ELECTRON MICROSCOPY OF BASOPHILIC COMPONENTS OF CYTOPLASM*

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It is reasonable to classify the basophilic component or components among the less well-defined structures of the cytoplasm. Some authors have chosen to refer to it as the basophilic material or substance rather than component perhaps in recognition of its indefinite and variable nature. In some cells after appropriate fixation and staining it appears in the form of filaments or threads (14, 12), in others as clumps or fine granules (3, 10, 15) and in some it is difficult or impossible to identify at all. The obvious deduction to be made from the light microscope image is that these various appearances reflect different organizations or arrangements of extremely small or submicroscopic units. Viewed thus it becomes one of the more interesting components of the cell to be explored by electron microscopy especially since so much in the nature of functional significance has come to be associated with it (5, 7).

As was indicated by J. W. Wilson in the introductory paper of this symposium (34), a few descriptions of the fine structure of the basophilic component have already appeared in the literature. These conflict in some respects but agree in showing that the basophilic component has a complicated structure at submicroscopic levels. Dalton (9) observed patterns of dense, parallel lamellae appropriately sized and shaped and properly located within the cells of liver, pancreas and stomach to represent the basophilic component. In similar studies on liver, pancreas and salivary gland cells of the rat, Bernhard and co-workers (4) found in thin sections the same arrays of long elements but they chose to interpret them as fibrils. These they identified with the basophilic ergastoplasm of Garnier (12).

From these studies it was very apparent that the basophilic components of several cell types are similar in being composed of lamellae or fibrillae in more or less parallel array. The earliest micrographs of Nissl bodies (12, 13, 22) made it appear that these particular basophilic components were going to be exceptions to this general rule but subsequent studies have reversed this first impression (13, 21). As improvements have been made in fixation and electron microscopy it has in fact become apparent that several of the earlier observations were in error. Thus the lamellae and fibrillae of the ergastoplasm have now been identified as longitudinal sections through canaliculi or sections cut normal to the parallel membranes of extremely thin sinuses (26), sacs (33), or flattened vesicles to be referred to hereinafter as cisternae (20).

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In the account which follows, these are described as parts of a highly complex and variable reticulum which is present in the cytoplasm of most cells (16, 17, 19, 20, 25). In whole cells it stains with haematoxylin and basic dyes and in sectioned cells it is especially prominent in basophilic regions of the cytoplasm. A small granular element is usually associated with the reticulum (16) and evidence is presented to show that it, more than any regular element of the reticulum, is responsible for the property of basophilia. Most of the information to be reviewed is drawn from the work of this laboratory and I wish to thank Dr. George Palade for permitting the use of results (and the micrograph in Fig. 15) which have previously appeared only in abstract (16).

THE BASOPHILIC COMPONENT IN THE CYTOPLASM OF CULTURED CELLS

Some of the earliest observations on the fine structure of cells by electron microscopy were made on thin cells obtained by *in vitro* culture procedures for the reason that techniques for cutting adequately thin sections were not then available (27). A further examination of such material demonstrated in the cytoplasm of most types of cells a system of strands and vesicles associated in the form of a complex reticulum (17, 20, 25, 28, 29) (Figs. 1, 2). It was evident from the presence of folds in the surfaces of some of the component elements that the system was clothed in a membrane which separated the contents of the system from the rest of the cytoplasm. It therefore came to be regarded as a finely divided vacuolar system capable of providing the cell with a very large intracytoplasmic membrane surface. The degree of reticulation was observed to vary from coarse to fine in cells of the same type, apparently in response to different physiological states. Since in most cells the elements of the system were excluded from the thin cortical or ectoplasmic layer of cytoplasm and therefore confined to the endoplasm, it came to be referred to as the endoplasmic reticulum.

In certain instances, and these did not seem to be confined to any single type of cell, some elements of the system were as large as or considerably larger than mitochondria. Whereas the finer strands and vesicles were usually between 50 and 100 mμ, some of these larger elements (now recognized as distinct entities) may be a micron or two across and so, entirely within the resolution range of the light microscope. It was not surprising, therefore, to find some evidence of the system in stained preparations (Fig. 3). This was brought out to better advantage with haematoxylin than with such basic dyes as toluidine blue or methylene blue but even with these latter something in the nature of a reticulation could be visualized. This is of course the image in a fixed cell and leaves some uncertainty as to whether a structure of similar character is present in the living unit. An answer to this was sought with phase contrast and darkfield microscopy and in both instances images were obtained of elements comparable to those in the fixed cell (25).

There is therefore ample reason to believe in the genuineness of the endoplasmic reticulum as a component of the cytoplasm and evidence to indicate that it is basophilic. It should be noted in support of this point that the various forms of its parts as for example the vesicles, are duplicated in electron micro-

scope images of the microsomal fraction isolated by centrifugation from the cells of rat liver (25, 32). In other studies, the same fraction has been shown to be identical with the basophilic component (6).

THE ENDOPLASMIC RETICULUM IN THIN SECTIONS OF CELLS

The above correlation of basophilia with this newly defined element of cell fine structure had been based entirely on evidence from cultured cells. Such material has recognized limitations and is invariably subject to the criticism that some artificial condition of culturing is responsible for the observed structures or their behavior. It was therefore very desirable that similar observations should be made on cells fixed *in situ*. Since really effective procedures for fixation and thin sectioning of such cells have been available only two or three years the available information is limited, but is nevertheless sufficient to provide the answers to two important questions:

a) Can a structure similar to the endoplasmic reticulum be identified in thin sections of cells in their normal tissue associations, and

b) If so, does the distribution of the system in the cytoplasm coincide with the property of basophilia?

The first of these problems has been investigated by simply comparing the fine structure evident in thin sections of a given cell type with that apparent in cultured cells of the same type. Sections have also been taken of the cultured cells themselves so that the third dimension of the structures defined in two dimensions in micrographs of the intact cell, might be observed. This comparative study has now included several kinds of cells and has demonstrated conclusively that the endoplasmic reticulum is present in all types examined (excluding erythrocytes) and is there in essentially the same form as in the cultured equivalents (20). Micrographs illustrating this point are shown in Figs. 4 and 5.

Figure 4 shows a small part of the margin of a thinly spread cell cultured from a rat sarcoma. Mitochondria are evident at the lower left and dense filamentous structures frequently encountered in rapidly proliferating cells are scattered about the field. The endoplasmic reticulum (*er*) is mostly represented by rounded (vesicular) elements which in places appear to be connected as beads on a string (er_1). Other components of similar density have irregular shapes and in places, also appear as overlapping groups of otherwise distinct elements (er_3). The predominant unit is round or elliptical in outline and from shadowed images of such preparations, it is recognized as a vesicular structure.

In Fig. 5 a portion of a thin section of another cell of this sarcoma is shown at the same magnification. The cell margins, nucleus and mitochondria are readily identified (see figure legends). Besides these there are numerous elements of diverse shapes and sizes, mostly bounded by a dense line representing a membrane. This encloses and separates from the cytoplasmic matrix a material of uniform density which serves to identify all these variously shaped components as parts of a single system. At higher magnifications it is very evident that these profiles represent sections through vesicular elements, the sizes of which coincide with those displayed by the vesicles of the endoplasmic reticulum (Fig. 4). This similarity plus others and the fact that these profiles are the only struc-

tures in the section remotely like those of the reticulum, identify the two as different views of the same cytoplasmic component.

As mentioned above, membrane-limited structures of this general nature have been found in the cytoplasm of all cell types thus far examined and this includes a number of protozoans (30, 31) as well as cells from a variety of vertebrates (2, 17) and invertebrates (11). The variations displayed in form and organization are fairly enormous and the actual range is considerably magnified by the fact that observations must be made on extraordinarily small parts of cells represented by thin sections. If one could see the system in its three-dimensional entirety in whole cells of all types (expanding on the narrow range of possibilities provided by cultured cells) it would be easier to recognize it as a unit system of the cytoplasm. The various degrees of reticulation, continuity and organization in different cells would be more readily seen as characteristic of a cell type or a particular phase in cell activity.

It is customary for parts of the system included in thin sections of normal cells to show in at least one dimension a greater uniformity than is displayed by elements of the system in cells of this sarcoma. This is the dimension of width of the elongate profiles. It appears that such profiles (Fig. 6) to a great extent represent sections cut at right angles to flat, lamellar vesicles or cisternae and that the uniformity they show in section derives from a tendency of the limiting membranes to retain a certain minimal distance one from the other.

Images of this nature are particularly prominent in cells of secretory epithelia. In the parotid mucous cell shown in Fig. 6 they are not as closely packed as in acinar cells of the pancreas (Fig. 9) but display a tendency toward parallel array or organization which is commonly encountered in other cells of this general type. Besides the long, slender profiles there are many circular ones which represent sections through vesicles or tubules or short connections between adjacent cisternae.

The characteristics of these sections through the reticulum are displayed to better advantage in Fig. 7. In certain places the membranous outline of the profiles is sharply defined. These represent regions where it is oriented in a plane normal to that of the section. In other places where the plane shifts to the oblique the outline loses its sharpness (er_1). There is a halo of diffuseness around each of the profiles and continuous with the matrix of the cytoplasm. The material contributing to this is discussed below. The circular profiles are observed in some cases to be in line with the general curvature or sweep of the elongate elements (er_2) and doubtless represent sections through channels between adjacent cisternae. The space between them, viewed in another aspect would be seen as holes or fenestrae in the flattened vesicles, and it is easily recognized that such interruptions in the continuity of these vesicles would give them the character of reticula.

The content of the vesicles is homogeneous. It is difficult to judge to what extent its density derives from osmium, but it may be assumed that the material natively present is highly solvated and that upon water evaporation only a slight residue remains.

This component of the cytoplasm would possibly be of less interest if it were

characteristic of only a few cell types. It might then be recognized as one expression of cell differentiation and studied in this connection. Actually, however, it is as universal in its occurrence as mitochondria and nuclei. The forms in which it appears are numerous, but each is easily recognized as a variation of the same finely divided vacuolar system of the cytoplasm. To some extent these various forms and their distribution in the cell are characteristic of different cell types and therefore products of differentiation. But to some extent also they reflect changes in functional or physiological states of the cell.

Some measure of the degree of variation with cell type is shown in Figs. 8 and 9, but this is small compared with that which may be found in a wider spectrum of cells and cell types (17).

Parts of three fibroblasts from the dermis of chick embryo skin are shown in Fig. 8. At this time (14 days incubation) in the differentiation of this tissue these cells are actively involved in the deposition of collagen and it is characteristic for them to show an excessive development of the endoplasmic reticulum. Sections which are cut vertical with respect to the epidermis usually show profiles of the reticulum similar to those in Fig. 7. These represent vertical sections through flattened vesicles and the upper and lower membranes are seen to fuse at the margins. This defines the system as a closed one. It is also apparent (as at er_1) that the cisternae at one level may continue into those at another, thus making the system a continuum.

In other cells, of which the acinar cell of the pancreas is a notable example, the elements of the endoplasmic reticulum may pervade the entire cytoplasm and the parts may be disposed in some degree of order. The micrograph in Fig. 9 is a picture of a part of a pancreas cell, fixed 1.5 hours after secretion has been induced by pilocarpin. Under these conditions the cell is usually filled with profiles of the nature shown here. The membranes are in close apposition and if fixation is not carefully controlled the vesicles collapse and in sections appear as solid sheets ["lamellae" of Dalton (9)] or fibrils ["fibrilles" of Bernhard (4)]. It should be noted that the continuity of the profiles is interrupted (arrows) where the section passes through fenestrae in the sheet-like elements of the system.

IDENTIFICATION OF ENDOPLASMIC RETICULUM WITH BASOPHILIC COMPONENT

If now, as suggested by the stained image of cultured cells, the endoplasmic reticulum is basophilic, then it should be possible in microscopy of sections to demonstrate a correlation between the distribution of these profiles representing the reticulum in section and the basophilic component. The fact just noted, that the cytoplasm of the exhausted pancreas cell, which is known to be strongly basophilic, is packed with elements of the reticulum, is good evidence in favor of the correlation. In this case it would seem that the material binding the basic dye must be either a part of these extraordinarily thin vesicular structures or at least closely associated with them.

It is recognized, however, that a more positive correlation would be established if it could be shown that similar concentrations of the endoplasmic re-

ticulum are distributed with the basophilia in cells where the latter component is restricted to one small and well-defined region of the cell. There are several kinds of cells, especially secretory units, which satisfy this requirement, but none is more useful than the albuminous cell of the parotid. Here, as is well known, the basophilic material is confined very largely to a thin layer along the basal pole of the cell as though pushed there by the large accumulation of zymogen granules which occupy about three fourths of the volume of the cell (Fig. 12). When these cells are examined by electron microscopy and attention is focused on the bottom quarter of the cytoplasm (Fig. 10) it is readily observed that the cytoplasm contains numerous profiles of the endoplasmic reticulum. These are in no detectable way dissimilar to those shown in previous micrographs. They appear to show the same tendency toward parallel array, they fall within the same dimension range, and they show similar accumulations of material on their exterior surfaces (Fig. 11). What is most significant is that such structures are very largely excluded from the apical parts of the cytoplasm where zymogen granules accumulate. Thus it appears that where the reticulum and associated materials are in greatest quantity the cytoplasm shows its strongest affinity for basic dyes.

The correlation can be more firmly established by experimentally displacing the basophilic component from its position in the resting cell and then by electron microscopy observing whether the endoplasmic reticulum is similarly distributed. When, for example, gland secretion is induced by the injection into the animal of pilocarpin, the secretory granules are discharged and the cell loses volume. At the same time the chromophil substance comes to be distributed in all parts of the cell and may be found as frequently between the nucleus and the free surface as along the basal border of the cell (Figs. 12 and 13). The electron microscope image of this experimental material depicts a similar new distribution of the profiles characteristic of the endoplasmic reticulum. As shown in the thin section in Fig. 14, a few elements persist along the basal margin of the cell, but as many more are found just within the free surface. The cell is entirely depleted of zymogen granules and, compared with that shown in Fig. 10, it is greatly reduced in size. The two were fixed and otherwise treated the same.

Again it appears that the reticulum and the basophilic component move together and are indeed identical. At very least it is justified to conclude from the material reported on here and elsewhere (4, 9, 33) that the material possessing the property of binding basic dyes (nucleoproteins) is frequently associated with elements of this newly defined system of the cytoplasm.

Once these are recognized as structures of the basophilic component it becomes possible to relate them to the light microscope image of the cytoplasmic chromophile substance wherever found. In liver cells, *e.g.*, the endoplasmic reticulum sometimes appears as skeins or bundles of long profiles. The size and shape of these bundles as they appear in sections is easily identified with the "discrete clumps" of basophilic material described by Deane (10). In plasma cells the basophile cytoplasm is filled with the membrane-bound elements of the

reticulum (18) and in neurons the masses of Nissl substance show small anastomosing tubules or sections through thin cisternae (21).

A SMALL PARTICULATE COMPONENT OF CYTOPLASM AND BASOPHILIA

We are interested now in inquiring into the location of the basophilic compounds (nucleic acids and nucleoproteins) with respect to the endoplasmic reticulum. Are they within the system as something which accumulates or functions on that side of the membrane? Or are such compounds perhaps a part of the membranes or possibly attached to or adsorbed on its surface from the surrounding cytoplasm? A partial answer to these questions has come from some observations and studies of Palade (16).

In a survey of the fine structure of several cell types he noted that it was common, in the more basophilic organizations of the endoplasmic reticulum for the outer surface of the membranes to be coated with a fine granular material (Figs. 16–18). At higher resolutions the granules appeared as discrete macromolecular units ranging in size from 10 to 30 mμ. A few clusters of such granules were usually scattered through the cytoplasm, but by far the greater concentration of them was found (especially in acinar cells of the parotid and pancreas and in plasma cells) associated with the reticulum. This did not necessarily make them responsible for the property of basophilia. However, as the survey expanded it was noted that, in rapidly growing cells such as those in embryonic tissue or in the basal layers of the epidermis and in the crypts of the intestinal epithelium in adults, where the cytoplasm is diffusely basophilic, the endoplasmic reticulum is *not* prominent and dense granules of the same kind are scattered throughout the cytoplasm (Fig. 15). This suggested very strongly that the granules, rather than any component of the reticulum, represented the basophilic material.

That they do indeed appear with striking regularity in the cytoplasms of cells and more especially in association with the reticulum is evident from an examination (at higher resolutions) of the various cell types in which the endoplasmic reticulum has already been noted. Fig. 16, for instance, depicts a small part of an acinar cell of the pancreas. The same long profiles of sections through cisternae run from top to bottom of the micrograph. Between them and closely applied to their surfaces are great numbers of these granules. The picture from the sarcoma cell cytoplasm (Fig. 17) is similar except that here, as might be expected in rapidly growing cells, the granules appear in free clusters as well as applied to the surfaces of the reticulum. In chick fibroblasts (Fig. 18) actively producing collagen, the distribution is confined largely to the membranes. And finally, in an entirely different cell type, striated muscle of a young growing animal (Amblystoma larva) the granules of similar character are almost all free in the matrix of the sarcoplasm (Fig. 21).

The association of these granules with basophilia is supported by some observations on particulate fractions of cells (8). Barnum and Huseby (1) have reported the separation of a U fraction, distinct from and smaller than microsomes and sedimentable at 95,000 g. One of the major characteristics is that it

is extraordinarily rich in nucleic acid. More recently with the ultracentrifuge Peterman and co-workers (23, 24) have identified four principal macromolecular components of the cytoplasm which contain about 45% ribonucleic acid. The sizes of the particles defined by their methods essentially coincide with those evident in the electron micrographs. It is entirely reasonable then to relate them to the particles of Palade and therefore to assign to the latter a high content of nucleic acid and the property of basophilia.

With the thought that it may be characteristic for ribonucleic acid to be associated with particles of this general nature, it is of some interest now to look at the fine structure of the nucleolus after identical procedures of fixation, etc. The morphology of this seems to vary considerably from one cell type to another and not all by any means are apparently as well preserved or as striking structurally as those found in the nuclei of muscle cells of Amblystoma larvae. A section through such material is shown in Fig. 19. A part of the nucleus passes diagonally across the micrograph and the nucleolus is located in the approximate center of this. The laminar structure, which is not at present interpretable, is repeated in sections taken at other angles with respect to the nucleus. Even at this relatively low magnification the end of the nucleolus (arrow) is seen as a fairly close packing of fine granules. In a further enlargement (Fig. 20) these stand out with greater clarity and it is possible to see that they are remarkably uniform. When measured they are found to match in size those evident in the sarcoplasm and elsewhere (150 Å) (Fig. 21). Since these granules appear to comprise a large part of the nucleolus it is not unreasonable to suspect them of being rich in ribonucleic acid and in this as in other respects to simulate the dense (basophilic) granules of the cytoplasm. That nucleoplasm in general is particulate has not been overlooked. However, even a cursory glance will show that the material outside the nucleolus is, by comparison, a heterogeneous mixture of ill-defined elements.

SUMMARY

Electron microscopy of thinly spread cells grown *in vitro* has defined, as a component of the cytoplasm, a complex reticulum of strands and vesicles which has come to be called the endoplasmic reticulum. The component is limited by a membrane similar in thickness (ca. 80 Å) to the plasma membrane of the cell. It separates the content of the strands and vesicles from the general matrix of the cytoplasm and gives to the whole component the character of a finely divided vacuolar system. The elements of the system are frequently large enough to be resolved by light microscopy and can be shown to have an affinity for basic dyes. The system has therefore been identified as a basophilic component of the cytoplasm (25).

With the techniques now available for thin sectioning the same system of vesicles and interconnecting canaliculi can be demonstrated in cells fixed *in situ* and studies of such material have defined the system as a universally occurring component of the cytoplasm. In its structure it varies greatly from one cell type to another and even within a single kind of cell under different phys-

iological states. In one form it is common for it to be made up of extraordinarily thin, flattened vesicles referred to as cisternae. In certain types of cells, particularly acinar cells of exocrine glands, these vesicles are organized in parallel arrays and such organizations have been shown to coincide with the distribution of the basophilic component. Thus in thin sections as in cultured cells the reticulum is identified with the basophilic material.

In very recent electron microscopy of thin sections, Palade (16) has observed that a dense granular component of the cytoplasmic matrix is usually adjacent to the membrane surfaces of the reticulum. In rapidly growing cells where the basophilia is diffuse and where elements of the reticulum may not be prominent, granules of this latter character (100–300 Å diameter) are abundant and scattered throughout the cytoplasmic matrix. This suggests therefore that in the other instances where the intensely basophilic component and the endoplasmic reticulum coincide, the latter structure owes its basophile properties to the quantities of the granular material which associate preferentially with its membrane surfaces. This conclusion finds support in studies of cell fractions (1, 23) which have defined small cytoplasmic particles that are extraordinarily rich in ribonucleic acid. It is also supported by the observation that parts of the nucleolus consist of closely packed granules of similar size and density. It appears then that the basophilic component when it exists as a distinct and localized entity of the cytoplasm, consists of a concentration of vesicular and canalicular elements of the endoplasmic reticulum with small basophilic granules associated. Where the basophilia is diffuse it seems to be an expression of the diffuse distribution of the small granules.

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PLATES

PLATE 1

FIGS. 1–3

FIG. 1. Low power electron micrograph of a part of a mesothelial cell cultured from a newborn rat. It was fixed over vapors of 2% OsO_4 for 24 hours and then washed and prepared for microscopy as described elsewhere (25).

The nucleus is identified at *n*, the cell margin at *cm*. Other borders of the cell are beyond the limits of the micrograph. Mitochondria, *m*, are numerous, lipid granules less so. The endoplasmic reticulum is the lacework of material of intermediate density in the ground substance of the cytoplasm. It is highly irregular in this cell, consisting, in the thinner margins, of very fine strands (er_1) and in the thicker parts of the cell of relatively large patches (er_2). These latter were occasionally observed in earlier studies of cell fine structure but were not satisfactorily interpreted until after similar cells had been examined in thin sections. They are now recognized as flattened vesicles or sinuses [sacs of Weiss (33)] to be referred to hereinafter as cisternae (20). In the cytoplasm at the left of the nucleus such structures are large and two or more appear to overlap (er_2). Below and at the right of the nucleus such elements are more finely divided as though larger continuous areas had become fenestrated. Presumably in the living cell these and the finer elements of the system are amoeboid and what is a large flattened "sinus" at one moment may through fenestration become a reticulum of canaliculi during the next moment. In the thinner margins of the cell the cisternae may be in a sense obliged to break up in order to "service" functionally a thinner and more widely spread cytoplasm. There are indications, as well, that the presence of these flattened structures is related to a particular functional phase in the life of the cell. They are especially common in cells which are actively synthesizing a product such as collagen, mucin or digestive enzymes.

The rectangle outlined is shown at higher magnification in Fig. 2. Mag. × 3,500.

FIG. 2. This micrograph of a marginal area of the cell in Fig. 1, serves to show the highly irregular structure of the system. It is certain that some of this irregularity is inflicted on the system during dehydration but in a large measure it probably represents the native state of the component. Certain parts of the system are easily large enough to be visualized by the light microscope and when stained with haematoxylin or basic dyes appear as a basophilic component of the cytoplasm (see Fig. 3). Mag. × 10,500.

FIG. 3. Photomicrograph of mesothelial cell stained with haematoxylin. The mitochondria (*m*) are easily identified and the less deeply stained elements of the ground substance which form a vaguely defined reticulum are properly sized and distributed to represent the endoplasmic reticulum (*er*). As in the electron micrograph of a similar cell, the coarser elements of the system are located more centrally in the cell. Mag. × 1,400.

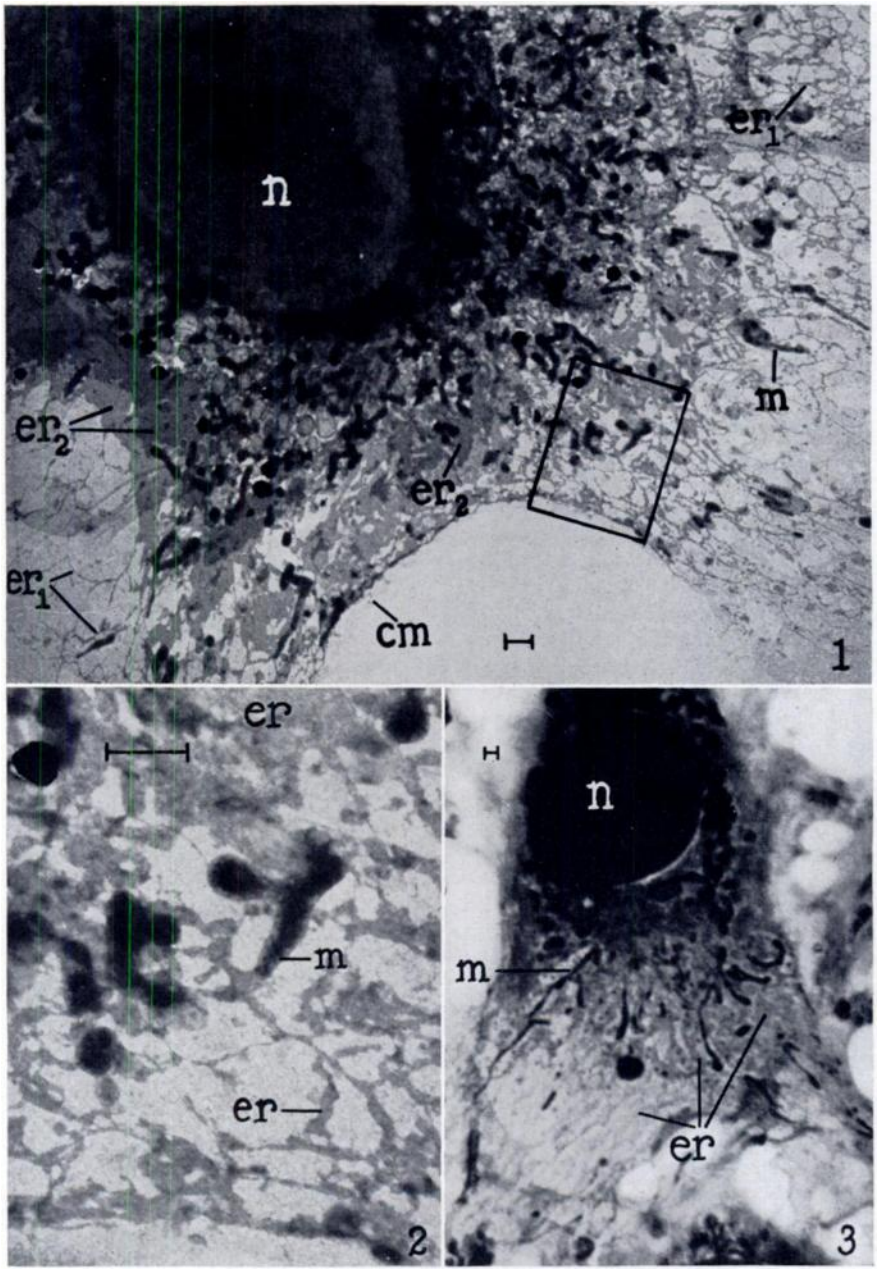


PLATE 1

PLATE 2

FIGS. 4–5

FIG. 4. Electron micrograph of a small marginal area of a thinly spread cell cultured from a rat sarcoma (4337) (28). The preparation was fixed in vapors of OsO_4 for 16 hours prior to washing and drying.

A short section of the cell margin is shown at *cm*. A few mitochondria (*m*) are present at the lower left, dense filaments (*g*) previously referred to as growth filaments from their common occurrence in growing cells, are scattered about the field, and lipid granules are identified at (*lg*). The picture shows otherwise only components of the endoplasmic reticulum. Rounded, vesicular units, as at (*er*), predominate. These vary greatly in size, from about 50 to 300 mμ, and are usually associated in strands (er_1 and er_2). Units with elliptical and irregular outlines are also evident as well as confused masses of vesicles (er_3). It is probable that some of the organization present in the native state is disrupted during preparation of the specimen. Mag. × 12,500.

FIG. 5. Micrograph of a cell of the same tumor in thin section. The tissue was fixed for 1.5 hours in 1% OsO_4 buffered at pH 7.8. The cell margin and membrane is indicated at (*cm*); the nucleus at (*n*); and mitochondria at (*m*). Profiles of irregular shape and size (*er*) characterized by dense outlines and homogeneous centers are identified as elements of the endoplasmic reticulum in section. The rounded elements are sections through vesicles or cross sections of canaliculi. The elongate shapes probably represent longitudinal sections of tubules, and profiles connected by a slender strand as at er_1 are taken to be sections through vesicles associated in strands as in Fig. 4. These interpretations of structures represented by the sections are based on several sets of micrographs of serial sections through cells of this tumor. In instances where the membranes surrounding the unit structure meet the plane of section obliquely, the shape is defined as that of flattened vesicles (er_2). In the cells of this tumor it is characteristic for the components of the endoplasmic reticulum to show great variation in size and form. The equivalent elements in the normal, cell-of-origin are generally more regular and frequently spatially organized. Mag. × 12,500.

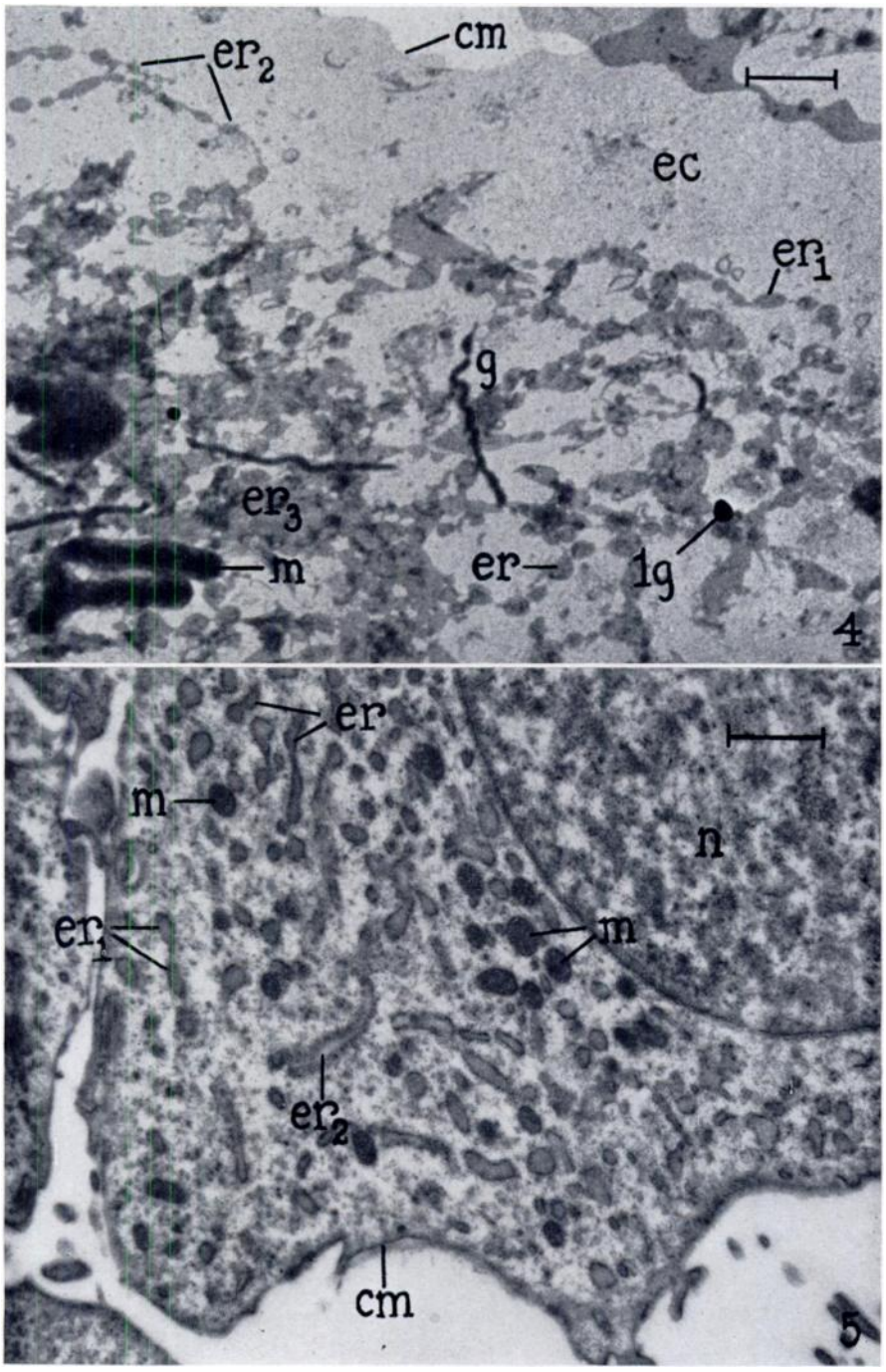


PLATE 2

PLATE 3

FIGS. 6–7

FIG. 6. Micrograph of part of a mucous cell of the parotid, with components labeled as in other figures. The section is cut obliquely to the basal-apical pole axis so that parts of the endoplasmic reticulum (*er*) and the nucleus appear considerably removed from the margin of the cell. The former (*er*) is represented by the long, slender profiles which are either sections lengthwise through canaliculi or transverse to the plane of flattened vesicles or cisternae.

In serial sections the long profiles have been observed to break up into and be continuous with a number (3 or 4) of short elements, a process recognized as a reticulation of the latter cisternae (26).

The structure at A closely applied to the mucous cell is assumed to represent a section through an arm of a myo-epithelial cell of the parotid.

The tissue was fixed for 30 minutes in 1% OsO_4 buffered at pH 7.5. Mag. × 12,600.

FIG. 7. Higher magnification of part of Fig. 6. It shows to better advantage portions of the endoplasmic reticulum (*er*). Certain of the profiles are sharply defined and represent sections of cisternae in which the plane of section is normal to the plane of the flattened vesicle. At er_1 several of these elements are cut obliquely and this accounts for the indistinctness of the images. Profiles which are not greatly elongated, as at er_2, are taken to represent either sections through tubular extensions from cisternae or transverse sections of independent canaliculi or vesicles. Where they are in line with the general contour plane of a cisterna they are probably sections through tubular extensions of same which connect with an adjacent cisterna.

The apparent granulation on the exterior surface (matrix surface) of the profiles is described further in subsequent figures. The interior of the vesicles is without structure. Mitochondria are indicated by *m* and cytoplasmic matrix by *ma*. Mag. × 26,400.

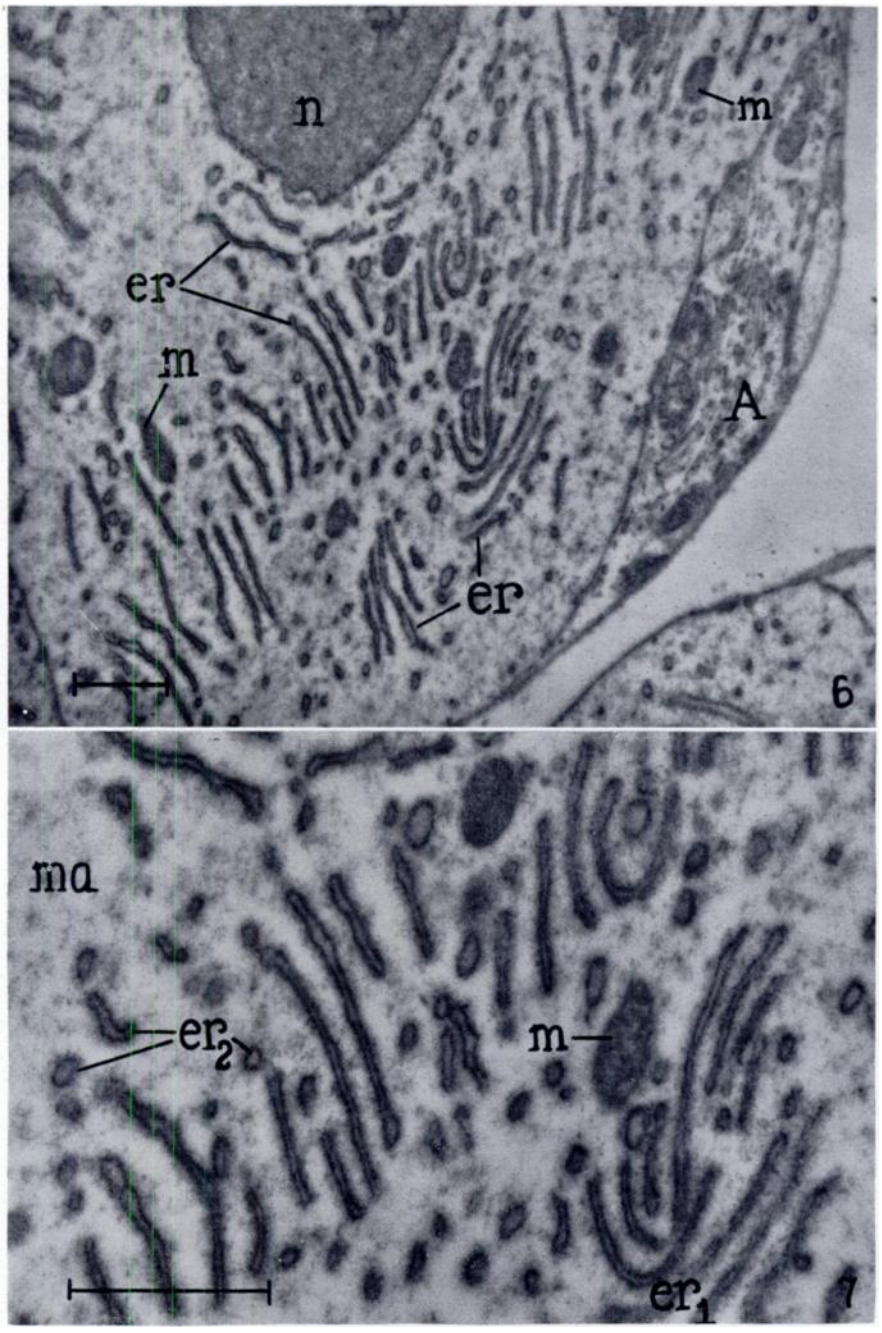


PLATE 3

PLATE 4

FIGS. 8–9

FIG. 8. Micrograph of parts of three fibroblasts from dermis of chick embryo skin (14 days incubation). The basement membrane of the epidermis, *bm*, passes across the upper left corner of the micrograph. Nuclei of separate cells are indicated at *n* and the third cell lies in between these two. Cell membranes or margins are designated by *cm*, mitochondria by *m*, and extracellular bundles of collagen fibrils at *c*. Long and short profiles of the endoplasmic reticulum (*er*) are prominent in all three cells. Most of these represent sections which cut the cisternae of the reticulum transversely. In these particular cells the cisternae are obviously oriented parallel to the basement membrane or in a plane parallel to that in which the cell is spread. Viewed in the whole cell they would probably resemble the overlapping structures at er_2 in Fig. 1. An additional point of interest and significance here is the evident connection at er_1 between these flattened elements at different levels in the cell. Micrograph from a study of collagen morphogenesis with George D. Pappas. Mag. × 16,000.

FIG. 9. Micrograph of a small portion of an acinar cell of pancreas of the rat. The cell, before fixation, had been stimulated to discharge its content of secretory granules, which leaves the cytoplasm packed with the long, s'ender profiles of the endoplasmic reticulum. This is an extreme example of the prominent development this cytoplasmic component may achieve. However, even in "resting" cells of this type it is customary for the basal part of the cell to be filled with such structures. Again these profiles represent vertical or transverse sections through cisternae. The space between the membranes varies (in this fixed specimen) between 150 and 300 Å but in most places remains close to 150 Å. At certain points (arrows) there are breaks in the continuity of the profiles and as mentioned elsewhere these mark holes or fenestrae in the cisternae. The granularity of the material on the surfaces facing the cytoplasmic matrix is shown to better advantage in Fig. 16. Mag. × 32,800

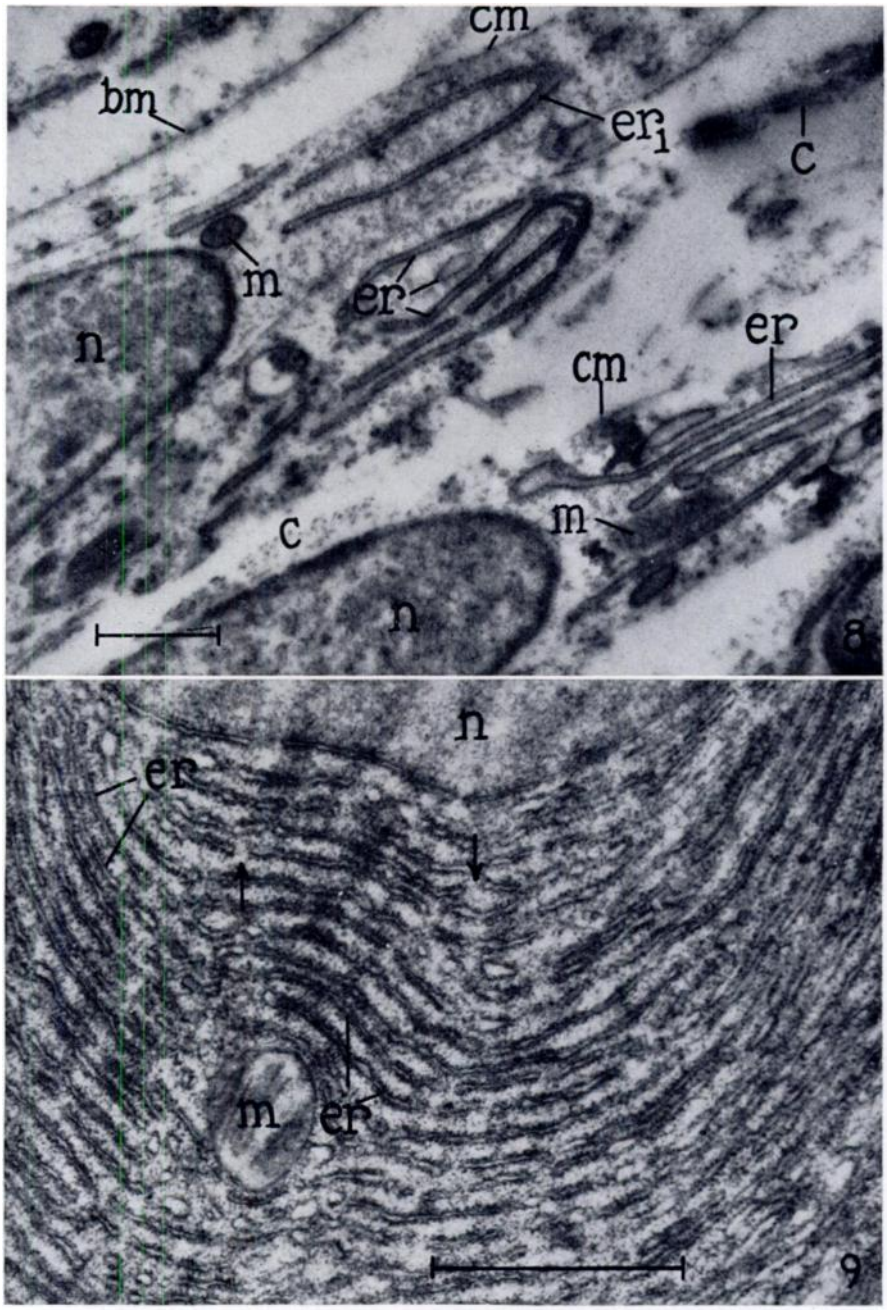


PLATE 4

PLATE 5

FIGS. 10–11

FIG. 10. Micrograph of section through albuminous or serous cell of the rat parotid. This is from a "resting" gland. The basal surface of the cell curves across the lower left and bottom of the micrograph; the apical pole and free surface are out of the micrograph. Zymogen granules (*zg*) fill the cytoplasm in the apical three fourths of the cell.

Long profiles of sections through cisternae of the endoplasmic reticulum (*er*) are localized very largely in the basal fourth or less of the cell. It is apparent that they tend toward parallel orientation. In their distribution they coincide with the basophilic component of the cytoplasm and seem likely to represent the deeply staining, parallel fibrils which have been repeatedly noted as structural units of the chromophil substance (12, 14). Cell margins are indicated at *cm*, nucleus at *n* and mitochondria at *m*. The tissue was fixed in 1% OsO_4 at pH 7.5 for 30 minutes. Mag. × 11,800.

FIG. 11. Micrograph showing at higher magnification profiles of endoplasmic reticulum in basal portion of parotid cell. This was similarly taken from a "resting" gland but was fixed in 1% OsO_4 for 24 hours instead of 30 minutes. A part of the material of the cytoplasmic matrix has been destroyed and removed by the long exposure to OsO_4. The profiles here are clearly identical in their major characteristics with those found in other types of cells (Figs. 5–9). Where the plane of section cuts the cisternae at right angles the membrane walls are densely defined (er_1); otherwide they overlap and the image is confused (er_2). A considerable amount of fine granular material is associated with the outside (matrix) surfaces of the cisternae. The basement membrane of the epithelium is at *bm*, the cell membrane at *cm*, and other parts as indicated elsewhere. Mag. × 21,000.

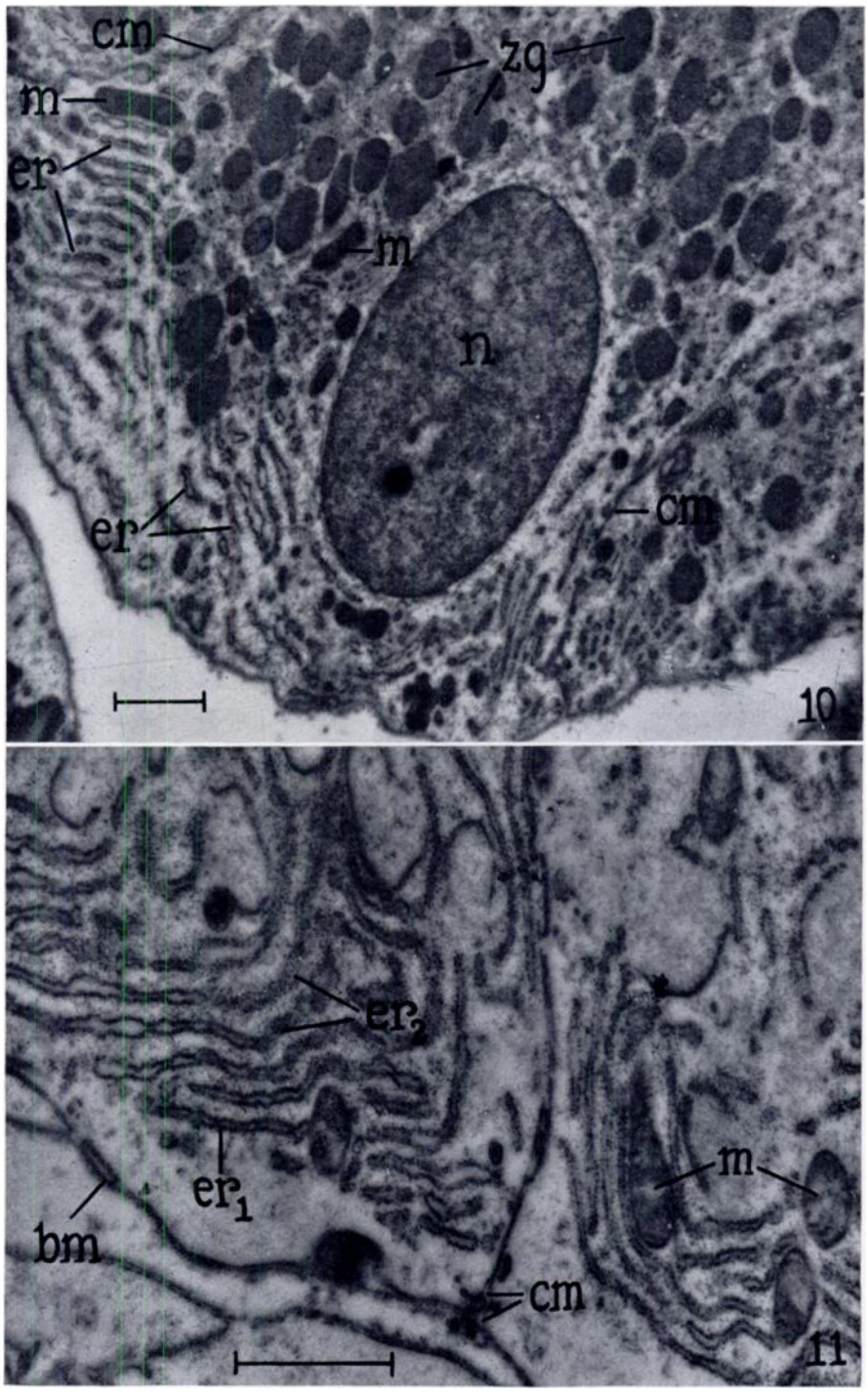


PLATE 5

PLATE 6

FIGS. 12–14

FIG. 12. Photomicrograph of section through "resting" parotid. Section was stained with toluidine blue and counterstained with eosin. A number of acini of serous cells are located around a duct. The deeply stained basophilic component is largely confined to the basal border of the cells (arrows). Mag. × 900.

FIG. 13. Photomicrograph of parotid fixed 1.5 hours after injection with pilocarpine. Each rat was given a total of 1.5 ml. of a 0.2% solution of pilocarpine in three injections at 15-minute intervals. The tissue pictured was taken 90 minutes after the first injection and fixed with 8% neutral formalin. The secretory granules have obviously been discharged and the basophilic component is now distributed in all parts of the cell (arrows). Mag. × 900.

FIG. 14. Electron micrograph of thin section through serous cell of rat parotid fixed 90 minutes after injection of pilocarpine into animal. The apical pole of the cell is at the top with its free surface bordering the lumen of a secretory capillary. Zymogen granules are no longer in evidence and elongate profiles of the endoplasmic reticulum (*er*) are now found in all parts of the cell. Other structures are labeled as elsewhere. The evidence from this (Fig. 14 with 13) and other comparisons indicates that parallel arrays of elements such as at *er* represent the basophilic component or chromophil substance of the light microscope image. Mag. × 12,400.

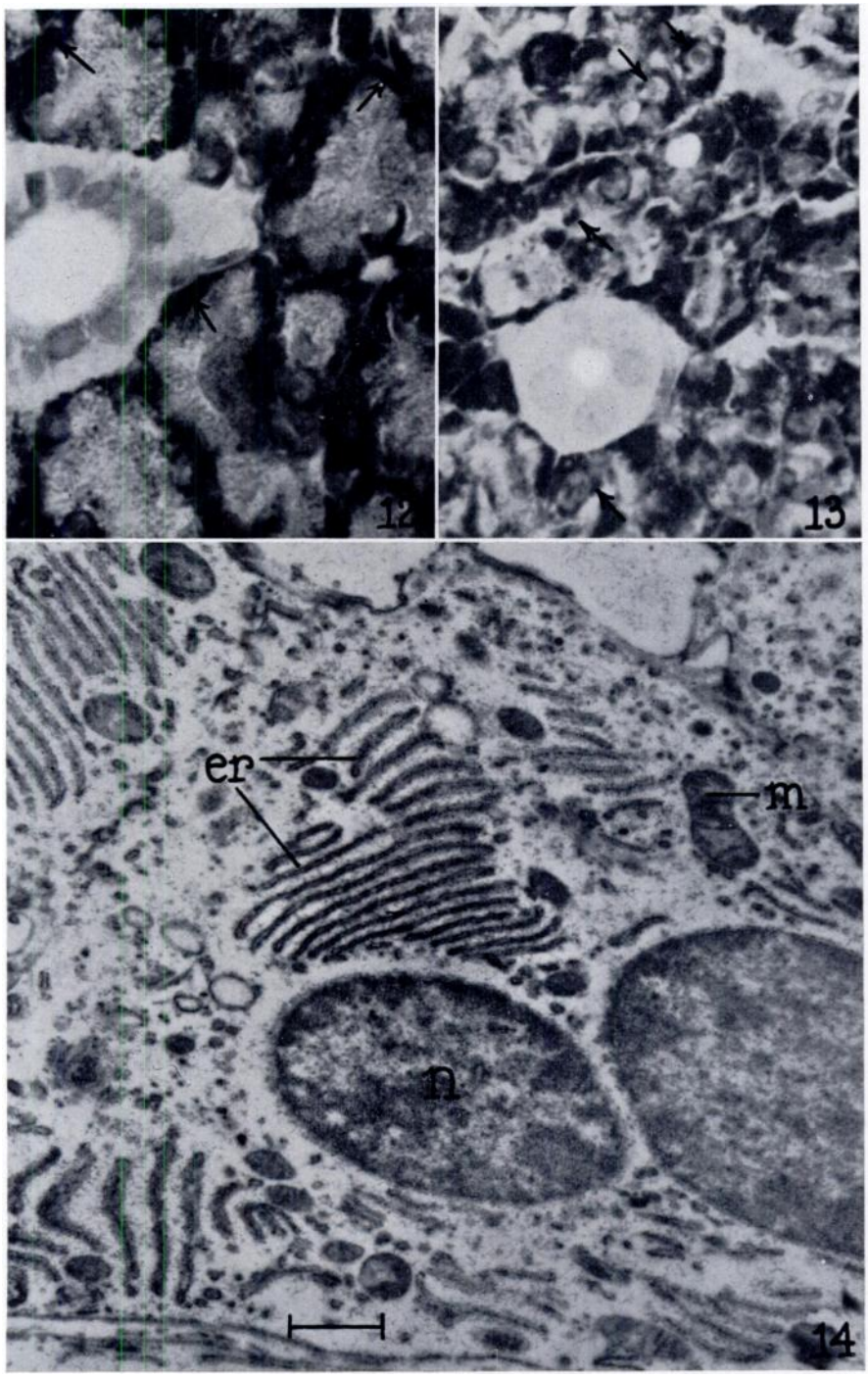


PLATE 6

PLATE 7

FIG. 15–16

FIG. 15. Micrograph of a small portion of epithelial cell from intestinal crypt of newborn rat. The basement membrane is at *bm*, nucleus at *n*, plasma membranes between adjacent cells at *cm*, mitochondria at *m* and endoplasmic reticulum at *er*. The same tissue, appropriately stained shows a diffuse basophilia even though elements of the endoplasmic reticulum are *not* present to any pronounced extent. Instead, the cytoplasmic matrix shows numerous small, dense granules similar to those usually associated with the endoplasmic reticulum. In this instance these granules vary considerably in size (80–200 Å) and show some tendency to associate in clumps (arrows). Many of the same granules are closely applied to the surfaces of such elements of the endoplasmic reticulum as are present. For reasons cited in the text they are thought to be the material responsible for the basophilia of the endoplasmic reticulum and the diffuse cytoplasmic basophilia of rapidly growing normal and malignant cells. The insert in the upper left shows a portion of the micrograph at a greater magnification (× 44,800). Arrows point to granules free in cytoplasmic matrix at *a*, on endoplasmic reticulum at *b*, and on nuclear membrane at *c*.

The cell was fixed in 1% OsO_4 for 2 hours. Mag. × 35,000.

FIG. 16. Micrograph showing a parallel array of profiles from the intensely basophilic region of a normal acinar cell of the pancreas. It is to be noted that the surfaces of the profiles (*er*, sections of cisternae) are covered with small granules (100–150 Å diameter) and that many units of the same general size and density are suspended on the cytoplasmic matrix (*ma*) between the profiles. The space between the membranes of the cisternae is extraordinarily narrow (ca. 200 Å). Parts of two mitochondria are at the right. Mag. × 63,000.

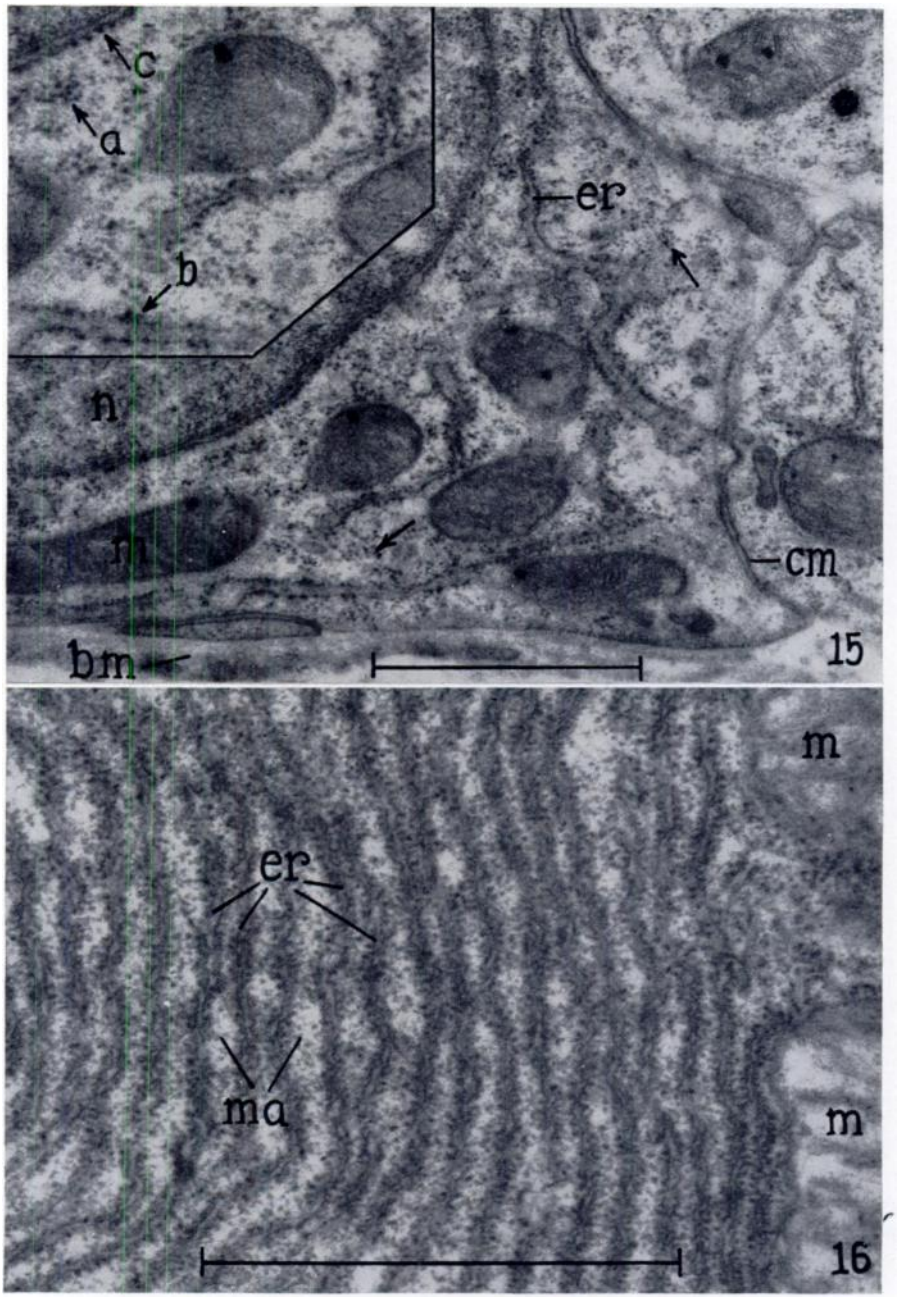


PLATE 7

PLATE 8

FIGS. 17–19

FIG. 17. Micrograph showing small area of rat sarcoma cell. When appropriately stained it shows a diffuse and relatively weak basophilia. The elements of the endoplasmic reticulum evident in profile appear as vesicles of irregular shape and size (*er*). They are seldom present in parallel arrays as in normal cells. The cytoplasmic matrix shows numerous clumps of dense granules (arrows) similar to those encountered in normal embryonic cells. Mag. × 54,000.

FIG. 18. Micrograph of part of a chick fibroblast similar to those shown in Fig. 8. It is an example of a cell which is differentiated in the sense that it is actively producing collagen and in which, and perhaps because of its maturity, the small granules are closely associated with the profiles of the endoplasmic reticulum (arrows). Mag. × 55,500.

FIG. 19. Micrograph showing a longitudinal section of striated muscle from a caudal myotome of an Amblystoma larva (Harrison stage 45). The nucleus occupies the center of the image and it shows to good advantage the complicated structure of a nucleolus (*nc*). Attention is directed especially to the granular character of the nucleolus (at arrow). (See Fig. 20). Mag. × 15,400.

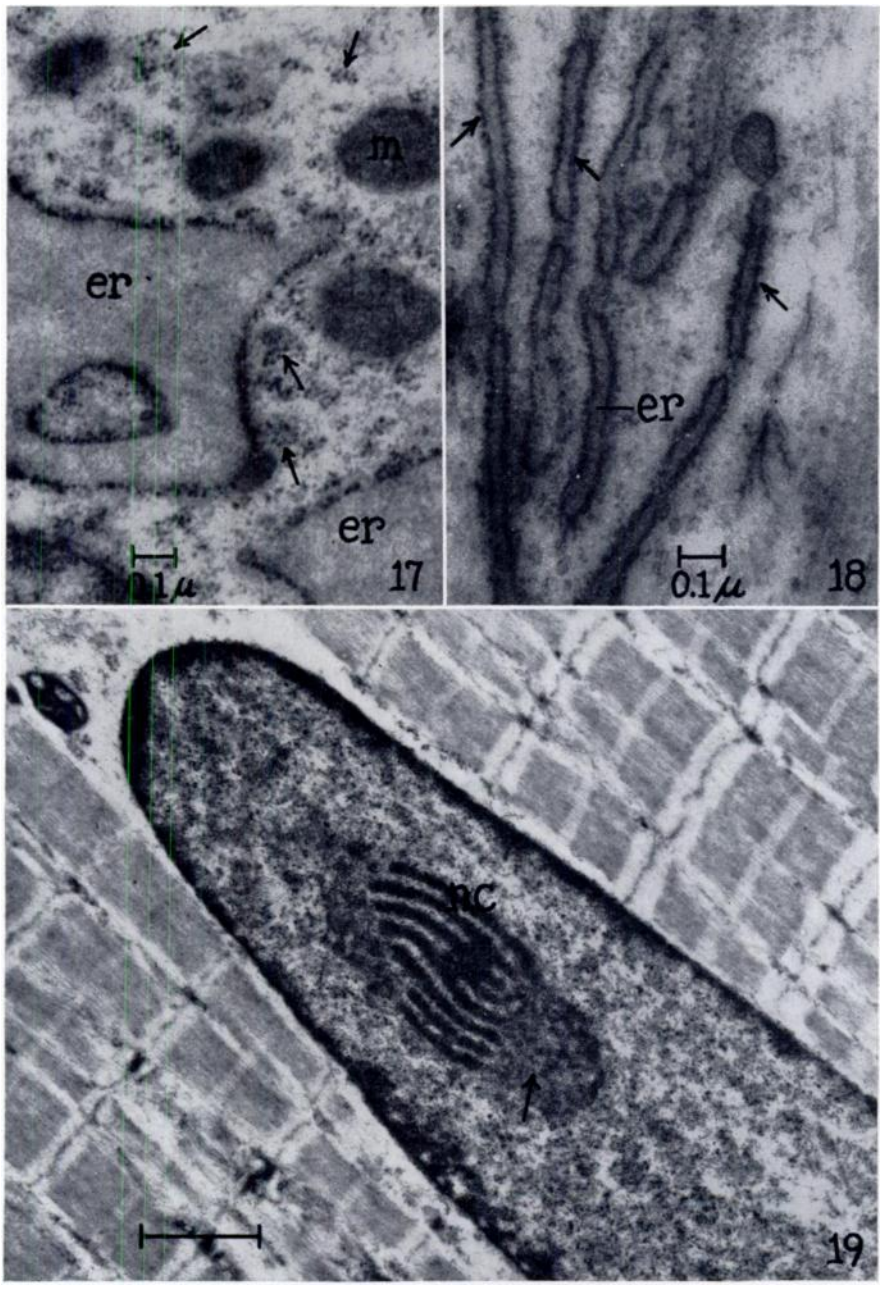


PLATE 8

PLATE 9

FIGS. 20–21

FIG. 20. Higher magnification of nucleolus in Fig. 19 presented to show packing of granules composing the two ends, and to some extent the central lamellar structures of the nucleolus. These particles are similar in size to those found on surface of endoplasmic reticulum and in clumps in cytoplasmic matrix of other cell types. They resemble very closely the granules found scattered through the sarcoplasm of the muscle cell containing the nucleolus (Fig. 21). In the sarcoplasm of myoblasts in younger embryos they are present in much greater concentration. They are assumed to represent the basophilic material (pyronin-positive, methyl green-negative) of the nucleolus which is rich in ribonucleic acid like the basophilic components of the cytoplasm. Mag. × 42,300.

FIG. 21. Micrograph showing myofibrils and elements of the sarcoplasm from a muscle cell of the same material shown in Figs. 19 and 20. It is reproduced at high magnification to show the fairly uniform size of the dense granules of the sarcoplasm (ca. 150 Å). When allowance is made for the difference in magnification they are recognized as being similar to the cytoplasmic and nucleolar (basophilic) granules described above. The membranous components (*er*) of the sarcoplasm are elements of the endoplasmic reticulum cut in oblique section. The Z bands of the myofibrils are indicated at *Z*. Mag. × 53,200.

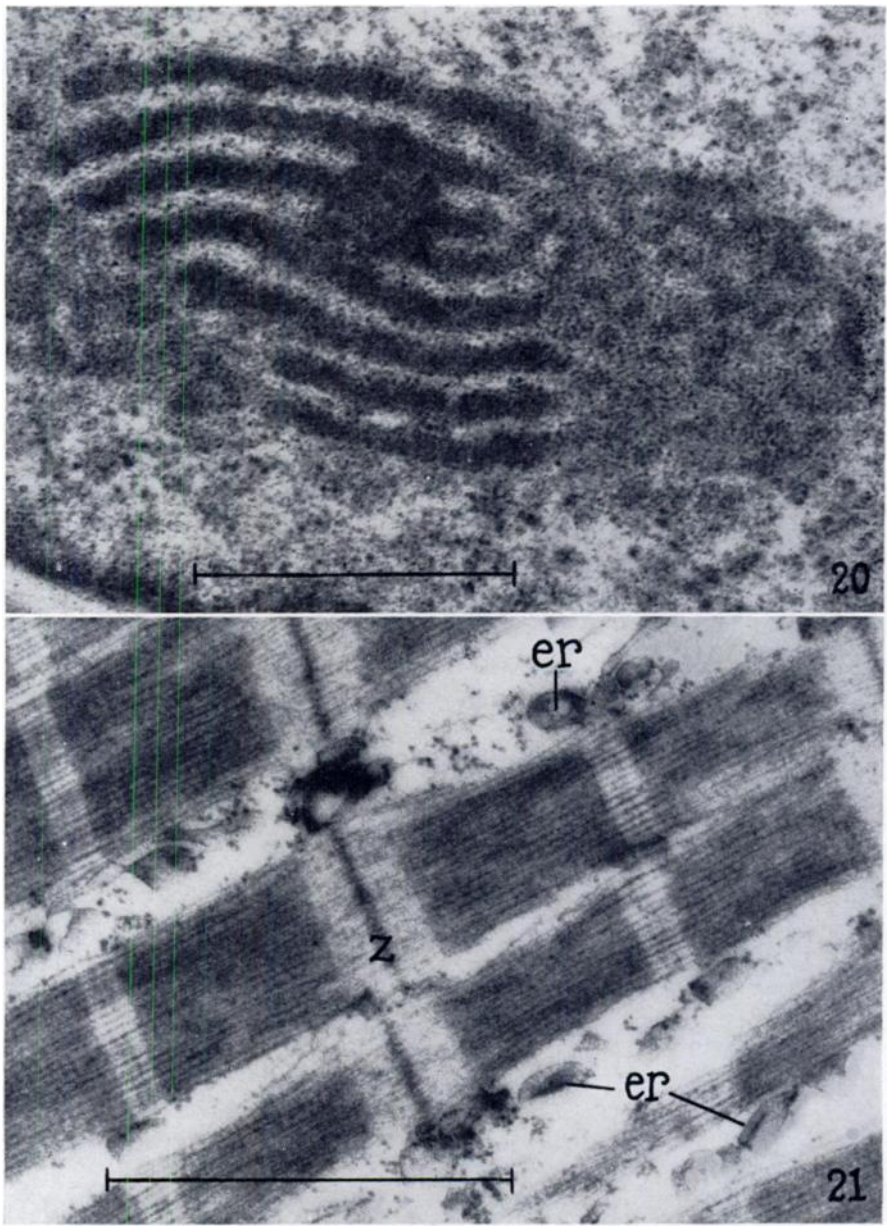
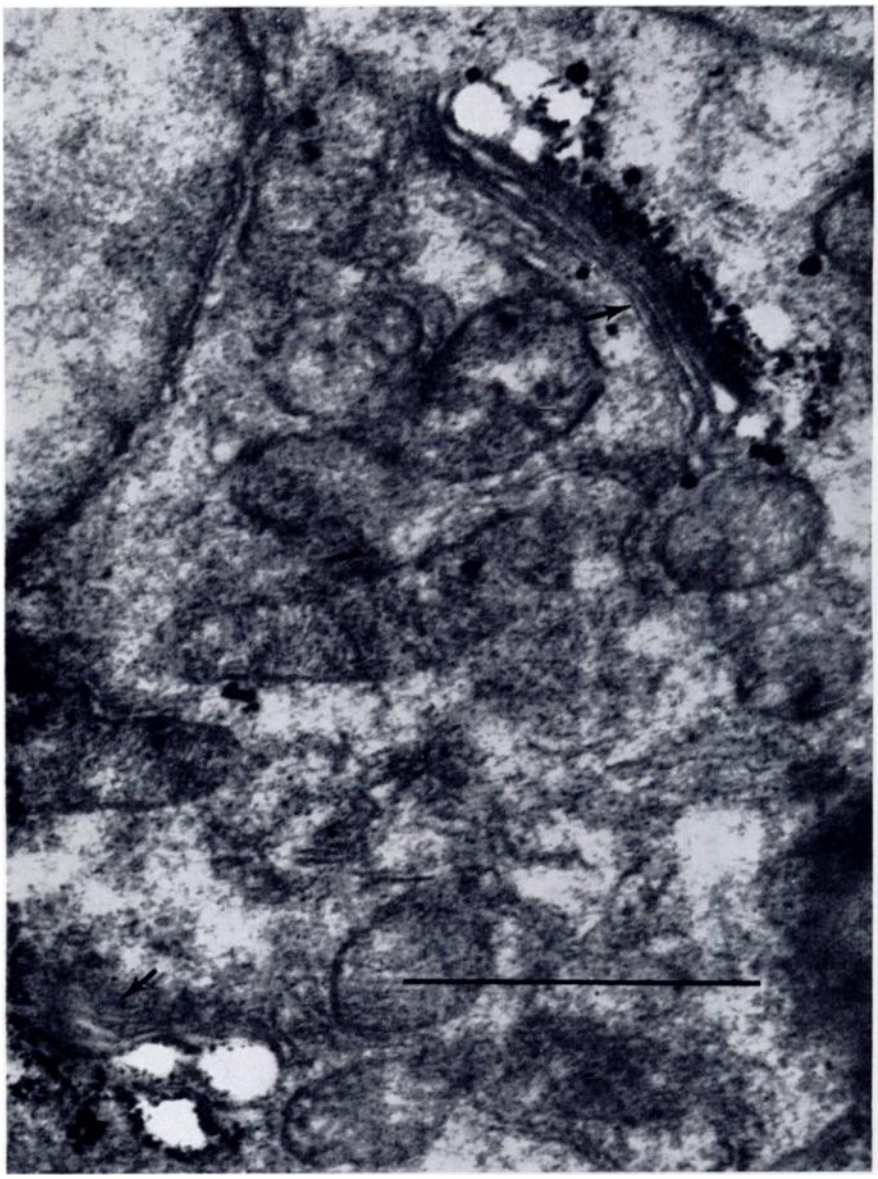


PLATE 9

DISCUSSION

A. J. Dalton, *National Cancer Institute, Bethesda, Maryland:* It should be pointed out that numbers of smooth double membranes measuring approximately 210 Å across are present in the Golgi zone of certain cell types—e.g., plasma cells and epithelial cells of the epididymis and duodenum. These newly



defined structures possibly represent a special form of the basophilic substance since they are not associated with the small (50 Å) granules described as characteristic of ergastoplasm by Sjöstrand and Palade. In the epididymis the membranes are regularly associated with groups of large vacuoles and numerous granules which average approximately 0.2 μ in diameter for the former and 400 Å for the latter. All three components together form what may be called the Golgi complex (Dalton and Felix '54, Am. J. Anat., *94:* 171–207). These three components show a consistent and characteristic topographic relationship to one another and to other cell constituents which is exemplified in the figure. The figure is of an electron micrograph of an epithelial cell of a duodenal gland of a mouse. The line near the bottom of the figure represents 1 μ. Two groups of double membranes arranged in a lamellar pattern (arrows) are separated by cytoplasm containing numerous mitochondria. The membranes are the more centrally located of the components of the Golgi complex. Peripheral to them is a row of small granules heavily impregnated with reduced osmic acid. At opposite ends of the groups of membranes are clusters of large vacuoles. The vacuoles are considered to represent the negative image of the Golgi substance as seen in ordinary histologic sections and the osmiophilic granules are considered to represent the osmiophilic reticulum of the classical Golgi apparatus. (The tissue from which this specimen was obtained was fixed by immersion in 1% osmic acid buffered at pH 7.4 with veronal-acetate buffer for 4 hours. It was then treated with 0.1% $KMnO_4$ (pH 7.0) for 30 minutes, washed in running tap water for 2 hours, immersed in an excess of 2% osmic acid in distilled water (pH 6.5) at 37° C for 18 hours, washed for 1 hour in running tap water, dehydrated and embedded.) These results on the morphology of intestinal epithelial cells are very similar to those obtained on epithelial cells of the epididymis and these latter results correlate well with chemical analyses (Schneider and Kuff '54, Am. J. Anat. *94:* 209–224) which demonstrated a high concentration of phospholipid and ribose nucleic acid in the isolated Golgi complex.

MARY L. PETERMANN, *Sloan-Kettering Institute for Cancer Research, New York, N. Y.:* We have been studying nucleoprotein particles, similar to those Dr. Porter has described, in the analytical ultracentrifuge. We find several kinds of particles, which can be distinguished by their sedimentation properties. In tissues such as pancreas and normal liver one kind predominates. Where the rate of cell division is high, as in regenerating liver and all the tumors we have examined so far, a second kind of particle is present in increased amounts. The nucleic acid content of these substances is very high, perhaps as much as fifty per cent.