THE GOLGI APPARATUS IN NEURONS AND EPITHELIAL CELLS OF THE COMMON LIMPET PATELLA VULGATA

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It is now established that the Golgi apparatus of vertebrate cells is a distinct organelle that can be seen in living cells (13, 9, 10, 22). With the aid of the light microscope the Golgi apparatus has also been observed in frozen-dried cells (40, 22) and in fixed material by the use of methods which do not involve impregnation (11, 12, 9, 23).

Within the limits of resolution of the light microscope it has been established that the Golgi apparatus of vertebrate cells consists of a chromophilic material enclosing chromophobic vacuoles (9, 10, 24, 26). In most somatic cells the chromophobic vacuoles are aligned in rows so that often the apparatus appears to be canalicular or tubular in form (11, 12, 1, 24, 25). On the other hand, in the male germ cells of vertebrates the vacuoles are irregularly arranged and generally embedded in a spheroidal mass of chromophilic material (26).

Studies with the electron microscope have revealed that the chromophilic material consists of a number of paired membranes which usually enclose a dense substance (9, 10, 41, 15, 26). The paired membranes are often folds of essentially a single Golgi membrane (26). The chromophobic component of the Golgi apparatus has been identified as a substance lying within dilations of the paired membranes (15, 26).

It is apparent from the above that in recent years a great deal of work involving both the light and electron microscope has been centered on the Golgi apparatus of vertebrate cells. By comparison relatively little such work has been done on the Golgi apparatus of invertebrate cells. This is particularly true in the case of the neurons of Mollusca. The nature of the Golgi apparatus of these cells has been in dispute for the past 50 years. The following theories have been advocated:

1. The Golgi apparatus is present in the form of discrete filaments or rods scattered around the nucleus (36, 21).

2. The filaments of Popoff (36) are osmiophilic granules as seen in different aspects; the fact that these are revealed by Golgi techniques is insufficient evidence to homologise them with the Golgi apparatus of vertebrate neurons (27).

3. The Golgi apparatus is present in the form of short rods, sinuous filaments, and occasionally as networks. The organelle originates from mitochondria (19).

4. The Golgi apparatus is present in the form of short curved rods (batonets) with attached archoplasm (6) or sometimes without archoplasm (28, 5, 29).

5. The rods (Golgi apparatus) of previous workers are artifacts caused by the distortion and impregnation of genuine inclusions which are vacuolar (spheroidal) in form and stain vitally with neutral red. There is no Golgi apparatus, only neutral red vacuoles, the "vacuome" (32).

6. The neurons contain spheroidal bodies (which stain vitally with neutral red or methylene blue and contain lipide) and mitochondria. The rods (Golgi apparatus) of previous workers are impregnated mitochondria. The classical techniques do not reveal the spheroids but these, in fact, are the true Golgi complement and are "Golgi bodies" (42).

7. The cells contain only spheroidal bodies and mitochondria as determined by Thomas (42). The classical methods sometimes impregnate both mitochondria (the rods of previous workers) and spheroids. The Golgi apparatus of these cells are the spheroids (7).

8. The classical methods either totally impregnate small spheroids or the outer sheath of larger spheroids which have a chromophobic medulla. The small spheroids correspond to the Golgi presubstance of Hirsch (16). The outer sheath and medulla of the large spheroids correspond respectively (*i*) to the dictyosomes (rods, batonets) and archoplasm of other workers and (*ii*) likewise to the Golgi externum and internum of Hirsch. The spheroids are the Golgi apparatus (18).

9. The rods or dictyosomes are artifacts caused by the distortion and impregnation of spheroidal bodies. The rods or dictyosomes seen in living cells are spheroidal bodies as seen in optical section. There is no Golgi apparatus, only spheroidal bodies, or "lipochondria" (39, 3).

10. (*i*) Techniques for the Golgi apparatus impregnate small granules and spheroidal bodies. The small granules are Golgi bodies and the spheroidal bodies the neutral red bodies of Parat (34).

(*ii*) The dictyosomes (rods, batonets) are distorted neutral red bodies. The latter are Golgi bodies (44).

(*iii*) The Golgi bodies (neutral red bodies) are lipochondria (44, 45).

From the above it is evident that various workers have differed not only in the interpretation of results but have, in fact, obtained quite different results. Parat (32), for example, states that the classical methods impregnate the neutral red vacuoles (spheroids), while Thomas (42), using one of the gastropod genera (*Helix*) studied by Parat, holds an opposite view and maintains that mitochondria are impregnated.

A variety of conflicting views are held about the identity and form of the Golgi apparatus in epithelial cells. Here the work has been carried out in many different classes of animals. Two principal theories have been advocated. One is that the Golgi apparatus is present in the form of discrete rods (batonets, dictyosomes) with or without associated archoplasm (20, 28, 4). The other theory maintains that the classical techniques impregnate spheroidal bodies (neutral red bodies) and that these should either be described as such (33–35), or referred to as Golgi bodies (38).

The main purpose of the present investigation is (*a*) to determine the identity and morphology of the Golgi apparatus in the neurons of the common limpet, *Patella vulgata*, and (*b*) to try to assess the validity of the various theories concerning the identity and morphology of the apparatus in Mollusca and particularly in gastropods. In addition an attempt has been made to identify the Golgi apparatus in epithelial cells of *Patella*. The results of this investigation have been compared with the study carried out on neurons of the same animal.

Material and Methods

Material was obtained from fresh specimens of *Patella vulgata*. The visceral hump was pressed backwards and a deep longitudinal incision made through the head. The two halves of the head were pinned back and the radula and other tissue removed to reveal underlying parts of the nervous system. The whole region was then flooded with an appropriate fixative (see below). The pleural, pedal, and visceral ganglia and their connectives were then carefully removed and transferred to fresh fixative. The tissue was next cut into small pieces and individual ganglia, about 1 mm. in diameter, transferred into fixative-filled tubes. During the examination of nerve cells by electron microscopy, adherent epithelial tissue was also observed.

Nervous tissue was treated by the following techniques:

Methods for Light Microscopy

1. Kolatchev's Method for the Golgi Apparatus.—Pieces of tissue were fixed in Champy's fluid for 24 hours, washed in tapwater for 24 hours, and then placed in 2 per cent osmium tetroxide solution at 37°C. for 24, 48, 72, and 96 hours. The ganglia were then variously embedded in paraffin and gelatin.

2. Methods to Show Spheroidal Bodies.—(*a*) Tissue was fixed in Champy's fluid for 24 hours and then divided into two lots. One group was washed for 24 hours and embedded in wax or gelatin. The other was postchromed in a saturated solution of potassium dichromate for 3 days at 37°C., washed 24 hours, and embedded in paraffin or gelatin. (There is less shrinkage in postchromed material).

(*b*) Ganglia were fixed in Helly's fluid for 24 hours and then postchromed in a saturated solution of potassium dichromate for 3 days at 37°C. The tissue was washed for 24 hours and embedded in wax or gelatin. Sections were stained with hot acid fuchsin and methyl blue (8).

(*c*) Tissue was prepared according to Baker's method (2) for lipides and the sections stained with a saturated solution of Sudan black in 70 per cent alcohol and counterstained with carmalum.

3. Methods for Mitochondria.—(*a*) Sections treated by method 2(*a*) above were bleached with a 1 per cent solution of potassium permanganate. The permanganate stain was removed with a weak solution of oxalic acid. The sections were then well washed and finally stained with Heidenhain's iron-haematoxylin. In some instances a short method of staining was employed (2 hours in mordant at 37°C. followed by haematoxylin at 37°C. for the same period). In other cases the sections were left in the mordant and stain for several days at room temperature. No difference in result was observed.

(*b*) As for method 2(*b*) above.

(*c*) Material was fixed in the same manner as in method 2(*b*), (Helly's and postchromed) but subsequently stained with Heidenhain's iron-haematoxylin either by the long or short method as described in 3(*a*) above.

The mitochondria were seen most clearly when material prepared by the methods described above (3 (*a*), (*b*)) were examined by phase contrast microscopy. They were difficult to see by

ordinary (direct) light microscopy. The best preparations were obtained by method 3(*b*), paraffin embedding being more satisfactory than gelatin. In the latter type of preparation both spheroids and mitochondria were revealed.

Methods for Electron Microscopy

4. Kolatchev's Method.—Material treated exactly as for method 1 above was dehydrated and embedded in a 1:3 solution of *n*-butyl methacrylate.

5. Palade's Method.—Tissue was fixed for times varying from 2 to 4 hours in a 1 per cent solution of osmium tetroxide buffered to a pH of 7.3 (30). The tissue was rapidly dehydrated (half-hourly changes in appropriate solutions) and embedded in a 1:3 solution of *n*-methyl and *n*-butyl methacrylate.

Epithelial tissue was fixed only in buffered osmium tetroxide (method 5) and examined by electron microscopy.

Material treated by methods 4 and 5 was sectioned on a modified version of the ultramicrotome of Hodge, Huxley, and Spiro (17). The sections were mounted on carbon films and examined on a Siemens Elmiskop I.

RESULTS

General

The ganglia of the nervous system of *Patella* are thickenings of the nerve cords. Each ganglion consists of a cortex, which is largely composed of cell bodies, and a medulla in which are numerous nerve fibres and a few cell bodies. The neurons vary greatly in size. The cell bodies of some measure only about 5 μ in diameter, while others measure about 25 μ. No significant difference in the cytology of the neurons of different ganglia was observed.

Observations on Neurons by Light Microscopy

Golgi Apparatus.—Kolatchev's method revealed in different cells an inclusion with the following appearances: (1) a network of fine black filaments, (2) a network of fine black filaments together with occasional discrete filaments, (3) discrete filaments. The inclusion, therefore, appeared in two principal forms, as a network (1 and 2 above) or as discrete elements (3 above). The word "network" will be used below to refer to appearances 1 and 2.

In most instances networks were observed and it was relatively rare to see cells which contained discrete filaments only. The networks were generally open in appearance and were formed of filaments anastomosing at widely spaced intervals (see Fig. 3). In some cells, however, the network was very complex at its periphery where there were numerous anastomoses (Figs. 4 and 5). When only discrete filaments were seen, these were usually either straight rod-like, or short curved bodies.

A dark, weakly osmiophilic material was sometimes observed either enclosed by the filaments of the network or lying in close association with the discrete filaments (Text-fig. 1 *c*).

The above described organelle was demonstrated most clearly in cells which had been postosmicated for 24 hours at 37°C. Cells that were postosmicated

for several days at 37°C. seemed to be less satisfactorily fixed. Furthermore, after long postosmication the filaments were generally thickened and tended to be granular in appearance. At the same time the darkish material associated with some of the filaments was most distinctly observed after more prolonged postosmication.

In very small neurons the organelle was restricted to a small region next to the nucleus (Figs. 1 and 2). In large neurons the network or discrete filaments were partially situated next to the nucleus, which was excentrically placed, but extended into a larger region of the cell body (Figs. 3 to 5).

The osmiophilic networks and separate filaments were not observed in all cells examined. They were not seen in neurons in which the cell body was largely occupied by the spheroidal bodies described in the next section. On the other hand networks were well developed in large neurons which contained few spheroidal bodies (Figs. 3 to 5). This suggests that in at least some instances (*cf.* below) the ability to demonstrate the Golgi apparatus may be correlated with the number of spheroidal bodies present in the cells and, therefore, probably varies with different physiological conditions of the cells.

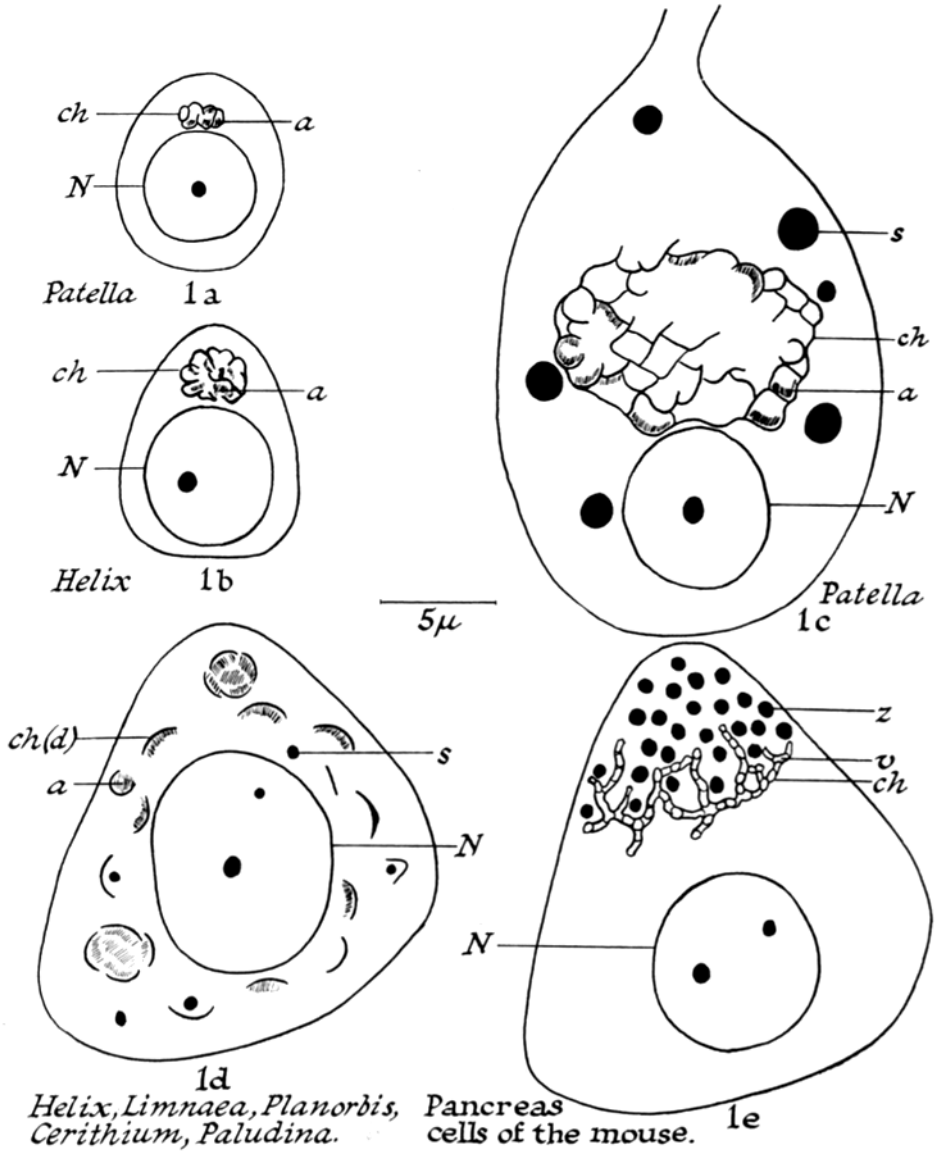
Not infrequently it was found that the networks and separate filaments were absent from one group of cells although present in another. Nevertheless, judging by the size of the neurons and number of spheroidal bodies they contained, the cells appeared to be in a similar physiological condition. Other cells were observed in which the networks and filaments were poorly defined due to under impregnation. In many instances, therefore, the absence of the osmiophilic material was probably due to imperfections in the impregnation technique.

There are obvious similarities between the osmiophilic *network* described above and the "classical" Golgi apparatus of vertebrate nerve cells. Both can be demonstrated by Kolatchev's technique. Each is present in the form of a network. Each, probably, reflects differences in the physiological condition of the cell. The position of the networks in neurons of *Patella* is similar to that occupied by the Golgi apparatus in young vertebrate neurons (37)

The discrete filaments are revealed by the same method that reveals the networks, they occupy the same part of the cell as the networks and they can be traced by intermediate forms to the networks. In our opinion, there is no doubt that the discrete filaments are directly related to the networks. Therefore in view of the evidence presented above, both networks and filaments are identified as the Golgi apparatus of the neurons of *Patella*.

The dark material which has sometimes been observed in this investigation in the immediate vicinity of the networks and filaments may also be part of the Golgi apparatus of these cells. Such material, however, is not seen in classical Golgi preparations of vertebrate neurons (from the study of which the term "Golgi apparatus" originates, 14). Its identity was only finally determined when the cells were examined by electron microscopy (p. 787).

Numerous attempts have been made in the present work to demonstrate the


ch
a
N
Patella 1a
ch
a
N
Helix 1b
5μ
s
ch
a
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Patella
1c
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a
s
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1d
Helix, Limnaea, Planorbis, Cerithium, Paludina.
z
υ
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Pancreas cells of the mouse.
1e

Text-Fig. 1

Golgi apparatus in neurons of *Patella* by methods (see methods 2 (*b*), (*c*); 3 (*a*), (*c*), outlined above) which do not entail impregnation procedures. These included prolonged staining with iron-haematoxylin and Sudan black and the examination of all preparations both by ordinary (direct) light and phase contrast microscopy. The results were negative.

Spheroidal Bodies.—In addition to the Golgi apparatus, Kolatchev's method distinctly revealed large numbers of osmiophilic spheroidal bodies ranging from about 0.5 μ to 2 μ in diameter. Such spheroids varied from light to dark brown in colour, but were rarely deep black as were the Golgi networks and discrete filaments. The majority of spheroids, both large and small, were homogeneous in appearance. The remainder, of large size, contained small vacuoles.

The number of spheroidal bodies in different cells was very variable. In some neurons, seen in sections 5 μ thick, they practically filled the cell body except for that part occupied by the nucleus. These neurons were generally of medium to large size and located in the outermost region of each ganglion. Other neurons, viewed in sections 5 μ thick, contained only four or five spheroidal bodies grouped around the Golgi apparatus (Figs. 3 to 5).

It should be emphasised that both in colour and morphology the spheroidal bodies were readily distinguished from the Golgi apparatus. In most instances

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Legend to Text-Figures

a, archoplasm.
ch, chromophilic component of the Golgi apparatus.
d, dictyosome.
d v, discrete Golgi vacuoles.
e, substance enclosed by Golgi membrane.
er, ergastoplasm (endoplasmic reticulum).
G m, Golgi membrane.
G ves, Golgi vesicle.
m, mitochondrion.
N, nucleus.
s, spheroidal body.
v, chromophobic or vacuolar component of the Golgi apparatus.
z, zymogen granule.

TEXT-FIG. 1. *Golgi apparatus in neurons of different genera of gastropods and in a mammalian somatic cell as visualised by classical impregnation techniques and studied by light microscopy.*

Text-Figs. 1 *a* and 1 *b* show that there is little difference in either the form or position of the Golgi apparatus in small neurons of *Patella* compared with that seen in young neurons of *Helix*.

Text-figs. 1 *c* and 1 *d* show the difference in both form and position of the Golgi apparatus in medium or large sized neurons of the marine *Patella* compared with that found in similar sized neurons of *Helix*, *Limnaea*, *Planorbis*, etc. It must be emphasized that occasionally neurons of *Patella* do contain discrete filaments (dictyosomes), but that they are located in the same region as the networks. The networks also vary in their complexity.

Text-figs. 1 *c* and 1 *e* are a comparison between the Golgi apparatus in neurons of *Patella* and in pancreas cells of the mouse. In both types of cells the Golgi apparatus is generally in the form of a network. Certain differences in the structure of the inclusion, however, are apparent. These are due to the limitations of the techniques and the resolution of the light microscope as shown in Text-fig. 2.

Text-figs. 1 *b* and 1 *d* are based on the work of Brambell and Gatenby (6), Monné (28), Boyle (5), and Moussa (29). Text-fig. 1 *e* is based on the writer's own observations (25).

too, these bodies were quite distinct topographically from the Golgi apparatus: they were grouped *around* the Golgi zone. This latter distinction was less obvious in cells containing many of them, for in such instances there appeared to be a widespread invasion by spheroids of the Golgi zone accompanied by a reduction or absence of the apparatus itself.

In cells fixed in Champy's fluid, and examined without the use of additional stains (method 2 (*a*)), the spheroidal bodies, again light to dark brown in colour, were readily observed. It was apparent that the colour of the spheroids was due to the osmium tetroxide present in the fixative. The Golgi apparatus was not revealed by this method and was only seen when the tissue was post-osmicated (Kolatchev's method).

The spheroidal bodies stained both with acid fuchsin (method 2 (*b*)) and Sudan black (method 2 (*c*)). The smaller spheroidal bodies stained more brightly with the fuchsin than the larger ones. Some of the larger ones contained unstained or very lightly stained vacuoles. All the spheroidal bodies coloured intensely with Sudan black and again some of the larger ones contained small vacuoles. However, unlike the results obtained by any of the other methods (1, 2 (*a*) and (*b*)), several of the larger spheroidal bodies were seen to consist of a heavily stained cortex and an unstained medulla.

Mitochondria.—As demonstrated by methods 3 (*a*), (*b*), and (*c*) above, the mitochondria were seen as small granules or, rarely, as minute rods. The actual localisation of the mitochondria varied in different neurons but in any one particular region of the cell body they were distributed at random. In preparations where both spheroidal bodies and mitochondria were demonstrated (methods 3 (*a*) and 3 (*b*); in the former instance the spheroidal bodies were seen as negative images) the latter inclusions were often seen as small granules lying at one pole of the spheroids.

Observations on Neurons by Electron Microscopy[1]

Golgi Apparatus.—Examination of Kolatchev preparations (method 4) revealed the Golgi apparatus with a form similar to that seen by light microscopy. However, the electron microscope showed that deposits of osmium (or osmium oxides) were often associated with small vacuoles free of metallic precipitates. Such vacuoles were below the limits of resolution of the light microscope. The Golgi apparatus, therefore, contains two of the main components found in vertebrate cells, namely, a chromophilic component which reacts with osmium tetroxide (as used in Golgi type methods), and a chromophobic component which does not so react and hence appears vacuolar.

A careful study was made of lightly impregnated cells in an attempt to ob-

[1] Only the fine structure of the Golgi apparatus is described here, since a full report on observations made on other cell components (spheroidal bodies, mitochondria, ergastoplasm, etc.) by electron microscopy will be made later.

serve details of the fine structure of the chromophilic component. In several instances, it was seen that this component consisted of strands or lamellae of higher density than the surrounding cytoplasm. In other cases, however, the individual lamellae were not revealed and the whole of the chromophilic material simply looked denser than the adjacent cytoplasm. In all cells examined it was apparent that the osmium had deposited unevenly on the chromophilic component of the Golgi apparatus (Fig. 6).

It has been stated (p. 783) that the Golgi apparatus was not seen by light microscopy in preparations where the cells were largely filled with spheroidal bodies. However, it was observed in Kolatchev preparations of such cells examined by electron microscopy. The organelle was confined to one pole of the nucleus and occupied a much smaller area than the Golgi apparatus of cells of similar size which contained few spheroidal bodies.

Spheroidal bodies and mitochondria were identified in Kolatchev preparations and were clearly distinct from the Golgi apparatus.

Examination of cells fixed in buffered osmium tetroxide revealed an organelle consisting of discrete groups of paired membranes (which usually enclosed an inner dense substance), vacuoles, and small vesicles. This organelle is identified as the Golgi apparatus for the following reasons:

1. It is present in the same region of the cell body as the networks and discrete filaments seen by light microscopy.

2. There is a general morphological similarity between the elongated groups of paired membranes and the Golgi filaments (forming either a network or present as discrete bodies) seen by light microscopy.

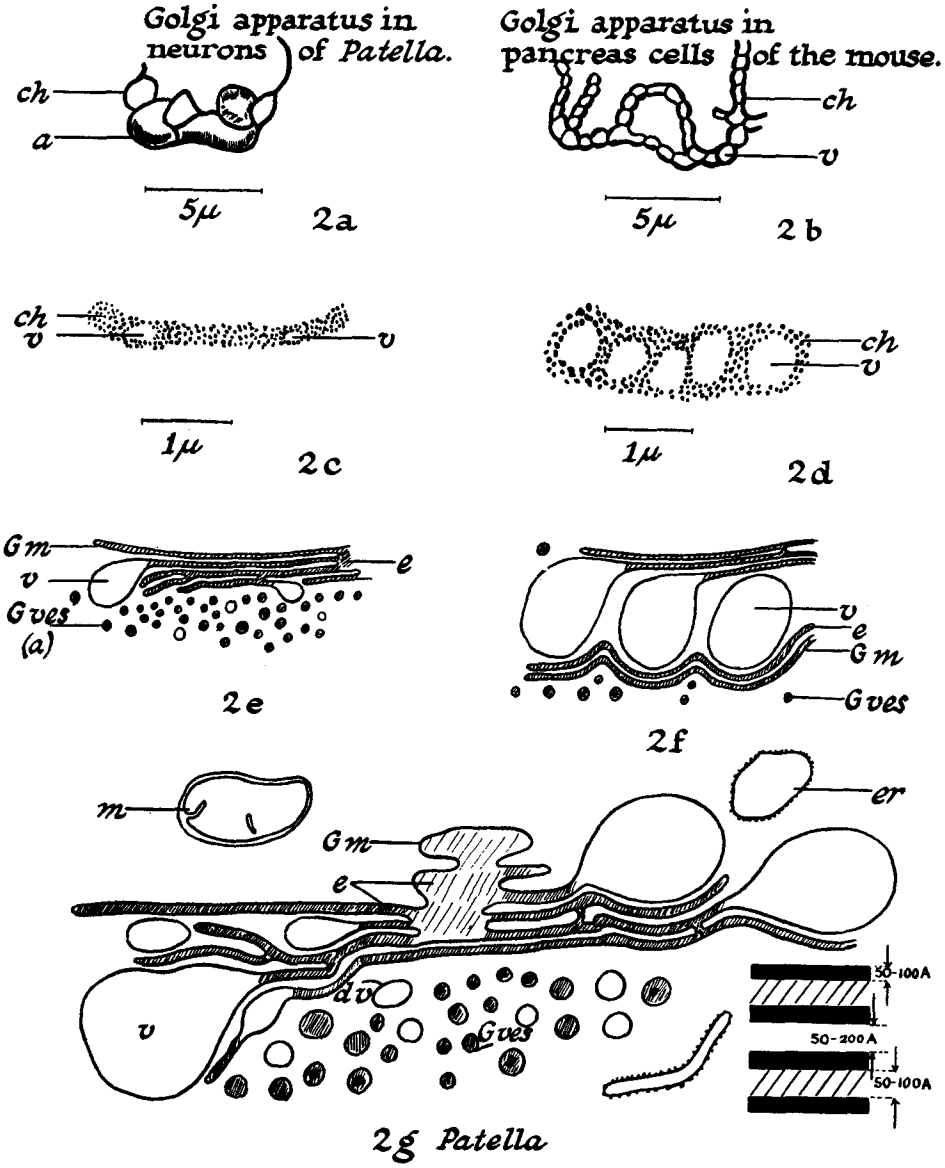
3. The thickness of the membranous structures is generally similar to that shown by the Golgi filaments (0.2 μ to 0.4 μ thick).

4. The paired membranes (together with the substance they enclose) and associated vacuoles closely resemble the lamellae and vacuoles of the Golgi apparatus as seen in Kolatchev preparations examined by electron microscopy.

5. The fine structure of this organelle is very similar to that described for the Golgi apparatus of vertebrate cells (see Introduction for references).

Further examination of the fine structure of the Golgi apparatus in *Patella* neurons revealed that the paired membranes of any one group branched and were linked by occasional anastomoses. Moreover, several pairs of membranes could be traced back to a common source suggesting that they were simply folds of what was essentially a single Golgi membrane (Fig. 8, which is one of three micrographs in a focal series of the same field; Text-fig. 2 *g*).

Whether the Golgi membrane seen in any one part of the cell was continuous with similar membranes seen in other regions was not determined owing to the difficulty of examining large numbers of ultrathin serial sections. However, judging from the results by light microscopy, it seems probable that, at least in cells containing a network, there is continuity of the membranes.



TEXT-FIG. 2. *Golgi apparatus in neurons of Patella and a mammalian somatic cell as seen by light and electron microscopy.*

Text-figs. 2 *a* and 2 *b* reveal the apparent absence of chromophobic vacuoles in neurons and the lack of an archoplasm in pancreas cells.

Text-figs. 2 *c* and 2 *d* show that chromophobic vacuoles are part of the Golgi apparatus in neurons of *Patella*, but that they are too small to be seen by light microscopy.

Text-figs. 2 *e* and 2 *f* show the similarity in the ultrastructure of the Golgi apparatus in the two diverse types of cells. Comparison with Text-Figs. 2 *c* and 2 *d* reveal that the chromophilic material of the Golgi apparatus corresponds to the paired membranes (together with the substance they enclose) and the chromophobic vacuoles to an osmiophobic substance lying within dilations of the paired membranes. The archoplasm corresponds to the Golgi vesicles which are present in both regions of cells. They are generally much more numerous in *Patella* neurons than in pancreas cells.

Text-fig. 2 *g* is the author's interpretation of the structure of either discrete filaments (dictyosomes) or part of the network seen in *Patella* neurons. The paired membranes are interpreted as membrane folds, and the substance they enclose (shaded) is regarded as being in a continuous phase.

Text-fig. 2 *b* is based on the author's own observations (25).

Text-figs. 2 *d* and 2 *f* are based on the work of Lacy and Challice (26).

The Golgi membrane seemed in these preparations to enclose two substances. One of these was typically dense in appearance and has been briefly referred to above. The other, distinguished by its lesser density, was the substance of the vacuoles, also mentioned above. The dense substance lay within the narrow folds of the Golgi membrane and next to the membrane, while the less dense material lay within dilations of such folds. The dilations varied greatly in size but were rarely within the limits of resolution of the light microscope. In any one Golgi filament the inner dense substance constituted a continuous phase and the other substance a discontinuous one (Text-fig. 2 *g*). Lying between the folds of the Golgi membrane there was a material identified as the cytoplasmic matrix (as distinct from the ergastoplasm or endoplasmic reticulum).

The thickness of the Golgi membrane and the space between membranes of the same fold varied from about 50 A to 100 A. The space between adjacent folds varied from about 50 A to 200 A. As seen in section, the number of membrane folds in a single array (filament) varied from two to five.

The Golgi vesicles appeared to arise from the ends of the folded Golgi membrane. They ranged in size from about 300 A to 0.1 μ in diameter. The smallest ones were usually homogeneous in appearance, while the larger ones contained a core of dense material. The vesicles were often remarkably numerous and were not only found in close proximity to the membranes, but were scattered about much of the Golgi zone (Fig. 7).

It seems most probable that the dark material (enclosed by the networks or lying near to the discrete filaments) seen in Kolatchev preparations by light microscopy was due to the weak impregnation of the Golgi vesicles (Text-fig. 2). Discrete vacuoles, about 900 A in diameter, were seen amongst the Golgi vesicles. These probably originated as dilations of the membrane folds.

The fact that elongate profiles (as opposed to circular profiles) were nearly always observed seems to prove that the membranous component of the Golgi apparatus is most frequently arranged in the form of parallel sheets or lamellae and not as tubes. Occasionally, however, numerous circular profiles were observed. These were close together and formed an elongate or curved body (Fig. 9). Such circular profiles did not appear to be discrete Golgi vesicles (compare Fig. 7 with Fig. 9) and may be transverse sections of numerous tubular processes.

Observations on Epithelial Cells by Electron Microscopy

General.—The epithelial cells were identified by their conspicuous brush border which was composed of finger-like protrusions of their free surfaces. Each of these protrusions was bordered by two closely applied membranes. The free surface of the cells was sometimes invaginated to form short canals opening to the exterior. Very dense granules were seen amongst the elements of the brush border, suggesting that the cells were absorptive epithelial cells and

were possibly part of the gut epithelium. Similar very dense granules, each about 2 μ in diameter and bounded by a distinct membrane, were seen within each cell. Mitochondria were also observed.

Golgi Apparatus.—These cells each contained an organelle lying to one side of the nucleus with an ultrastructure similar to that shown by the Golgi apparatus in *Patella* neurons. This inclusion, therefore, is identified as the Golgi apparatus (Fig. 10).

Certain differences are seen in the general form of the Golgi apparatus in these epithelial cells as compared with that described for the Golgi apparatus in the neurons. In the epithelial cells the Golgi apparatus is a compact organelle, which, judging from the evidence by electron microscopy alone, is not either in the form of a network or as discrete filaments. The Golgi vacuoles are again seen to be dilations of the paired membranes, but are generally of larger size than seen in neurons. Some are within the limits of resolution of the light microscope. The Golgi vesicles are generally much less numerous than seen in neurons (compare Fig. 7 with Fig. 10).

DISCUSSION

It is evident from the present work that the Golgi apparatus in neurons of *Patella*, as seen by light microscopy, consists basically of filaments. These filaments usually anastomose to form an open network. In a few cells they remain discrete. Occasionally, a dark material is associated with the filaments. This material, as shown by the evidence from electron microscopy, is probably composed of Golgi vesicles and may be regarded either as part of the Golgi apparatus or a secretory product.

The filaments in the neurons of *Patella* are similar to the filaments, rods, batonets, dictyosomes, etc. seen in neurons of other gastropod animals and identified by various workers as true Golgi elements (36, 19, 21, 6, 28, 5, 29). Similarly, the dark, weakly osmiophilic material in the neurons of *Patella* may be identified with the archoplasm observed by Brambell and Gatenby (6), Moussa (29), Boyle (5), and Monné (28). The present results, therefore, are in agreement with the conclusions reached by the above mentioned workers.

Significant differences appear to exist between the general form and position of the Golgi apparatus in the neurons of *Patella* and the form and position of this organelle in the neurons of gastropod genera studied by the workers referred to above. In *Patella* the Golgi apparatus is usually present as a network lying to one side of the nucleus. On the other hand, in neurons of all previously studied gastropod genera (*Helix*, *Limnaea*, *Planorbis*, *Cerithium*, *Paludina*) the Golgi apparatus is typically in the form of discrete elements (often remarkably numerous) scattered around the nucleus (Text-figs. 1 *c* and *d*). There is, however, a close similarity in the form and position of the Golgi apparatus in young neurons of *Helix* and in small, possibly young, neurons of *Patella*.

According to Brambell and Gatenby (6), the Golgi apparatus in young neurons of *Helix* consists of filaments or rods lying close to each other to form a compact structure lying to one side of the nucleus. These workers state that during development the filaments separate from each other and gradually come to lie around the nucleus. As shown by Text-figs. 1 *a* and *b*, the Golgi apparatus in small neurons of *Patella* is very similar, both in its general appearance and position, to the Golgi apparatus seen in young cells by Brambell and Gatenby. It would appear from this that the main differences referred to in the preceding paragraph only emerge during development. In *Patella* the apparatus does not fragment and encircle the nucleus, but simply seems to extend into more of the cell body as the cell increases in size.

It is noteworthy that the Golgi apparatus has the same general form and position in the neurons of five genera of gastropods and yet is different in the neurons of the marine-dwelling *Patella*.

The view that the Golgi apparatus in neurons of Molluscs is an artifact (32–35) or is represented by lipochondria (42, 7, 44, 45, 18, 39) is not supported by the results obtained in this investigation. Probably the lipoidal bodies (spheroids) have been confused with the Golgi apparatus because they are revealed by Golgi methods. Further errors of identification (18) are also partially due to the fact that some of the lipoidal bodies, like the Golgi apparatus, consist of chromophilic and chromophobic components.

The results obtained in the study of epithelial cells of *Patella* are generally in support of "classical" views held by Krjukowa (20), Beams and King (4), and Monné (28). However, it must be emphasised that it seems doubtful whether rod-like bodies (dictyosomes) would be seen by impregnation techniques. Rather, it would seem that the structure would resemble the appearance of the Golgi apparatus as seen in intestinal epithelial cells of mammals (9).

Golgi Apparatus in Somatic Cells of Mammals and in Neurons of Patella

It has previously been demonstrated that when mammalian somatic cells are treated by Aoyama's silver techniques and examined both by light and electron microscopy, the Golgi apparatus consists of two components: chromophilic material enclosing chromophobic vacuoles (24–26). When similar cells were fixed in buffered osmium tetroxide solution and examined by electron microscopy, the chromophilic component was found to correspond to paired membranes together with a dense substance they enclosed. The chromophobic component was seen to correspond with an osmiophobic substance lying within dilations of the paired membranes. Lying next to the paired membranes were small vesicles of the kind described previously by Dalton and Felix (10), Sjöstrand and Hanzon (41), and others. These vesicles were not detected in cells prepared by Aoyama's method and examined by light microscopy

Examination of neurons of *Patella* prepared by Kolatchev's method and

examined by light microscopy has shown that the Golgi apparatus consists of two components: chromophilic filaments (strongly osmiophilic), and archoplasm (weakly osmiophilic). When similar preparations were examined by electron microscopy, it was found that the filaments consisted of a chromophilic material enclosing chromophobic vacuoles. When neurons fixed in buffered osmium tetroxide solution were examined, the chromophilic material was found to correspond to paired membranes (usually enclosing an inner dense substance) while the chromophobic vacuoles were seen to correspond to a substance lying within dilations of the paired membranes. Numerous small vesicles were observed next to the membranes and identified with the archoplasm of light preparations.

When all the above results are compared, it is apparent that the Golgi apparatus in neurons of *Patella* has exactly the same structure as the Golgi apparatus in somatic cells of mammals. The reason for the different appearance of the inclusions when seen by light microscopy is simply because (*a*) the chromophobic vacuoles in mammalian cells are generally visible by light microscopy, whereas they nearly all lie below the limits of resolution of the light microscope in neurons of *Patella*, and (*b*) the classical techniques do not reveal an archoplasm in mammalian somatic cells, probably because the Golgi vesicles are much less numerous than they are in the neurons of *Patella* (Text-figs. 2 *a* to 2 *f*).

There is, then, evidence to support the view that silver and osmium impregnation techniques are capable of revealing the same organelle, the Golgi apparatus, in the cells of animals belonging to the widely diverse phyla Chordata and Mollusca.

In this paper the paired membranes of each Golgi filament have been interpreted as folds (lamelliform or tubular) and branchings of essentially a single Golgi membrane. The evidence from light microscopy has shown that in many cells the Golgi filaments anastomose to form a network. Therefore, in such cells it seems probable that the Golgi membrane of any one filament is continuous with the membranes of other filaments. If this is true, then the inner dense substance enclosed by the membranes is not only in a continuous phase in each filament but also within the whole of the Golgi network. This concept of the structure of the Golgi apparatus in neurons of *Patella* is similar to that outlined by Palay and Palade (31) for the "agranular reticulum" of mammalian neurons. Palay and Palade felt it hazardous to identify the "agranular reticulum" with any previously described cytoplasmic organelle. However, they pointed out that the membranous elements of the agranular reticulum were similar to the Golgi membranes described by Dalton and Felix (10). Palay and Palade also stated that the membranes of the agranular reticulum showed small dilations and were associated with numerous small vesicles. There can be little doubt, therefore, that the agranular reticulum is, in fact, the Golgi apparatus of mammalian neurons. It would appear, therefore, that the Golgi apparatus is virtually identical morphologically in neurons of *Patella* and mammals.

SUMMARY

1. In view of widely diverse views held about the identity and structure of the Golgi apparatus in neurons of Mollusca, particularly gastropods, a study has been made on neurons of the common limpet, *Patella vulgata*, both by light and electron microscopy. A report is given also of observations made on epithelial cells of *Patella* by electron microscopy.

2. As revealed by Kolatchev's method, the Golgi apparatus in neurons consists basically of black filaments lying to one side of the nucleus. The filaments generally anastomose to form networks of various complexity. Rarely some cells contain only discrete filaments. Associated with some of the filaments is a weakly osmiophilic substance identified as archoplasm. Kolatchev's method also revealed spheroidal bodies (neutral red bodies, "lipochondria," etc.).

3. It has not been possible to demonstrate the Golgi apparatus using either iron-haematoxylin or Sudan black.

4. Examination of Kolatchev's preparations by electron microscopy has revealed that some of the Golgi filaments consist of chromophilic and chromophobic components. The chromophilic component consists of dense lamellae.

5. After fixation in buffered osmium tetroxide solution and examination by electron microscopy, it has been concluded that (*a*) the chromophilic component of the Golgi apparatus corresponds to a system of paired membranes (which usually enclose an inner dense substance), (*b*) the chromophobic component corresponds to a substance lying within small dilations of the paired membrane, and (*c*) the archoplasm corresponds to numerous small vesicles.

6. The paired membranes branch, anastomose, and can often be traced back to a common source. They are interpreted as lamelliform folds, and occasionally tubular processes, of essentially a single Golgi membrane. In cells containing a Golgi network it is suggested that the membrane extends through the whole of the apparatus in such a way that the substance it encloses may be regarded as being in a continuous phase.

7. Epithelial cells of *Patella* contain a juxtanuclear Golgi apparatus with an ultrastructure similar to that described for neurons.

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EXPLANATION OF PLATES

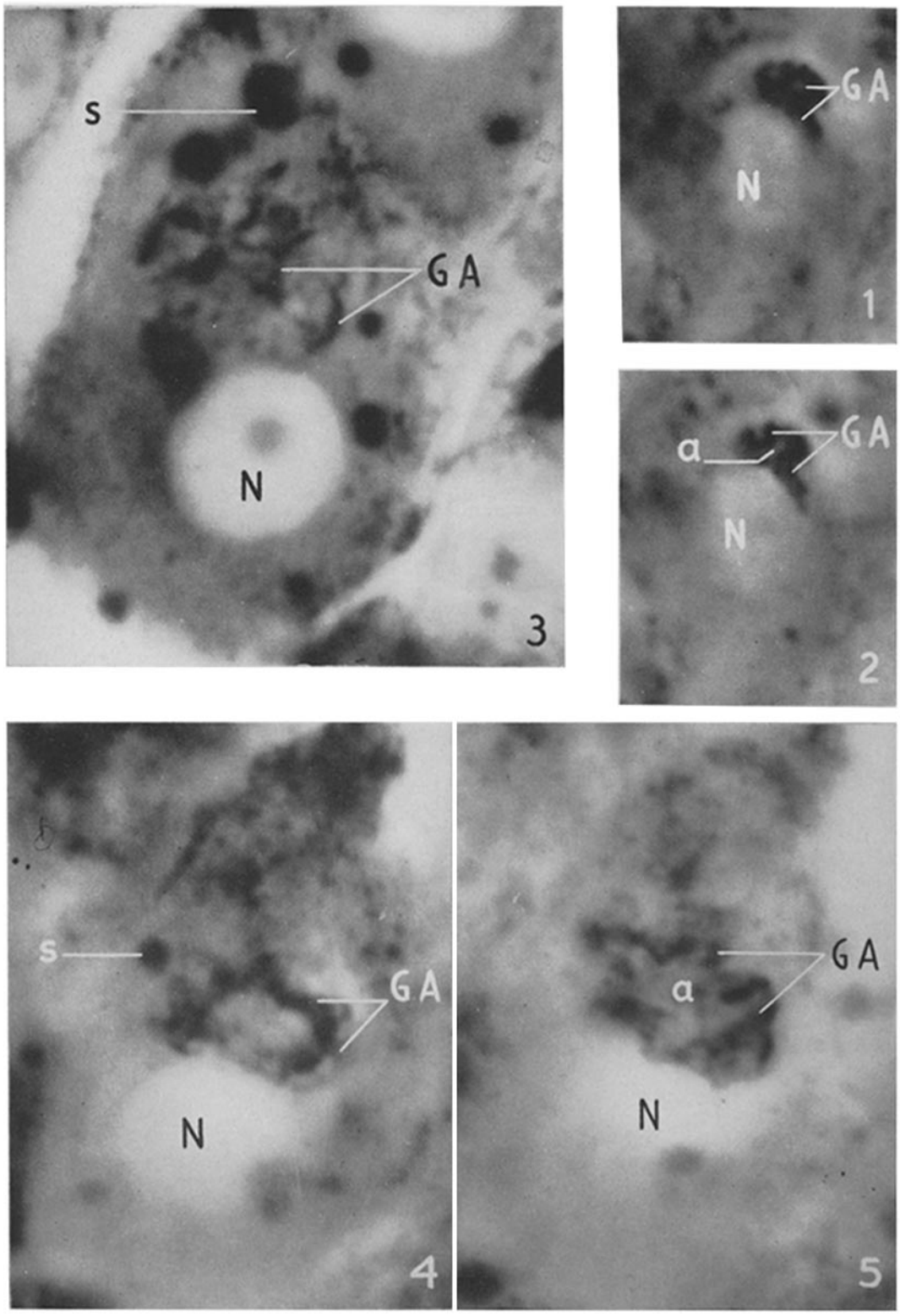
PLATE 246

FIGS. 1 to 5. Photomicrographs of neurons treated by Kolatchev's method.

FIGS. 1 and 2. Photomicrographs of the same small neuron taken at different focal planes. The Golgi apparatus (*GA*) is a small compact structure lying near the nucleus (*N*). *a*, archoplasm. × 4,800.

FIG. 3. The Golgi apparatus (*GA*) in a large neuron. The organelle is mainly in the form of an open network, although some discrete Golgi filaments are also present. Part of the organelle lies next to the nucleus (*N*), but the rest extends into much of the cell body. Spheroidal bodies (*s*) have also been revealed by the technique, but are readily distinguished from the Golgi apparatus. × 5,000.

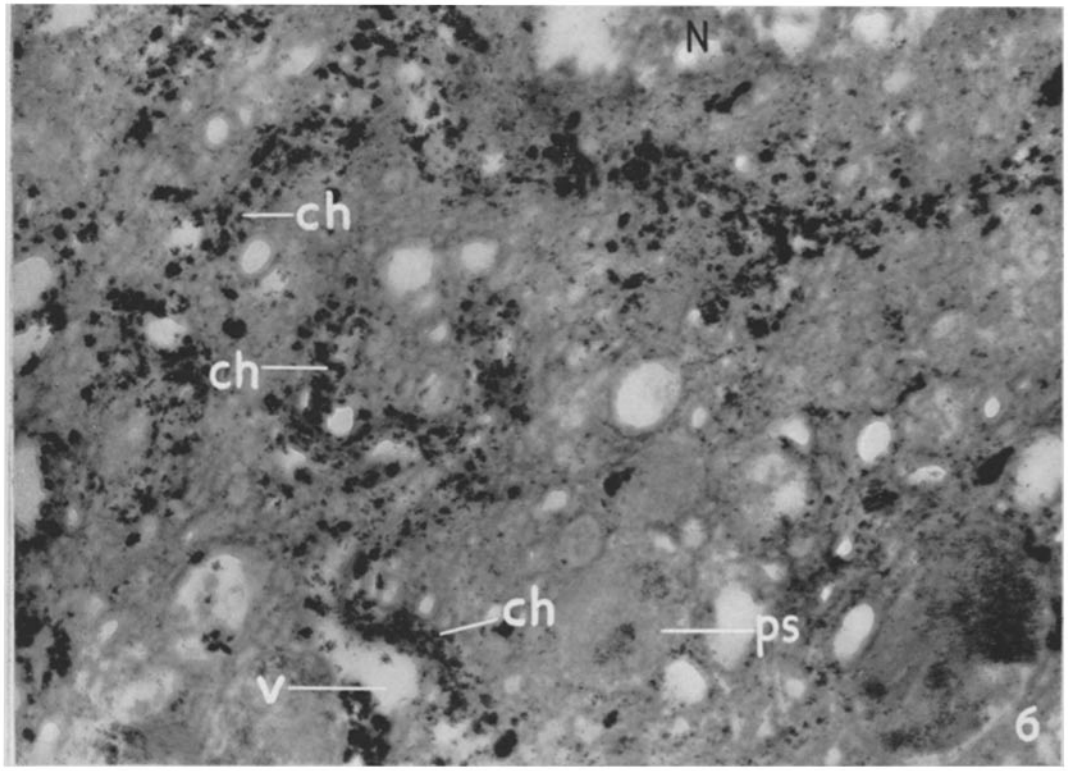
FIGS. 4 and 5. Photomicrographs of the same large neuron as seen at different focal levels. The Golgi apparatus (*GA*) is in the form of a more complex network than shown in the previous figure. Associated with parts of the network is a weakly osmiophilic substance identified as archoplasm (*a*). This substance appears darker than the surrounding cytoplasm but less dark than the filaments of the network. It can be seen clearly in Fig. 5. × 5,000.

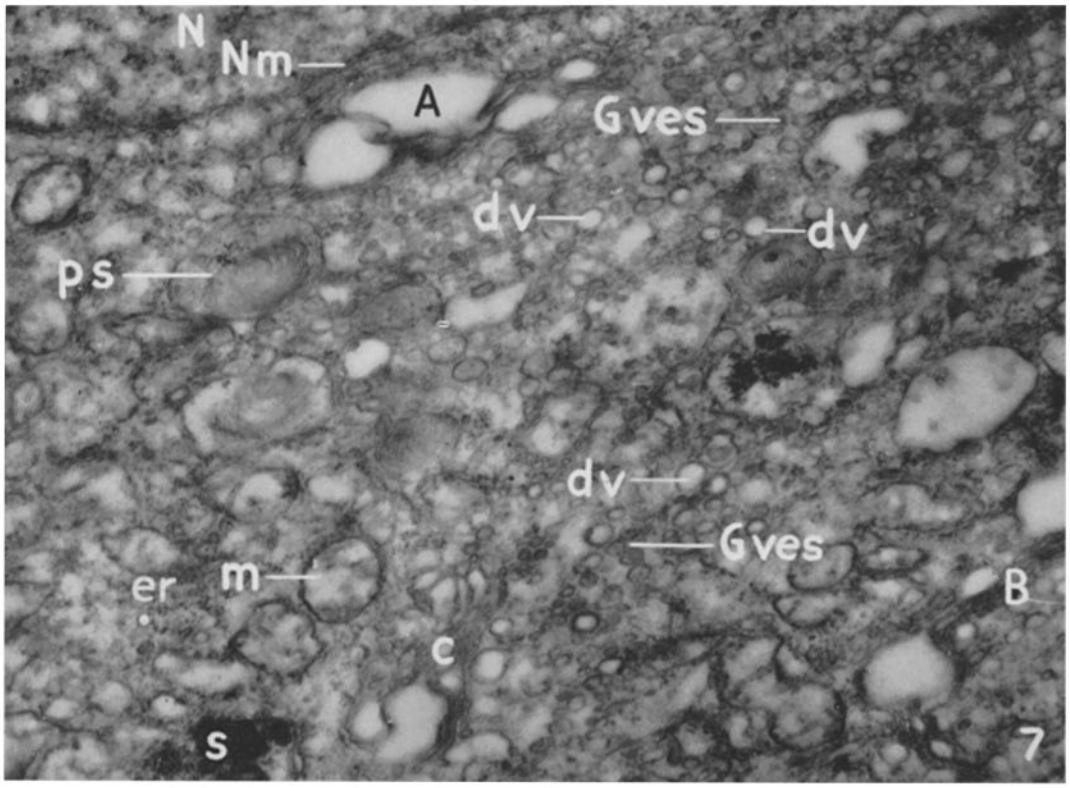


(Lacy: Golgi apparatus in neurons)

FIG. 6. Electron micrograph showing part of the Golgi zone of a medium sized neuron treated by Kolatchev's method. Some of the Golgi filaments (present either as discrete elements or as part of a network) seen in light microscopical preparations are now shown to consist of a chromophilic component (*ch*) blackened with osmium (or osmium oxides) enclosing chromophobic vacuoles (*v*). Several partially impregnated inclusions can be seen within which are traces of an internal coiled membrane (as in inclusion *ps*). These inclusions appear to be of the same kind as those labelled *ps* in Fig. 7 below. *N*, nucleus. × 24,300.

FIG. 7. Electron micrograph of part of the cell body of a neuron fixed in buffered osmium tetroxide solution. The region shown is the same as that illustrated in Fig. 6, *i.e.* a region lying to one side of the nucleus (*N*) and which, as shown also in Figs. 1 to 5, contains the Golgi apparatus. In this micrograph can be seen apparently discrete groups of paired membranes with intercalated "vacuoles" (as at ABC); masses of small vesicles (*G ves*); and some discrete vacuoles (*dv*). Detailed comparison with Kolatchev preparations shows that the paired membranes and intercalated "vacuoles" correspond respectively to chromophilic and chromophobic components of the Golgi apparatus. The small vesicles probably account for the "archoplasm" seen by light microscopy. At one point the outermost membrane of the Golgi filament *A* is only about 100 A from the outer nuclear membrane (*Nm*). Mitochondria (*m*), a spheroidal body (*s*), and inclusions (*ps*), containing an inner coiled membrane, can be seen within or around the Golgi zone. The latter inclusions may be the secretory antecedents of the spheroidal bodies. The ergastoplasm (*er*) is concentrated mainly towards the periphery of the cell body (on this scale the cell membrane lies about 25 mm. from the bottom of the micrograph). × 29,400.



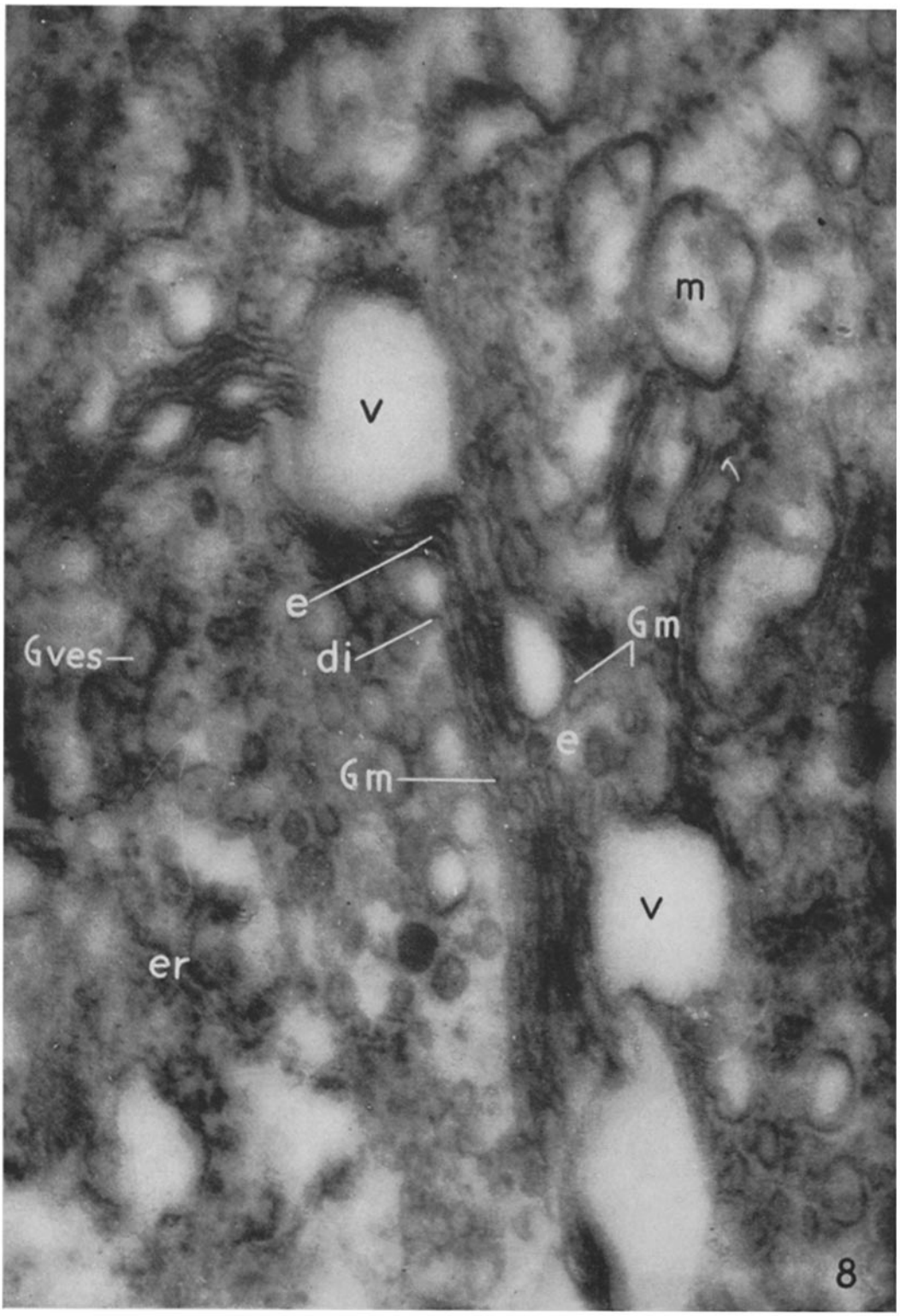


(Lacy: Golgi apparatus in neurons)

PLATE 248

FIG. 8. Micrograph showing in detail the ultrastructure of the Golgi filament marked *B* in Plate 247, Fig. 7.

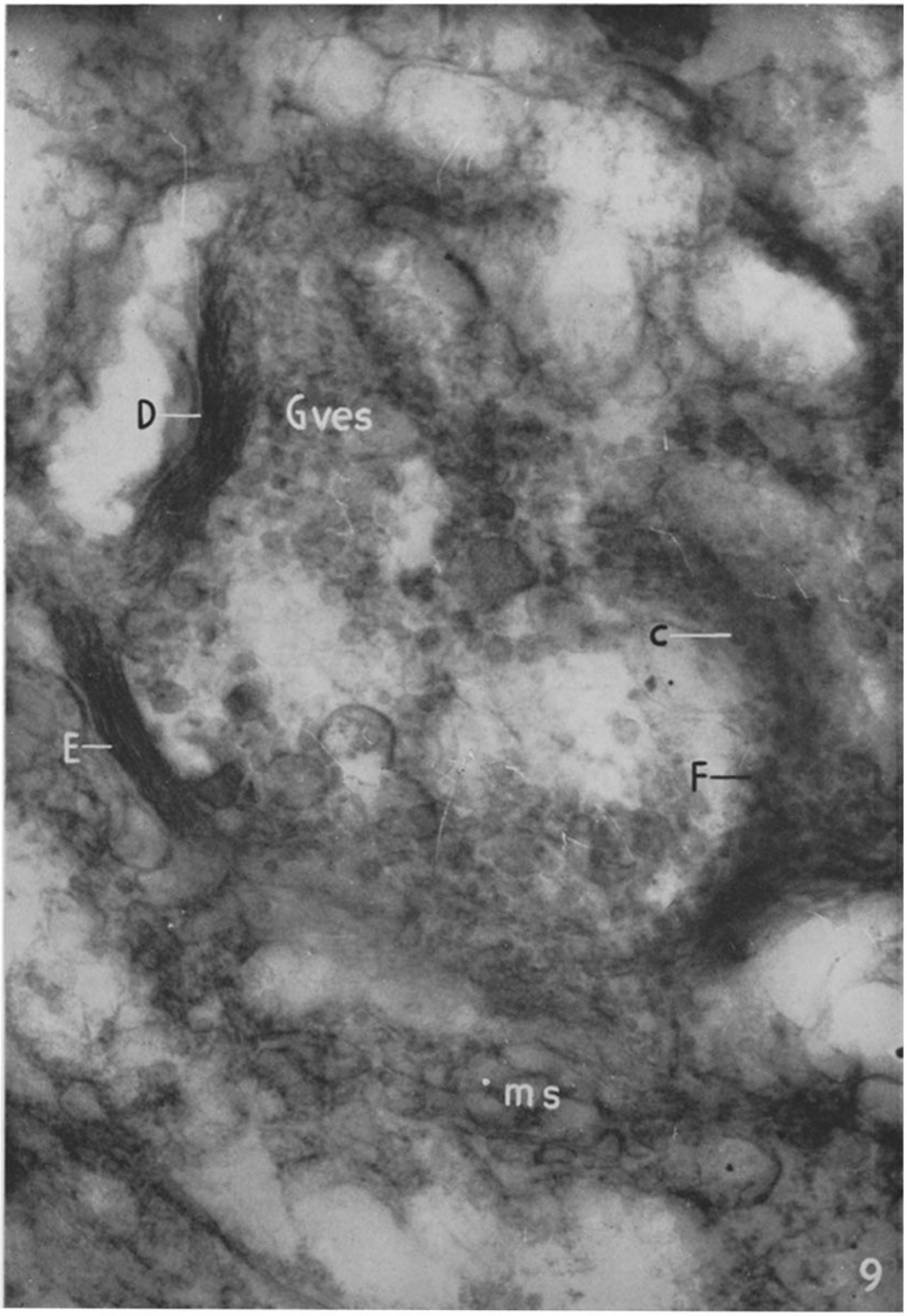
Several of the paired membranes can be traced back to a common source and are interpreted as folds of essentially a single Golgi membrane (*Gm*). The intercalated "vacuoles" referred to in Fig. 7 are now seen to be due to an osmiophobic substance (*v*) lying within dilations (*di*) of the membrane folds. A further substance (*e*) is enclosed by the Golgi membrane. This micrograph is one of three in a through focus series taken of the same field and at an initial magnification of 20,000. All three micrographs show that several of the paired membranes originate from a common source. The three micrographs show also that the substance enclosed by the Golgi membrane varies in its density at different foci. Golgi vesicles (*G ves*), mitochondria (*m*), and parts of the ergastoplasm (*er*) can be seen. × 92,000.



(Lacy: Golgi apparatus in neurons)

PLATE 249

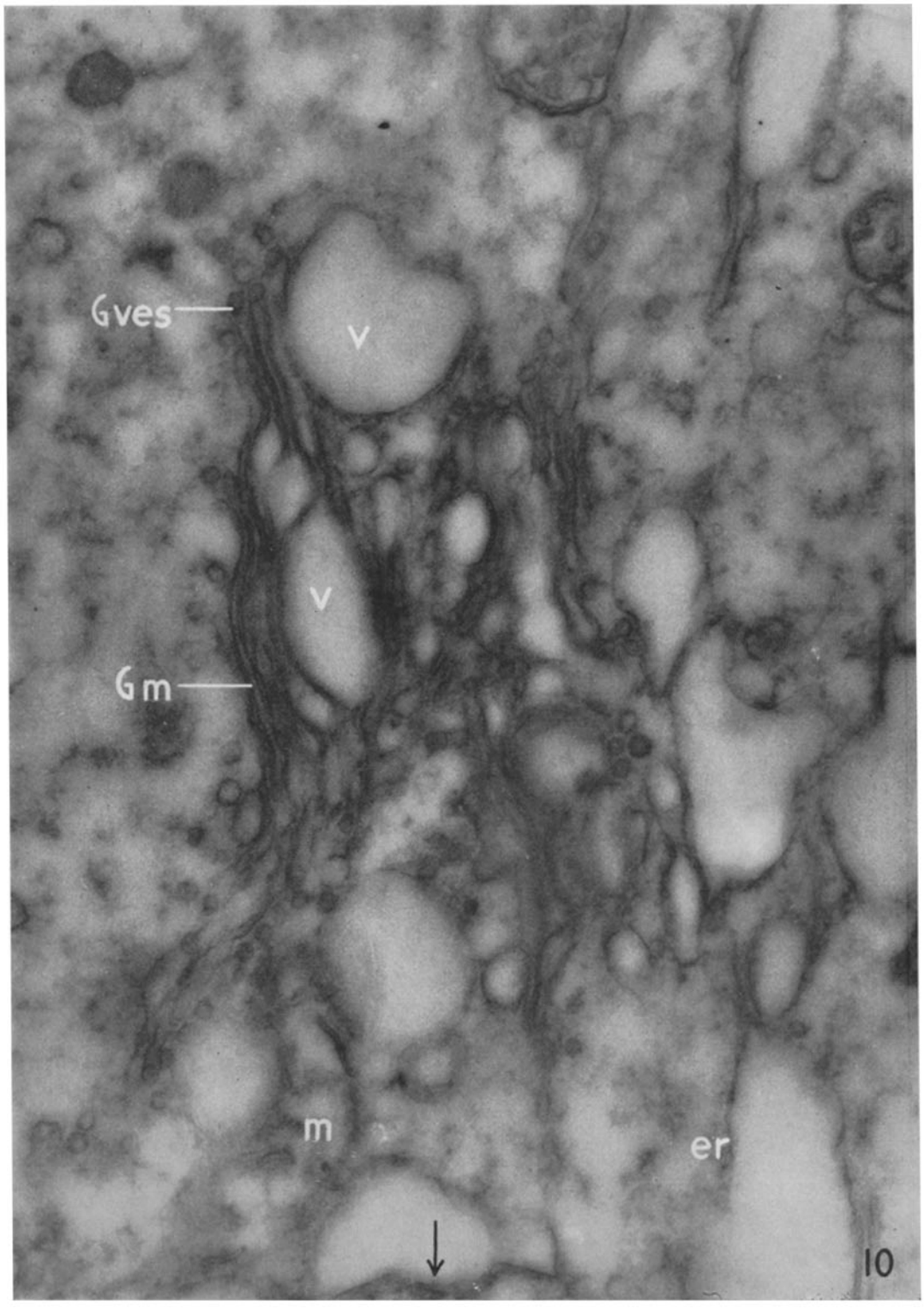
FIG. 9. Micrograph showing three Golgi filaments in part of a small neuron lying in the medullary region of a ganglion. In general appearance they closely resemble the "classical" curved or banana-shaped form of Golgi dictyosomes. Filaments *D* and *E* are composed of several pairs of parallel membranes which lack the dilations seen in the preceding figure (Fig. 8). Filament *F* is composed mainly of circular profiles (as at *c*) and some membranes. The circular profiles may be transverse sections of Golgi membranes arranged in the forms of tubes, although usually they are present as lamellae. Numerous Golgi vesicles (*G ves*), and a further membrane system (*ms*), can be seen. Tissue fixed in buffered osmium tetroxide solution. × 46,500.



(Lacy: Golgi apparatus in neurons)

PLATE 250

FIG. 10. Electron micrograph of an organelle identified as the Golgi apparatus in an epithelial cell fixed in buffered osmium tetroxide solution. It lies to one side of the nucleus (the position of the nucleus is shown by the arrow) and has an ultrastructure similar to that described for the Golgi apparatus of neurons. There are, however, certain differences in its general appearance as compared with the Golgi apparatus of neurons. These are (1) its compact form, (2) the relatively large amounts of osmiophobic substance (*v*), and (3) the presence of only a few Golgi vesicles (*G ves*). *m*, mitochondria, *er*, ergastoplasm. *Gm*, Golgi membranes. × 52,700.



(Lacy: Golgi apparatus in neurons)