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Fine Structure of Cytoplasm: The Organization of Membranous Layers

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WHEN climbing the ladder of levels of organization of living matter, one finds nowadays that at least indications of steps are present along the whole ladder. Some steps may be weak and might break when exposed to too critical attention. Light microscopy has furnished information regarding a great number of subcellular structural elements, the so-called cell organelles, as well as of cytoplasmic differentiations of a more diffuse character. The most impressive of the cell organelles which are localized in the cytoplasm are the mitochondria and the Golgi apparatus. Basophilic regions of the cytoplasm represent more-diffusely distributed parts which long ago were called the ergastoplasm by Garnier. The reason for this term was the idea that these regions were especially active parts of the cytoplasm. In the exocrine pancreas cell, a filamentous or lamellar structure was observed within the basophilic regions of the cytoplasm, "die Basalfilamenten" of Heidenhain, or "die Basallamellen" of Zimmermann. These various components of the cytoplasm were considered immersed in the ground substance of the cytoplasm, a component which was assumed to lack any higher degree of specialized organization.

The submicroscopic organization of the regularly occurring cell organelles was completely unknown, however. A few cell components, such as the myelin sheath of peripheral nerve fibers and the outer segments of the retinal receptors of the vertebrate eye, were assumed to consist of alternating layers of lipid and protein molecules. This assumption was based upon polarization-optical data of these strongly birefringent components.

Electron microscopy has made possible the fairly close analysis of the submicroscopical organization of these various components. In some cases, the combination of electron-microscope data with polarization-optical and x-ray diffraction data has made it possible to propose models containing some features of the molecular architecture of these components. In this latter respect, the results can be considered rather crude.

On the other hand, electron microscopy has not revealed any definite new structural component of the cytoplasm. It has helped in bridging the gap between the molecular and the organizational levels of the cell organelles by making supramolecular elements available for direct observation.

The most striking feature of the ultrastructural organization of the cytoplasm is the frequent occurrence of membranous components in various cell organelles, as well as in the ground substance of the cytoplasm. Membranes of various dimensions and different organization appear to represent a common and basic principle of organization in the cytoplasm.

Some examples of such membranous elements are presented here, together with some arguments which justify an interpretation of some of the structural patterns as reflecting a certain molecular structure of the membranes. Also, the probability that the observed structural patterns represent preformed patterns existing in the intact living cell is discussed. Finally, some ideas are presented regarding the functional significance of the various membranous elements.

When evaluating the structural patterns observed by means of the electron microscope, it is wise to account for a certain degree of deformation introduced by preparatory techniques. When comparing two patterns, sometimes one pattern easily might be imagined as derived from the other as a result of disorganization or deformation. In such a case, the second pattern is selected as probably more directly related to the *in vivo* pattern. When a certain variation in the patterns is observed, the most frequently occurring pattern is selected as the most representative.

The chapter on the biochemistry of mitochondria by Lehninger (p. 136) makes it quite natural to start this survey with some electron micrographs of mitochondria. For this purpose, mitochondria may be chosen from almost any type of cell. Let us choose those of the retinal receptors of the eye. The first figure shows a schematic drawing of the rod type of receptor in the guinea pig retina. These receptors are segmented cells with each segment structurally organized in a characteristic and different way (Fig. 1). The outer segments which are most remote from the pupil of the eye contain the photochemically active molecules, such as rhodopsin in the rod cells. Here, the primary reactions in converting electromagnetic energy into chemical energy take place. The inner segment contains all of the mitochondria of the receptor and can be looked upon, therefore, as the energy-generating center of the cell.

The rod and cone fibers connecting the inner segment with the synaptic bodies are organized like unmyelinated nerve fibers. The vitreous end of the receptor cell forms the synaptic body where the synaptic contacts between receptors and nerve cells are located.

Figures 2 and 3 picture the inner segment, *IS*, of a cone cell in the perch retina. Most of the inner segment consists of a dense aggregation of closely packed mitochondria which together form the so-called ellipsoid.

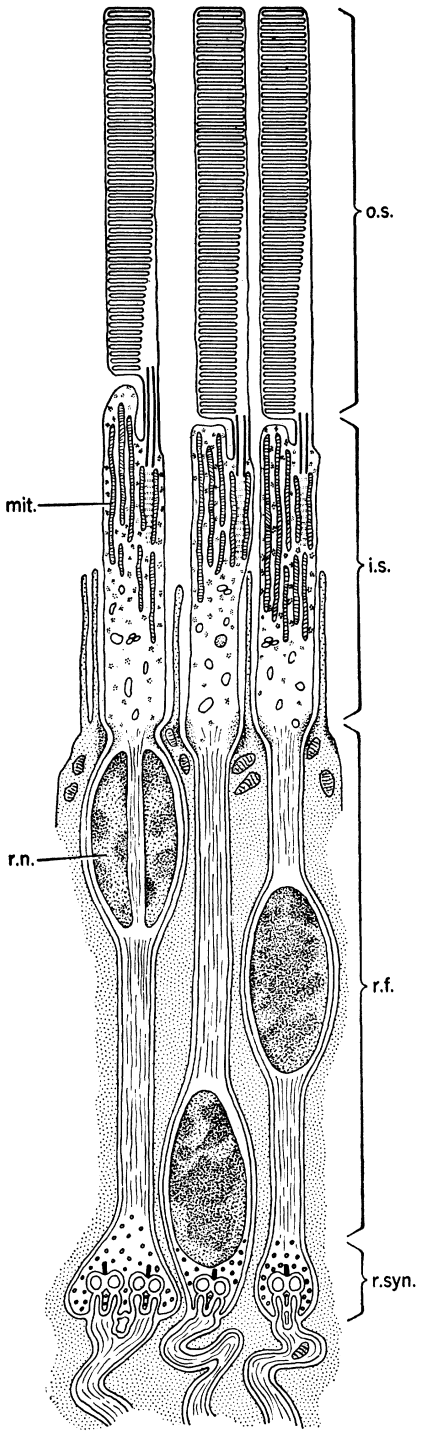

FIG. 1. Schematic drawing of retinal rods in the guinea pig eye. *o.s.*, outer segment; *i.s.*, inner segment; *r.f.*, rod fiber; *r.syn.*, rod synaptic body; *mit.*, mitochondria; *r.n.*, rod nucleus [from F. S. Sjöstrand, Intern. Rev. Cytol. **5**, 455 (1956)].

Each mitochondrion is bounded by a surface membrane, and a great number of inner membranes or platelets are oriented in a fairly parallel fashion in the interior. All of these membranes appear to be triple layered.[1–4] It is striking to note the rather constant spacing of the layers of these mitochondria membranes.

In 1952, when we first presented our structural model of mitochondria, Palade[5] proposed a different model consisting of a single-layered surface membrane which showed local infoldings which he called "cristae mitochondriales." These cristae left a central space in the mitochondrion free. After confirming our observations on the triple-layered character of the surface membrane, Palade changed his model by enveloping his original model in a peripheral opaque layer. He assumes[6] that this layer represents the surface membrane of the mitochondrion and that an outer space or chamber extends between the two opaque layers located at the surface of the mitochondrion. This space is continuous with spaces which extend in the cristae. An inner mitochondrion chamber forms a continuous central space. This interpretation differs from our own.

Our interpretation of the mitochondrial pattern is based upon certain observations that we had made on some typical lipoprotein systems—namely, the outer segments of retinal receptors[7,8] and the myelin sheath of peripheral nerves.[9,10] In both cases, polarization-optical data, and, in the myelin sheath, x-ray diffraction data had permitted the proposal of models showing alternating layers of lipid and protein molecules. X-ray diffraction data revealed some dimensions for the thickness of the layers in the myelin sheath and of the double layer of dried, mixed nerve lipids. For the latter, a value of 67.4 A was obtained.[11]

A discussion of the observations made on the outer segments of retinal receptors gives a necessary background for our interpretation of the mitochondrial pattern. In electron micrographs of sections (Figs. 4–6) oriented parallel to the long axis of the outer segments of rods and cones, alternating light layers and pairs of opaque layers are seen, the two opaque layers of a pair being fused along their rims. They thus bound a light interspace which, in guinea pig rods, has a thickness of 70 to 80 A. These pairs of layers form triple-layered disks, the total thickness of which varies from 100 to 250 A with the species and with the type of receptor, but remains constant for any one type of receptor. These disks are the unit structure of the outer segments.

The outer segments are 40% lipid in content.[12] More than 80% of these are phospholipids. The polarization-optical data have revealed that presumably the lipid molecules are oriented parallel to the long axis of the outer segment, and that the protein molecules form transversely oriented layers.[13]

When fragmenting isolated outer segments, it is possible to obtain round disks of varying thicknesses and also fragments of such disks.[7] The thicknesses of the disks isolated from the guinea pig retina are multiples of 140 A—that is, multiples of the thickness of the triple-layered unit disk which can be observed in sections and which measures 140 A in thickness in the

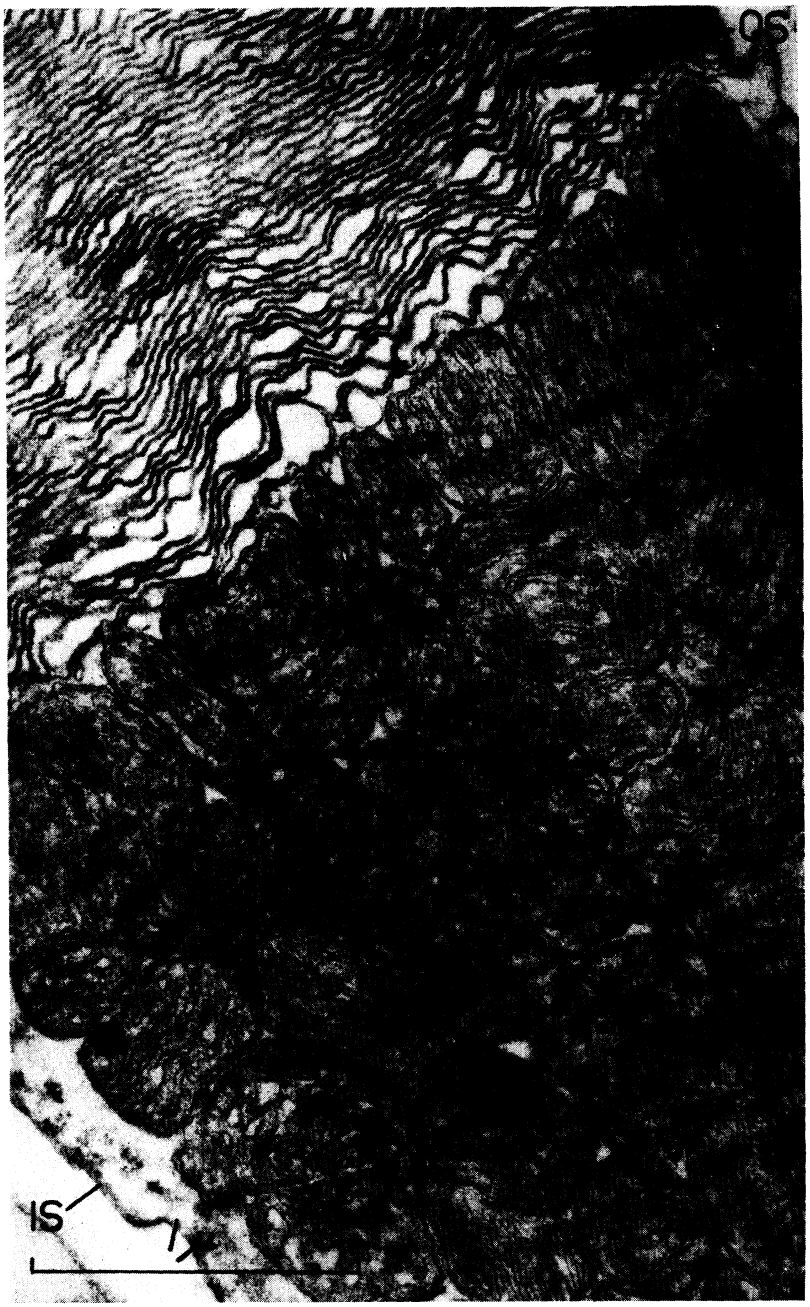

FIG. 2. Survey picture of the boundary between the outer, *OS*, and inner, *IS*, segments of a cone cell in the perch retina. Most of the inner segment consists of a dense aggregation of mitochondria [from F. S. Sjöstrand, *Ergebnisse der Biologie* (Springer-Verlag, Berlin, 1958), Vol. XXI, p. 128]. ×44 000.

guinea pig retina. The thickness of the thinnest membrane fragments is about 30 A. These fragments are osmiophilic. The thickness of the osmiophilic layers of the unit disks as observed in sections through guinea pig retinas is 30 to 40 A. There seem to be no doubt that these components in fragmented and in sectioned material are identical. The 140-A thick disks which were obtained by fragmentation could be demonstrated to consist clearly of two thin membranes, and under favorable conditions they could be split completely into two thinner components with a thickness of 70 A at the edge.

The fact that the thicknesses of the various disks obtained by fragmentation represent multiples of 140 A makes it justifiable to conclude that, when the outer segments are fragmented in piles of unit disks of different thicknesses and when these piles are dried on a supporting film, the interspaces between the triple-layered disks contribute insignificantly to the thicknesses of the piles. This means that these interspaces

FIG. 3. Higher magnification of a region at the border between the inner and outer segments of a cone cell in the perch retina. Several mitochondria are observed densely aggregated. The triple-layered surface membrane and inner membranes show certain similarities to the triple-layered disks of the outer segment [from F. S. Sjöstrand, *Ergebnisse der Biologie* (Springer-Verlag, Berlin, 1958), Vol. XXI, p. 128]. ×65 000.

are filled mainly with an aqueous, ionic medium containing little or no lipids. The lipid molecules, therefore, are located presumably in the triple-layered disks. Their localization (Fig. 7) in the less-opaque space bounded by the 30- to 40-A thick osmiophilic layers was assumed because the dimension of this space, 70 to 80 A, could well accommodate a double layer of lipid molecules. Furthermore, the osmiophilic layers had shown a rather remarkable tensile strength in the fragmentation experiments which appeared to point to a protein nature. The total volumes of the opaque layers and of the spaces surrounded by these layers are estimated to be about equal. This observation is in accordance with the determined figure of 40% for the concentration of lipids in percent of dry weight.

The similarity of the mitochondrial pattern and that of the outer segments of retinal receptors is rather striking. Values as high as 40% were reported for the lipid content of mitochondria, and they showed positive birefringence after freeze-drying,[14] which could be assumed to be due to oriented lipid molecules. The triple-layered membranes of the mitochondria were interpreted as representing two protein layers sandwiching a double layer of lipid molecules (Fig. 8). This interpretation is based upon a series of evaluations of indirect evidence and upon a generalization which well might be erroneous.

The myelin sheaths of peripheral nerves also show a characteristic layered structure. Furthermore, peripheral nerves can be fragmented into thin membranous fragments.[15,16] In this instance, polarization-optical data, as interpreted by Schmidt,[13] revealed that the lipid molecules were oriented radially and that protein molecules formed concentric layers around the axis cylinder. X-ray diffraction data by Bear, Palmer, and Schmitt[11] were interpreted as showing a repeat period of 171 A in amphibian nerves, and 185 A in mammalian nerves. The repeat period was interpreted as containing two bimolecular leaflets of lipids, each about 67 A thick, and a layer of proteins about 25 A thick. A concentrically layered structure could be observed in the electron micrographs, obtained in 1952,[9,10] of osmium-fixed, sectioned peripheral nerves. Opaque layers about 25 A thick are arranged concentrically in the myelin sheath with a definite periodicity. In each period, a fainter opaque line is seen to halve the main period. The mean periodicity was estimated at 120 A in the electron micrographs of 20 nerve fibers.

Although the x-ray diffraction and the electron-microscope data did not coincide as to the length of the period, the over-all scheme predicted from the former data appears to be pretty well confirmed by the latter. From the dimensions, it was concluded that the opaque layers correspond to the protein layers, and the less opaque layers to bimolecular leaflets of lipids. The recent extensive work by Fernández-Morán and Finean[17] on this problem seems to support this interpretation.

A membranous component which is frequently present in the basophilic regions of the cytoplasm is discussed next. These membranes are particularly abundant in the excretory pancreas cells, the salivary gland cells, the plasma cells, and other types of cells in which a somewhat intense protein synthesis takes place.

In the exocrine pancreas cells (Fig. 9), most of the ground substance of the cytoplasm is filled with this type of membrane.[1,18] In osmium-fixed material, the membrane is easily identified by the numerous opaque particles that are attached to one surface. These particles have an average diameter of 150 A. Their form varies; the particles are more or less irregularly, angularly shaped. The membrane to which these particles are attached consists of an osmiophilic component which is about 40 A thick. These membranes are arranged in pairs and a pair bounds a narrow space or, as in the thyroid epithelium, rather large, irregularly shaped spaces. They thus appear to divide the cytoplasm into two different parts or compartments.[19]

Opaque particles of identical appearance, as those attached to the membranes, frequently occur free in the cytoplasm. Palade and Siekevitz[20] clearly demonstrated that the opaque particles are responsible for the RNA content of the microsome fraction from pancreas tissue. The terms microsomes, ribosomes, or microsomal

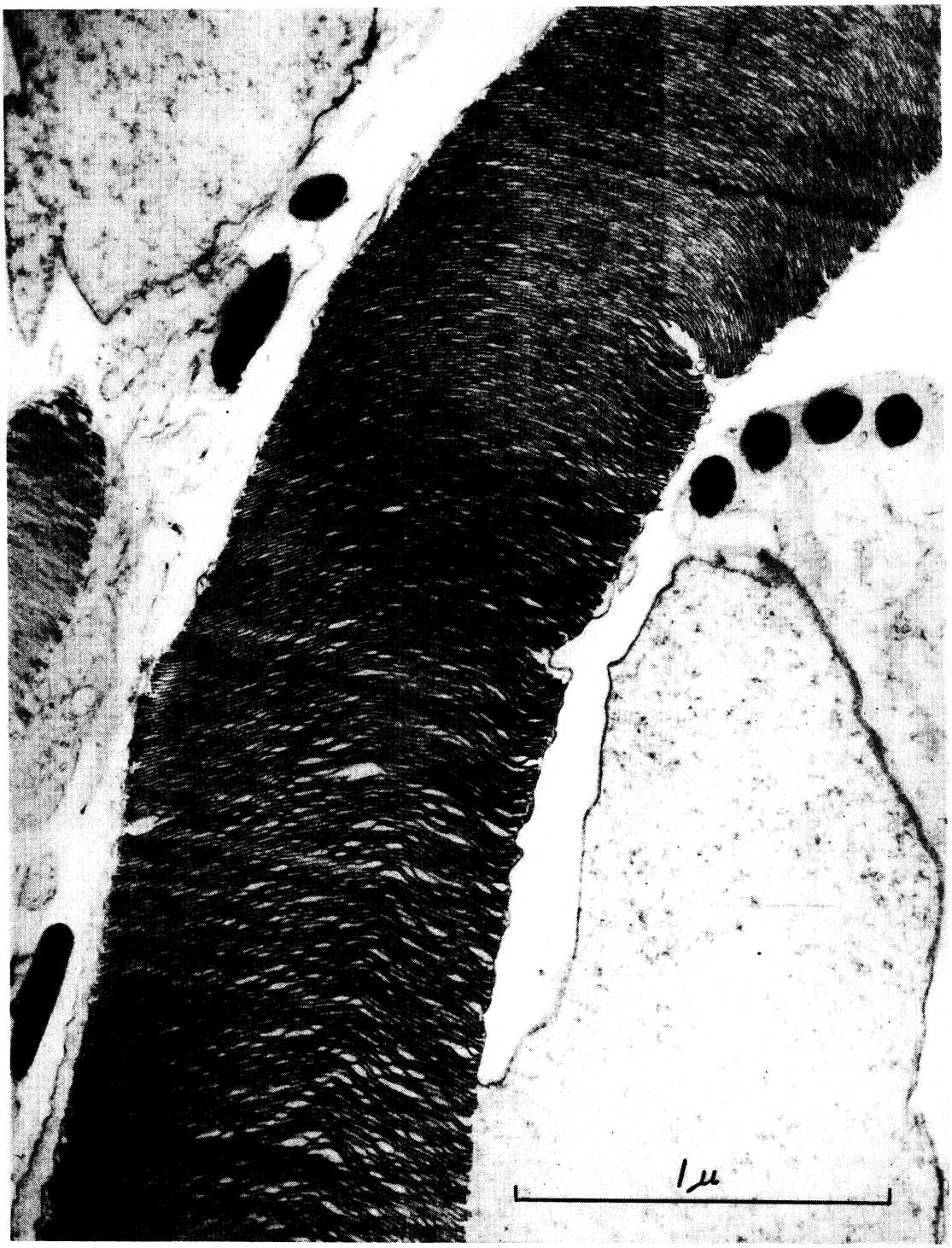

FIG. 4. Longitudinal section through the outer segment of a retinal rod cell from the perch eye showing the uniformly layered structure of the outer segment. ×59 000.

particles now seem to be used to classify this component which represents only a minor part of the classical microsome fraction.

At this point, it seems justifiable to make some comments on what is known about the fixation and embedding artifacts that are introduced when fixing

FIG. 5. Longitudinal section through a retinal-rod cell of the perch eye showing the triple-layered disks, which represent the elementary component of the outer segment [from F. S. Sjöstrand, *Ergebnisse der Biologie* (Springer-Verlag, Berlin, 1958), Vol. XXI, p. 128]. ×82 000.

and embedding labile living matter. The tissue is fixed in a solution of osmium tetroxide, dehydrated in a graded series of concentrations of ethyl alcohol, and then transferred to methacrylate which is polymerized before the tissue can be sectioned.

At the beginning, we were very much concerned about this problem and, therefore, we intentionally chose to analyze such structural components about which a little was known from an ultrastructural point of view through polarization-optical and x-ray diffraction analysis. In the outer segments of retinal receptors and in the myelin sheath, patterns in complete harmony with polarization-optical data obtained from fresh, unfixed material were observed by means of electron microscopy, as mentioned in the foregoing. Regarding the exocrine pancreas cells, there were no data available regarding birefringence of the cytoplasm *in vivo*. Living exocrine pancreas cells in mice were analyzed in polarized light and we found that the cytoplasm was birefringent.[1] When analyzed, this birefringence proved to be negative with the axis oriented perpendicularly to the cell membrane, a result that could fit in very well with the electron-microscope picture.

When the direct study of living material is excluded, the most rational way of checking the reliability of such a preparatory technique is to change the technique so radically that it appears unlikely that the two techniques could produce identical distortions. The second preferred preparatory technique is then preservation by means of freeze-drying. In fact, we proceeded in the opposite direction and studied frozen-dried material first[1] and later checked the observations made on exocrine pancreas cells by means of osmium fixation and birefringence analysis.

The freeze-drying technique is a rather delicate procedure with many pitfalls. The observations made on frozen-dried, sectioned material, stained with phosphotungstic acid, were rather surprising.[21] Similar structural patterns could be observed in the ground substance of the cytoplasm as well as in the mitochondria, with the remarkable exception that the patterns appeared as negative patterns, as compared to those obtained after osmium fixation (Figs. 10 and 11). These results mean that these patterns probably are preformed and exist in the living cell. At the same time, however, they may make it necessary to re-evaluate the interpretation of the molecular structure assumed to be responsible for these patterns.

One feature of the frozen-dried specimen must be noted. The so-called RNA particles do not show up.

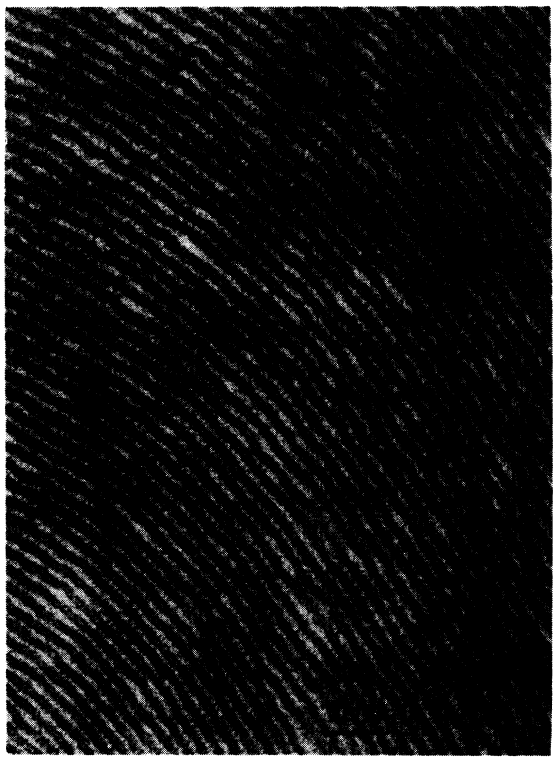

FIG. 6. Section oriented perpendicularly to the triple-layered disks of the outer segment of a cone cell in the perch eye. Notice the tendency to a uniform blackening of the whole triple-layered disk due to a staining of the middle layer [from F. S. Sjöstrand, *Ergebnisse der Biologie* (Springer-Verlag, Berlin, 1958), Vol. XXI, p. 128]. ×87 000.

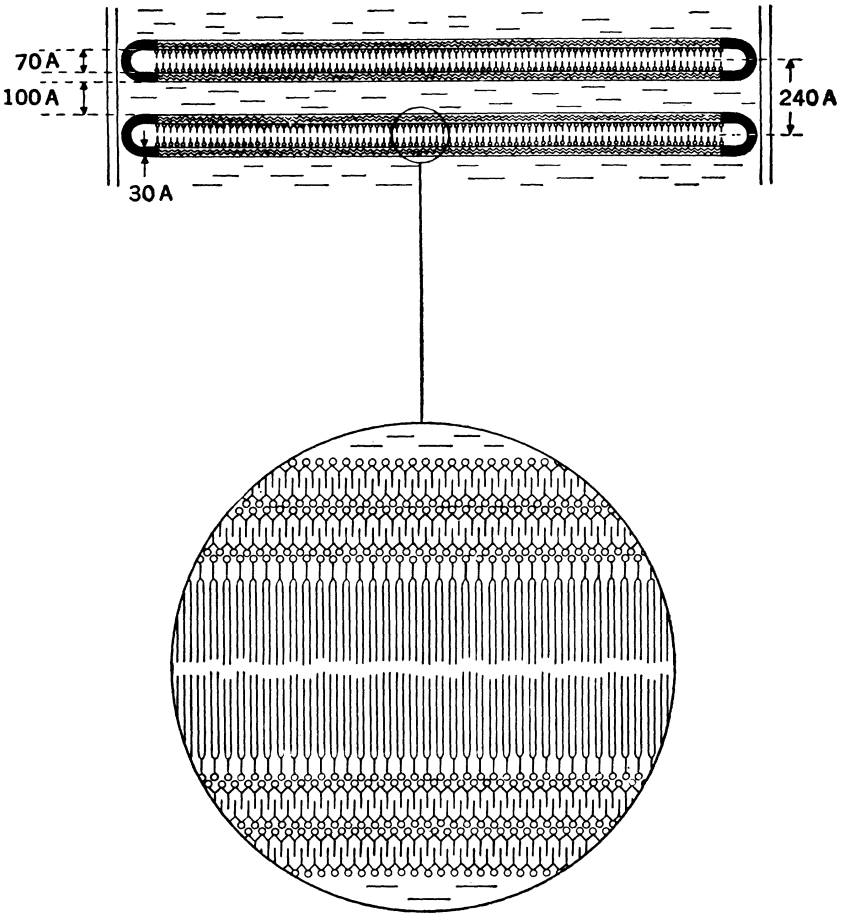

FIG. 7. Schematic drawing to illustrate a proposed interpretation of the localization and orientation of lipid and protein molecules in the triple-layered elementary disks of the outer segments of retinal receptors [from F. S. Sjöstrand, *Ergebnisse der Biologie* (Springer-Verlag, Berlin, 1958), Vol. XXI, p. 128].

The cytoplasm between the cytoplasmic membranes appears homogeneous even after long periods of staining. One cannot exclude, therefore, the possibility that these particles are artifacts formed through an aggregation or precipitation in connection with the preparatory procedure. This possibility has to be tested more extensively than has been done to date.

Even if some doubts can be raised regarding the particulate form of the RNA-protein components in the cytoplasm of intact living cells, there is little or no doubt that these particles indicate the approximate location of RNA in the cytoplasm. The firm connection of the particles with the cytoplasmic membranes, on the other hand, can be questioned as due to secondary absorption. This would explain why such particles are attached to the outer opaque layer of the nuclear membrane in cells where the RNA is located close to the nucleus. Furthermore, it explains why the particles are absent when the cytoplasmic membrane is located very closely to mitochondria where the amount of RNA easily can be assumed to be inadequate for the formation of such RNA particles.

Another structural component of the cytoplasm is the so-called *Golgi apparatus*. This component has been interpreted by light microscopists as an apparatus involved in secretory mechanisms, and it has been extensively studied in glandular cells. The secretory products were assumed to be either produced or accumulated in this region.

Electron microscopy has revealed another type of cytoplasmic membrane as the main component of the Golgi apparatus.[22–24] These membranes are arranged in pairs also and bound a space that can be either very narrow, about 100 A or less in width, or very wide appearing as large vacuolar, irregularly shaped spaces (Figs. 12 and 13). The membranes consist of one osmiophilic component, 60 A thick. Presumably, a less osmiophilic component separates the membrane pairs, because the space between the pairs frequently is very constant, measuring about 60 A in width.

It is rather striking that the zymogen granules which contain the secretory products of the pancreatic cells show a definite topographic relationship to the Golgi apparatus. The zymogen granules can be divided into two types—the fully-developed granules and the precursor granules. A complete series of intermediate-type granules bridges over from the one type to the other. The fully developed zymogen granules are spherical

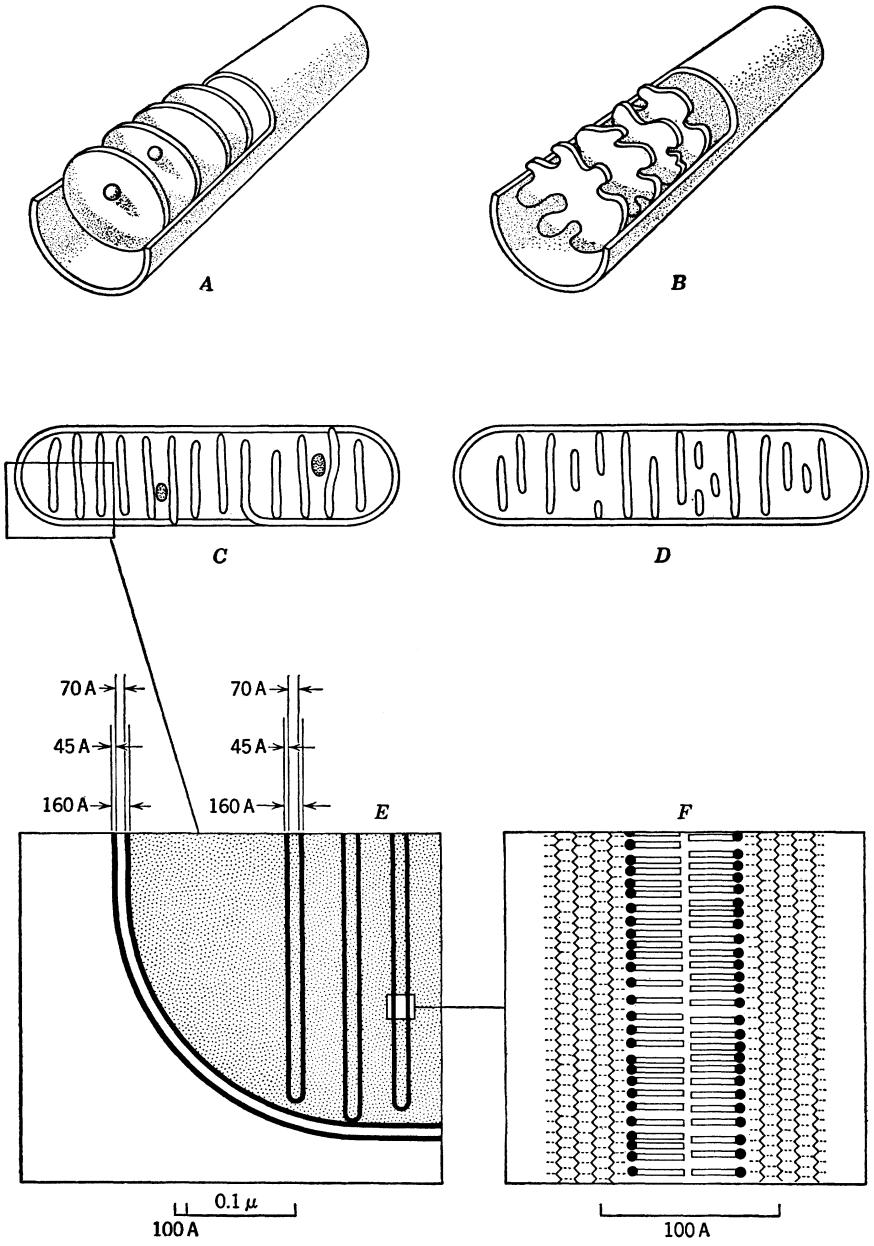

FIG. 8. Schematic drawing which illustrates an interpretation of the three-dimensional appearance of the mitochondria and of the molecular architecture of the mitochondria membranes. The three-dimensional representation *A* and *B* is based on an interpretation of the patterns observed in sections which can be, for instance, like the ones shown in *C* and *D*. *E* gives some commonly observed dimensions of the mitochondrial membranes, and *F* a scheme of the proposed arrangement of lipid and protein molecules in the membranes [from F. S. Sjöstrand, in *Fine Structure of Cells*, Proc. Symp. VIII Cong. Cell. Biol., Leiden, 1954 (Noordhoff, Ltd., Leiden, 1955), p. 16].

and of high density, and are bounded by a surface membrane about 50 A thick. The precursor granules are irregularly shaped and of lower opacity, but are bounded by a surface membrane which shows outpocketings and bulgings (Fig. 13). The precursor granules are found only in connection with the Golgi apparatus where the fully developed zymogen granules are either rare or completely absent. Furthermore, there is a definite polarity of the Golgi apparatus with the precursor granules arranged on the apical side of the Golgi membranes. On the basal side, the type of cytoplasmic membranes described earlier reaches close to the Golgi membranes. A zone of granulated cytoplasm, however, separates the territories of these two types of membranes.

Turning to the problem of the functional significance of these various kinds of membranes, the mitochondrial membrane is considered first. The article by Lehninger (p. 136) introduces the present concept regarding the importance of the structural organization of the mitochondria. The biochemical data point to a structural factor as important in connection with coupled phosphorylation. The electron transport seems to depend upon an organization of macromolecular particles. The

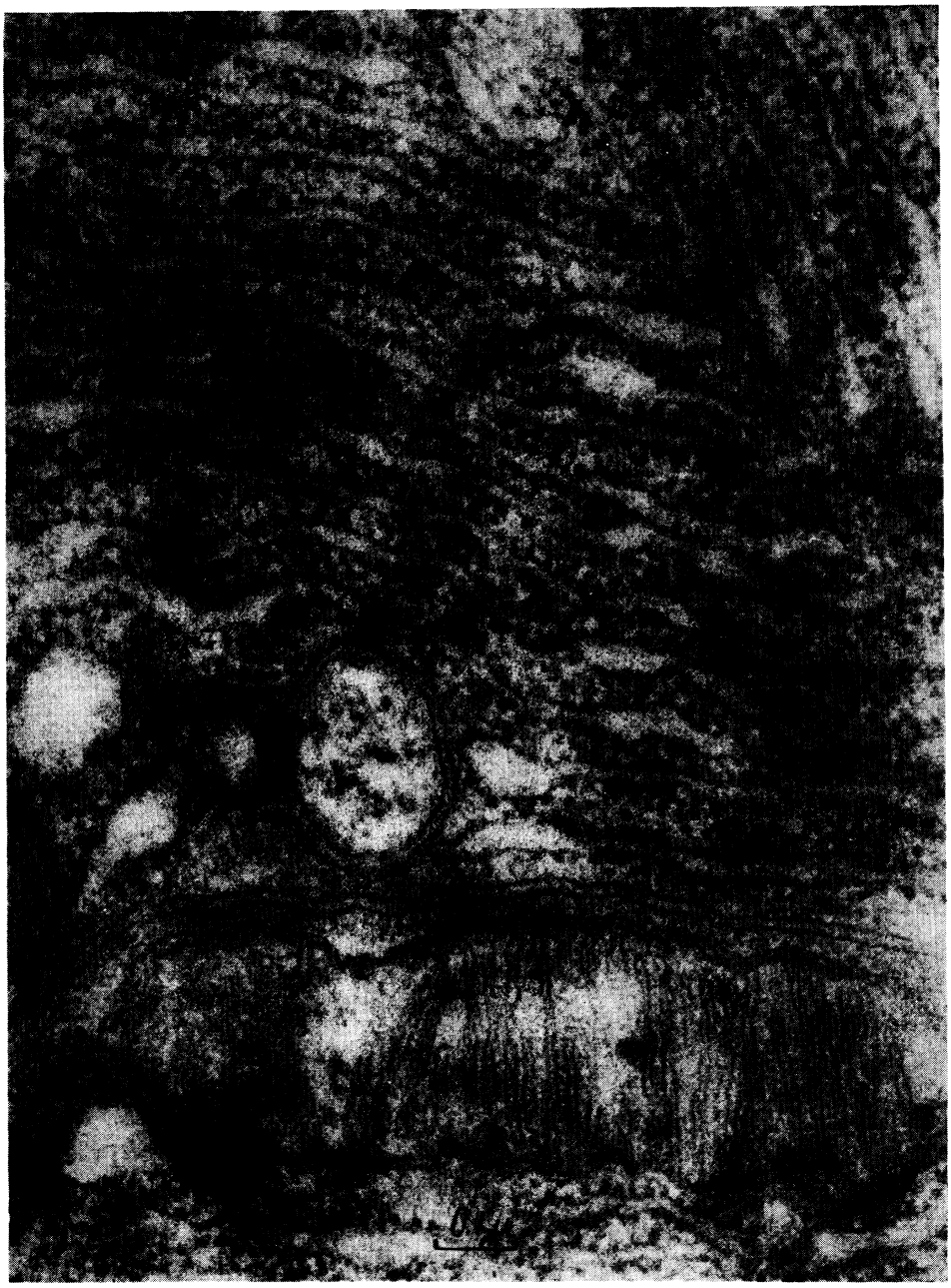

FIG. 9. Cytoplasmic membranes in the basophilic cytoplasm of exocrine pancreas cells. Notice the opaque 150-A particles attached to the one surface of the membranes which are arranged in pairs. In the lower part of the picture a cell boundary represented by two mutually parallel lines, and below the boundary a mitochondrion [from F. S. Sjöstrand and V. Hanzon, Exptl. Cell Research **7**, 393 (1954)]. ×108 000.

smooth coordination of various enzymatic reactions was imagined as being the result of a topographic arrangement of the enzyme molecules according to certain patterns. The membranes of the mitochondria were assumed to be composed to a great extent, or altogether, of mitochondrial enzyme molecules oriented and spread out in layers in combination with layers of oriented lipid molecules.

The experiments done so far to isolate the membranous components of the mitochondria from the ground substance have been rather crude from a morphological point of view, but are interpreted as supporting this idea. The mitochondrial membranes are considered to be enzymatically active where the organization of the enzyme molecules in a plane increases the chances for a well-coordinated sequence of enzyme-substrate interactions. This organization is thought to be important for a rapid step-wise degradation of substrate molecules, and possibly for their step-wise synthesis.

One may assume that the synthesis of the secretory products in the exocrine pancreas cells takes place

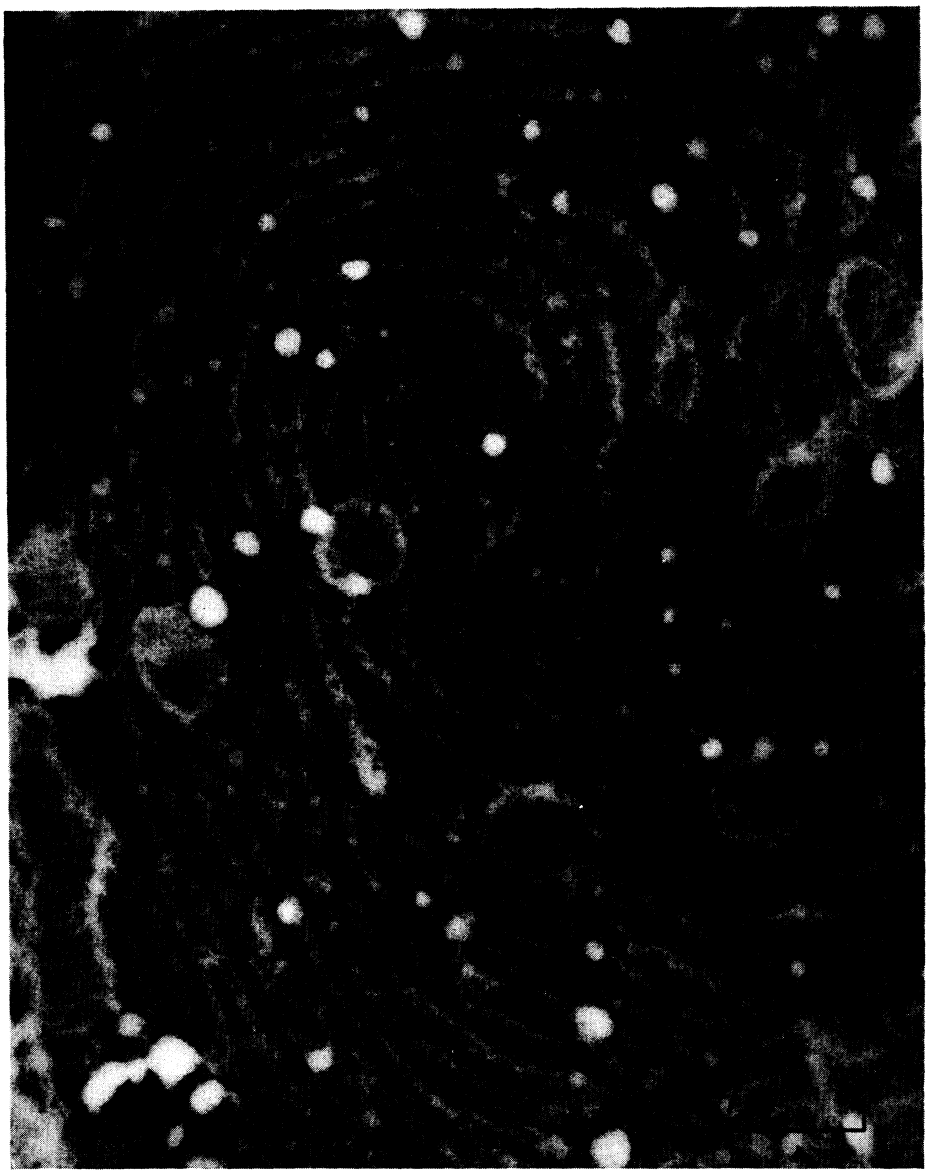

FIG. 10

FIGS. 10–11. Sections through exocrine pancreas cells preserved by means of freeze-drying. The methacrylate section was stained with phosphotungstic acid before the examination in the electron microscope. The cytoplasmic membranes are visible as well as the mitochondria but the pattern is a negative one as compared to that shown in Fig. 2. The spaces between the membranes (Fig. 11) are uniformly filled with material in contrast to the lack of material, except the opaque 150-A particles, after osmium fixation. No such particles can be observed in these pictures. *Mi*, mitochondria; *CM*, cytoplasmic membranes; *V*, ice crystal vacuole [from F. S. Sjöstrand and R. F. Baker, J. Ultrastructure Research 1, 239 (1958)]. ×30 000 (Fig. 10); ×56 000 (Fig. 11) (Fig. 11 is on opposite page).

in steps. First, an enzyme-containing membrane is formed which is able to synthesize the secretory products (Fig. 14). This membrane is manufactured in the Golgi apparatus from material delivered by the cytoplasm as represented by the RNA particles. These particles swell, fuse, and mix with lipids, and extensive hydration results in the formation of vacuoles bounded by a thin membrane consisting of lipoproteins. The vacuoles collapse and pairs of membranes are formed which disintegrate into fragments forming the surface membrane of the precursor granules. The surface membrane then synthesizes the secretory products which are successively accumulated in the granule. Raw material can be delivered all of the time by the RNA-rich cytoplasm.

The foregoing is a wild hypothesis that could explain the morphological observations made so far. It is derived, however, from still pictures; the various stages

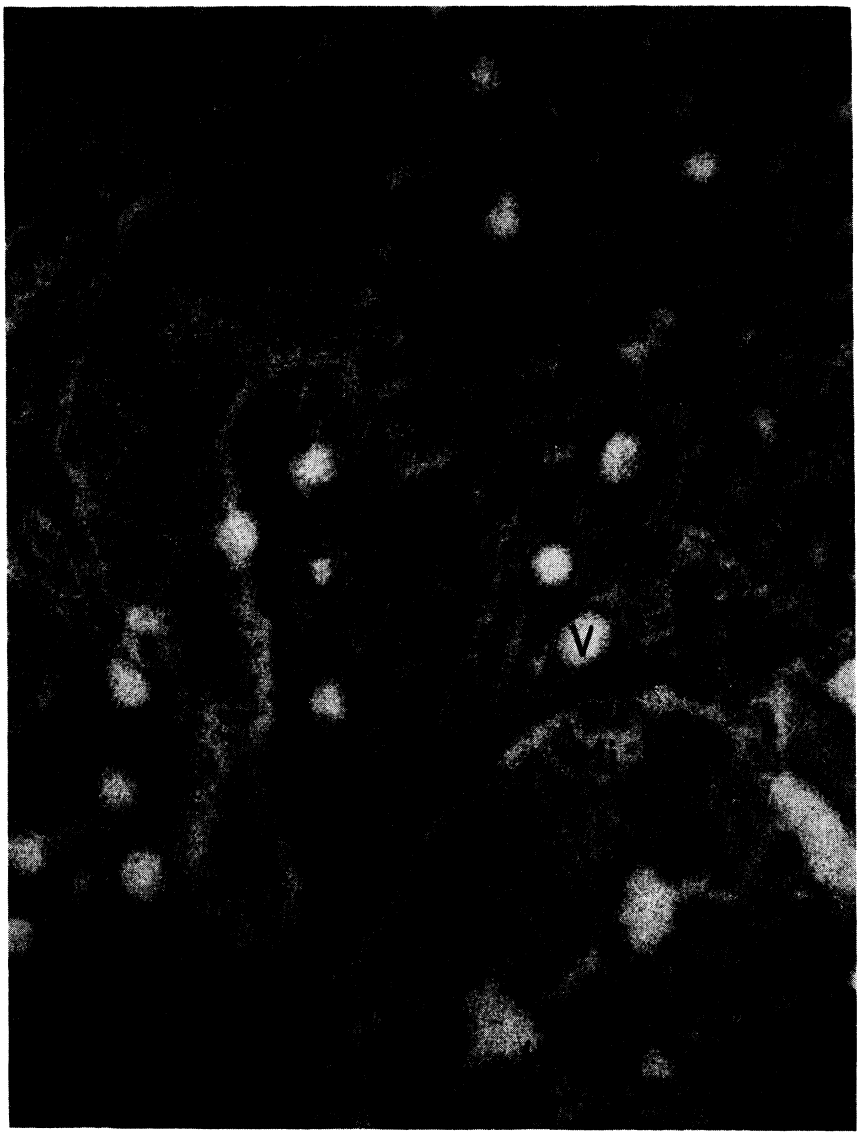

FIG. 11 (for legend, see Fig. 10).

have been selected subjectively from these pictures. Experimental evidence remains to be presented. One thing seems to be quite clear—namely, that the Golgi membranes can form vesicles or the membranes of secretory granules. This latter process is demonstrated better by the goblet cells in the intestinal epithelium. Holmberg,[25] in our laboratory, found that, after administration of diamox, an inhibitor for carbonic anhydrase, the Golgi apparatus in the ciliary epithelium of the eye partially disintegrated into vesicles and the whole cytoplasm became loaded with vesicles. These reversible changes could be described in a quantitative way and be correlated with a temporary 60% inhibition of the secretion of aqueous humor.

In certain types of cells, the plasma membrane appears extremely folded. It is not quite clear whether these folds are tight infoldings of the plasma membrane or whether they represent interdigitating ridges of the cell surfaces of adjacent cells. Zetterqvist,[26] in our laboratory, demonstrated that the plasma membrane can appear rather different at various parts of the surface of the same cell. In the intestinal epithelium (Fig. 15) the free surface of the cells forms a number of cylindrical processes, the so-called brush border. The plasma membrane bounding these processes appears as a 105-A thick triple-layered component (Fig. 16). Where two epithelial cells are in close contact, the plasma membrane appears as an osmiophilic layer 60 to 70 A thick. Between the two osmiophilic layers of the two adjacent epithelium cells, there is a light space with a very uniform thickness of about 100 A.

On the other hand, the plasma membrane which

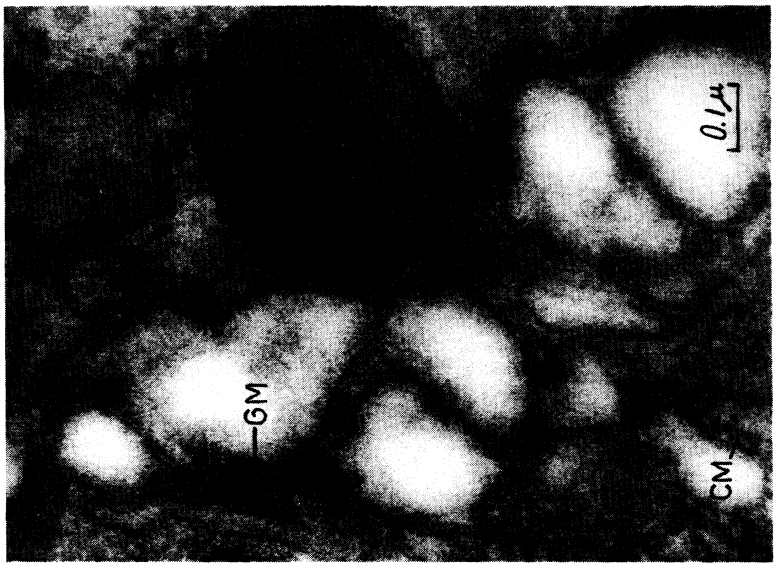

FIG. 13. Higher magnification of a part of Fig. 12. For explanation of indications, see Fig. 12. ×83 000.

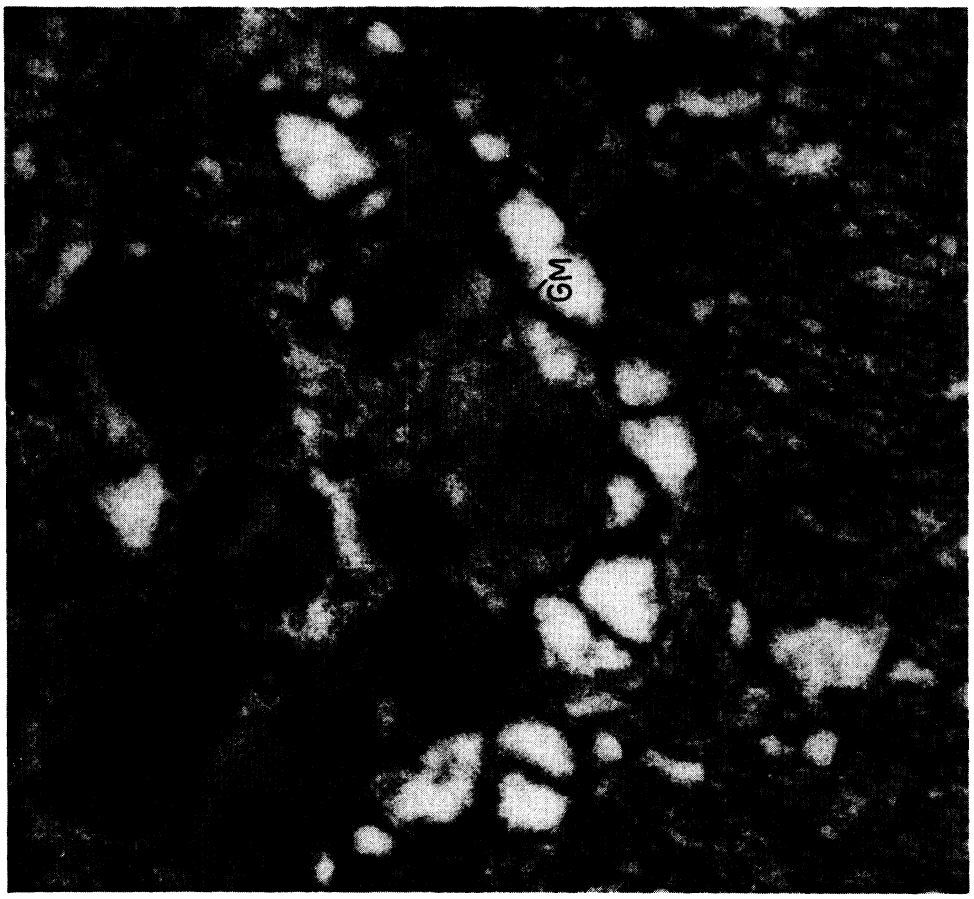

FIG. 12. A Golgi apparatus in an exocrine cell of the mouse pancreas. *GM*, Golgi membranes; *GR*, granules on the basal side of the Golgi apparatus; *CM*, cytoplasmic membranes; Z_0, prozymogen granules that appear to develop from the Golgi membranes; Z_1, prozymogen granules which can be interpreted as intermediate stages between Z_0 and Z_2, which are fully developed zymogen granules [from F. S. Sjöstrand and V. Hanzon, Exptl. Cell Research **7**, 415 (1954)]. ×44 000.

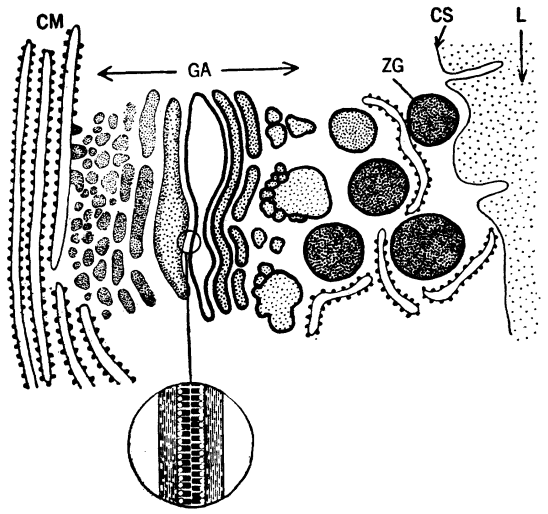

FIG. 14. Schematic drawing illustrating a hypothesis which aims at interpreting the functional significance of the Golgi apparatus of the excretory cells of the pancreas as an assembly line for membrane material with the power to synthesize the secretory products of the exocrine cells from raw material in the cytoplasm. *CM*, cytoplasmic membranes; *GA*, Golgi apparatus with formation of membranes from material delivered by the cytoplasmic membranes; *ZG*, zymogen granules formed from precursor granules in the Golgi apparatus; *CS*, cell surface facing the lumen of the excretory duct, *L* [from F. S. Sjöstrand, UNESCO Symp. Patterns of Cellular and Sub-Cellular Organisation, Edinburgh, 1957 (to be published.)]

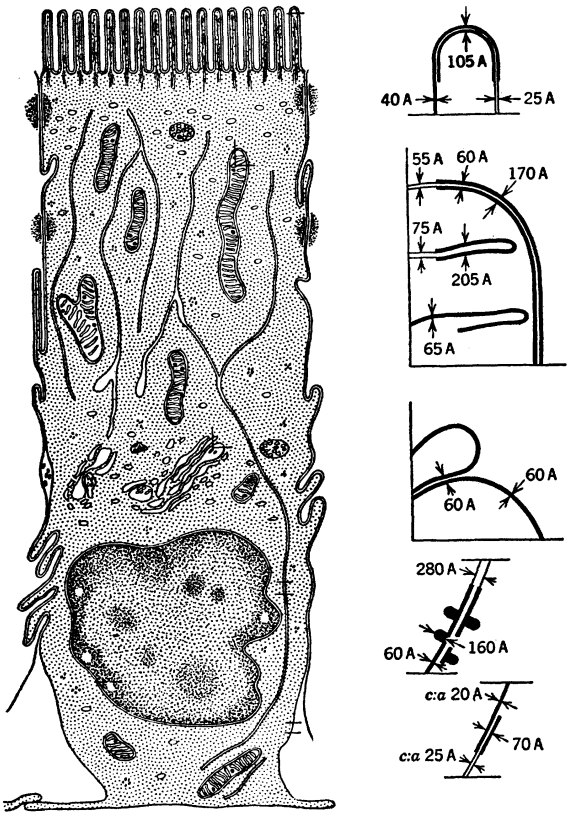

FIG. 15. Schematic drawing of a cylindrical epithelial cell lining the small intestine in the mouse. Notice the so-called brush border at the upper surface of the cell which faces the lumen of the gut [from H. Zetterqvist, *The Ultrastructural Organization of the Columnar Absorbing Cells of the Mouse Jejunum*. Thesis, Stockholm (1956)].

covers the surface of the cell facing the intercellular spaces and the basement membrane appears as a triple-layered structure which measures only 70 A in thickness. This structure consists of two opaque layers, each about 25 A thick, separated by a 20-A thick light interspace. A similar appearance has been reported recently by Robertson[27] for the plasma membranes of the Schwann cells. Robertson has interpreted this approximately 70-A thick triple-layered structure as representative of the whole plasma membrane (unit membrane) and which consists of two protein monolayers sandwiching a double layer of lipid molecules.

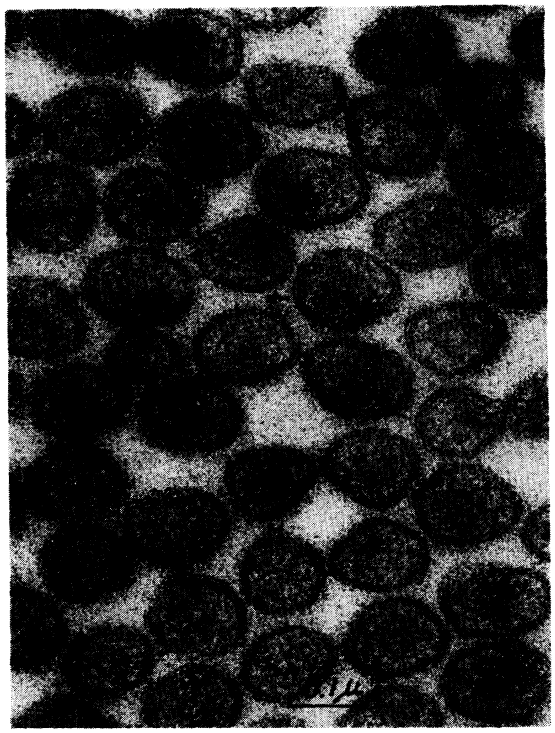

FIG. 16. Transversal section through the cylindrical processes of the brush border zone in an intestinal epithelial cell. The processes are bounded by a triple-layered surface membrane with an average total thickness of 105 A [from H. Zetterqvist, *The Ultrastructural Organization of the Columnar Absorbing Cells of the Mouse Jejunum*. Thesis, Stockholm (1956)]. ×103 000.

This interpretation is based on a great deal of evidence and it is discussed further by Schmitt (p. 455). It is assumed that the consistency of the width, about 100 A, of the light interspaces between two cells in close contact indicates the presence of some binding material, such as lipids or mucopolysaccharides.

Consider now the formation of membranous material in the outer segments of retinal receptors. It is seen, in the double cones of the perch retina, that the disks of

FIG. 17. The peripheral parts of two outer segments belonging to two twin cones of the perch retina. The outer segment shown in the lower half of the picture demonstrates that the less opaque interspaces in the triple-layered disks are open, and that the opaque layers of adjacent disks are continuous. At the opposite side of this segment, the conditions are those observed in the outer segment shown in the upper half of the picture. The less opaque interspaces are closed. This shows that the pile of disks is formed by a continuous membrane that is repeatedly folded. In other types of receptors, no such obvious folding can be observed but the disks are mutually connected through tube-like stalks which are continuous with the opaque layers of the disks. ×100 000.

FIG. 18. Retinal receptors from an eye of a four-day-old kitten. At α, a repeatedly folded membrane is observed which appears to represent a stage of the development of the outer segment. In the cat, the retinal receptors are differentiated after the birth. ×18 000.

FIG. 19. Synaptic contact between a synaptic body, *SB*, of a retinal rod of the guinea pig eye, and a bipolar nerve cell, *B*, through the dendrite, *D*. The dendrite enters the synaptic body in an invagination of the plasma membrane and makes contact with two vacuoles also located in the same invagination [from F. S. Sjöstrand, J. Ultrastructure Research 2, 122 (1958)].×41 000.

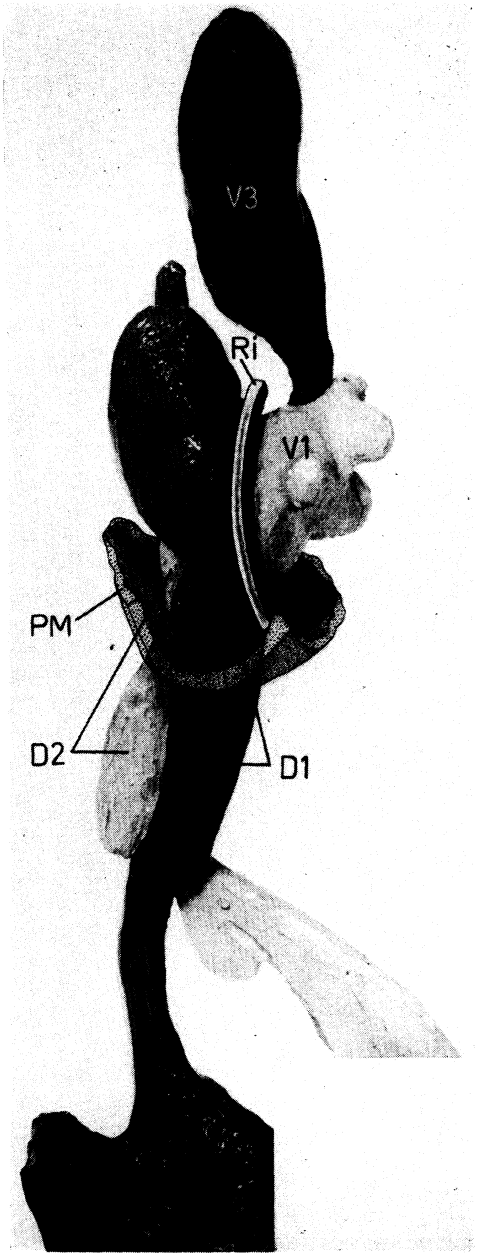

FIG. 20

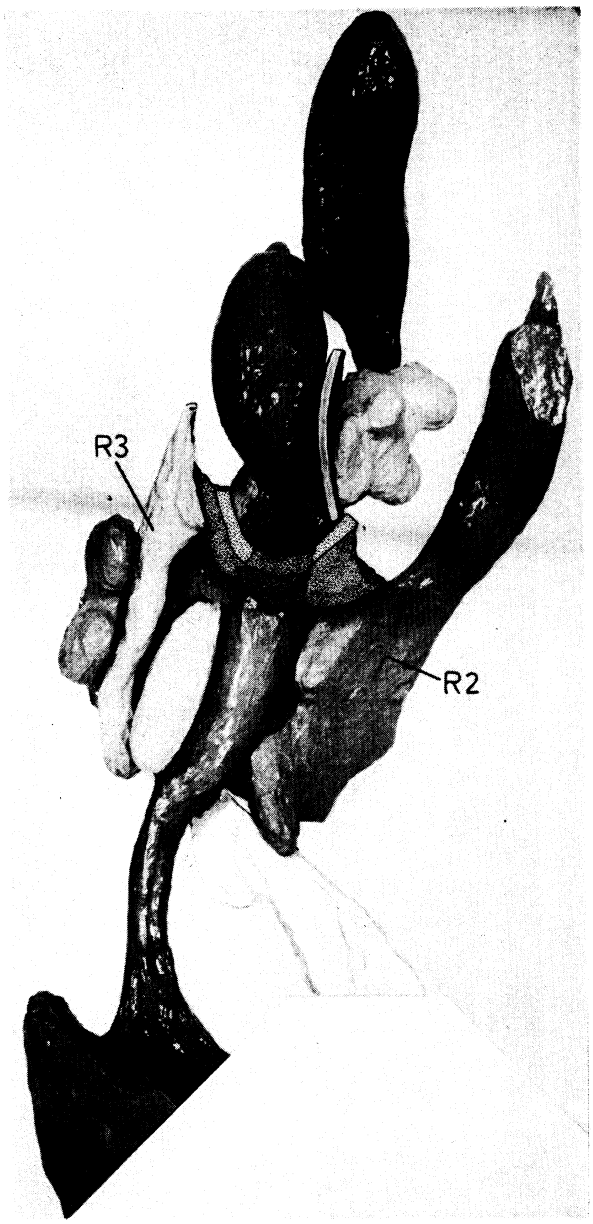

FIG. 21

FIGS. 20–21. Three-dimensional reconstruction of the synaptic body of a retinal rod of the guinea pig eye made from a series of 40 sections according to the classical technique of the three-dimensional reconstructions for series of sections. Two dendrites, D1, and D2, from nerve cells are shown entering into synaptic contact with the synaptic body of the receptor. The plasma membrane bounding the synaptic body has been removed with the exception of a fragment, PM, in order to show the large vacuoles and the synaptic ribbon which, together with synaptic vesicles and granules, constitute the elementary components of the retinal synaptic bodies. In Fig. 21, some of the extensions from adjacent synaptic bodies, R2 and R3, of receptor cells are shown making contact with the surface membrane of the reconstructed synaptic body [from F. S. Sjöstrand, J. Ultrastructure Research 2, 122 (1958)].

the outer segments appear as folds of one continuous membrane (Fig. 17). If one looks at the outer segments of the kitten retina a few days after birth, one can see the outer segments in the process of being formed. They seem to be formed through a folding of a membrane (Fig. 18), each fold being transformed into a disk later on, each disk remaining associated with its neighbors through a tube-like connection located close to the center of the disk at the end of a deep incision.

It well might be that the mitochondrial membranes

are formed in the same way, which explains the fact that, in 1 to 10%, the light interspace of the inner mitochondrial membranes is continuous with the light interspace of the outer mitochondrial membrane. This would give one explanation of why this kind of contact between outer and inner membranes is so rare.

Recently, we succeeded in obtaining quite extensive series of serial sections. From one series of 40 sections with an average thickness of 250 A, the author has made a three-dimensional reconstruction of the synaptic connections and the inner structure of the synaptic bodies of the retinal rods in the guinea pig eye.[28] This reconstruction has made it possible to reveal the very complicated arrangement of various membranous components of this part of the cell (Figs. 19–21) and to draw the first primitive circuit diagram (Fig. 22) of the synaptic contacts of a receptor cell. This technique is, of course, of interest when analyzing the central nervous system, but it is necessary also for a detailed description of the topographical relations between different structural components of the cytoplasm.

One now may survey some of the types of membranes as observed in the cytoplasm (Fig. 23). Distinction can be made between the cytoplasmic membranes which, after osmium fixation, are associated with the RNA particles, the infolding of the plasma membrane, and the Golgi membranes. These various types of membranes easily can be distinguished morphologically and a neutral terminology is proposed for them: α-, β-, and γ-cytomembranes.[29]

As assumed by Porter and Palade and accepted by most American electron microscopists, all of these types of membranes, as well as the nuclear membrane, the centrosphere, and various types of vesicles in the cytoplasm, have been considered to represent different aspects of one continuous canalicular system, the so-called endoplasmic reticulum, extending throughout the whole cytoplasm. There are no evidences that we have been able to confirm that might allow the conclusion that these various components form a continuous system. We represent an opposite standpoint in accepting morphological differences as indicating differences regarding function, and we stress that the cytoplasm is differentiated into a limited number of structurally and presumably functionally different components.

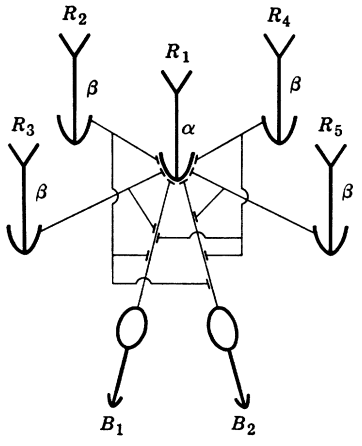

FIG. 22. A trial to make a circuit diagram of the synaptic contacts of a retinal receptor in the guinea pig eye showing the extensive inter-receptor contacts. R_1–R_5, receptor cells; B_1 and B_2, bipolar nerve cells [from F. S. Sjöstrand, J. Ultrastructure Research **2**, 122 (1958)].

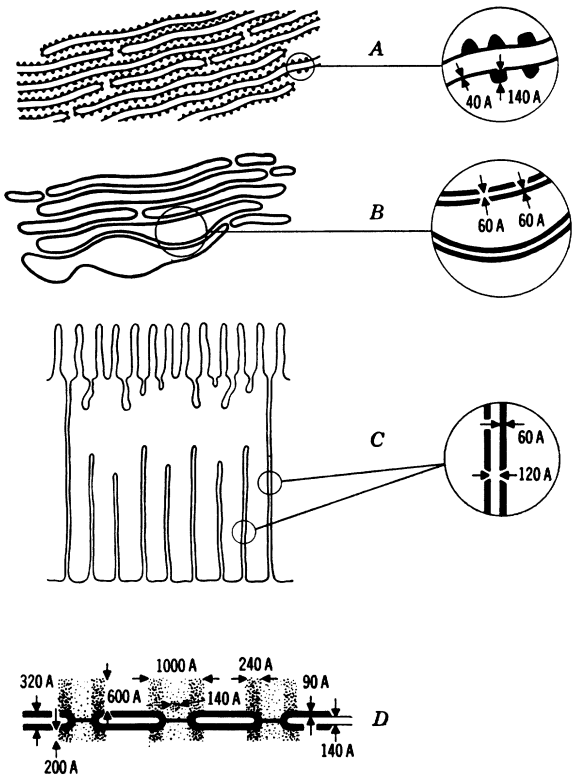

FIG. 23. Schematic presentation of some of the types of membranes that appear in cells. A. α-cytomembranes; B. Golgi membranes (γ-cytomembranes); C. β-cytomembranes; D. nuclear membrane [according to B. A. Afxelius, Exptl. Cell Research **8**, 147 (1955)]. Some characteristic dimensions of the membranes are presented [from F. S. Sjöstrand, *Methods in Enzymology* (Academic Press, Inc., New York, 1957), Vol. IV, p. 391)].

For the future, it is probably less important to extend one's knowledge of the structure of cells to more and more types of cells. The structural patterns are repeated in a rather monotonous way. It is more important by far to try to analyze further the molecular architecture of the various components of the cytoplasm by using a variety of techniques. What appears most important, however, is to try to supplement the collected geometrical data with biochemical data. It is necessary then to work on fractions of homogenized cells, to improve the techniques of fractionation, to work out methods that make it possible to identify in the fractions the various cell components, and to estimate their relative concentrations in a quantitative way.

Membranes appear as the most common structural principle so far. When dealing with the cytoplasm, one gets the impression that the construction of a surface membrane with certain properties represents one of the first steps in the creation of life, and that this principle has been applied in a rather monotonous way by nature.

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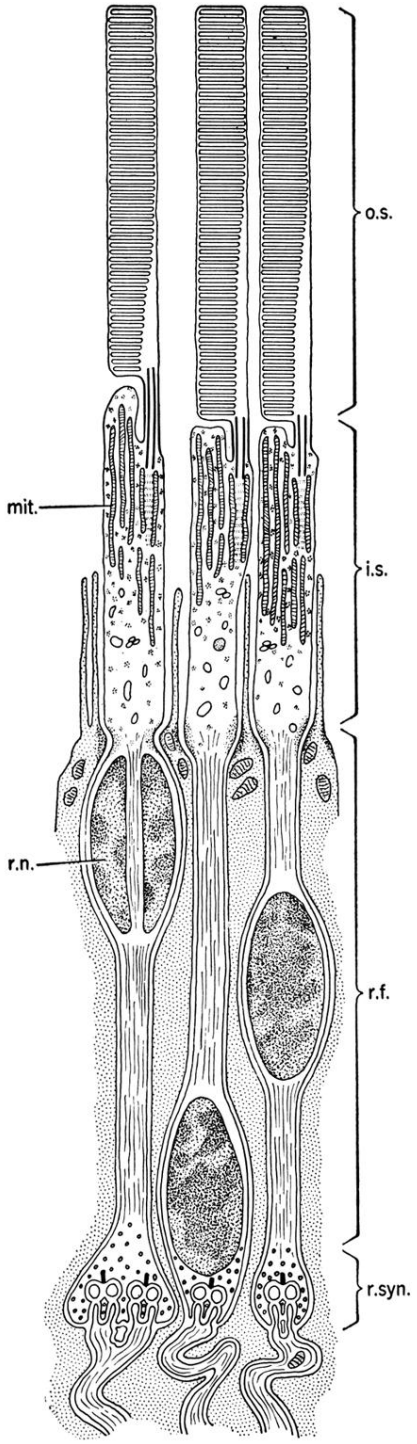

FIG. 1. Schematic drawing of retinal rods in the guinea pig eye. *o.s.*, outer segment; *i.s.*, inner segment; *r.f.*, rod fiber; *r.syn.*, rod synaptic body; *mit.*, mitochondria; *r.n.*, rod nucleus [from F. S. Sjöstrand, Intern. Rev. Cytol. **5**, 455 (1956)].

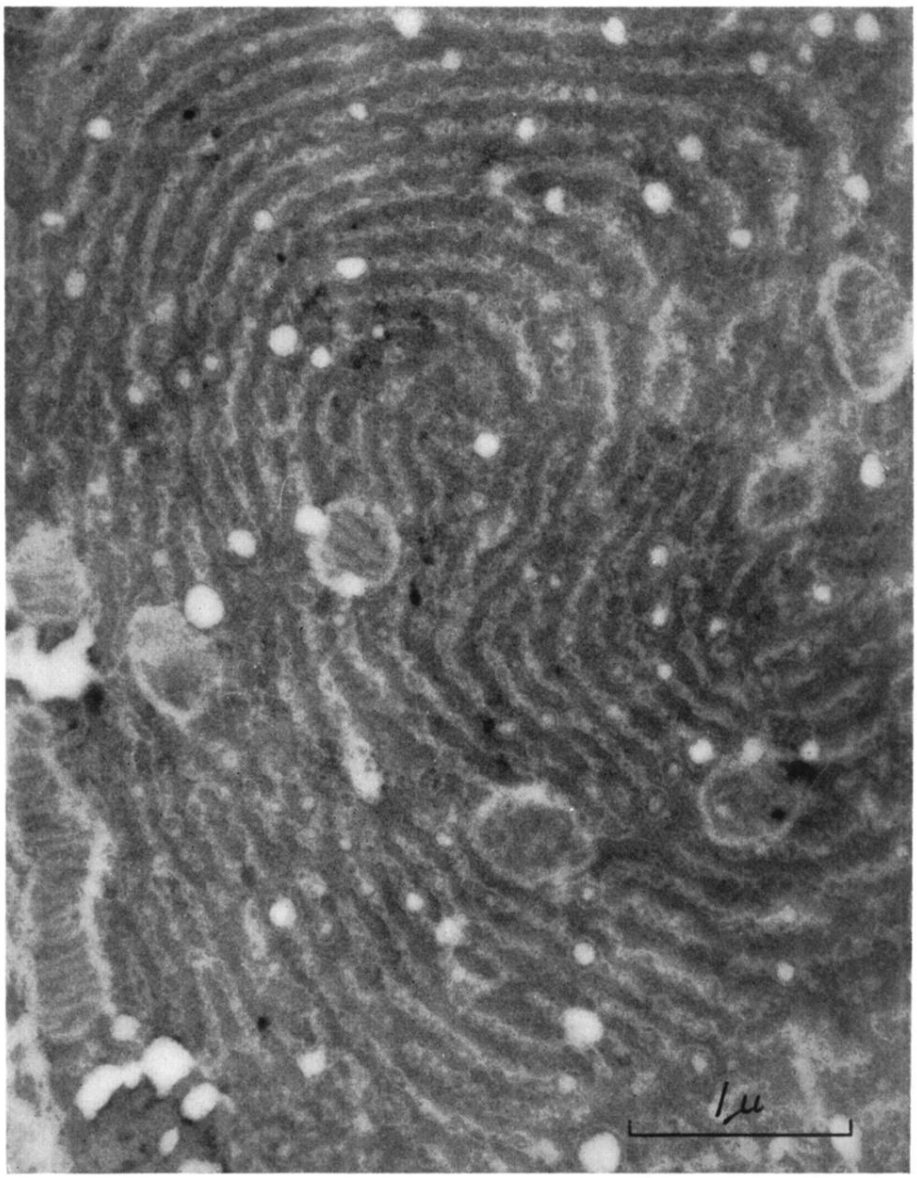

FIG. 10

FIGS. 10–11. Sections through exocrine pancreas cells preserved by means of freeze-drying. The methacrylate section was stained with phosphotungstic acid before the examination in the electron microscope. The cytoplasmic membranes are visible as well as the mitochondria but the pattern is a negative one as compared to that shown in Fig. 2. The spaces between the membranes (Fig. 11) are uniformly filled with material in contrast to the lack of material, except the opaque 150-A particles, after osmium fixation. No such particles can be observed in these pictures. *Mi*, mitochondria; *CM*, cytoplasmic membranes; *V*, ice crystal vacuole [from F. S. Sjöstrand and R. F. Baker, J. Ultrastructure Research **1**, 239 (1958)]. ×30 000 (Fig. 10); ×56 000 (Fig. 11) (Fig. 11 is on opposite page).

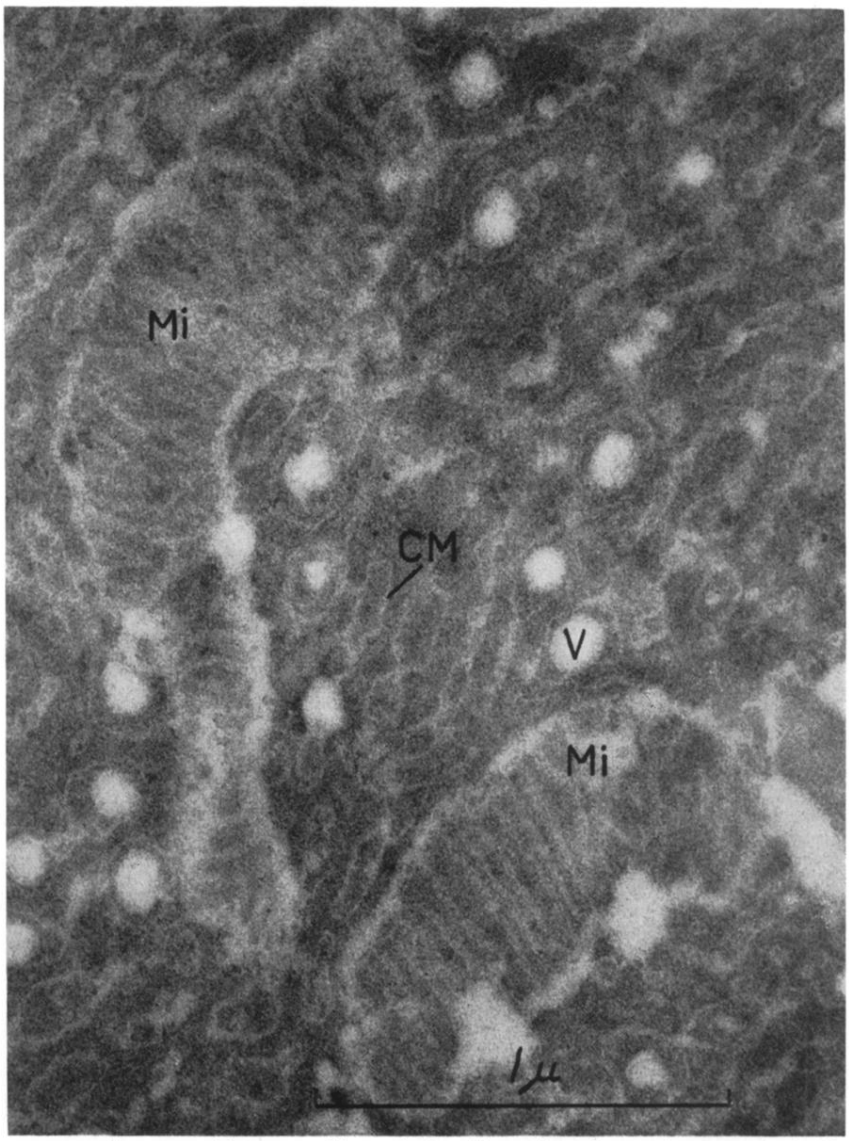

FIG. 11 (for legend, see Fig. 10).

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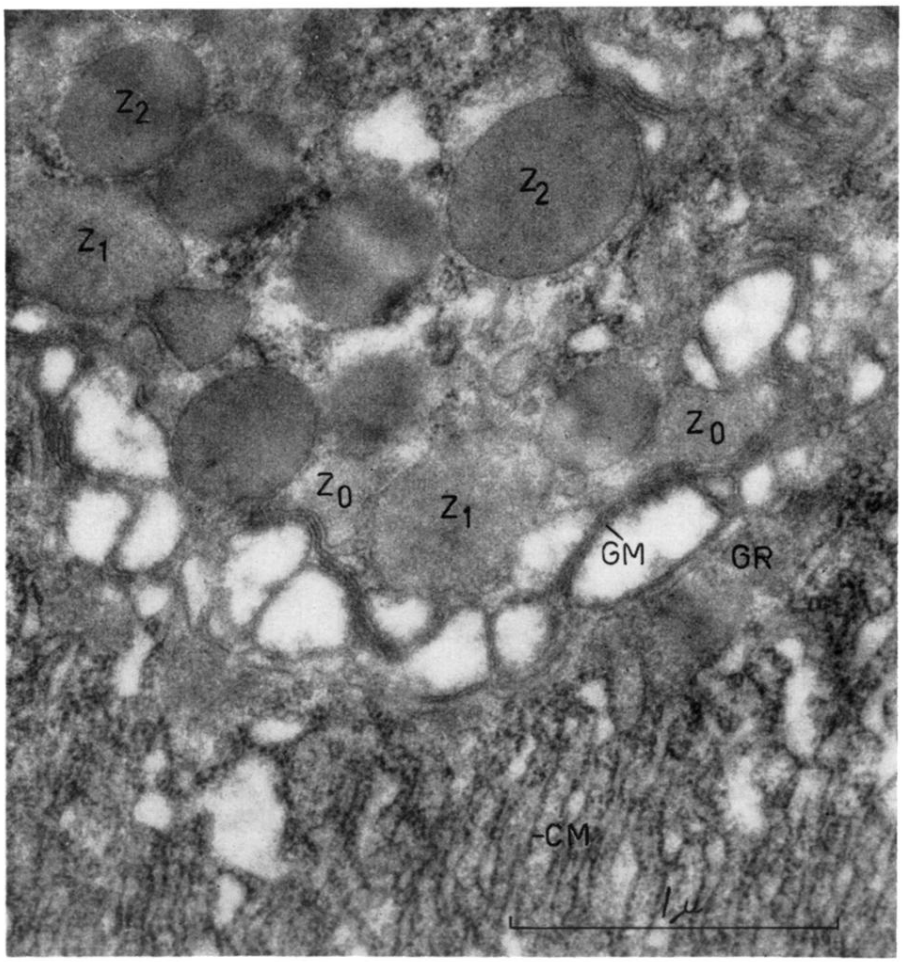

FIG. 12. A Golgi apparatus in an exocrine cell of the mouse pancreas. *GM*, Golgi membranes; *GR*, granules on the basal side of the Golgi apparatus; *CM*, cytoplasmic membranes; Z_0, prozymogen granules that appear to develop from the Golgi membranes; Z_1, prozymogen granules which can be interpreted as intermediate stages between Z_0 and Z_2, which are fully developed zymogen granules [from F. S. Sjöstrand and V. Hanzon, Exptl. Cell Research **7**, 415 (1954)]. ×44 000.

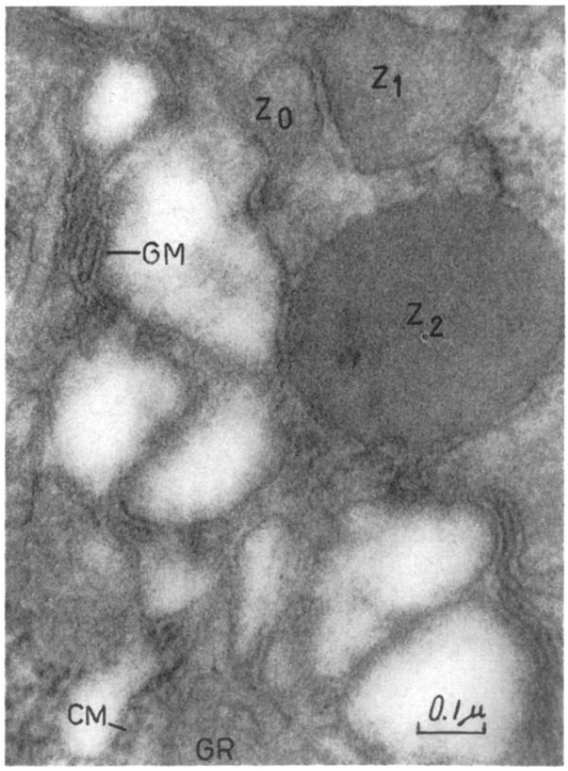

FIG. 13. Higher magnification of a part of Fig. 12. For explanation of indications, see Fig. 12. ×83 000.

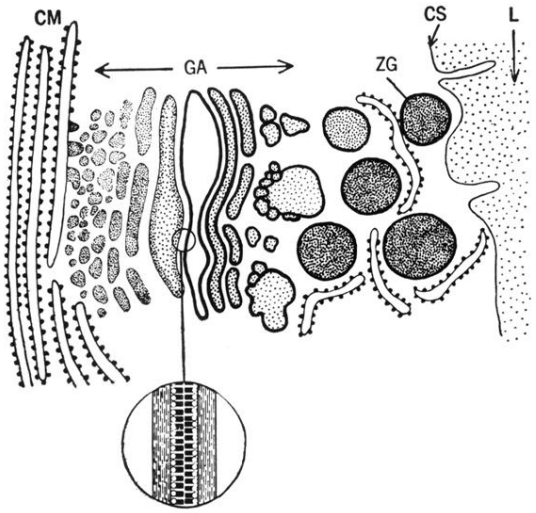

FIG. 14. Schematic drawing illustrating a hypothesis which aims at interpreting the functional significance of the Golgi apparatus of the excretory cells of the pancreas as an assembly line for membrane material with the power to synthesize the secretory products of the exocrine cells from raw material in the cytoplasm. *CM*, cytoplasmic membranes; *GA*, Golgi apparatus with formation of membranes from material delivered by the cytoplasmic membranes; *ZG*, zymogen granules formed from precursor granules in the Golgi apparatus; *CS*, cell surface facing the lumen of the excretory duct, *L* [from F. S. Sjöstrand, UNESCO Symp. Patterns of Cellular and Sub-Cellular Organisation, Edinburgh, 1957 (to be published.)]

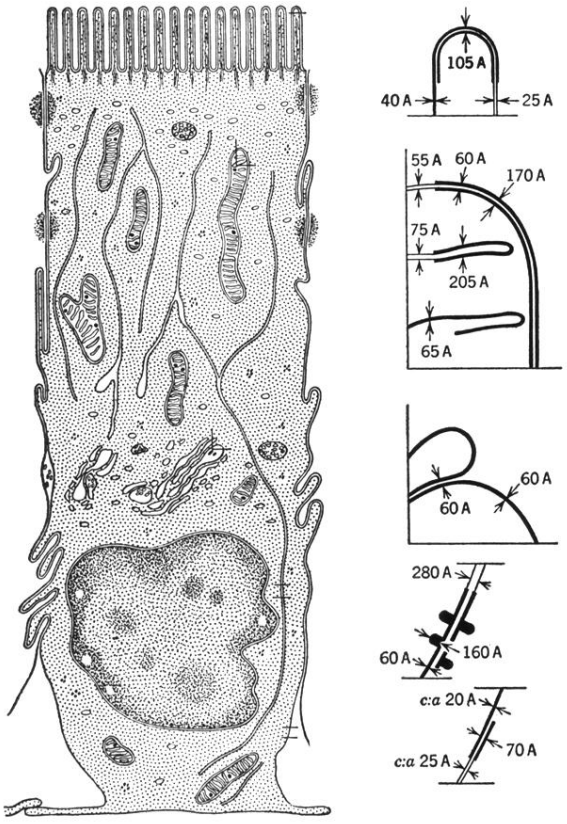

FIG. 15. Schematic drawing of a cylindrical epithelial cell lining the small intestine in the mouse. Notice the so-called brush border

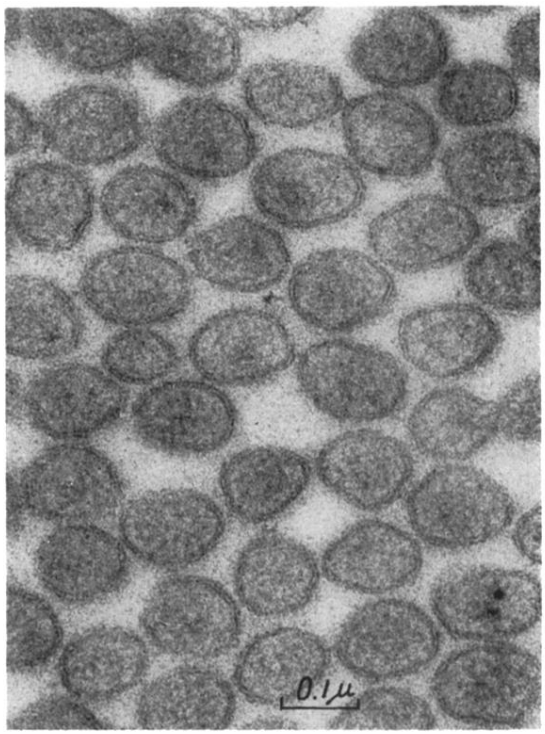

FIG. 16. Transversal section through the cylindrical processes of the brush border zone in an intestinal epithelial cell. The processes are bounded by a triple-layered surface membrane with an average total thickness of 105 Å [from H. Zetterqvist, *The Ultrastructural Organization of the Columnar Absorbing Cells of the Mouse Jejunum*. Thesis, Stockholm (1956)]. ×103 000.

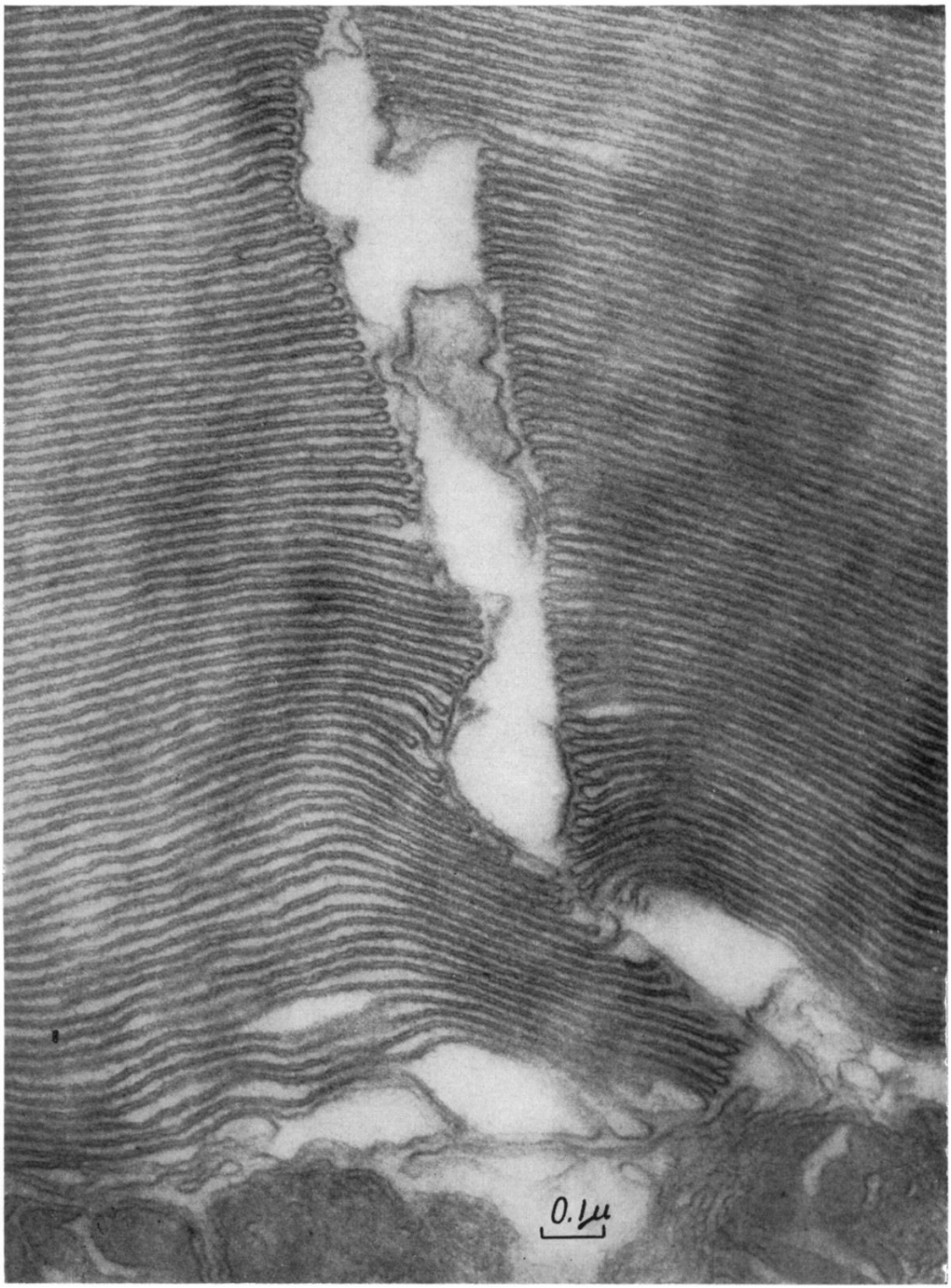

FIG. 17. The peripheral parts of two outer segments belonging to two twin cones of the perch retina. The outer segment shown in the lower half of the picture demonstrates that the less opaque interspaces in the triple-layered disks are open, and that the opaque layers of adjacent disks are continuous. At the opposite side of this segment, the conditions are those observed in the outer segment shown in the upper half of the picture. The less opaque interspaces are closed. This shows that the pile of disks is formed by a continuous membrane that is repeatedly folded. In other types of receptors, no such obvious folding can be observed but the disks are mutually connected through tube-like stalks which are continuous with the opaque layers of the disks. ×100 000.

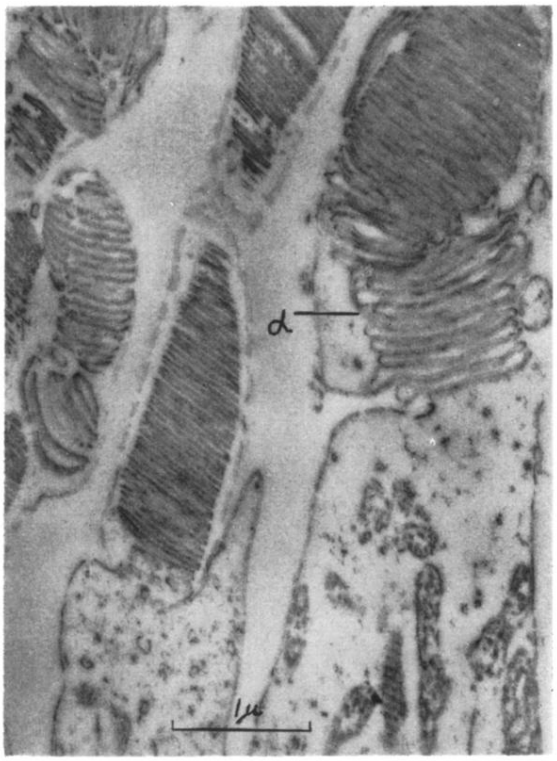

FIG. 18. Retinal receptors from an eye of a four-day-old kitten. At α, a repeatedly folded membrane is observed which appears to represent a stage of the development of the outer segment. In the cat, the retinal receptors are differentiated after the birth. ×18 000.

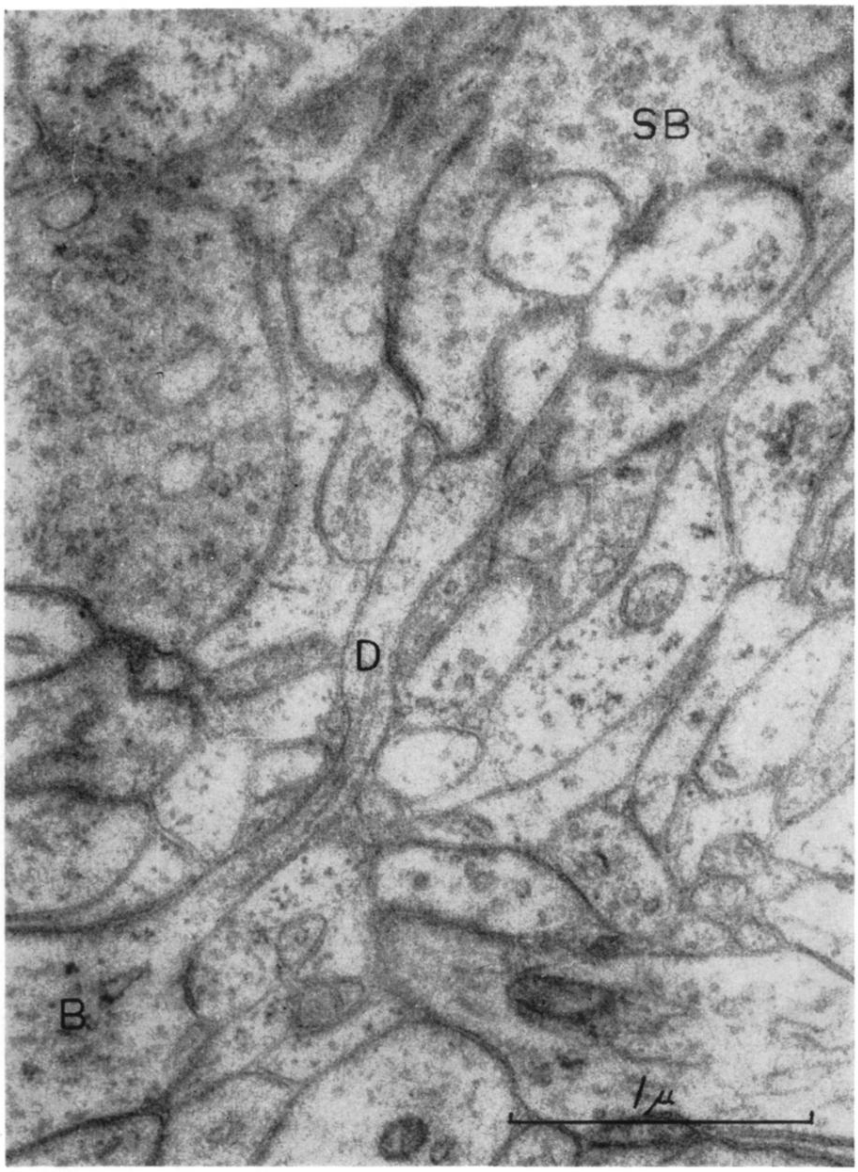

FIG. 19. Synaptic contact between a synaptic body, *SB*, of a retinal rod of the guinea pig eye, and a bipolar nerve cell, *B*, through the dendrite, *D*. The dendrite enters the synaptic body in an invagination of the plasma membrane and makes contact with two vacuoles also located in the same invagination [from F. S. Sjöstrand, J. Ultrastructure Research **2**, 122 (1958)].×41 000.

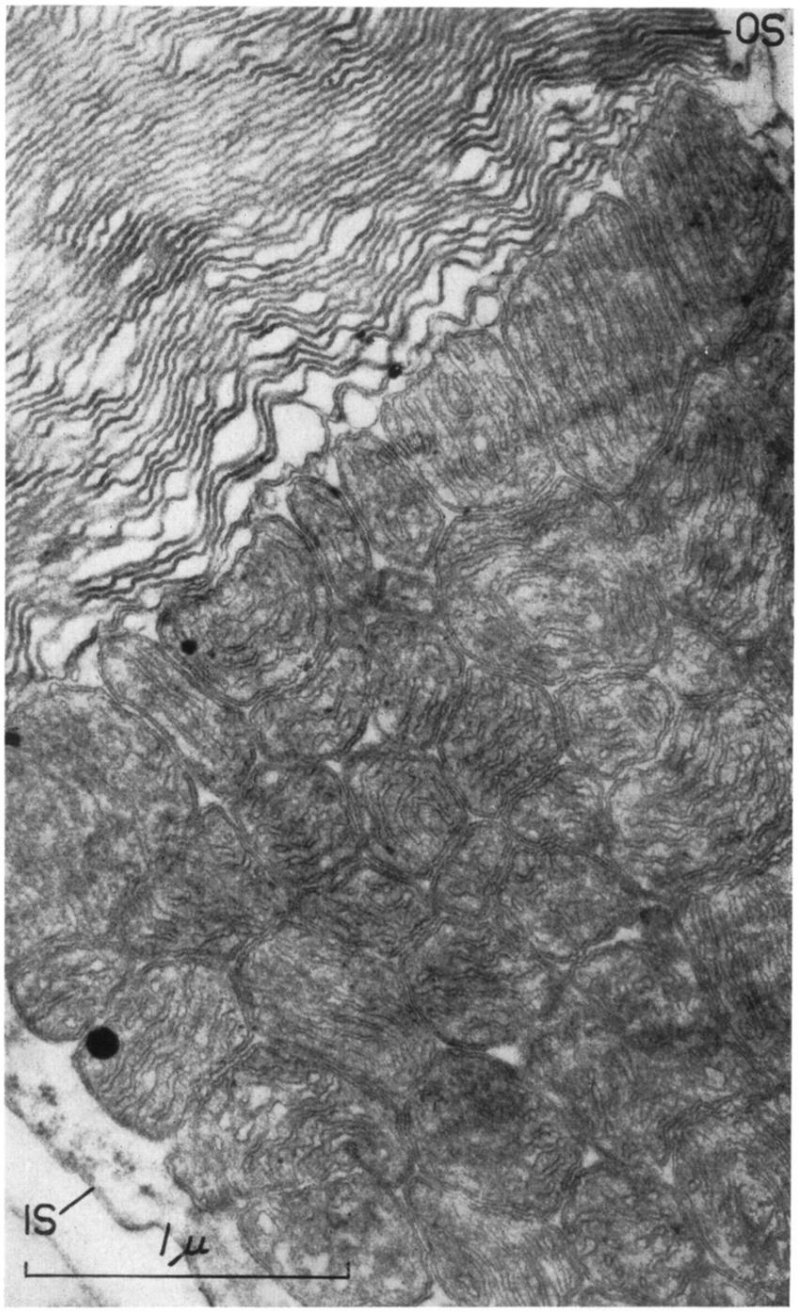

FIG. 2. Survey picture of the boundary between the outer, *OS*, and inner, *IS*, segments of a cone cell in the perch retina. Most of the inner segment consists of a dense aggregation of mitochondria [from F. S. Sjöstrand, *Ergebnisse der Biologie* (Springer-Verlag, Berlin, 1958), Vol. XXI, p. 128]. ×44 000.

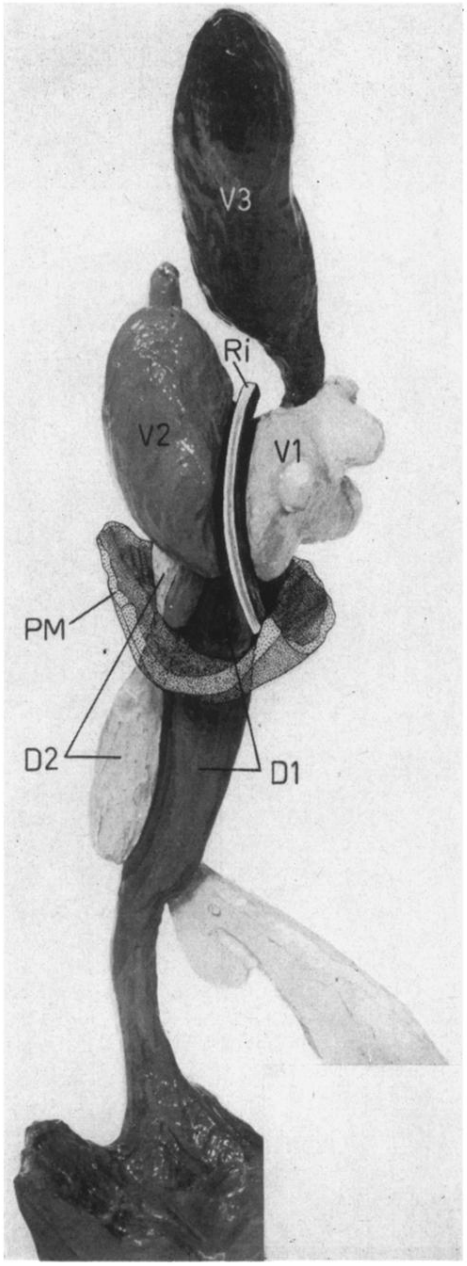

FIG. 20

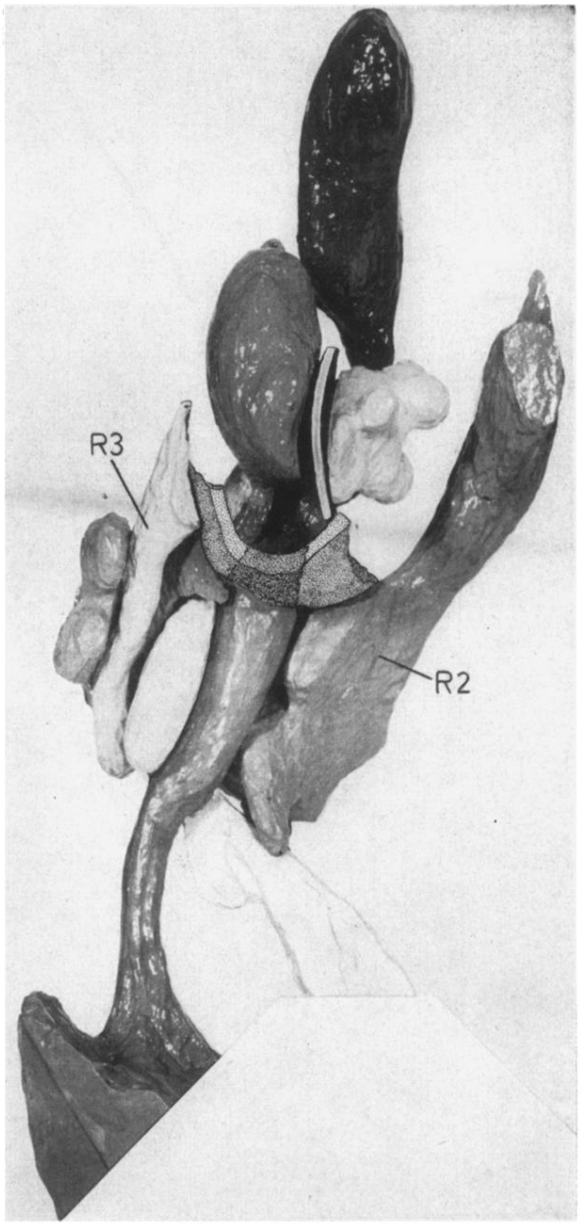

FIG. 21

FIGS. 20–21. Three-dimensional reconstruction of the synaptic body of a retinal rod of the guinea pig eye made from a series of 40 sections according to the classical technique of the three-dimensional reconstructions for series of sections. Two dendrites, D1, and D2, from nerve cells are shown entering into synaptic contact with the synaptic body of the receptor. The plasma membrane bounding the synaptic body has been removed with the exception of a fragment, PM, in order to show the large vacuoles and the synaptic ribbon which, together with synaptic vesicles and granules, constitute the elementary components of the retinal synaptic bodies. In Fig. 21, some of the extensions from adjacent synaptic bodies, R2 and R3, of receptor cells are shown making contact with the surface membrane of the reconstructed synaptic body [from F. S. Sjöstrand, J. Ultrastructure Research 2, 122 (1958)].

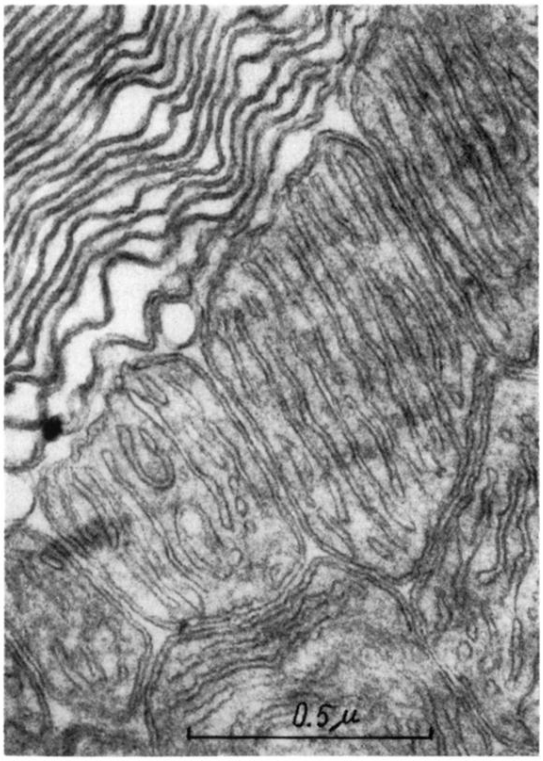

FIG. 3. Higher magnification of a region at the border between the inner and outer segments of a cone cell in the perch retina. Several mitochondria are observed densely aggregated. The triple-layered surface membrane and inner membranes show certain similarities to the triple-layered disks of the outer segment [from F. S. Sjöstrand, *Ergebnisse der Biologie* (Springer-Verlag, Berlin, 1958), Vol. XXI, p. 128]. ×65 000.

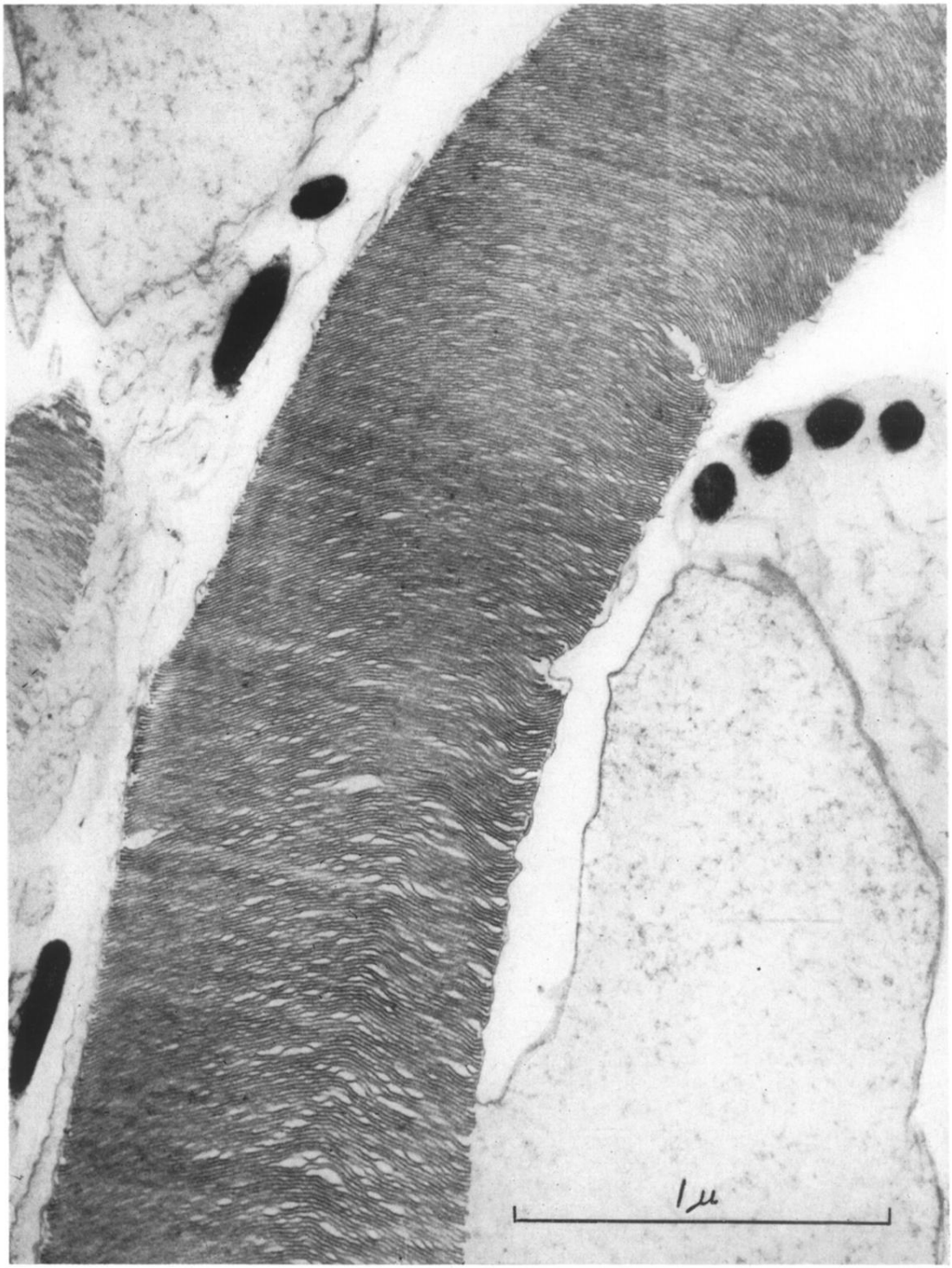

FIG. 4. Longitudinal section through the outer segment of a retinal rod cell from the perch eye showing the uniformly layered structure of the outer segment. ×59 000.

FIG. 5. Longitudinal section through a retinal-rod cell of the perch eye showing the triple-layered disks, which represent the elementary component of the outer segment [from F. S. Sjöstrand, *Ergebnisse der Biologie* (Springer-Verlag, Berlin, 1958), Vol. XXI, p. 128]. ×82 000.

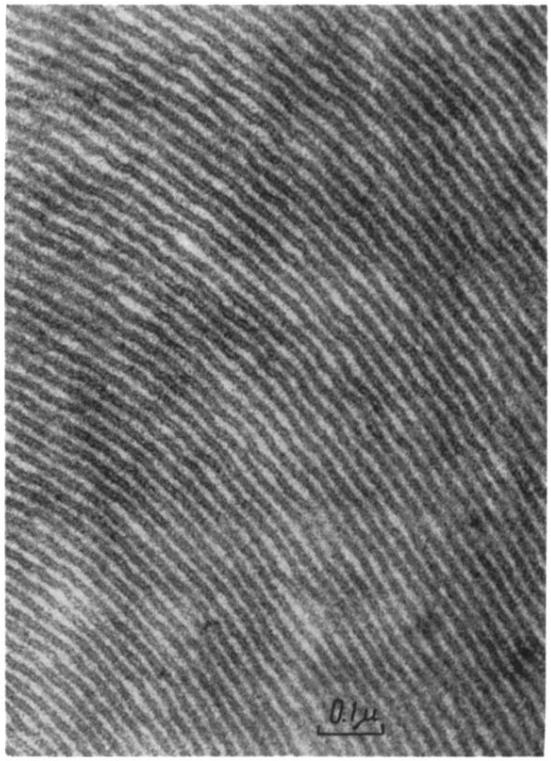

FIG. 6. Section oriented perpendicularly to the triple-layered disks of the outer segment of a cone cell in the perch eye. Notice the tendency to a uniform blackening of the whole triple-layered disk due to a staining of the middle layer [from F. S. Sjöstrand, *Ergebnisse der Biologie* (Springer-Verlag, Berlin, 1958), Vol. XXI, p. 128]. ×87 000.

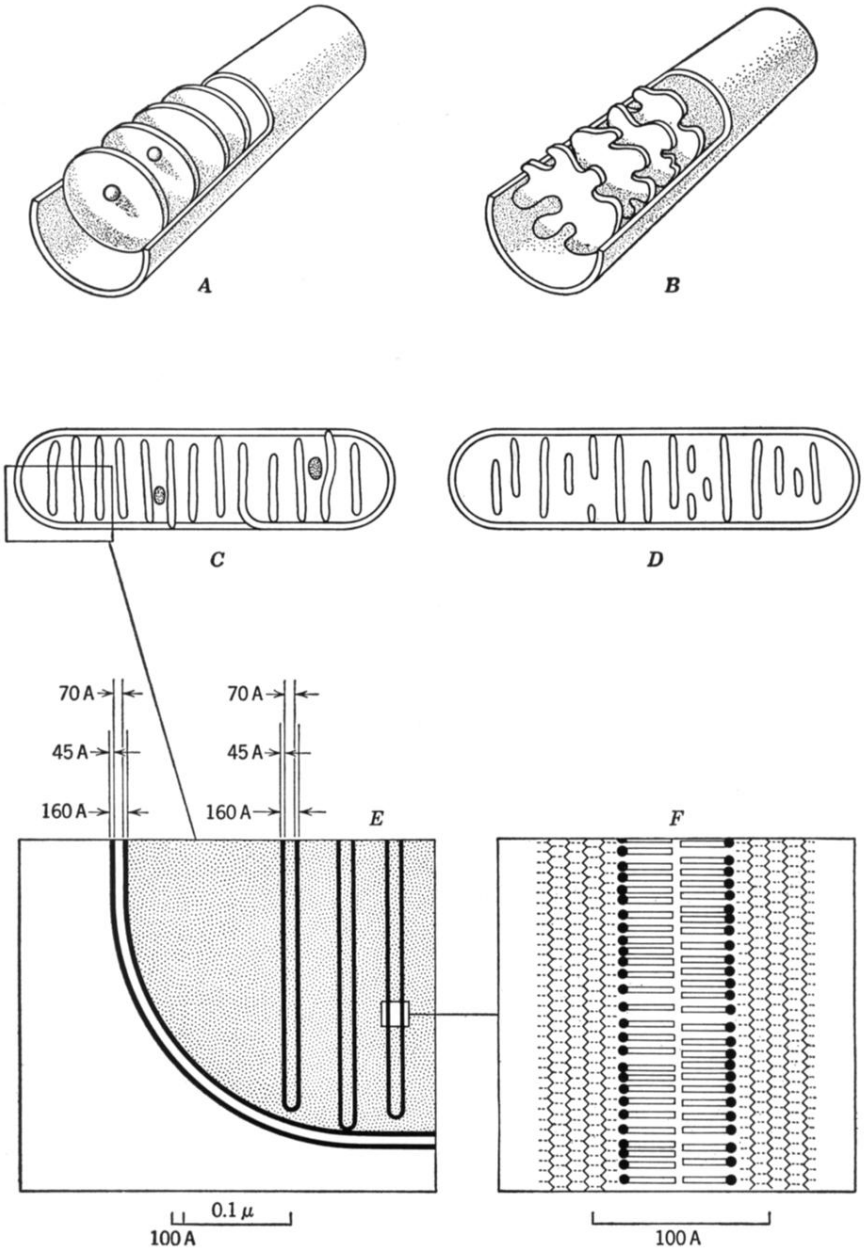

FIG. 8. Schematic drawing which illustrates an interpretation of the three-dimensional appearance of the mitochondria and of the molecular architecture of the mitochondria membranes. The three-dimensional representation *A* and *B* is based on an interpretation of the patterns observed in sections which can be, for instance, like the ones shown in *C* and *D*. *E* gives some commonly observed dimensions of the mitochondrial membranes, and *F* a scheme of the proposed arrangement of lipid and protein molecules in the membranes [from F. S. Sjöstrand, in *Fine Structure of Cells*, Proc. Symp. VIII Cong. Cell. Biol., Leiden, 1954 (Noordhoff, Ltd., Leiden, 1955), p. 16].

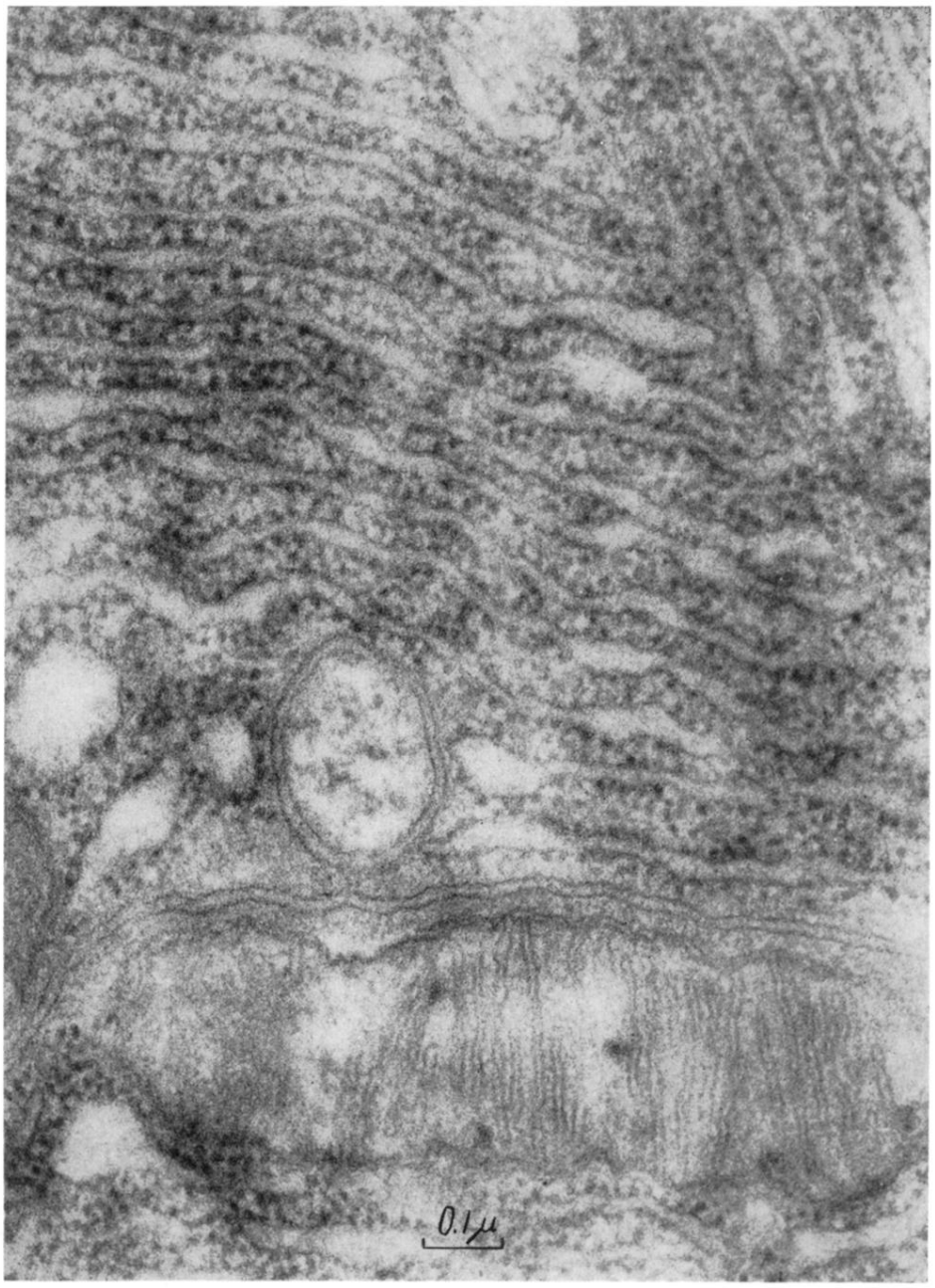

FIG. 9. Cytoplasmic membranes in the basophilic cytoplasm of exocrine pancreas cells. Notice the opaque 150-A particles attached to the one surface of the membranes which are arranged in pairs. In the lower part of the picture a cell boundary represented by two mutually parallel lines, and below the boundary a mitochondrion [from F. S. Sjöstrand and V. Hanzon, Exptl. Cell Research **7**, 393 (1954)]. ×108 000.