancreas cell, in the course of which, immediately subsequent to the discharge of the secretory granules, the centrioles assumed a temporary position in the region of the Golgi apparatus. Later, as the process of elaboration of a new batch of secretory granules got under way, the centrioles moved away from the Golgi apparatus, back to the usual epithelial position at the distal end of the cell. This suggests that for a brief time following discharge of secretion there is a sort of resting period, when the normal proximo-distal flow of materials through the cell is interrupted—the cell then being in the same physiological relation to the blood-stream as is normally characteristic of the cells of the adrenal cortex. The consequent movement of the centrioles to a region near the centre of the main mass of cytoplasm, adjacent to the Golgi apparatus, brings about an orientation of cytoplasmic components that is much like that of the cells of the adrenal cortex—and of unpolarized cells in general. To date, these interesting results of Tschassownikow remain unconfirmed.}

The above discussion is presented as an attempt to bring some order to the chaotic and unsatisfactory state of our knowledge concerning the relation of the cytological structure of tissue cells to their function in the organism as a whole. It is, of course, obvious that the relation of Golgi apparatus and centrioles must be examined in a number of other cell types—e.g. the impermeable epidermis, the hepatic epithelium—and that there must be more exact studies of histogenesis before it can be considered as an established fact that, on the basis of orientation of the cytoplasmic components, all vertebrate tissue cells are divisible into two main groups; such orientation being a consequence of the relationship of the cell to the body fluids.\footnote{The possibility of classifying vertebrate tissue cells into these two groups was briefly indicated five years ago (Pollister, 1933\,a). It seems pertinent to repeat here that this classification is also supported by evidence from a study of chondriosomes in the same cell types. In the polarized epithelia these rod-like bodies are oriented with their long axes parallel to the course of flow of materials through the cell, that is from blood capillary to lumen, fig. 17, Pl. 17. In the unpolarized type, where there is considerable volume of endoplasmic cytoplasm, chondriosomes are arranged with their long axes radial to the point where the centrioles are located. It now seems likely that these facts are explicable by the assumption that the watery phase of the cytoplasm is in the form of channels}
Some students of the Golgi apparatus have considered it to be of fluid consistency, while to others it has appeared solid (see especially Bowen, 1926a, for discussion of this point). Evidence from the amphibian tissues favours the second view. The thin lamella is an extremely unlikely form to be assumed by a material more fluid than its surroundings. A liquid under such conditions rather would be expected to approximate a spherical shape. Consideration of the normal form of the lamellae in the various types of cells leads to the conclusion that the substance is not a highly plastic solid, for such folds as occur are merely slight bends, rarely over forty-five degrees. Only under conditions of pressure, such as that exerted by distention of the stomach, do we find instances of the plates being actually folded back upon themselves; and here the normal form is resumed upon removal of the distorting force and the return of the cell to its usual shape. It seems probable that the lamellae are of the same elastic nature as has been demonstrated for the Golgi apparatus (acroblast) of the spermatid of Gerris (Pollister, 1930). In this experiment the cell was ruptured, allowing the Golgi apparatus, a sac-like structure to one end of which the acrosome is attached, to escape into the surrounding medium, Ringer's fluid. There the apparatus remained unchanged in form for nearly an hour, though completely separate from the remainder of the cell, which had promptly disintegrated. The form of this isolated Golgi apparatus could be altered by pressure, and it regained its original shape when the distorting force was removed. From all this it was concluded that the sac-like Golgi apparatus (acroblast) of the spermatid of Gerris was a solid, with the property of elasticity. The assumption that the Golgi apparatus of amphibian tissues consists of lamellae of a solid, elastic nature seems thoroughly compatible with its normal appearance and which are parallel to the line of flow of materials through the epithelial cell (see Jasswold, 1925), and radial to the centre of the cytoplasm in leucocytes, &c. (the latter giving rise to the appearance of an aster). The restriction of the chondriosomes to the more stainable, presumably less aqueous cytoplasm between these watery channels will explain the definite orientation of the chondriosomes in these cells.
Summary.

The Golgi apparatus has been studied in nearly all tissues of larvae and adults of a number of Amphibia. In every case the apparatus is lamellar in structure. In all epithelia, except the simple squamous and the specialized cells of the liver and peptic glands, the Golgi apparatus approximates the form of a vertical collar, most commonly encircling the distal part of the nucleus and projecting into the distal end of the cell. In leucocytes and fibroblasts the Golgi apparatus takes the form of a horizontal collar near the approximate centre of the main cytoplasmic mass. The Golgi apparatus of smooth muscle-fibres and of myocardium is developed from the condition in leucocytes by elongation of the ring. The apparatus is greatly extended throughout the central region of the cytoplasm in cartilage and bone cells, and in odontoblasts; and in these types there is a condition where the width of the Golgi plates approximates the thickness, resulting in a filamentous structure. In nerve-cells of the spinal cord and medulla, and in the smaller nerve-cells of cranial, spinal, and sympathetic ganglia, the Golgi apparatus is in the form of a single complex plate-work. In large cranial ganglionic cells of larvae the apparatus consists of a considerable number of separate platelets.

Measurements of the lamellae that are the basis of structure of the Golgi apparatus, in all types of cells of all animals studied, shows that the thickness is very uniform, approximating 0.2 micron, regardless of the shape of the apparatus as a whole. Data on the form of the apparatus, and on the mode of distortion of the lamellar structure when the cell shape is altered by pressure, indicate that the lamellae have the properties of an elastic solid.

On the basis of orientation of centrioles and Golgi apparatus all cells of Amphibia that have been studied may be assigned to one of two groups: A, the epithelial, or physiologically polarized type; or B, the leucocyte, or physiologically unpolarized type.
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EXPLANATION OF PLATES

All the figures were outlined with the Abbé camera lucida, with projection at table level. The actual magnification, after reduction in reproduction, of all the figures is (as fig. 1) × 1,525, unless otherwise stated. In most of the drawings the details of nuclear structure have been purposely omitted. Unless otherwise stated, all figures are from Kolatchev preparations. Epithelial cells are oriented with the free surface towards the top of the plate.

PLATE 17.

EXPLANATION OF FIGURES.

Fig. 1.—Pancreatic duct, 19 mm. larva, Amblystoma punctatum. ×1,525.
Fig. 2.—Gall-bladder, 19 mm. larva, Amblystoma punctatum.
Fig. 3.—Mucous cell, oesophagus, 15 mm. larva, Rana pipiens.
Fig. 4.—Like fig. 3, cross-section through Golgi apparatus.
Figs. 5 a–d.—Successive focal planes of goblet-cell, intestine, 17 mm. larva, Amblystoma opacum. ×1,000.
Figs. 6 a–c.—Successive focal planes perpendicular to plane of fig. 5. ×1,000.
Fig. 7.—Goblet-cell, intestine, Necturus maculosus. ×1,800.
Fig. 8.—Non-mucous cell, intestine, Necturus maculosus. ×1,800.
Fig. 9.—Sections perpendicular to long axis, non-mucous cells, intestine, 17 mm. larva, Amblystoma punctatum. ×1,000.
Fig. 10.—Cells of superficial epithelium, stomach, Amphiuma tridactylum. ×800.
Fig. 11.—Like 10, transverse to long axis of cell.
Fig. 12.—Like 10, Da Fano preparation. ×800.
Fig. 13.—Like 11, Da Fano preparation.
Fig. 14.—Superficial epithelium, stomach, 17 mm. larva, Amblystoma punctatum. Cell distorted by distension of stomach.
Figs. 15 a–d.—Successive focal planes, proximal segment of mesonephric tubule, Necturus. ×1,000.
Fig. 16.—Pronephric tubule, 17 mm. larva, Amblystoma opacum.

Fig. 17.—Distal segment, mesonephric tubule, Necturus. ×1,000.

Fig. 18.—Distal segment, mesonephric tubule, Necturus. ×1,000.

Fig. 19.—Ciliated cell, neck of mesonephric tubule, 19 mm. larva, Amblystoma punctatum.

Fig. 20.—Ciliated cell from trachea, 19 mm. larva, Amblystoma punctatum.

Plate 18.

Figs. 21 and 22.—Mucous cells, upper oesophagus, 19 mm. larva, Amblystoma punctatum.

Fig. 23.—Superficial cell, pharyngeal epithelium, fat droplets grey, Golgi apparatus black, 19 mm. larva, Amblystoma punctatum.

Fig. 24.—Like 23, from deeper layer of cells.

Fig. 25.—Ameloblast, left side of cell was in contact with tooth, 19 mm. larva, Amblystoma punctatum.

Fig. 26.—Cell of deeper layer of two-layered epithelium, branchial chamber, 17 mm. larva, Amblystoma punctatum.

Fig. 27.—Like 26, section perpendicular to epithelial surface.

Figs. 28 and 29.—Superficial cells, epithelium of urinary bladder, Necturus.

Fig. 30.—Deeper cell, epithelium of urinary bladder.

Fig. 31.—Epithelium of lung, 19 mm. larva, Amblystoma punctatum.

Fig. 32.—Cross-section of branch of external jugular vein, 17 mm. larva, Amblystoma opacum. ×800.

Fig. 33.—Vertical section, peritoneal epithelial cell, 17 mm. larva, Amblystoma opacum. ×1,000.

Fig. 34.—Surface view of part of peritoneal cell, Golgi apparatus and enclosed mass of pigment granules (latter lighter grey), 17 mm. larva, Amblystoma punctatum. (Only part of cell shown, since cell boundaries were not distinct.) ×800.

Fig. 35.—Hepatic epithelium, Amphiuma tridactylum, dark marginal line indicates secretory surface, adjacent to bile canaliculus. ×800.

Fig. 36.—Like 35, Da Fano preparation. ×800.

Plate 19.

Figs. 37 a-c.—Successive sections through zymogenic cell of gastric gland, 14 mm. larva, Triturus torosus. Large spheres are fat droplets; smaller spheres are secretory granules adjacent to secretory canaliculus.

Fig. 38.—Acinar cell of pancreas, 17 mm. larva, Amblystoma opacum.

Fig. 39.—Pancreatic acinar cell, 14 mm. larva, Triturus torosus.

Fig. 40.—Non-granular leucocyte, hepatic capsule, 17 mm. larva, Amblystoma opacum. ×1,800.

Fig. 41.—Eosinophilic leucocyte, capsule of liver, Amphiuma tridactylum. ×1,000.
Fig. 42.—Non-granular leucocyte, peri-hepatic zone, Amphiuma tridacylum. Shows both Chondriosomes (grey filaments) and Golgi apparatus. x 1,000.

Fig. 43.—Non-granular leucocyte, ventricle, 17 mm., Amblystoma opacum.

Fig. 44.—Non-granular leucocyte, peri-hepatic zone, adult Amblystoma opacum. Da Fano preparation.

Fig. 45.—Non-granular leucocyte, between aorta and notochord, 19 mm., Amblystoma punctatum.

Fig. 46.—Non-granular leucocyte, from leucopietic zone ventral to aorta, 17 mm., Amblystoma punctatum.

Fig. 47.—Non-granular leucocyte, perihepatic zone, 17 mm., Amblystoma opacum.

Fig. 48.—Like 47.

Fig. 49.—Non-granular leucocyte, perihepatic zone, 17 mm., Amblystoma opacum. Drawn to show collar-like Golgi apparatus in edgewise view. Fig. 49 is oriented approximately at right-angle to figs. 40–8.

Fig. 50.—Non-granular leucocyte, leucopoietic zone of testis of Necturus. Centrosome in centre of perforation in collar-like Golgi apparatus.

Fig. 51.—Non-granular leucocyte, perihepatic zone, Amphiuma tridactylum. Fixed in Helly's fluid, stained with iron hematoxylin, showing centrosome at centre of aster, and the two centrioles on surface of centrosome.

Fig. 52.—Cell of head mesenchyme, Amblystoma punctatum, Harrison's stage 43.

Fig. 53.—Fibroblast, atrioventricular valve, 17 mm. larva, Amblystoma opacum.

Fig. 55.—Cell of blastemal rudiment of trunk vertebra, 19 mm. larva, Amblystoma punctatum.

Figs. 56 and 57.—Two views of sub-perichondrial cartilage cells, 17 mm. larva, Amblystoma punctatum.

Fig. 54.—Portion of fibroblast, that was flattened between peritoneum and ventral rectus muscle, 17 mm. larva, Amblystoma punctatum.

Fig. 58.—Cartilage cell from centre of branchial bar, 19 mm. larva, Amblystoma punctatum. x 800.

Fig. 59.—Portion of notochordal cell, 19 mm. larva, Amblystoma punctatum. Golgi apparatus in form of filamentous network.

Figs. 60 and 61.—Two different aspects, surface and transverse, of perichondrial cells. Nucleus not included in 60. 17 mm. larva, Amblystoma opacum.

Fig. 62.—Germinal cell from gonadial ridge, 17 mm., Amblystoma opacum. Golgi apparatus flattened in one plane between nucleus and cell membrane.

Plate 20.
GOLGI APPARATUS IN AMPHIBIA

Fig. 63.—Like 62, but more typical condition, Golgi apparatus interpreted as like that in 62, but on the surface of a sphere, the idiozone.

Fig. 64.—Smooth muscle-fibre, intestine, 17 mm. larva, Amblystoma opacum. Simple, collar-like Golgi apparatus. \(\times 1,000\).

Figs. 65 and 66.—Two aspects of Golgi apparatus of uncontracted smooth muscle-fibre, circular layer of intestinal muscularis, adult Amblystoma punctatum.

Fig. 67.—Like 65 and 66, cross-section of fibre.

Fig. 68.—Contracted smooth muscle-fibre, longitudinal layer of intestinal muscularis, adult Amblystoma punctatum.

PLATE 21.

Fig. 69.—Portion of myocardium, Necturus, showing part of Golgi apparatus on surface of nucleus.

Fig. 70.—Like 69, but more complicated Golgi apparatus. \(\times 3,050\).

Fig. 71.—Myocardium, Necturus, showing Golgi apparatus in region at end of nucleus.

Fig. 72.—Myocardium, Necturus, portion of Golgi apparatus drawn with great care to show details of twisting of the ribbon-like structure under conditions of fibre contraction.

Fig. 73.—Peripheral non-fibrillar myocardium and adjacent fibrillar region with which it is continuous, Necturus. Note especially collar-like Golgi apparatus.

Fig. 74.—Hair-cell, of crista ampullaris, 19 mm. larva, Amblystoma punctatum. (Isolated oval grey mass above nucleus is clump of granules of pigment.)

Fig. 75.—Supporting cell from vicinity of cell shown in 74.

Fig. 76.—Glandular cell, chorioid plexus of fourth ventricle, 19 mm. larva, Amblystoma punctatum.

Fig. 77.—Cell of non-nervous portion of lining of ampulla, 19 mm. larva, Amblystoma punctatum.

Fig. 78.—Ciliated cell from lining of fourth ventricle, 19 mm. larva, Amblystoma punctatum.

Fig. 79.—Sheath cell and nerve fibre. Vagus nerve, 19 mm. larva, Amblystoma punctatum. (Light grey granular mass is clump of pigment.)

Fig. 80.—Nerve cell, small mesenteric ganglion, adult Amblystoma punctatum.

Figs. 81 and 82.—Small nerve-cells, vago-glossopharyngeal ganglion, 19 mm. larva, Amblystoma punctatum.

Fig. 83.—Nerve cell, spinal cord, 19 mm. larva, Amblystoma punctatum.

Fig. 84.—Large nerve cell from dorso-lateral area of vago-glossopharyngeal ganglion, 19 mm. larva, Amblystoma punctatum. \(\times 800\).

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