1957

Keith Porter, 1956

The Rockefeller University

THE SUBMICROSCOPIC MORPHOLOGY OF PROTOPLASM*

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IT is perhaps not uncommon for anyone preparing a Harvey Lecture to go back to the published accounts of other series to see whether in content or in plan there was any established pattern. In a cursory inspection I cannot say that I found any, but I did notice that in recent years a large number of the lectures have dealt with the chemistry and biological activity of enzymes, nucleoproteins, or hormones. This is, of course, a reasonable reflection of the great advances being made in biological chemistry and of the fact that more and more biological materials are being described in terms of their chemical composition. At the same time it indicates that relatively less attention and interest is being focused on the spatial arrangement of these materials in biological systems. This fact, if such it is, must be regarded as unfortunate because we cannot hope to comprehend the activities of the living cell by analysis merely of its chemical composition or the properties of its component molecules. With this oft-repeated thought in mind, one may regard the advent of the electron microscope as extremely fortunate for, in its powers of resolution, it bridges rather well the gap between the limits of light optics and the resolutions of physical and biological chemistry. It is certain to attract a large number of devoted users who will seek to relate the form and function of cells at the macromolecular level.

There are, of course, more restricted reasons for wanting to study cells with the greater resolutions of electron microscopy. Initially, I think, we simply wanted to see what there might be in the optically empty parts of protoplasm. To some extent this curiosity has now been satisfied and others are taking its place. We have, for example, studied the morphogenesis of one or two structures of the cytoplasm as well as extracellular fibers,

* Lecture delivered March 15, 1956.

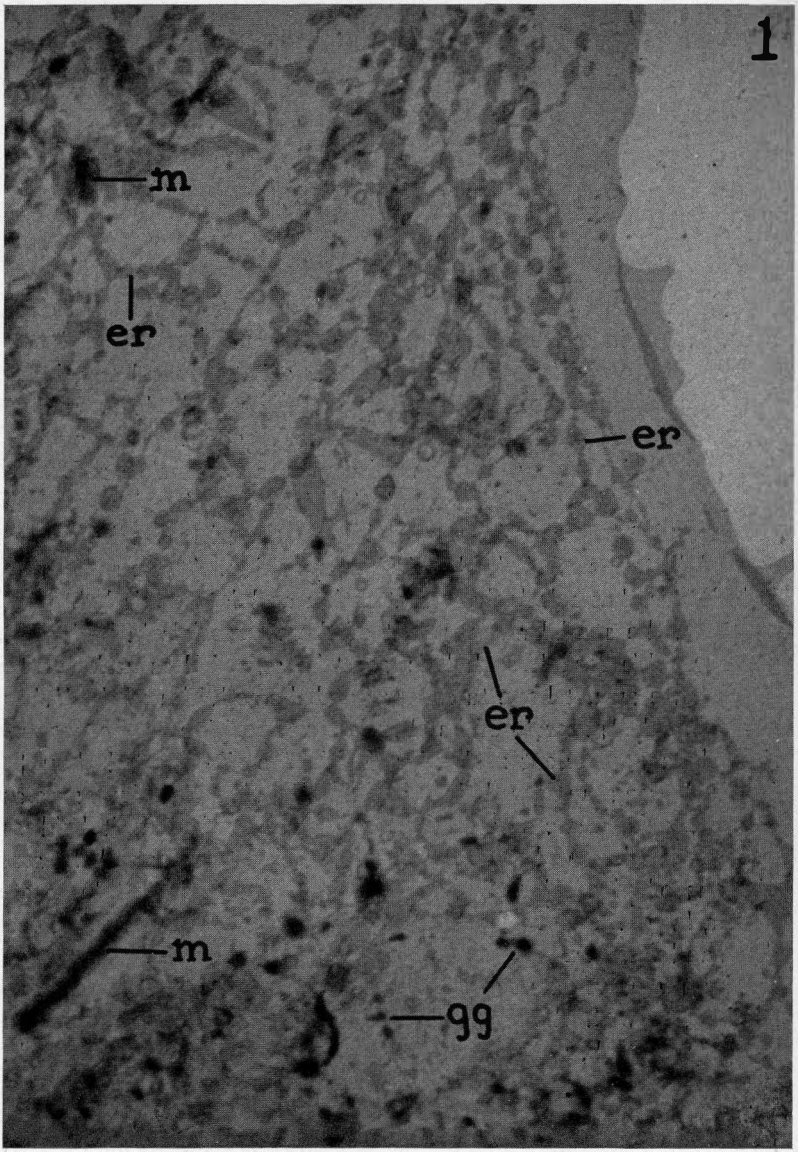


FIG. 1. Electron micrograph of a marginal area of a thinly spread tumor cell grown *in vitro* from an explant of a rat endothelioma, 4337.[7] The edge of the

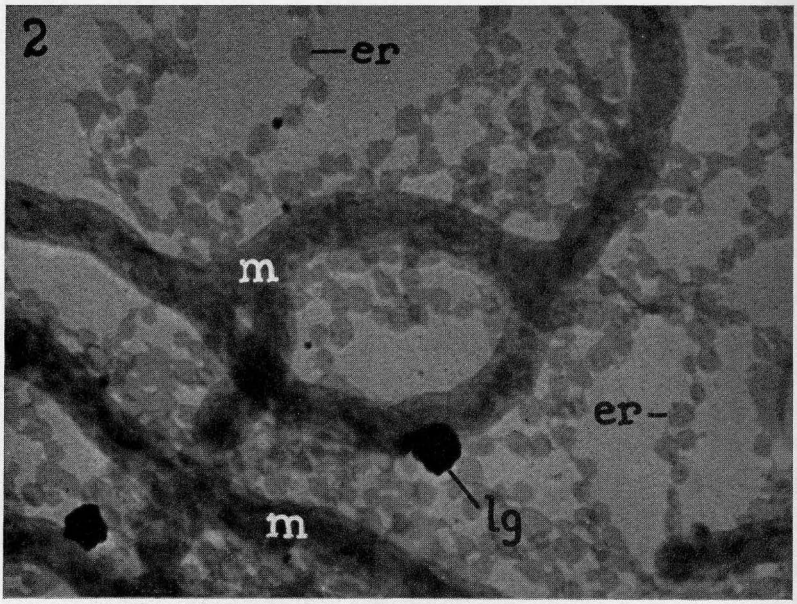
and I like to believe that we shall eventually learn something about the structural basis of the over-all form and organization of cells and cell aggregates. Thus far we have contributed essentially nothing to the elucidation of this latter problem, but I should like to take a moment to consider it because I intend to return to it again later on.

The various forms or shapes that cells can adopt and maintain, both in external appearance and in internal distribution of optically visible and biologically active components, are of course well known. Among the cells of metazoa, existing as naked protoplasts, some are long and slender, others cuboidal, and others may have many cilia or pseudopodia, and so on. These features, which normally find most elegant expression in the intact tissue, are retained to some characteristic degree even under conditions of *in vitro* cultivation. In their external form protozoa are even more remarkable, and distortions induced in such cells or their contents by external forces disappear as soon as the force is removed. The same tendency to retain their organization is shown by egg cells in which a pattern of formative factors finds expression in development. There appears to exist, therefore, beyond the limits of optical resolutions an "elastic" framework, undefinable except in terms of its apparent influence on the form and functional properties of the cell and organism.

Needless to say, the topic is a favorite one for theoretical discussions. It is, for example, common to assume, as Needham[1] has, that cell form is the expression of a paracrystalline state within the cytoplasm. Others postulate the same thing at varying orders of magnitude and speak of the pattern as residing in an invisible cytoskeleton or framework or space lattice of interacting particles.[2,3] The choice of words is perhaps of slight significance—the general concept appears the same. It is a concept that has been

cell crosses the figure at the upper right; the center is out of the image at the lower left.

The cytoplasm contains great numbers of round or oval elements (100 to 300 mμ in diameter) connected in most cases to form strands (*er*). These in turn appear to be part of a complex, and in this case irregular, reticular structure, known as the endoplasmic reticulum. A few mitochondria are indicated by (*m*). Small dense bodies (*gg*) have been described as especially common in rapidly growing tumor cells.[8] ×9000.


2
er
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er
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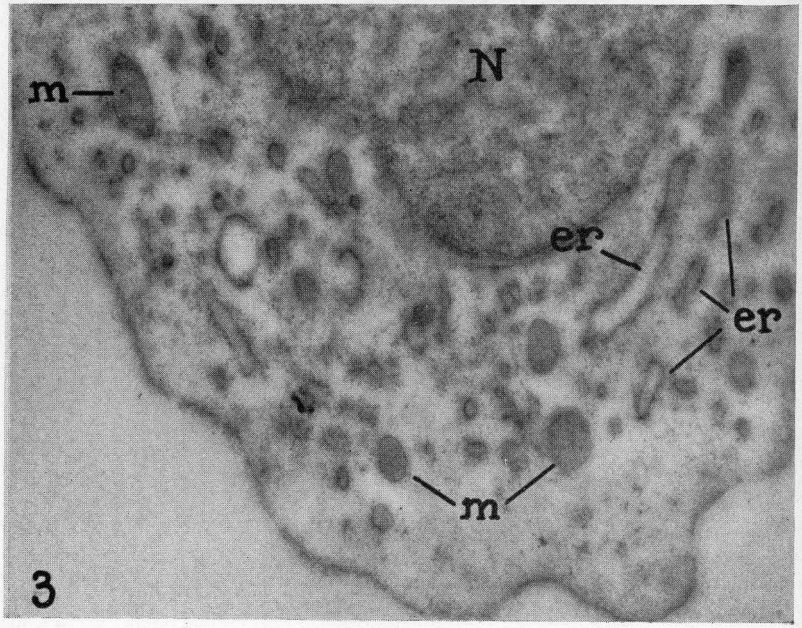
m
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3

difficult to explore by light microscopy, and, even with the resolutions now available, success is not certain. It is distinctly possible that the structural units assumed to be involved will not retain their organization through the preparation procedures of electron microscopy. Perhaps also, since we are not sure what we are looking for, it may take us a while to recognize it even if resolvable and not displaced. Despite these considerations it is probably not too early to contemplate some of the structures we have defined, to see if any of them could be regarded as "cytoskeletal" in function.

First I should like to review a few of our observations on a membrane-limited component of the cytoplasm which, before electron microscopy, was essentially unknown, and in so doing point out certain relationships that exist between it and other elements of the cell. I shall then give some attention to cilia and their associated structures and finally consider briefly one or two observations on centrioles and components of the mitotic spindle. Some of the material to be presented will be drawn from collab-

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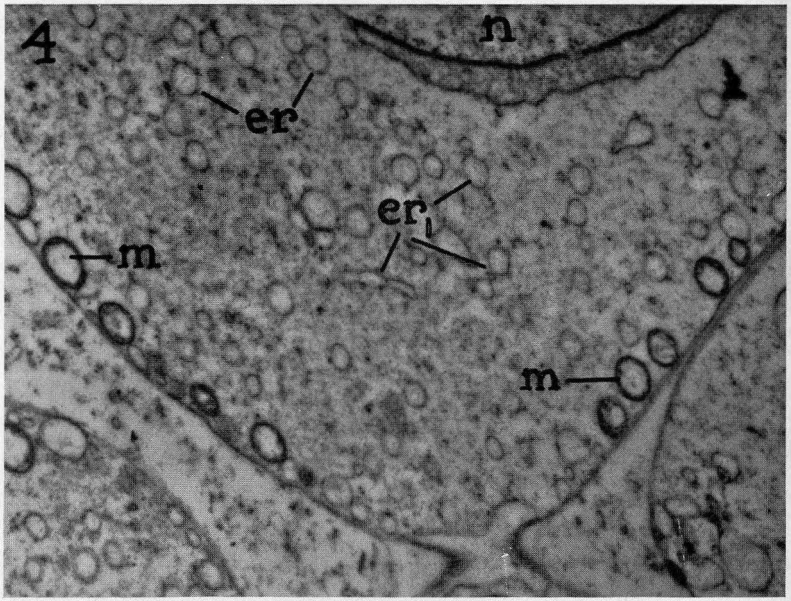
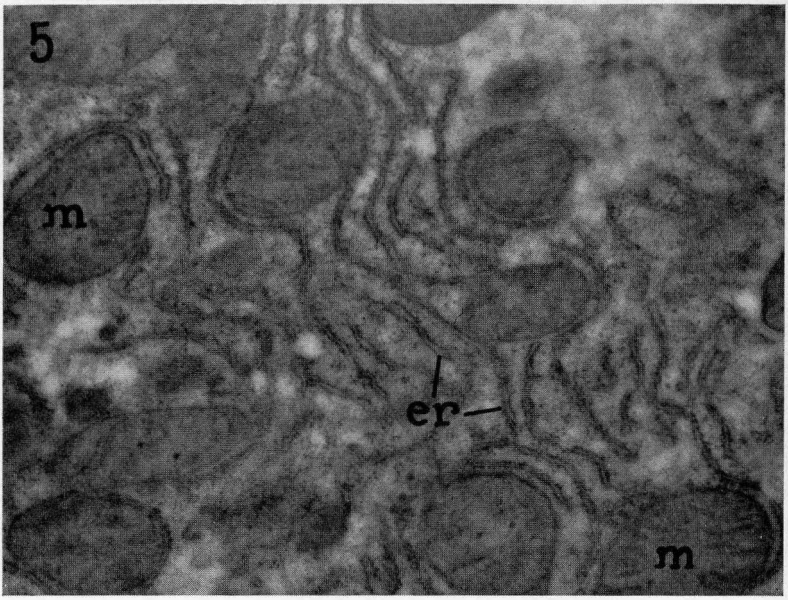
FIG. 2. This micrograph shows a small area of a macrophage grown *in vitro* from a monocyte of chick buffy coat. The endoplasmic reticulum (*er*) appears as a network of vesiculated strands, the vesicles of which have flattened in drying. Thus in this preparation, which has been lightly "shadowed" with chromium, they appear wafer-shaped. The strands, here vesiculated, are sometimes relatively smooth in outline and represent slender tubules or canaliculi. The transformation to the form depicted here seems a normal one which is reversible. Other inclusions of the cytoplasm shown are large mitochondria (*m*), also flattened in drying, and lipid granules (*lg*) which are extremely dense possibly because of pronounced osmiophilia. ×20,000.

FIG. 3. Micrograph of a part of a thin section through a chick monocyte. For this preparation the buffy coat, from centrifuged chicken blood, was fixed in buffered OsO_4, dehydrated, embedded in *n*-butylmethacrylate, and sectioned. A portion of the same buffy coat was used as a source for the macrophage (monocyte) shown in Fig. 2. Besides the nucleus (*N*) and sections through mitochondria (*m*), the figure shows circular or oblong profiles which represent sections through the vesicular members of the endoplasmic reticulum (*er*). The evident variation in size and density is doubtless related to the size variation of the original vesicles and to whether the section represents an equatorial or peripheral sector of the vesicle. In one or two instances the profiles appear in a row and depict rare occasions when the plane of section coincides with the long axis of a vesticulated strand. ×24,000.


4
n
er
er$_i$
m
m


5
m
er
m

orative studies made with George E. Palade and D. W. Fawcett, and I am pleased to acknowledge their help and stimulation.

It is not essential here, I think, to describe techniques or instrumentation; they are adequately covered in a number of recent publications. We are the slaves of both in the sense that we have considerable faith in what they show us. Investigators still appear on the fringes of our society who question the authenticity of what the rest of us accept as factual, but such terrorists are neither numerous nor long-lived. They soon join us in extolling the virtues of OsO_4 and our new gadgets. Actually, of course, the electron microscopist is not so unconcerned about the artifacts in osmium-fixed material as these remarks would suggest, and some reasonably serious work has been done to define the action of the fixative as a chemical reagent.[4,5] We attempt also, on occasion, to distinguish between fact and fiction in the osmium-fixed preparation by using alternative procedures. To be very brief about it, the general conclusion is that the electron microscope image of the OsO_4-fixed cell is remarkably faithful to the original morphology and that much can be learned from examining it.

It will help the presentation to go back a bit to some of our earliest observations on cell fine structure. These stemmed from the idea that cells as they grow in tissue culture and spread out on the cover glass might be thin enough for the differential penetra-

FIG. 4. Micrograph showing part of a rat spermatid. The nucleus is indicated at *n* and marginally located mitochondria by *m*. Circular or oval outlines scattered throughout the cytoplasm (*er*) represent sections through vesicular elements of the endoplasmic reticulum. These are occasionally connected (as at er_1) and doubtless represent places where the section includes a segment of longer vesiculated strands. It is suggested, therefore, that the system is not so discontinuous as it appears in this single thin section and may even consist of randomly oriented strings of vesicles (possibly connected to form a lattice) not unlike those depicted in Fig. 2. For further evidence see Palade.[16] ×24,000.

FIG. 5. This represents a small portion of a thin section through the cytoplasm of a liver cell. Mitochondria with their internal cristae[17] are indicated at *m*, and ribbon-like, cisternal elements of the endoplasmic reticulum at *er*. These, it will be noted, are of fairly uniform thickness (400 A.) in places but otherwise of variable lengths and thicknesses, determined in part by the obliquity with which they pass through the section. The membranes defining these elements are smooth on the lumen side but studded with RNA-rich granules on the matrix (cytoplasmic) side.[15] ×30,000.

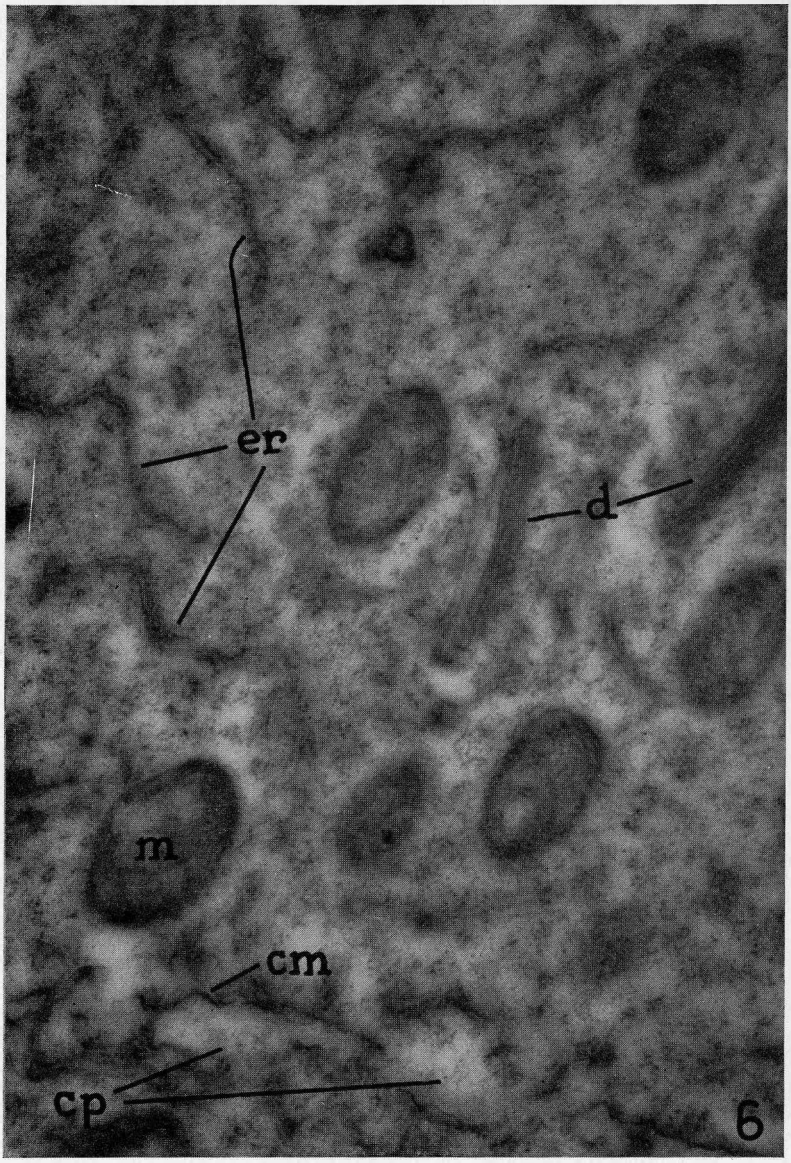


FIG. 6. Micrograph showing cytoplasm of cell from growing region of onion root tip. The cell depicted is a product or recent division as shown by the

tion of electrons and consequent image formation. The idea proved a sound one, and in the period before we learned to cut adequately thin sections preparations of cultured cells provided some useful and new information. The first electron micrographs ever taken of cells were not remarkable for what they depicted, but among other things our attention was attracted to a certain vaguely defined network of lacework of densities in the cytoplasm.[6] As techniques and facilities improved, we obtained better images of this same component. It seemed to consist of strands or strings of vesicular bodies tied together to form a reticular structure (Fig. 1). The same or a similar component was observed in the cytoplasms of a variety, but limited number of cell types that could be grown in suitable form for electron microscopy (Fig. 2). It was usually excluded from the thinnest margins of the cell, i.e., the region representing the more rigidly gelled cortex or ectoplasm, and so confined to the endoplasmic portion of the cytoplasm. Out of this distribution and form, the structure came to be referred to as the endoplasmic reticulum.[9] The name, applied as it was without knowledge of the function and very limited knowledge of the structure, is obviously of slight significance. The concept of it (derived from cultured cells) as a finely divided vacuolar system has survived to the present time and is apparently as valid now as it was when only whole cells were studied. I might reasonably add that without these early observations on cultured cells our concepts of the system would probably be as incomplete and inaccurate as those of other investigators who first encountered the system in sections of liver and pancreas cells and saw it as an organization of double membranes[10] or filaments.[11,12] The excuse for their error may be evident as we go along. It was subsequently found that this reticular system could be discerned in living cul-

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presence of new "cell plate" across the bottom of the figure (*cp*) and developing cell membrane (*cm*). Mitochondria are indicated at *m*, and slender tubular elements, identified as parts of the endoplasmic reticulum, at *er*. In this material the latter do not organize in skeins though they frequently are paired (see also Fig. 40). The small dense granules of the cytoplasmic matrix, as usual in embryonic cells,[20] show no special affinity for their surfaces. Small arrays of parallel membranes (*d*) have not been identified but may be properly referred to as dictyosomes. ×45,000.

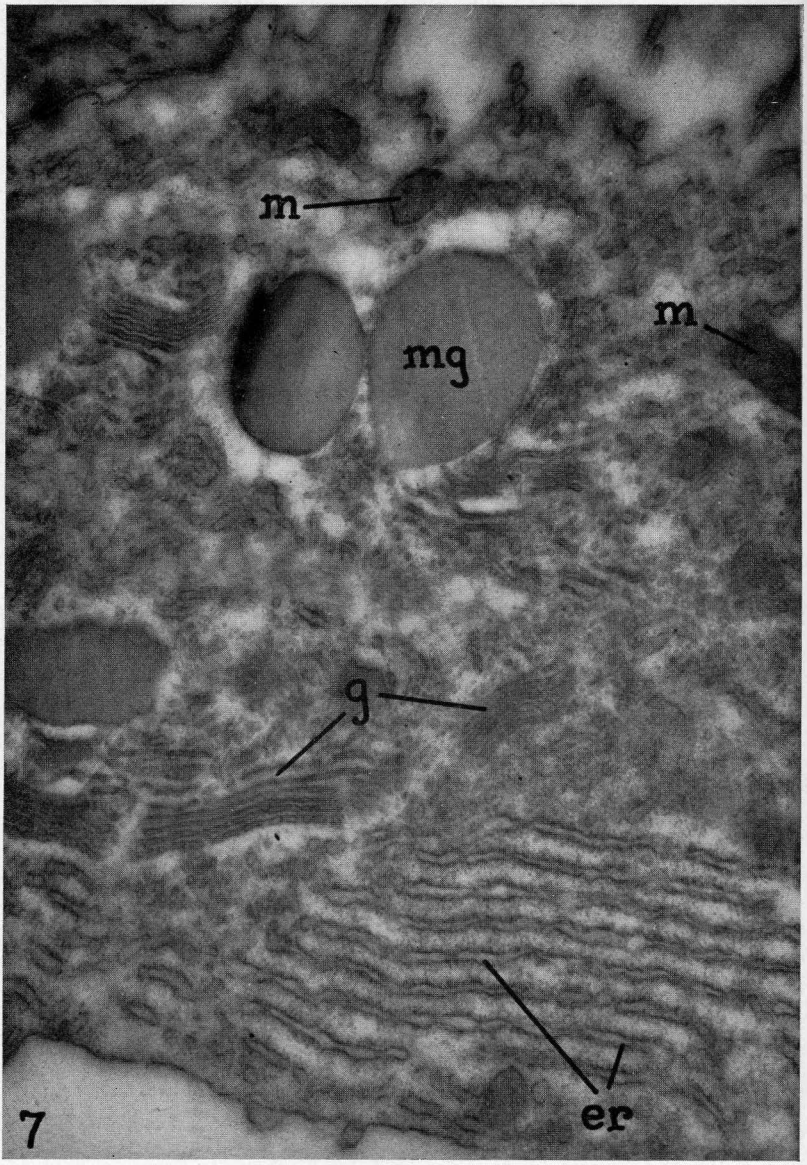

FIG. 7. This micrograph shows a portion of a mucus-secreting cell found in the olfactory epithelium of the frog. The free surface of the cell is at the top

tured cells by phase contrast microscopy, if one knew what to look for, and its staining properties suggested that it represented or had associated with it the basophilic or RNA-rich material of the cytoplasm.[9]

The discovery of adequate techniques for fixation of cells in tissue blocks[13] and for embedding[14] and thin sectioning[10,15] opened the way to the study of this and other structures in any and all types of cells. This was a tremendous step forward. In thin sections of chick monocytes fixed *in situ* (Fig. 3), it was not difficult to identify elements corresponding to the vesicles in the image of the equivalent cell (Fig. 2) grown *in vitro*. They appeared in profile as membrane-limited, usually discrete, units (Fig. 3). Occasionally where the plane of section coincided with a string of vesicles or strand of the reticulum, the continuity of the system was also evident. The content of the vesicles appeared homogeneous, particulate-free, and frequently of lower density than the surrounding matrix of the cytoplasm. This suggests that the content is either not fixed by the OsO_4 solutions used or is in many instances simply a dilute aqueous solution of metabolites of small molecular size that can diffuse freely from the fixed material.

One measure of the significance of such a component to the form and function of cells is the generalness of its occurrence; if it is ubiquitous we may consider it an essential element of the cytoplasm. Observations on cultured cells suggested that it is indeed a commonly occurring component, but we have to remember that the number of cell types studied was small and that those examined were selected for their thinness—a condition that might reasonably produce some features of the morphology observed. For

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of the figure, and basal surface at the bottom. Mucin granules are indicated at *mg*, and mitochondria at *m*. The parallel array of elongated profiles located near the basal pole of the cell (*er*) represent vertical sections through flattened or lamellar vesicles (500 to 1200 A. thick) characteristic of the endoplasmic reticulum in this and other protein-secreting cells. The membranes marking these profiles appear extraordinarily thick and dense because their outer surfaces are coated with small particulates (ribonucleoprotein (RNP) granules) of the cytoplasmic matrix.[20] Other arrays of parallel profiles evident in the micrograph (*g*) represent longitudinal sections of fine cisternal elements, identified as part of the Golgi material in these cells. ×17,000.

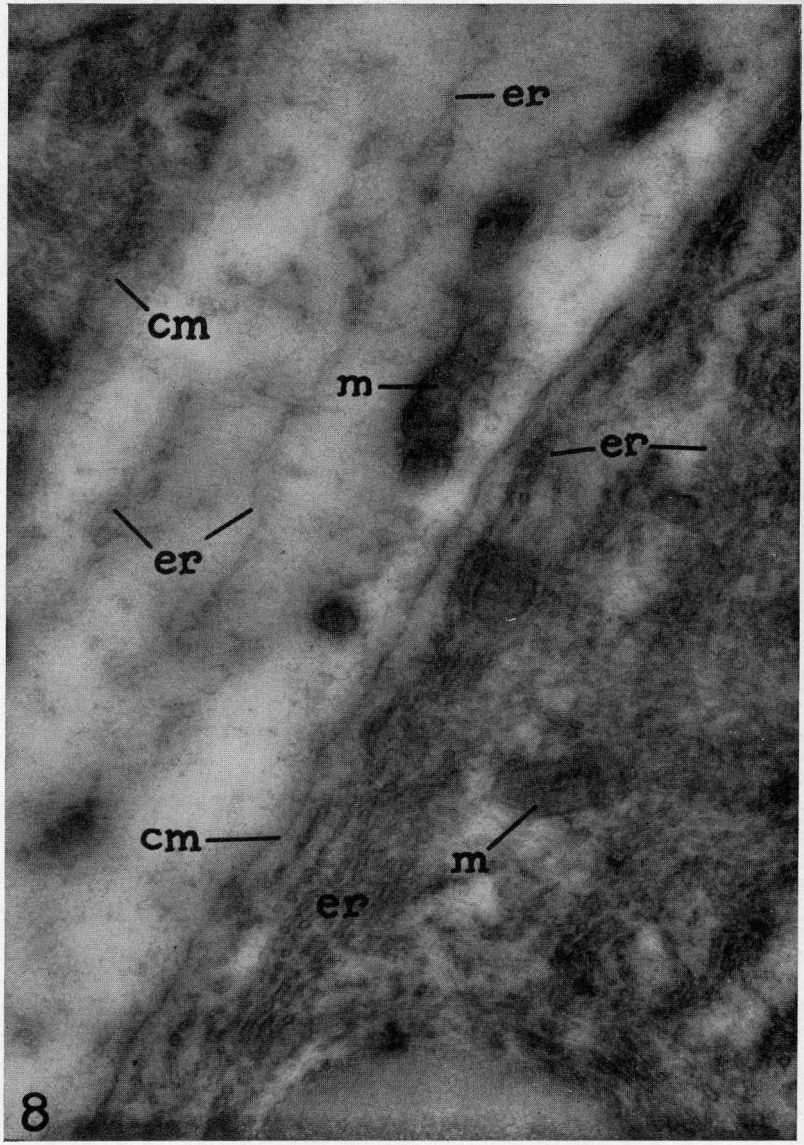

FIG. 8. Micrograph depicting adjacent cells in the olfactory epithelium of the frog. The lighter area crossing the figure from lower left to upper right represents a portion of a sensory cell just within the free surface of the epithelium.

these and several other reasons it was important to explore thin sections of a wider variety of cell types, preserved *in situ,* i.e., in their normal tissue relationships. The results were more interesting than anticipated and showed that such systems of closed vesicles, identified as homologous on the basis of their size, their membranous walls and homogeneous content, and their location within the cell, do in fact exist in all types of cells except mature erythrocytes. This apparently applies as well to all forms of animal and plant life.

In some instances, as for example in spermatids of the seminal epithelium of the rat (Fig. 4), the elements of this membrane-limited system appear as circular profiles scattered quite uniformly throughout the cytoplasm.[16] The general absence of other shapes indicates that most of the members of the system are vesicular, and whether all are connected to form a reticulum of vesiculated strands such as shown in Fig. 2 could be determined by examining an extensive series of consecutive sections. The presence, however, of a few strings in the image (er_1, Fig. 4), representing presumably the few instances where the plane of section coincided with a strand (as well as similar appearances in other micrographs), suggests that all may be linked together into a tridimensional lattice.

Another configuration of the system is observable in the cytoplasm of liver cells (Fig. 5). Here a majority of the elements are ribbon-like (cisternae) and generally not vesiculated. The individual members of the system are extraordinarily slender in one dimension (thickness, 400 A.) and fairly uniform in this dimension throughout their visible lengths. The "ribbons," whose other

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The bordering regions of the image (upper left and lower right) show parts of two adjacent sustentacular cells. The separating cell membranes are indicated at *cm.*

The endoplasmic reticulum (*er*) of the sensory cell is extremely tenuous in its form and slight in total amount. Its appearance here is similar to that observed generally in axonic and dendritic processes.[25] In the sustentacular cell cytoplasm, on the other hand, the reticulum is complex and abundant in amount. It is composed of fine (diameter, 400 A.) canaliculi continuous in a close, tridimensional lattice. This pattern, in various degrees of compactness, is also encountered in other cells which possess and retain a characteristic and independent form (e.g., apical poles of crown cells, Fig. 19). Mitochondria are indicated at *m.* ×27,000.

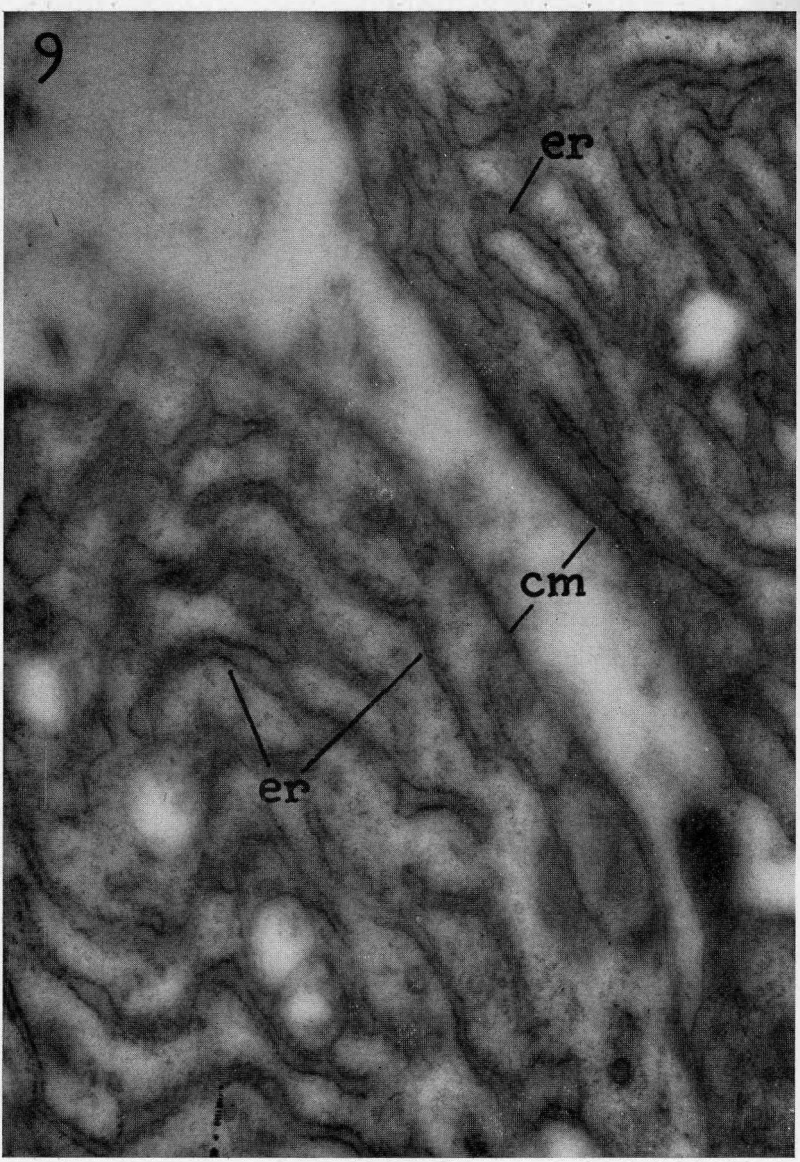

FIG. 9. Portions of two fibroblasts producing fibrous stroma in Jensen rat sarcoma. The cytoplasm of these cells, like other cells involved in protein

dimensions are not defined, appear to be loosely bundled into skeins which are of undetermined length. Dense granules attached to the outer (matrix) surfaces are rich in RNA[18] and together with the cisternae constitute the "clumps" of basophilic material seen in appropriately stained preparations of liver.[19]

A fairly special form seems to be characteristic of the reticulum observed in the cytoplasm of cells of the growing onion root tip (Fig. 6). Here extremely fine canalicular elements constitute the system. These are not arranged in bundles or indeed in any recognizable organization, but are uniformly present, thus attesting to the apparent ubiquity of the reticulum as a component of plant and animal cell cytoplasm.

One of the more striking representatives of this membrane-limited system is characteristic of cells involved in protein synthesis. Here the larger and more prominent members of the system adopt the form of flattened vesicules or cisternae.[21,22] The dimensions of these vary considerably, and since their form is extremely labile and responsive to minor changes in the extracellular environment,[23] what is at one time a continuous lamellar structure may at another develop many fenestrae or even disintegrate into a layer of discrete vesicles. Usually these cisternae are organized into parallel arrays of a few to many members (Figs. 7 and 14), and such arrays characteristically coincide with the basophilic portions of the cytoplasm as, for example, along the basal poles of exocrine cells of the pancreas or parotid glands.[22,24] They thus represent the ergastoplasm of Garnier. In this, as in the case of liver mentioned above, the property of basophilia probably resides in the small dense granules which cover the outer or matrix-facing surface of the limiting membranes.[18,20] Other parallel arrays of tubular or

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synthesis,[22] shows a rich development of the endoplasmic reticulum (*er*). Here the members of the system have the form of irregular cisternae all connected together to form a complex reticulum. In this instance the content of the system is more dense than the surrounding matrix of the cytoplasm, and the contained material responsible for this density may represent a precursor of collagen. Just at the cell membrane (*cm*) fine fibers are polymerizing out of an otherwise amorphous material produced by the cells. Small dense RNP granules cover the outer surfaces of the *er* membranes, as in all cells synthesizing proteins. ×21,000.

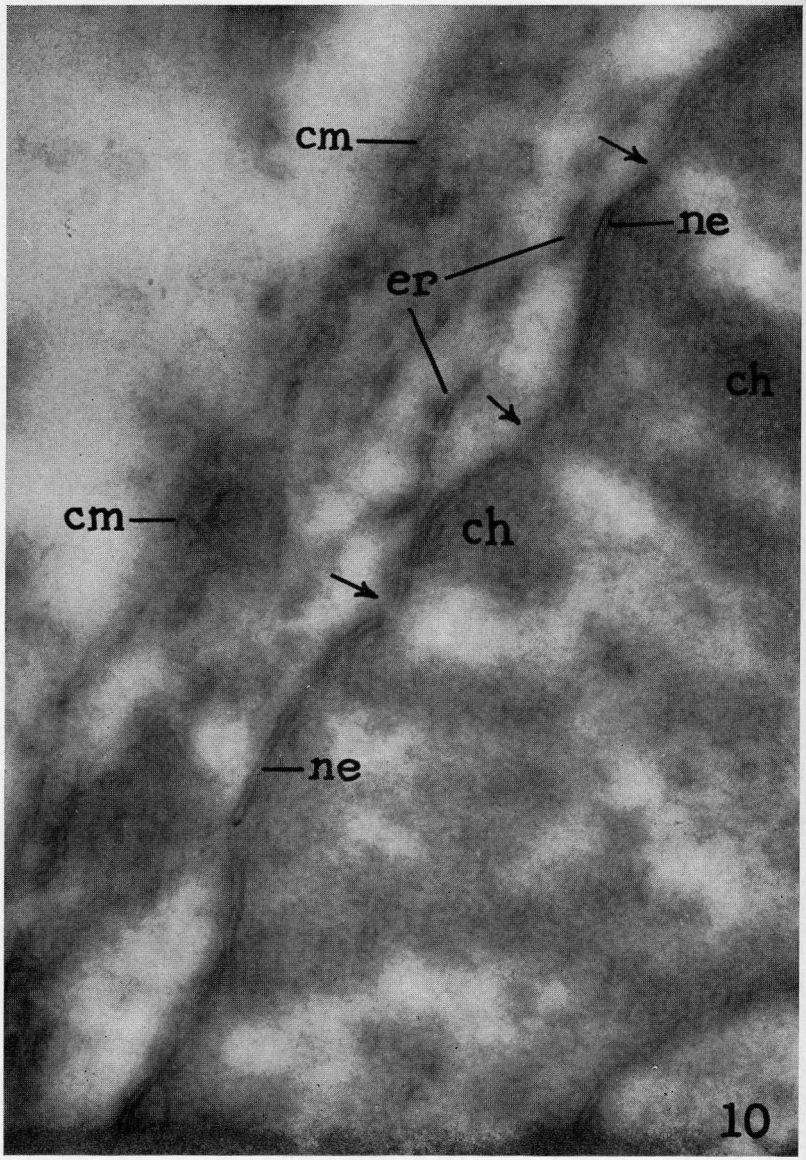

FIG. 10. This micrograph shows a portion of the nucleus and cytoplasm of a sustentacular cell in frog olfactory epithelium. The cell membrane (*cm*) sep-

cisternal units also encountered in the cytoplasm, although apparently continuous with and part of the endoplasmic reticulum[16] (see below), are distinctive in not having particulate-studded surfaces (Figs. 7, 8, 27, 28).

These are perhaps descriptive of the major configurations the system may adopt. In other cells, however, the cisternae may be more numerous, thinner, and more closely packed. In still others the reticulum may appear for the most part as a close, three-dimensional lattice of small canaliculi (Fig. 8).

From such observations as these another feature of the system emerges, which is that in each type of cell it tends in its major development to show a characteristic form. This, as suggested above, may vary somewhat with the physiological state of the cell but seems to revert to a characteristic appearance under more or less normal conditions. The system may be regarded, therefore, as a mark of differentiation, and distinctions between cell types are readily made on the basis of its form alone (Fig. 8).

It is obvious from this same figure that where the functional differences between cells is pronounced the reticulum may appear very different. Conversely, among cells having a somewhat similar function the form of the system is fairly similar. For example, in cells actively synthesizing protein for secretion it is characteristic, as mentioned above, for the system to achieve its greatest development and to appear as parallel arrays of cisternae or flattened vesicles. This holds true not only for the exocrine, enzyme-producing cells of the digestive glands (especially parotid,[22] body chief cells

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arates the sustentacular from the dendritic process of an adjacent sensory cell. The nuclear membrane or envelope is indicated at *ne*, and dense chromatin masses within the nucleus at *ch*. The cytoplasm between the cell membrane and the nuclear envelope contains numerous profiles of small canalicular elements of the endoplasmic reticulum (*er*) (see Fig. 8). It can be noted that the nuclear envelope (*ne*) consists of two membranes separated by a space measuring about 350 A. At several points along the envelope the continuity of the profile is interrupted by pores or openings (arrows), and at these points the less dense regions of the nuclear content are continuous with the continuous phase (the matrix) of the cytoplasm. It is interesting because of their apparent relationship (see below) to note also that the over-all thickness of the nuclear envelope (500 A.) is identical with the diameter dimension of the small canalicular elements of the endoplasmic reticulum. ×30,000.

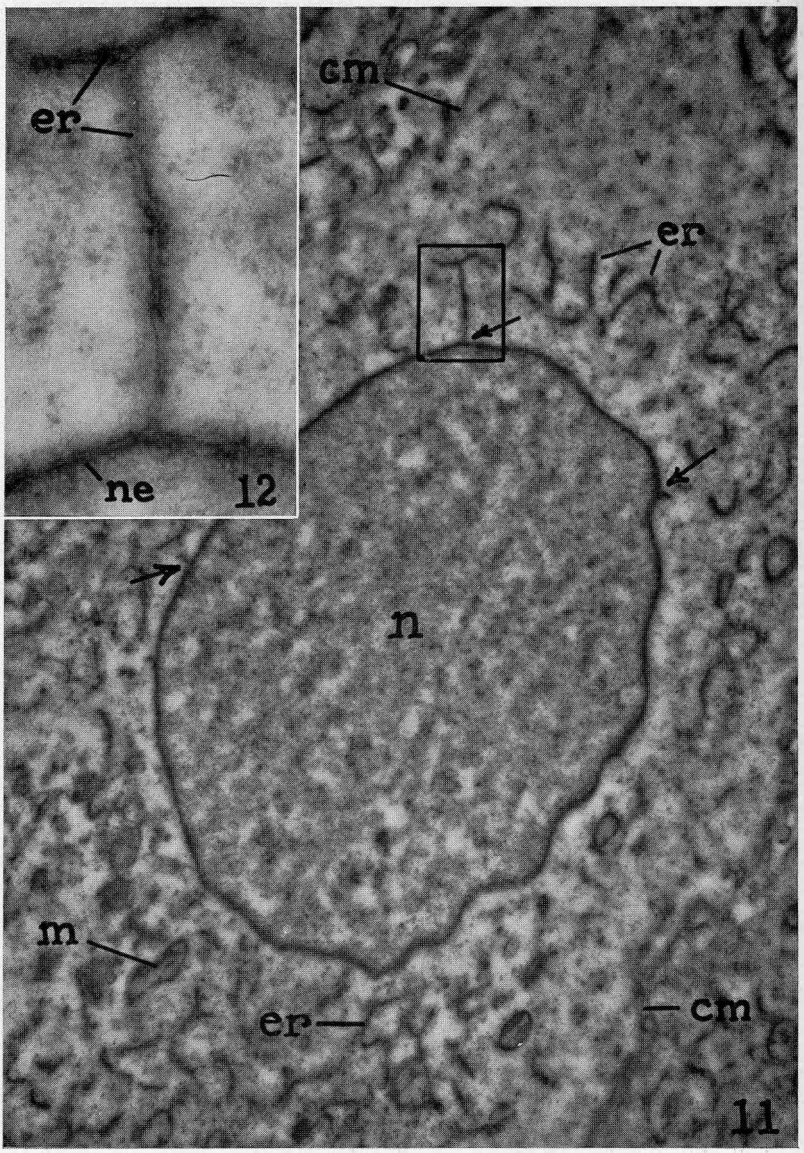

FIG. 11. Low-power micrograph of a section through a cell of the Jensen rat sarcoma. The nucleus is indicated at *n*, and the cell margin, where included in

of glandular stomach, and exocrine pancreas[22,24,26]) but also for other protein-producing cells such as plasma cells[20] and fibroblasts (Fig. 9).[22]

I have reviewed, then, some of the more general features of the form and distribution of the endoplasmic reticulum. It should not be judged from what I have said that a fibroblast contains only the cisternal elements of the type just mentioned or that a nerve cell shows only the characteristic slender strands. In the region of the cell center, for example, there are localized variants of this membrane-limited system[16] which are too complex to take up in this brief survey (e.g., Golgi material, Fig. 7, or numerous small vesicles of the cytocentrum). In several respects these are similar in all types of tissue.

Other variants of the reticulum may assume some specific relation to another formed element of the cell, descriptive of the general integration of the system into the morphology of the whole unit. The nuclear membrane, e.g., as Watson[27] has shown, is, in its structure, not unlike a large flattened unit of the reticulum. It appears in profile as a two-membraned envelope, with a fairly uniform space between the membranes (Fig. 10). There are fenestrae or pores in this envelope as there are occasionally in the large cisternae of the cytoplasm. But the most significant reason for identifying this structure with the reticulum is shown in Figs. 11 and 12, where the space within a strand of the cytoplasmic system is seen to be continuous with the space between the membranes of the nuclear envelope.

A further example of a structural correlation between the reticulum and another component of the cell is observed in striated muscle. Here the system is confined very largely to the sarcoplasm

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the micrograph, at *cm*. Besides a few mitochondria (*m*) it is typical for the cytoplasm of these cells to show a reticulum (*er*) composed of slender tubular members. These branch and appear to form an open lattice. At a few points within the section (indicated by arrows) strands of the reticulum appear to fuse with the surface of the nucleus. ×7,000.

FIG. 12. Greater enlargement of a point of continuity between the tubular element of the endoplasmic reticulum (*er*) and the nuclear envelope (*ne*). It is evident that the less dense space in the image, between the membranes of the nuclear envelope, is continuous with the cavities of the reticulum. ×32,000.

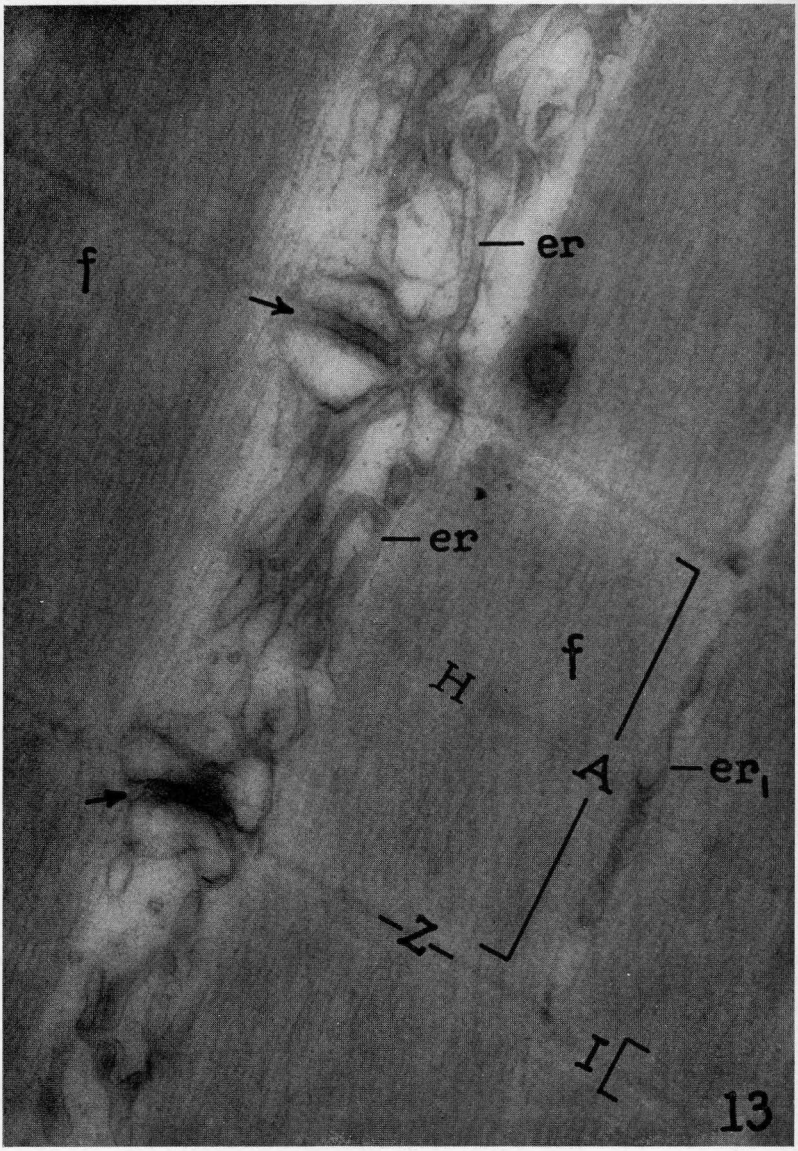

FIG. 13. Portion of longitudinal section of a muscle cell from a caudal somite of a 12-mm. *Amblystoma* larva.[28] Myofibrils (*f*) run from lower left to upper

between the myofibrils and shows its integration into the over-all structural pattern by the presence of special differentiations opposite the various bands of the myofibrils (Fig. 13). Since these are repeated in each sarcomere, the reticulum (known as the sarcoplasmic reticulum) is essentially segmented in its organization. The existence of this system, noted initially in electron micrographs of fowl muscle,[29] has since been reported in a variety of other types.[28,30,31] Several other instances of such integration might be cited in a more leisurely discussion of the system, but these may suffice to make the point.

I might now summarize this brief review of the observations thus far made on this newly defined component of cells. At resolutions provided by the electron microscope the cytoplasms of all cell types are found to contain what I like to call a finely divided vacuolar system, also known as the endoplasmic reticulum. This varies in form among different cells, both in the character of the elements comprising the system as well as in relative amount, general organization, and distribution. In these structural variations the system appears to be an expression of cellular differentiation.

A few comments regarding its function are appropriate, though we are poorly informed in this regard. In cells actively engaged in protein synthesis, as mentioned above, it achieves a prominent development and is assumed therefore to be important to the metabolic processes involved. We were encouraged very early to relate the system to the microsomal fraction of Claude[32] because of the size and membranous character of the vesicles seen in cultured

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right, and the A, I, Z, and H bands of these are so indicated. Elements of the sarcoplasmic reticulum[29] (the endoplasmic reticulum of muscle) are evident in the sarcoplasm between the myofibrils (at *er* and er_1). Along the center of the figure, at *er*, the system is pictured as it spreads over the surface of a myofibril. Canalicular elements comprising it run roughly parallel to the long axis of each A band. Opposite the I band these expand into large vesicles which are separated from equivalent structures in the adjacent sarcomere by a uniform space, approximately 500 A. wide (arrows). At the H band, the longitudinal membranes of the reticulum fuse to form an irregular channel that is continuous laterally across the muscle cell in each sarcomere. It has been suggested[28] that, if potentials develop across the membranes limiting this system, the system might serve for the internal transmission of excitatory impulses. ×42,000.

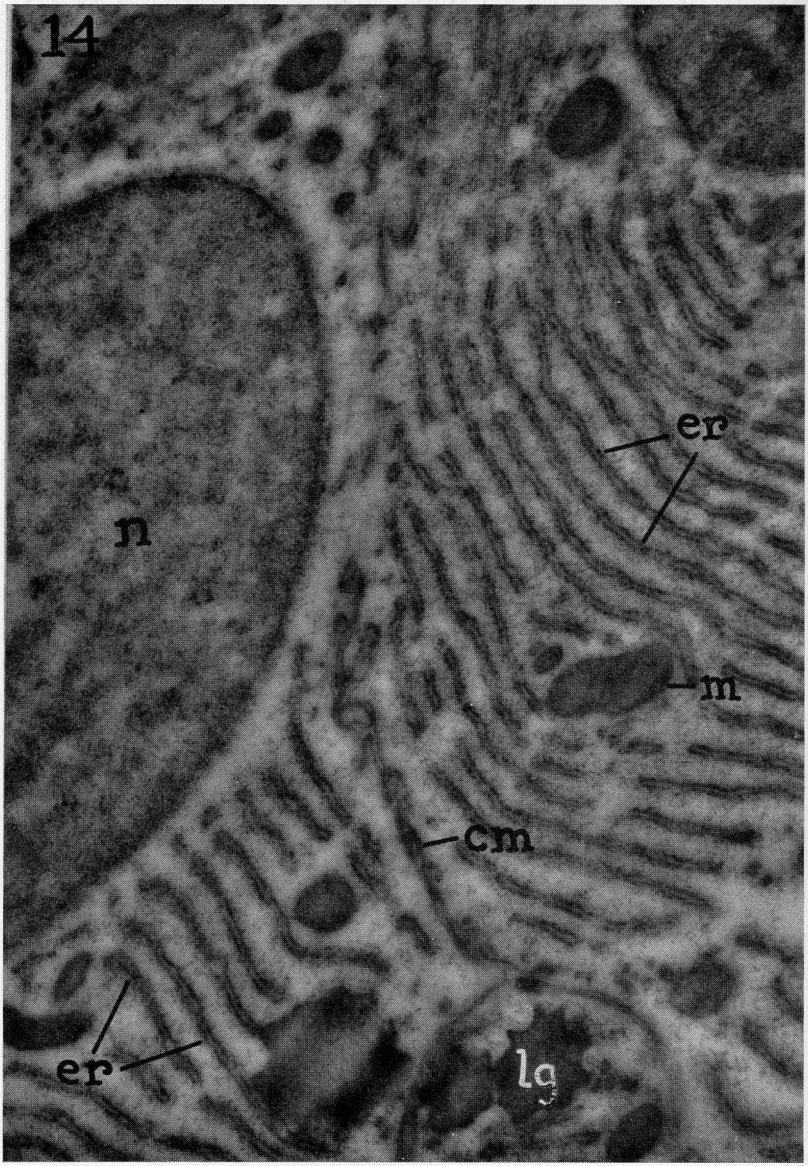

FIG. 14. Portions of two rat parotid cells showing profiles of cisternae in parallel array. Nucleus (*n*) and mitochondria (*m*) are readily identified. The

cells.[9] More recently this correlation has been proved beyond doubt in investigations by Palade and Siekevitz[18] and to some extent also by Kuff *et al.*[33] Thus, biochemical studies, past and present, on the microsomal fraction apply to this system and define it as being rich in RNA (when granules are attached)[32] and in phospholipid (because of membranes) and as containing, in liver, glucose-6-phosphatase,[34] DPNH cytochrome c reductase,[35] and an esterase.[36]

The system obviously may provide the cell with a large internal membrane surface for the orderly distribution of enzymes.[9] It also adds a new compartment to the cytoplasm for the segregation of metabolites. Finally, in those instances where it exists as a continuum, and these seem to predominate, the membranes may conceivably transmit impulses (see legend of Fig. 13), and the enclosed phase may provide for the more rapid diffusion of metabolites from one part of the cell to another, thus rendering more uniform the intracellular environment for other cell components.

These are possible roles it may play in the metabolic life of the cell. But what can be said regarding its form and the factors controlling this? Are these inherent in the system itself; does it hold the initiative in determining not only its own shape and organization but also that of the cells of which it is a part? The answers to these vastly interesting questions are not at the moment very apparent.

Certain configurations the system adopts do seem to suggest that it can exert an organizing influence over relatively large distances through the cytoplasmic matrix. For example, in mucous cells of the rat parotid and elsewhere it is not uncommon to encounter

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dense bodies at the bottom of the figure with the crenated appearance represent lipid granules (*lg*). The double membrane between the cells may be seen at *cm*. The slender, "double-membrane"[10] profiles which fill the cytoplasm are sections through lamellar vesicles (cisternae) of the endoplasmic reticulum (*er*) (see also Fig. 7). They are approximately 600 A. thick and are separated in some instances by uniform spaces as wide as 2000 A. The parallel arrangement is characteristic of glandular cells; the spacing, however, varies greatly among different cells and possibly also in different phases of physiologic activity. It is conceivable that a polarity develops across these membranes much as across the cell membrane, and that this operates to influence the spacings between the vesicles. ×18,000.

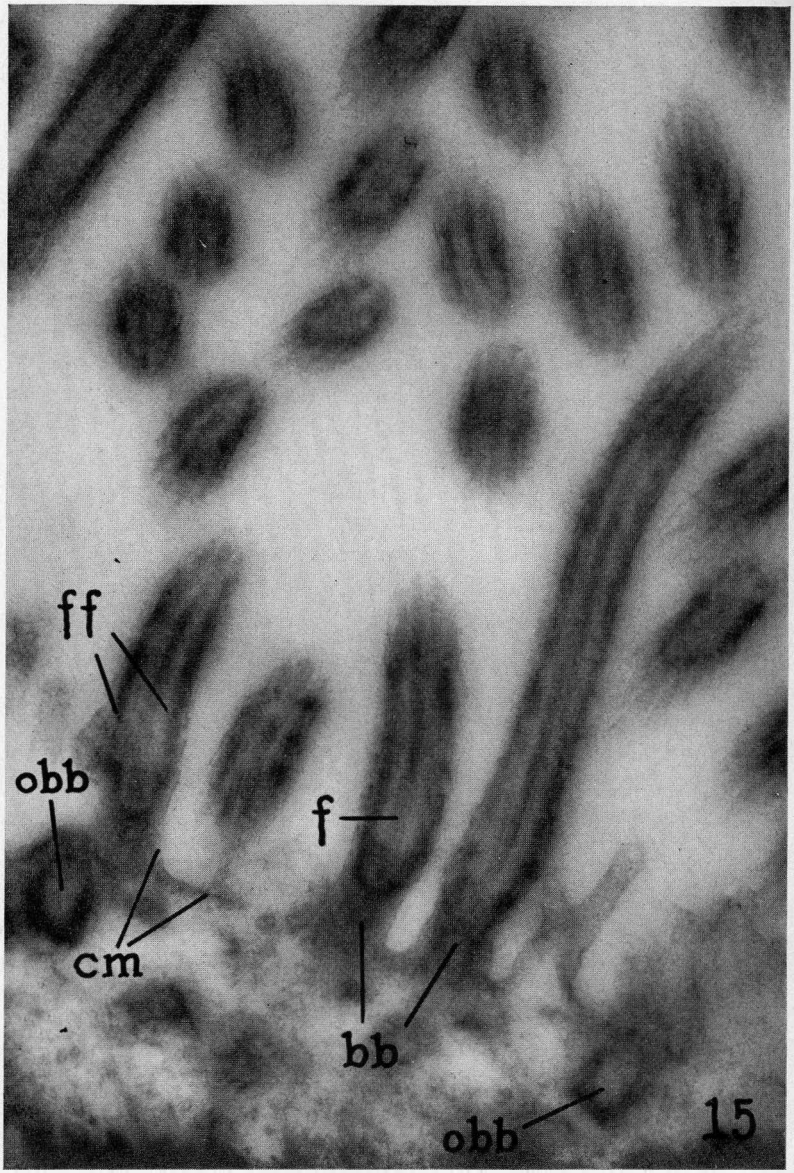

FIG. 15. Micrograph showing a small segment of the free surface of a ciliated epithelial cell in the oviduct of *Xenopus laevis*, African toad. Cilia are cut

extensive parallel arrays of large flattened vesicles or cisternae (Fig. 14). These elements have a thickness of approximately 60 mμ but are separated and apparently held more or less parallel at distances of two to three times this measure (i.e., 150 to 200 mμ). Higher magnifications fail to show any organization of resolvable particulates between the cisternae which might serve as a structural basis for their separation. So we are left to assume that some degree of polarity is established in the resolvable and unresolvable units of the adjacent matrix and that repulsive forces operating in a limited space serve to keep these lamellar elements at approximately even distances, one from the other. If this represents a common property of such membrane-limited elements,[23] the combination of a specifically patterned reticulum and a reactive matrix could conceivably influence the form of cells of which they are a part.

An eminent physicist among electron microscopists recently accused biologists in the field of indulging in a "free-wheeling" approach to the study of their material. To some extent his accusation is justified. Miscellaneous observations on a greater variety of materials are appearing with ever-increasing frequency, and the rate will doubtless double many times as more microscopes are made available. In spite of this, however, I doubt if our progress is or will be as disordered as he views it. Actually I think we are making a reasonable attempt to search out similarities and repeating designs in the fine structure of biological materials even as complicated structurally as tissue cells. In recognizing these, and

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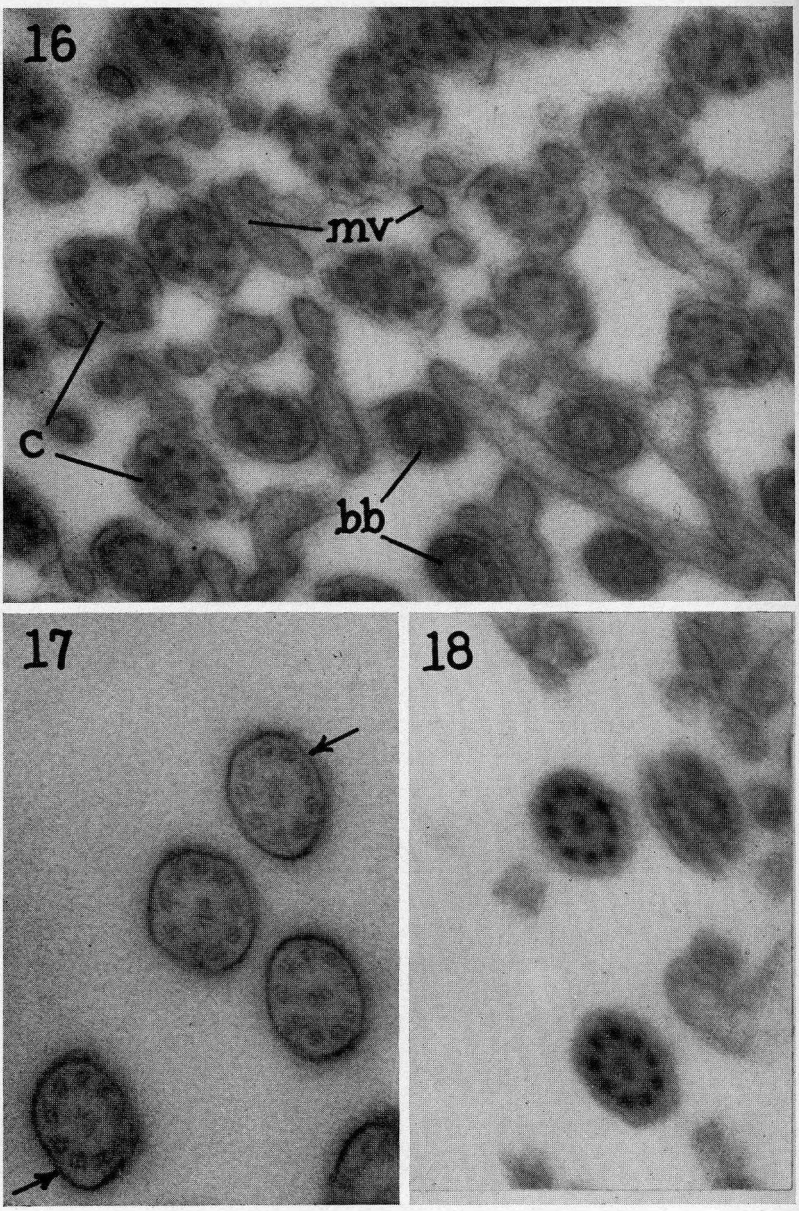
longitiudinally or obliquely. Where the plane of section coincides with the base of a cilium and includes as well a portion of the shaft, it is possible to see that the limiting membrane of the cilium is continuous with the plasma membrane of the cell (e.g., at *cm*). Within the shaft of the cilium there are slender longitudinal densities, one on either side (just under the membrane) (*ff*) and one in the middle which in places appears double (*f*). These represent longitudinally oriented filaments. At the base of each cilium where it joins the cell, there is a basal body (*bb*) of special design consisting of a transverse basal plate and, proximal to this, extensions of the peripheral filaments. Oblique sections of basal bodies are evident at *obb* and illustrate the cylindrical form of the whole structure. Short striated fibers, extending from the basal body into the cytoplasm, are present in this material but not illustrated. ×40,000.


16
mv
c
bb
17
18

more especially the variations related to function, we may hope to gather some clues to the possible roles of these new structures.

I should like now to direct your attention to some observations on the form and internal structure of cilia which in part are drawn from separate studies made in collaboration with D. W. Fawcett[37] and A. W. Sedar.[38] My purpose in doing this is to show you another and more remarkable recurring pattern of fine structure and to point out certain similarities between filamentous elements of cilia and similar components of the cytoplasm. It will be implied that they perform analogous roles in these two different locations.

In their simplest form, cilia are slender extensions of the protoplast, usually about 0.2 μ in diameter and of variable lengths up to many microns. They are able to execute a helicle or pendular motion, and in so doing they move the immediate environment over the cell or epithelial surface. They are found very widely throughout both plants and animals. Where they occur on isolated cells they are referred to as either flagella or cilia, and here, by their motion, they ordinarily move the cell through its environment.

The presence of minute filaments in cilia was recognized about fifty years ago by Koltzoff[39] using the light microscope, and the

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FIG. 16. Cross section of cilia as part of the paryngeal epithelium of the frog, *Rana pipiens*. The plane of section is close to the cell surface at the lower right and so includes transverse sections of ciliary basal bodies (*bb*). Where a cilium shaft is cut, as at *c*, 9 peripheral (~150 A. in short diameter) and 2 central filaments (150 A. in diameter) are seen in cross section. The peripheral ones are evenly spaced with respect to one another and essentially so with respect to the central pair and the limiting membrane. Somewhat oblique orientation of the section as well as the compression of cutting account for the oval shape and departure from symmetry. The intervening bodies are profiles of microvilli (*mv*) and folds extending from the free surface of the epithelial cell. Note that the peripheral filaments are double in structure and the two central filaments single. ×50,000.

FIG. 17. Cross sections of cilia of *Paramecium multimicronucleatum* showing essentially the same structural detail seen in Fig. 16. The ciliary membrane is here intact, and the limits of the filaments are more clearly depicted. Thus the double structure of the peripheral units is more easily seen (as at arrow). The matrix material around the filaments is without evident structure.[37] ×53,000.

FIG. 18. Cross sections of cilia on epithelium of human Fallopian tube. The spatial distribution of the filaments is unusually well preserved. ×50,000.

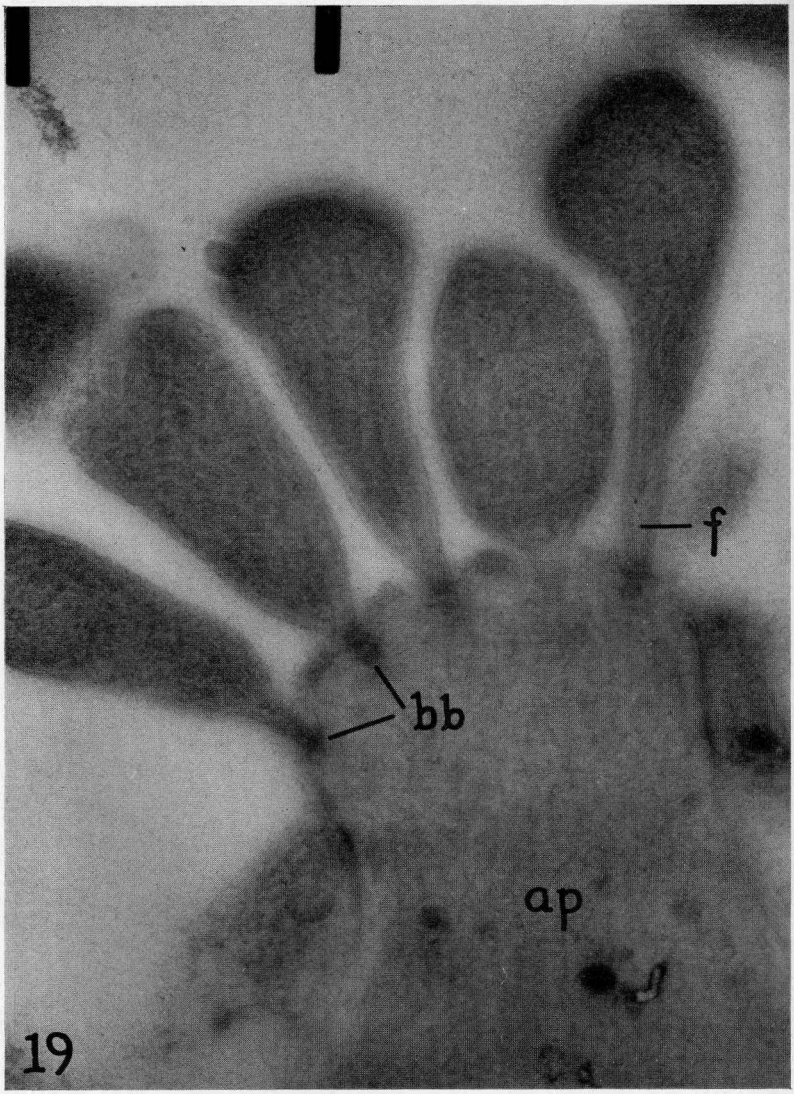

FIG. 19. Portion of crown cell and associated crest of modified cilia from the saccus vasculosus of a small aquarium fish, *Hyphessobrycon rosaceus*. The apical pole (*ap*) only of the cell is shown at the lower right of the figure. From the free surface of the cell included in the thin section, five "swollen cilia" project

observation has been repeated several times since. It was therefore not entirely surprising to find them in the first electron micrographs of cilia published by Ruska[40] and later by Jakus and Hall ten to fifteen years ago.[41] The number of component filaments was reported from these early studies to be 11 in most instances, though because of the nature of the preparations it was difficult to determine the constancy of this figure.

Longitudinal sections of cilia taken some years later[37] confirmed the existence of longitudinal filaments and showed a number of other features (Fig. 15). It is evident, e.g., that the cilium has a membrane continuous with the plasma membrane and that it contains, apart from the longitudinal filaments, a homogeneous matrix in which no molecular order or elements of fine structure have been resolved. The filaments are observed to continue into basal bodies which reside within the cortex of the cell. These latter have characteristically a dense margin. In some forms studied, striated fibers appear to take their origin from the basal body and to extend to a considerable depth into the cell.[37,38] The number and prominence and indeed presence of these is not constant. The basal body is, however, a constant feature. I shall return to a consideration of it in a few moments.

When cross sections of the cilia are examined, one obtains a much better picture of the number, structure, and arrangement of the component filaments. It then becomes evident, as shown in Figs. 16, 17, and 18, that each cilium possesses 9 of these arranged around a central pair. The cilium observed in such preparations is essentially cylindrical in form, except for distortions induced by sectioning, is limited by a membrane, and, except for the filaments, shows no structure. The central pair provides for two possible axes of symmetry[37]—one bisecting the two, the other coinciding with

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into the ventricular cavity. These are limited by a membrane and seem to owe their "swollen" form to the presence within them of large numbers of small vesicles. A typical basal body (*bb*) may be noted at the point where the "cilium" joins the cell (see also Fig. 20), and distal to this there is some evidence of slender filaments (*f*) typically encountered in longitudinal sections of cilia. These do not, however, extend far into the head of the "cilium" and seem to be replaced, in some instances, by longitudinally arranged rows of small vesicles. The function of these modified cilia is unknown. ×11,000.

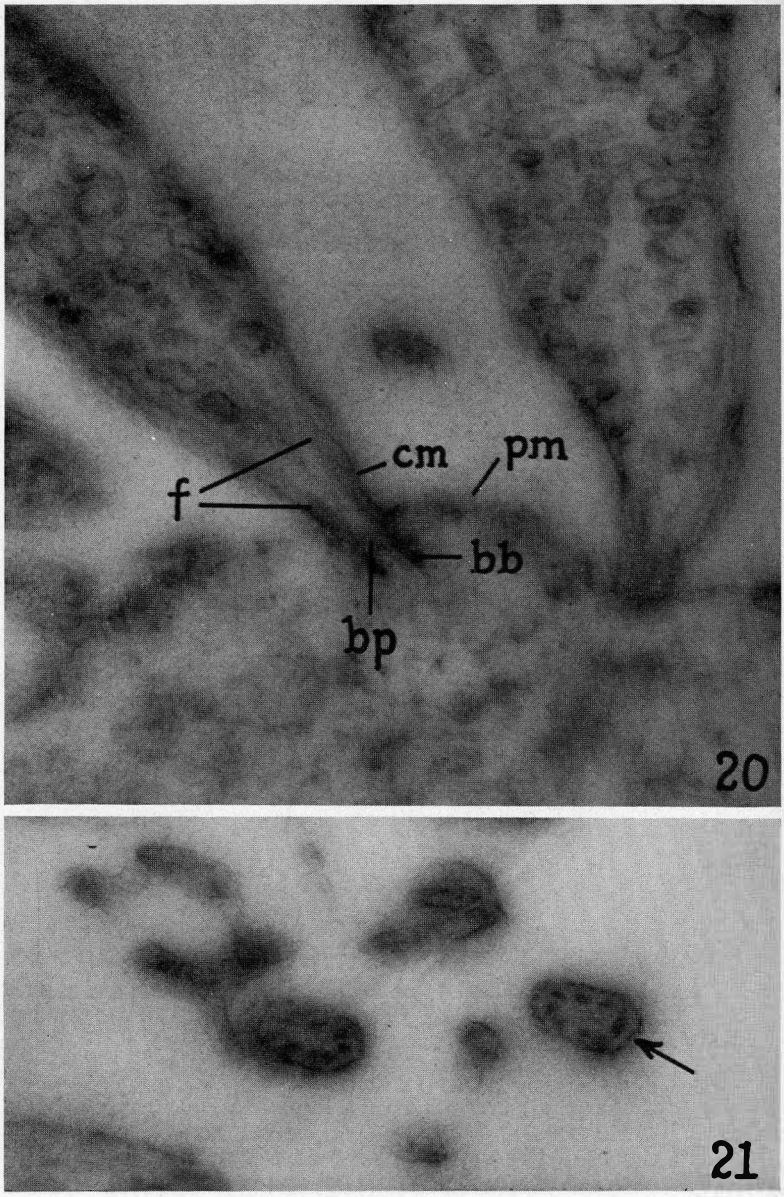

FIG. 20. Micrograph of basal portion of crown cell "cilium" to show detailed

them. In some instances, where the cilia are all beating in one direction, the plane of bilaterality is identically oriented over a relatively large ciliated surface.[37,42] In its pendular motion it has been shown to bend in the plane of bilaterality that bisects the central pair. Micrographs of higher resolution demonstrate that each peripheral filament is made up of two parts and that each unit in the pair is the equivalent in size (~150 A.) of one of the central filaments. Essentially then, each cilium has 10 pairs of filaments.

A large variety of cilia have now been examined in ours and other laboratories, and in all instances this pattern has been found to be repeated. This statement can be broadened to include all flagellae, whether as part of sperm cells or zoospores of algae,[43] for in them as well, the core, the axial unit of the flagellum, has this same arrangement of filaments. From its ubiquity and regularity one gains the impression that there must be something extraordinarily fundamental in this organization. The reason for 9 pairs of peripheral filaments is not evident, and 7 or 11 would serve as well for the more plausible theories of motion.[42] Clearly, however, Nature has found 9 a satisfactory number and has retained it for all forms from protozoa (Fig. 17) to primates (Fig. 18).

I have already commented on the cylindrical form of cilia. The average diameter is 0.2 μ, but the length may vary from a few to 150 μ.[44] This slender form is not only maintained, but the cilium may display in its motion some stiffness or rigidity suggesting that its content is under turgor pressure or at times undergoes gelation.

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structure of basal body, etc. Membrane (*cm*) limiting "cilium" is obviously continuous with plasma membrane (*pm*) of cell. Filaments (*f*) of "cilium" extend proximally through transverse density, representing basal plate (*bp*), and into cell for short distance to become part of basal body (*bb*). (See Figs. 15, 22, and 29 for similar structure.) Distally the filaments are lost from view, apparently because they vesiculate. ×29,000.

FIG. 21. Cross section of modified "cilia" cut distally to the cell surface. Again the limiting membrane of the "cilium" is evident, and just within this the peripheral ring of 9 filaments characteristic of cilia (two are crowded together at arrow). In favorable places each filament can be seen to be double, further confirming the cilia-like nature of these crown cell projections. Central filaments are absent. ×40,000.

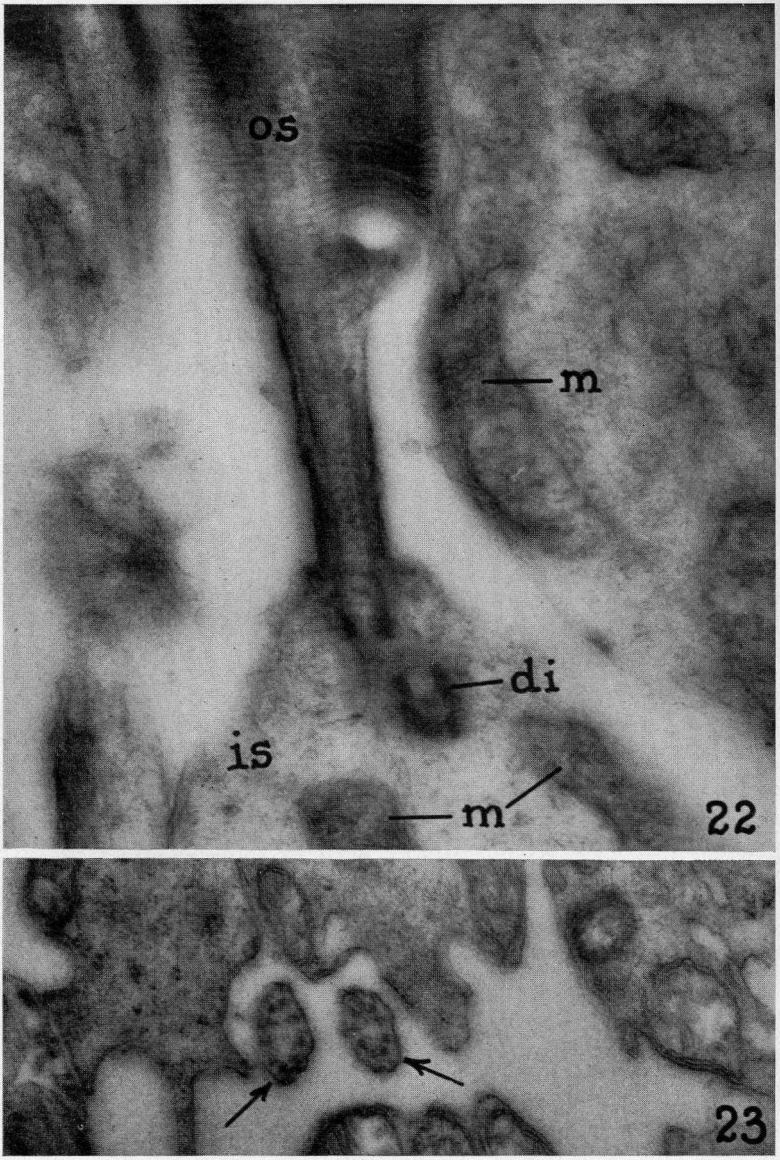

FIG. 22. Longitudinal section of connection between outer (*os*) and inner segments (*is*) of rod cell in retina of 3-week-old rat. The connection is ob-

In searching here for a framework or "cytoskeleton" that might be involved in maintaining this cylindrical form, the filaments are an obvious candidate, and there are one or two features of their structure and arrangement which suggest that they play such a role.

It has already been noted that they occur in pairs, and it appears as well that they are constructed in the form of tubules—or at least have a surface component that reacts with osmium. Paired tubules, closely adherent laterally as these are, or twisted about one another, might be regarded as structural elements possessing some rigidity and possibly capable of maintaining an orientation in the fluid matrices of cilia and cytoplasm. It should, in addition, be noted that these peripheral filaments are equally spaced at 500 A. (center to center) and that from their centers to the ciliary membrane the distance is approximately one-half this value. It seems, therefore, as though each has organized around itself as center, a layer of matrix material ~250 A. thick, which possibly serves to maintain these uniform distances.[37] When bundled together around a central pair possessing similar properties, this series of 9 has presumably no choice but to form a cylinder. I would point out that these phenomena of orientation and spacing between ciliary filaments are reminiscent of those encountered in the cytoplasm involving the cisternae of the endoplasmic reticulum.

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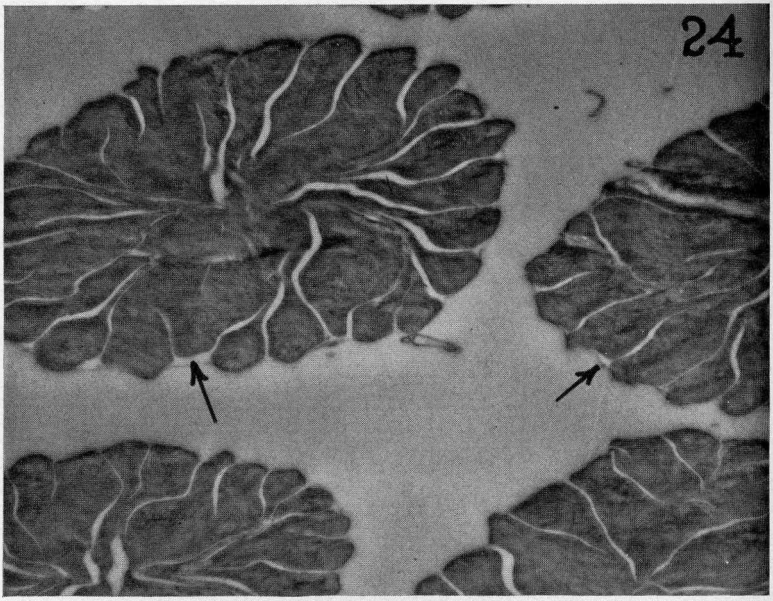
viously very similar morphologically to the cilia in Figs. 15 and 29. The membrane limiting it is continuous with that of the inner segment. The shaft contains longitudinally oriented filaments, and at the point where it connects with the inner segment there is a transverse density or basal plate below which the filaments extend for a short distance to form a basal body. Where the outer segment shows the developing organization of "double-membrane" discs (Sjöstrand[49]) the filaments are lost to view. The suggestion from available evidence[51] is that they segment and that during differentiation each division is translated into a disc. The dense body marked (*di*) represents the oblique section through a diplosome, the second of two, the other being the basal body of the "cilium." Mitochondria are at *m*. ×32,000.

FIG. 23. Section of another part of the same retina that provided the specimen shown in Fig. 22. This cuts transversely through the ciliary connection between outer and inner segments and shows (at arrows) the typical cross-sectional design of cilia (Figs. 16–18) except that the central pair of filaments is missing. ×36,000.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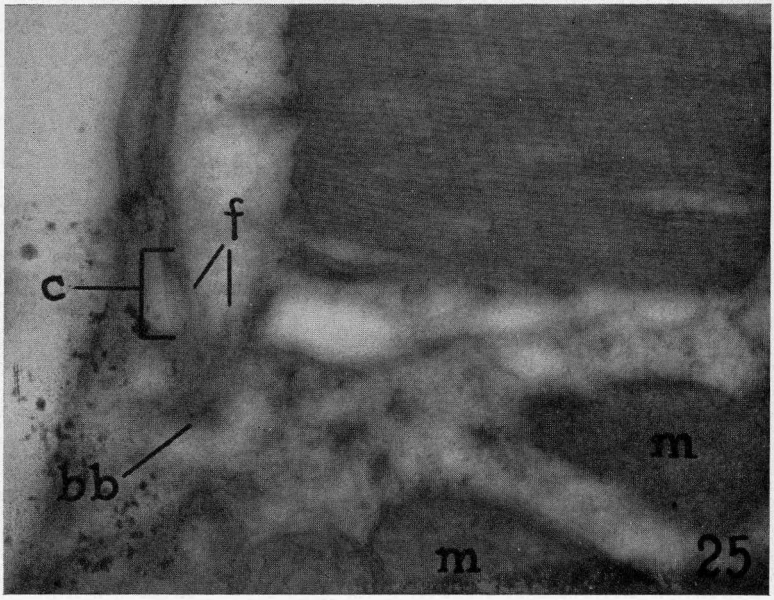
f
c
bb
m
m
25

If there were a pathology of these organelles we might test this hypothesis of the influence of these filaments on the form of the cilium by looking for units with a deficient number of filaments or cilia with abnormally formed filaments. Unfortunately for our purposes such pathological types have not been encountered, if indeed they exist. But the search for unusual cilia has not been futile, for Nature has found some remarkable things to do with these subdivisions of the cell.

It has been known for some time that certain cilia or cilia-like processes of cells are nonmotile and probably functionally different as well. Such modifications of the typical vibratile unit are especially common on cells of the nervous system and sensory organs. Thus, in some animals, cells of the ependyma lining the cavities of the central nervous system are ciliated. The sensory cells in the olfactory epithelium bear long cilia, which are usually nonmotile.[44,45] Several cell types of the inner ear carry on their free surfaces a variety of hairs, of which some have the fine structure of cilia.[46] Lateral line organs of amphibia and fish contain ciliated cells—to mention only a few. In their capacity to form cilia or cilia-like processes these cells are not unlike many cells of the embryonic ectoderm from which, after all, they are derived. But the similarity between the derived form and cilia is quite remote in some instances, and to suggest a relationship on the basis of light microscope evidence is to test one's credulity (see Arey).[47]

I am introducing these electron microscope observations on these bizarre variants of cilia to illustrate, that in some instances,

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FIG. 24. Cross section through outer segments of retina of the leopard frog, *Rana pipiens*. The lamellar units which are stacked to form this part of the rod are multilobed structures rather than plain discs. Their form and organization account for the deep furrows or crevices which penetrate to the center of what is still roughly a cylinder. The whole is surrounded by a thin membrane (arrows). ×5000.

FIG. 25. Longitudinal section of connection between outer and inner segments of rod, found in retina of adult frog. The portion of the figure representing the connection and indicated at *c* shows some evidence of longitudinal filaments (*f*). The equivalent of the basal body, present only in oblique tangential section, is marked *bb*. Lamellar, double-membraned units (ca. 120 A. thick), making up the outer segment, occupy the upper right quadrant of the figure. Mitochondria are at *m*. ×39,000.

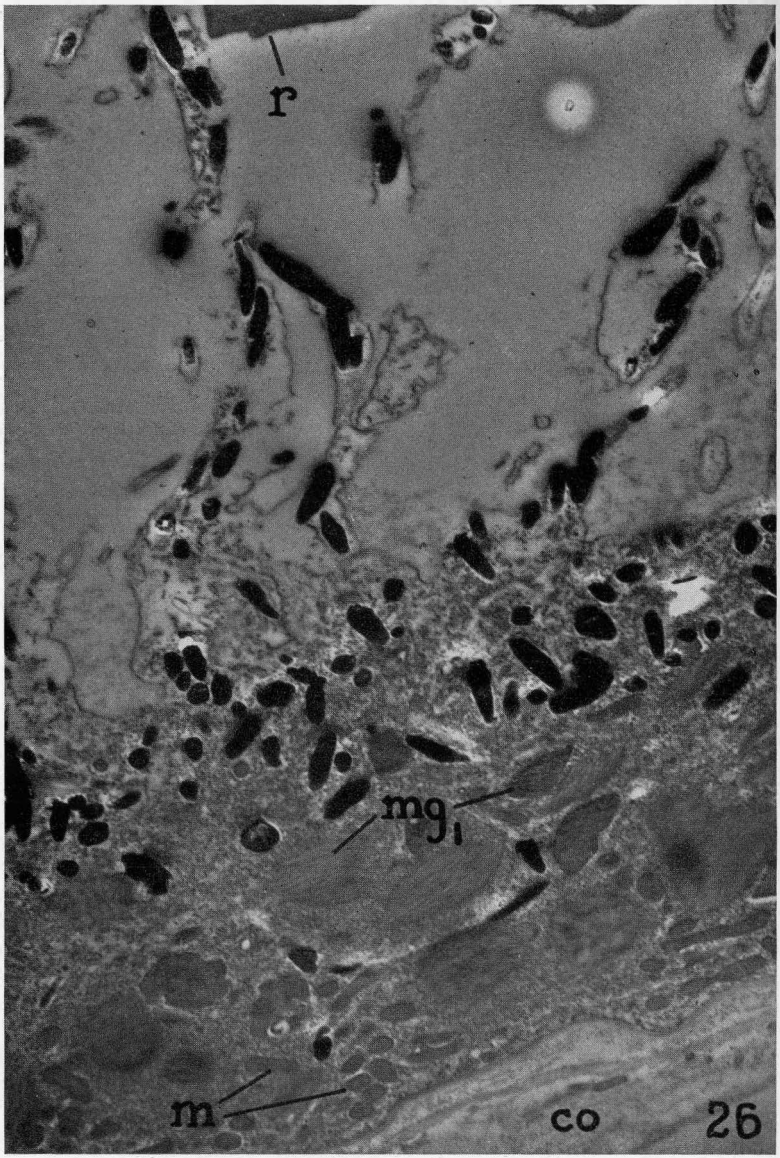

FIG. 26. Micrograph showing a portion of a cell of the pigmented epithelium as found in the retina of the frog, *Rana pipiens*. The major part of the cell

cilia may develop internal components that are similar to certain structures found within the cytoplasm of the cell and, to show further, that these replace and seem to be derived from the peripheral filaments of the 9 and 2 configuration. It becomes evident that, with the disappearance of the filament "framework" and the development of derivatives, the cilium changes its form.

One of these derived forms is found on what are called crown cells of the saccus vasculosus, a vascularized infolding of the ependyma of the third ventricle of the fish's brain. The cells in question are readily identified in either light or electron microscopes by the "crown" of small projections which cover their free surfaces. At higher resolutions the projections are in turn identified as cilia with enormously swollen ends which are filled with vesicles (Fig. 19). Bargmann, who has studied such cells extensively and recently published a paper on their fine structure,[48] proposes that the modified cilia are secretory, but there is little evidence to support this view (Fig. 19).

In so far as one spheroidal vesicle can be said to resemble another in electron micrographs, these encountered here within these modified cilia are reminiscent of the vesicles found within the cytoplasm and described above as elements of the endoplasmic reticulum. They occasionally appear in rows running lengthwise of the larger sac (formed by the cilium membrane) containing them, but they seem not to give the latter any special shape. The ciliary filaments identified at the base of the organelle appear to end before they proceed far distally into the structure (Fig. 20). Favorable micrographs depict a fragmentation of filaments (which are like small canaliculi), and one may assume that the small vesiculated fragments grow to give the units shown in the outer end of the sac. The filaments, so to say, seed the development of vesicles. Growth phenomena cannot of course be watched in the

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occupies the lower half of the figure and rests upon the choroid across the lower right (*co*). Long slender pseudopodia extend from the free surface and interdigitate with the outer segments of rods and cones (tip of rod at *r*). The dense, mostly oblong profiles are pigment granules. The ellipsoidal structures deeper in the cell, toward the basal surface, (mg_1) represent the "myeloid bodies" of Kühne[52] (see also reference 47). The fine structure of these is shown in Figs. 27 and 28. Mitochondria are at *m*. ×6600.

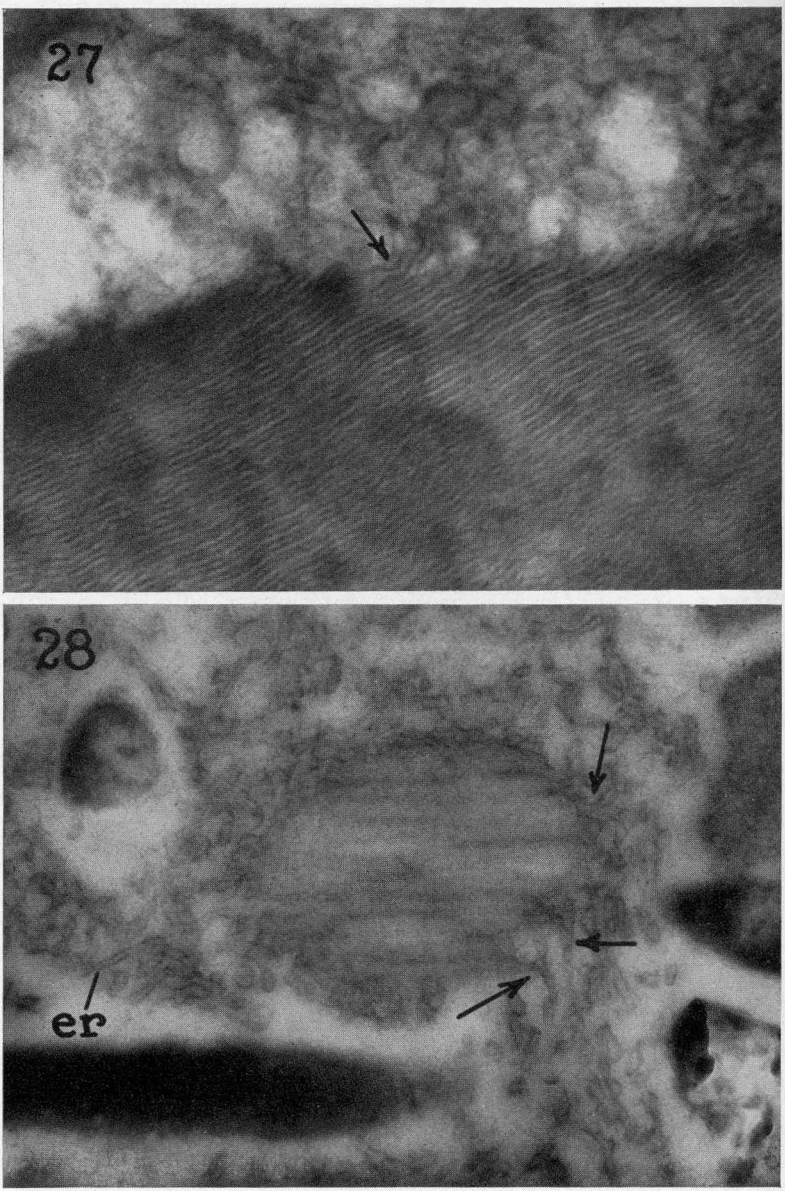

FIG. 27. Margin of myeloid body showing its lamellar structure and con-

electron microscope; hence evidence for sequences must be collected piecemeal and assembled in what seems a logical form. If further support is needed for the contention that these are cilia it may be found in cross sections through the base of the stalks (Fig. 21).

A second variant of the orthodox cilium, and the most extraordinary I know anything about, is found in the retina of the eye. This tissue, as is well known, is constructed of several cell layers, the outermost of which is photosensitive. The visual cells of this layer are modified at their apical ends into the form of rods or cones, and these names are applied to the cells. Electron microscopy of the apical or outermost segments, principally by Sjöstrand,[49] has shown them to be made up of innumerable discs, each consisting of two closely approximated membranes, separated by a distance of 140 A. These units are stacked up like thin wafers to form a cylindrical body, and the whole is enclosed in a thin membrane. A few students of the histogenesis of the retina (see Mann[50]) have expressed the belief that the rod and cone outer segments are modified cilia, but this interpretation seems not to have been widely accepted. However, some recent electron microscope observations of De Robertis[51] have provided evidence of the relationship, and Figs. 22 and 23 confirm his findings.

The first (Fig. 22) shows longitudinal sections through outer and inner segments of rod cells from the retina of a 3-week-old rat. Differentiation is partly completed. The connection between the two parts is obviously like a cilium, and the proximal end is a typical basal body. It is to be noted that the ciliary filaments do not

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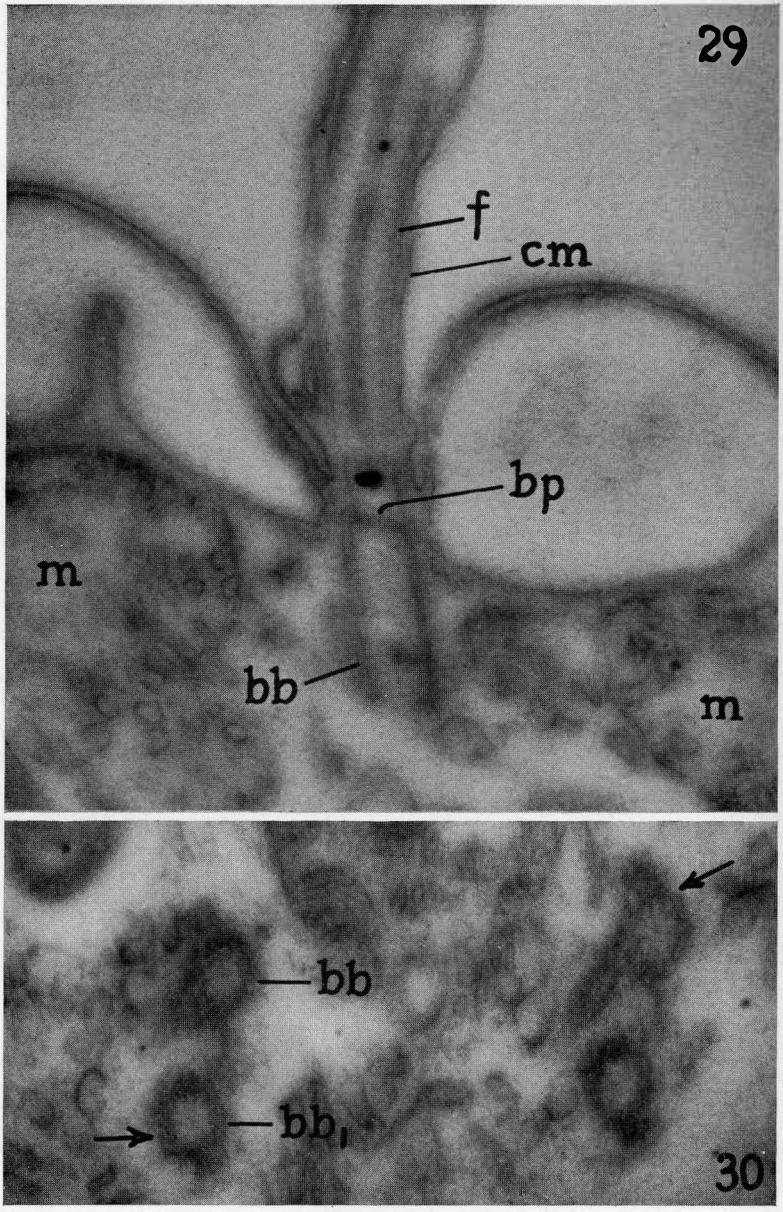
tinuity between membranes of lamellae and those of endoplasmic reticulum. The lamellar units are constructed of two membranes (paired dense lines ~60 A. thick) separated by a space of about 30 A. Each double-membraned unit is in turn separated from the next by a space of ~70 A. Thus the lighter lines are alternately thick and thin. They are organized in arrays which in outline are rhomboidal. The arrow designates a region providing evidence of continuity between the membranes of the lamellae and those limiting the channels of the endoplasmic reticulum.

FIG. 28. Small section of myeloid body cutting obliquely through lamellae. Arrows point to additional evidence of continuity between membranes of lamellae and small tubular members of endoplasmic reticulum (*er*). ×60,000.


29
f
cm
bp
m
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m
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bb1
30

extend far into the laminated or light-sensitive portion, presumably because they have been "used up" in the differentiation of the double-membrane discs. Actually, as the filaments are followed into the outer segment they seem to become discontinuous, and the resulting segments are seen to be continuous with horizontally oriented tubules which are in turn continuous with the expanding lamellar structures. This is interpreted to mean, as other observations have indicated,[51] that the ciliary filaments "seed" the development of the lamellar units. Again, an examination of a cross section (Fig. 23) provides all that is needed to convince one that the outer segment and its connection to the inner segment constitute a modified cilium.

The extent and complexity of this modification is in some instances very remarkable. In the young rat (Figs. 22 and 23) the light-sensitive element is relatively small (2 μ in diameter), but in the frog and other amphibia it achieves a much greater size (8 μ in diameter, 30 μ in length). The double membrane structures in the outer segments are not simple discs but are lobed, and the stacking of these create furrows in the otherwise cylindrical body (Fig. 24). The large number of double-membraned units making up the rod is indicated in longitudinal section, and presumably these all provide surfaces for the dispersal of light-sensitive com-

FIG. 29. Vertical section of cortex and cilium of *Paramecium multimicronucleatum*. The shaft of the cilium comprises the expected filaments (*f*), a homogeneous matrix, and a limiting membrane (*cm*) which is continuous with that of the pellicle. At its proximal end the cilium is continuous with a basal body (*bb*) or kinetosome residing in the cell's cortex. The 9 peripheral paired filaments of the cilium appear to connect through the basal plate (*bp*) with a similar number of fibrils or filaments arranged longitudinally in the wall of the basal body. These are not so easily visualized as the filaments in the cilium because they are surrounded by an amorphous or diffuse material continuous with the cortical cytoplasm. The entire basal body, from basal plate to proximal end, has the form of a cylinder 150 mμ in diameter and 375 mμ long. ×72,000.

FIG. 30. Section taken in tangential plane through cortex of same microorganism showing 2 pairs of kinetosomes or basal bodies. One (*bb*) is the basal body of a cilium, the other (bb_1) is referred to as the accessory basal body. In at least two of the cross sections (arrows) a peripheral ring of densities may be noted which, on careful examination, is found to number 9. The cylindrical form of the basal body is obvious from the cross and longitudinal sections. ×54,000.

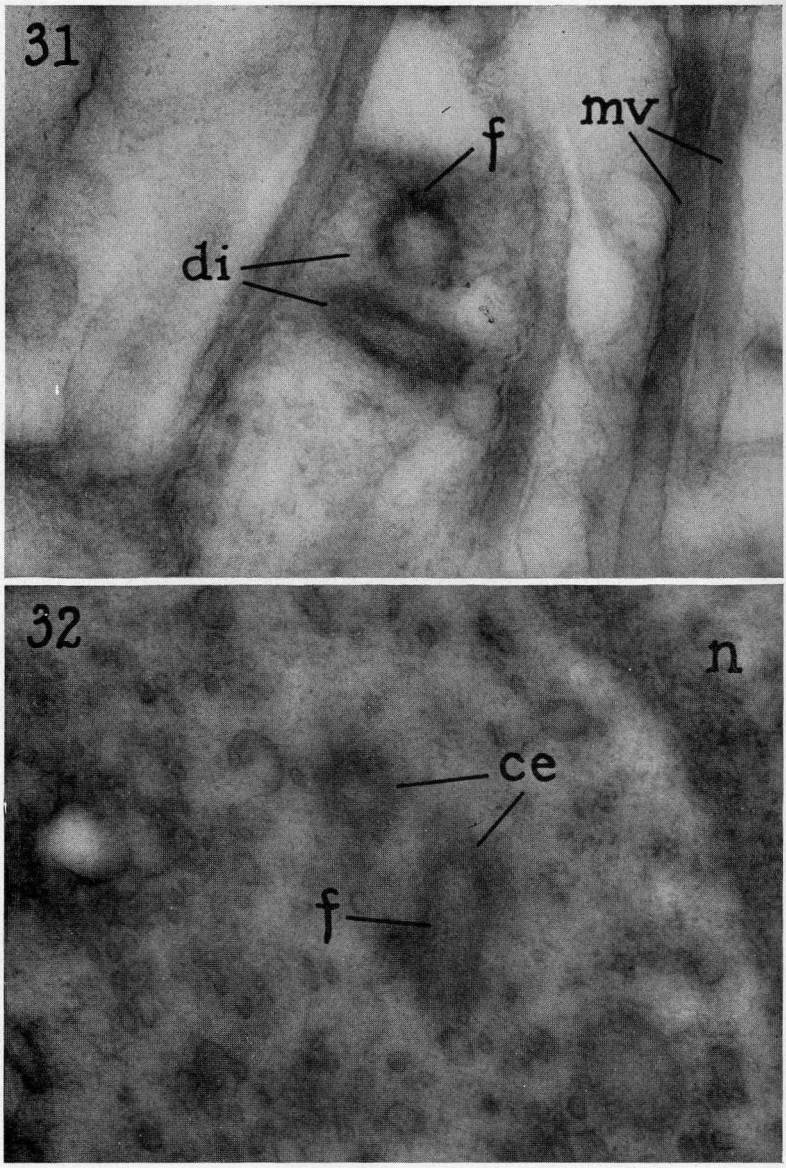

FIG. 31. Section through apical pole of unidentified cell in retina of the frog, *Rana pipiens*. The diplosomes (*di*) illustrated are in the center of the figure.

pounds. The "cilium" in this completely differentiated and complex unit is reduced to a vestige of its former self, but it is there and is apparently the only patent protoplasmic connection between outer and inner segments (Fig. 25).

It was suggested above that these lamellar and vesicular elements found within the modified cilia resemble certain differentiations of the endoplasmic reticulum; in other words, that the structural elements within the modified cilia are duplicated in the cytoplasm. Evidence for this in the case of the crown cell "cilia" is not hard to find, nor is the suggested analogy probably very significant. The lamellar units (double-membraned discs) which fill the other segments have a more distinctive structure, however, and their equivalent in the cytoplasm occurs less commonly. It is found, among other places, in the cells of the pigmented epithelium which faces and is closely applied to the outer portion of the visual cells. Long, slender pseudopodia from the free surfaces of these epithelial cells extend down between the outer segments of the rod cells and carry pigment granules with them (Fig. 26). It is assumed that the pigment, thus located, prevents or reduces lateral light scatter among the photoreceptors.[47,53] Whatever the function of the pigment, the pseudopodia extend in the light and withdraw in the dark, and the cell may be regarded as responsive to light stimulation.

Each is cut obliquely with respect to its long axis, but the upper of the two is shown near to cross section and the lower is nearly longitudinal. It is evident, therefore, that their long axes are oriented at 90° to one another. A fine tracery of filamentous elements (*f*) may be observed in the dense walls of these cylindrical structures. Microvilli are indicated at *mv*. ×50,000.

FIG. 32. Micrograph of centrioles encountered in leucocyte (neutrophil) of rat.* The nucleus (*n*) of the cell is shown in part at the upper right. The centrioles (*ce*) are in the middle of the centrosphere region of the cell. Filaments arranged longitudinally in the wall of the centriole are shown faintly at (*f*). Since one centriole (the upper) appears in cross section and one in nearly longitudinal section in the same plane, it is evident that their axes are oriented at 90°. ×50,000.

* Grateful acknowledgment is made to Dr. Eichi Yamade for permission to use the micrograph in Fig. 32 which he took while a visiting investigator in our laboratory. Figures 33, 34, and 40 are from a collaborative study with Dr. James Caulfield.

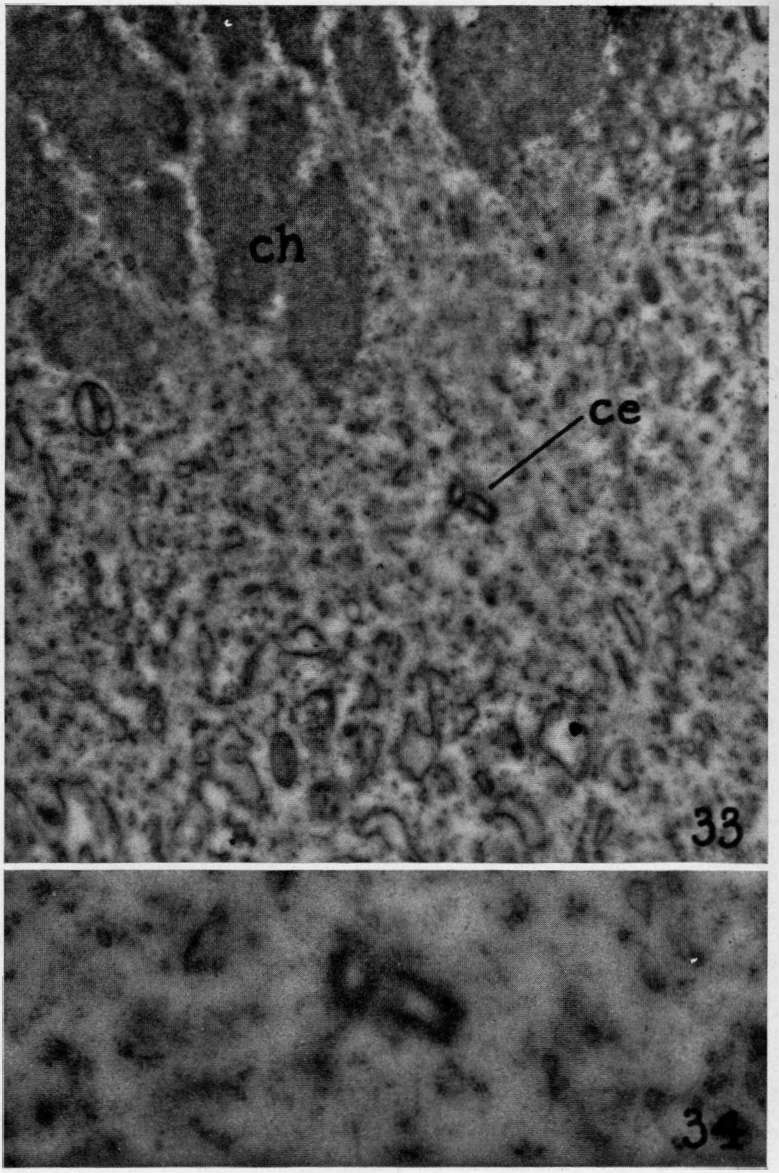

FIG. 33. Section through dividing cell of Jensen rat sarcoma. Anaphase chromosomes (*ch*) appear in the section at the upper left; a pair of centrioles

It is therefore not entirely surprising to find in the cytoplasm of these cells structures which are fundamentally similar to those in the outer segments of the visual cells (as well as in chloroplasts). The structures referred to appear as parallel arrays of closely packed, lamellar vesicles showing thickness and spacing dimensions similar to those in the rods and cones and presumably possessing a similar property. What is most important to the present discussion is that the units of these arrays are continuous with the otherwise canalicular members of the endoplasmic reticulum (Figs. 27 and 28). Thus it appears that an intracytoplasmic system of membranous structures, existing as a special differentiation of the endoplasmic reticulum, may in its form be duplicated in the outer portions of cilia. In the cilia they appear to arise from the ciliary filaments and thence to adopt a form which certainly influences the shape of the whole unit. We now might reasonably ask whether the complex membranous systems of the cytoplasm are similarly derived, i.e., whether there is in the undifferentiated cell a unit of structure similar to the double peripheral filament of the cilium which might contribute to the formation of the endoplasmic reticulum. There is some evidence suggesting this, and even though there is not time to present it fully or properly, I am going to show enough to indicate why our interest has been attracted to this problem and the possibilities of exploring it further.

It is first important to recall an early conclusion of cytologists that centrioles, diplosomes, and ciliary basal bodies are homologous

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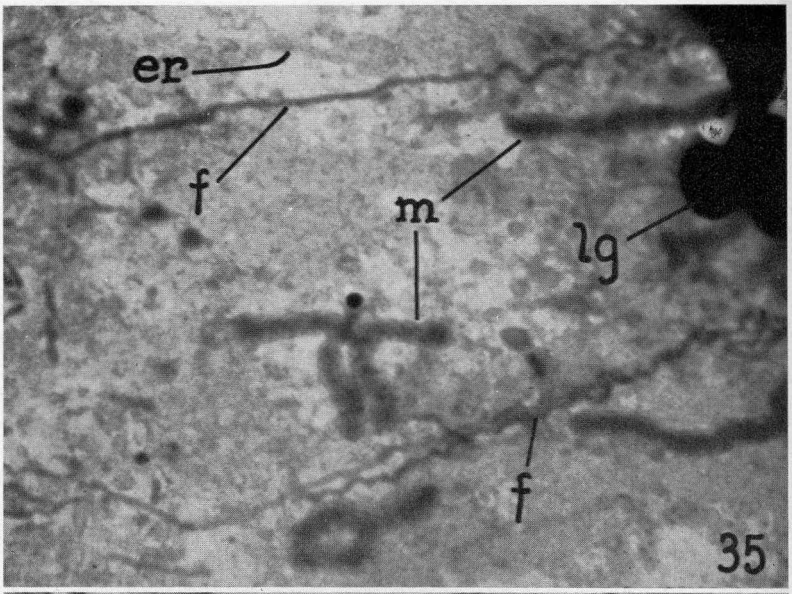
near the center of the figure (*ce*).* ×14,000.

FIG. 34. Enlargement of centrioles in Fig. 33. The one at the right appears much like a basal body. The other is cut more nearly transversely. Again it is evident that the axes are oriented essentially at right angles. In the transverse section there are small dense loci representing some of the 9 filamentous structures in the wall of the centriole. ×36,000.

* Bernhard and DeHarven have recently published (1956. *Compt. rend.* **242**, 288) a brief description of centrioles in dividing thymocytes which essentially coincides with that presented here. They noted double "fibres" in the wall of a cylindrical body but failed to recognize the similarity of this structure to that of basal bodies or that of centrioles described earlier by Burgos and Fawcett.[86]



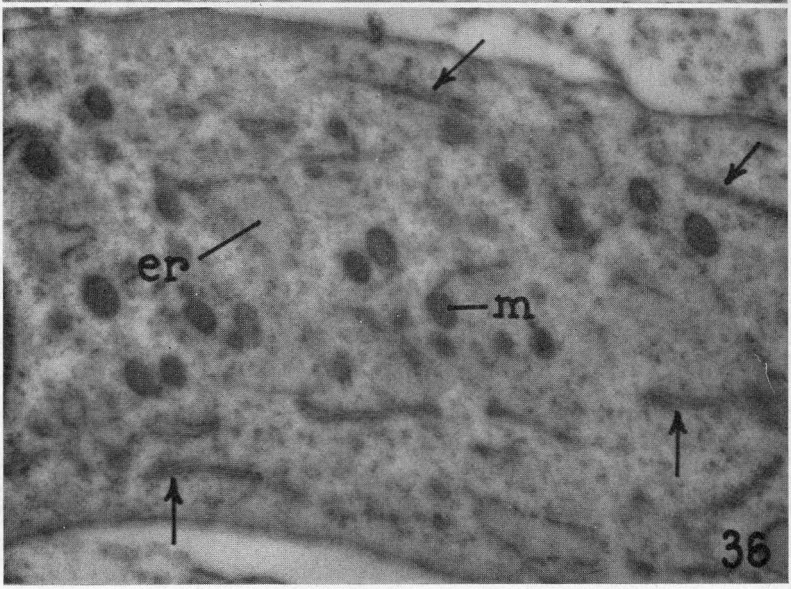


FIG. 35. Small portion of the margin of a thinly spread cell, grown *in vitro* from explant of rat endothelioma (4337).[56] Mitochondria are indicated at *m*,

structures. All are approximately the same size, have the power of self-duplication, and derive one from the other under certain circumstances. As pointed out a moment ago, the basal body is the intracellular terminus of the cilium (Fig. 29). From it the peripheral filaments of the cilium take their origin, and in it their plan of distribution seems to be inherent. These bodies may best be described as short cylindrical structures (150 mμ in diameter and 375 mμ long) composed of a moderately dense wall, in which there may usually be discerned 9 special loci, continuous with the ciliary filaments (Fig. 30). The center of the basal body is less dense and shows no central structures. The whole is without evidence of a membrane, and the margin grades off into the surrounding matrix. The intracellular end of the basal body is apparently open; the other (distal) end usually shows a cross wall or basal plate at the point where the cilium begins. At the intracellular end, the wall of the cylinder may be continuous with a large variety of structures, frequently fibrous, which extend to various depths into the cytoplasm or surrounding cortex. Assuming that we are able to define the true limits of a basal body, we can say that they are morphologically similar in different cells and situations but not identical. Basal bodies, in protozoa known as kinetosomes, are remarkable for a number of reasons, as Lwoff[54] and others have shown, but two are outstanding: they reproduce very precisely, and

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lipid granules at *lg*, and endoplasmic reticulum at *er*. In addition to these the cytoplasm contains a number of long dense filaments (*f*) 75 to 100 mμ in diameter which, together with small dense granules at left of figure, are common in cells of rapidly growing cultures of this and other tumors (see "Fine Structure of cells" for references[56]). They have been seen also in normal cells of growing cultures of embryonic tissue. It is to be noted that two of these dense filaments are in close association as though paired and further that at some points along their length the form is that of an open helix. ×9500.

FIG. 36. Thin section of cytoplasm of 4337 tumor cell from a transplanted tumor.[56] Besides the usual organelles the micrograph shows several fine filamentous elements which, when cut in section, appear as slender canaliculi. When examined carefully these are found to be, in most instances, paired structures (arrows). The size of the paired unit (75 to 100 mμ) is similar to that of the dense filaments in the cultured cell. The double nature of the structure (seen to better advantage in Fig. 39) reminds one of the peripheral filaments in cilia. Mitochondria (*m*) and endoplasmic reticulum (*er*) are indicated. ×21,000.

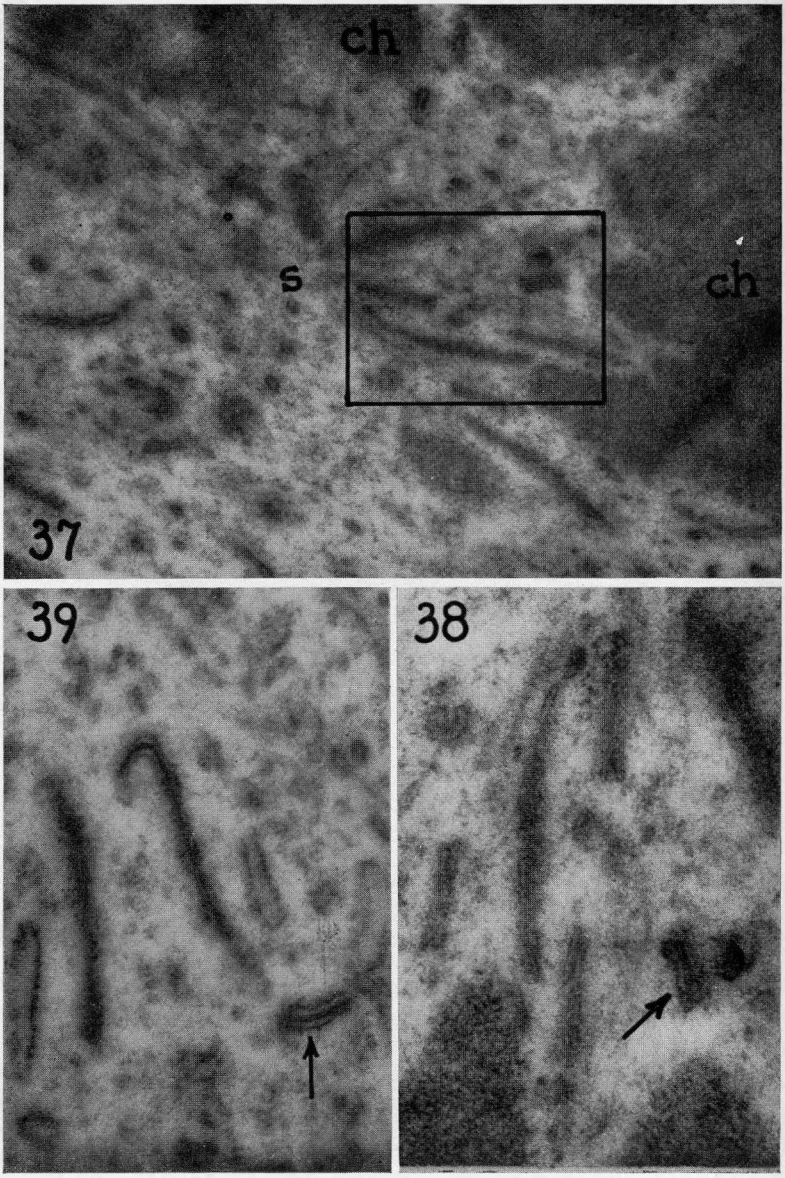

FIG. 37. Micrograph of thin section through Jensen sarcoma cell (rat) in

the progeny duplicate the complex patterns of arrangement present in the cortex of the parent cell.

The relatives of basal bodies, i.e., centrioles and diplosomes, are not so readily examined by electron microscopy because they are encountered very infrequently in thin-section samples of a cell. Enough have been located, however, and recorded by ourselves and others to provide evidence of the fundamental similarity of all (Figs. 31–34).

Centrioles, it will be recalled, are small densely staining bodies (ca. 0.2 μ) of wide-spread occurrence. They have not been identified in cells of the higher plants, which may simply mean that they are present in some less obvious form. They are perhaps best known as the central bodies in the astral system of the dividing cell, but they usually persist as paired bodies (also referred to as diplosomes) during the interphase when they reside in the cell center (at one side of the nucleus) or at the apical pole of the cell near its free surface. In sperm cells the centriole has been identified with the basal body or blepharoplast connected to the axial filaments of the flagellum. In electron microscope studies of this latter material, Burgos and Fawcett[55] first depicted and recognized the structural similarity between centriole and basal body. Thus centrioles show in cross section a ring of 9 peripheral loci resembling those in cilia and basal bodies, and the whole structure is cylindrical in form. Though the perfect cross section is not shown in any of the figures used here as illustrations, the peripherally arranged densities and filaments are evident in Figs. 22, 31, 32,

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anaphase of division.[56] Portions of chromosomes (*ch*) are shown along the top and the right of the figure. The pole of the spindle is in the region of *s*. Spindle fibers radiate from this point. Within the denser strands, thus oriented, it is possible to identify a pair of fine canaliculi or filaments. The surrounding density represents a condensation of fibrous material from the spindle matrix. The area in the rectangular outlined is enlarged in Fig. 38. × 16,000.

FIG. 38. Enlargement of small outlined region of Fig. 37, to show paired tubular elements in spindle filaments.[56] They are individually about 30 mμ in diameter, though this value obviously varies considerably from one to another. They focus on the pole of the spindle. ×43,000.

FIG. 39. Paired tubular elements in the cytoplasm of an endothelioma (4337) cell.[56] Note the similarity between the unit cut obliquely (arrow) and one similarly sectioned in Fig. 38 (also indicated by arrow). ×50,000.

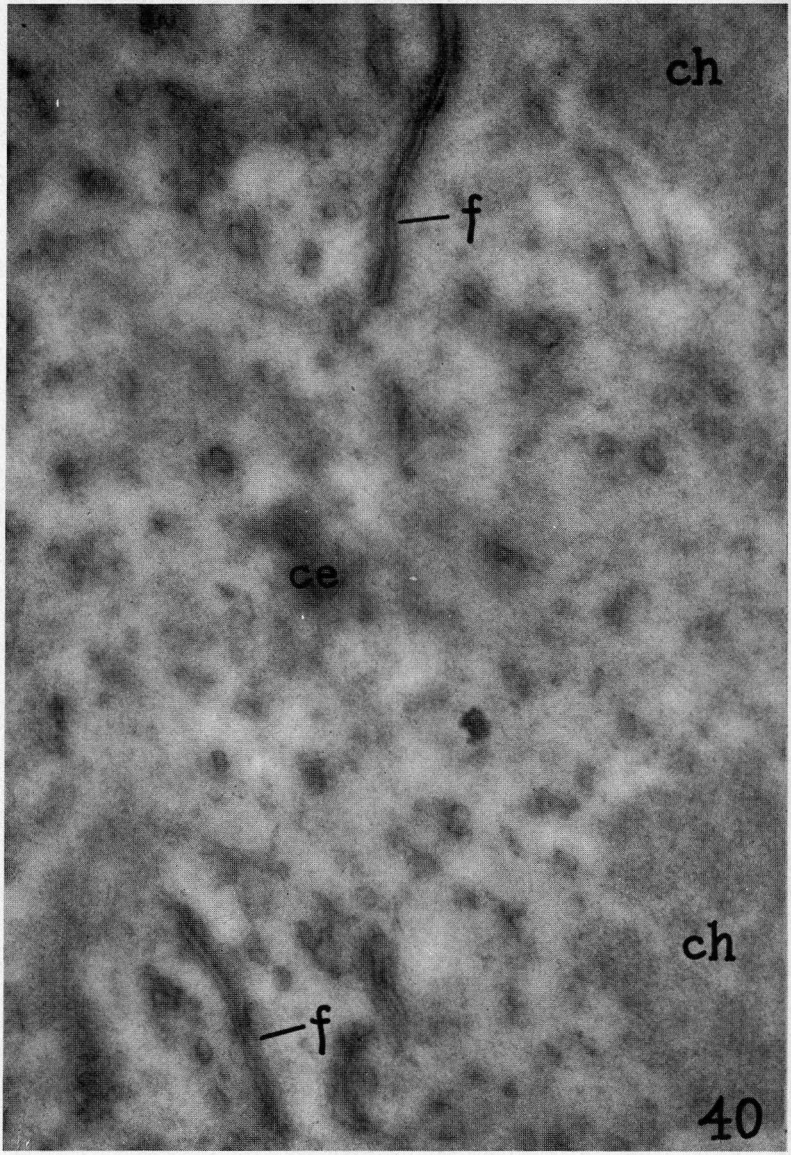

FIG. 40. Double tubular structure of spindle filaments (*f*) in metaphase of an endothelioma (4337) cell. The section appears to cut close to the region of

and 34 and number 9 in other more favorably oriented micrographs.

Actually in Fig. 14 of the Burgos-Fawcett paper, the proximal centriole is shown in cross section, and the distal centriole, or basal body of the flagellum, in longitudinal section. The longitudinal axes of the two centrioles are therefore oriented at right angles to one another. It is interesting to notice that the paired centrioles (with one a basal body) in the spermatid duplicates in essence the arrangement found in the developing rods of the rat's retina (Fig. 22). Furthermore, the orientation of the centriolar axes at 90° is repeated in all situations thus far encountered where two centrioles are in close proximity (Figs. 22, 31, 32, and 34).

With this similarity between basal bodies and centrioles established it is perhaps not unreasonable to ask whether double filaments (fine canaliculi) such as those extending from basal bodies into the shaft of the cilium ever appear associated with centrioles.

It must be admitted that this question was not immediately asked when the ciliary filaments were encountered or the close similarity between basal bodies and centrioles was recognized. It was stimulated rather by some additional observations on rapidly multiplying tumor cells, which I shall now review very briefly.

Some years ago in a study of cultured cells from an endothelioma of the rat, we noted at times large numbers of dense filaments[7,8] (Fig. 35). Later, when sectioning techniques were perfected, the same tumor was examined again, with the object of learning more about these filaments and their relation to other cell components. It was difficult to find them because they could not be recognized unless they happened to coincide with the plane of section, but where encountered they usually appeared as paired elements resembling tiny tubules or canaliculi (Figs. 36 and 39). In dividing cells, they were encountered more frequently and appeared to be involved in the spindle and aster structure and to focus on the pole of the spindle (Figs. 37, 38, and 40). They are generally larger (individually ~400 A. in diameter) than the filaments of cilia and occasionally show some evidence of vesicula-

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the centriole (*ce*), and the (double) filaments are oriented toward this point. Chromosome at *ch*. ×50,000.

tion along their length, as though transforming into elements of the endoplasmic reticulum.[56] Thus far no fortunately oriented section has shown them all connecting with the centriole. It may be that such a connection, if ever existing, is very transitory. It could also be, of course, that the double tubular structure is a coincidence and not to be confused with ciliary filaments. I should perhaps mention that structures of the same character have been found in dividing cells of normal tissues of the rat, mouse, salamander, and onion root tip.

I believe most cytologists would agree that centrioles seem at times to be the center of considerable organization in cells. If now we accept these double filaments as homologs of the ciliary filaments and acknowledge that they can contribute to specific formations of the endoplasmic reticulum as the ciliary filaments contribute to the content of modified cilia, we have in them a means whereby the centriole and related structures could influence the form of the whole cell.

With this extreme note of conjecture I shall end this somewhat disjointed presentation. I have tried to acquaint you with some of the submicroscopic systems that we are finding in cells and that appear, because of their ubiquity and relationships, to have some very real significance in the life of the cell. The "facts" were contained in the micrographs. The fabric of speculation against which they have been projected is thin indeed and will have to be rewoven many times before it will stand much wear.

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