OBSERVATIONS ON A SUBMICROSCOPIC BASOPHILIC COMPONENT OF CYTOPLASM

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It is customary to recognize in cells, as a major division of living cytoplasm, an optically empty ground substance or hyaloplasm in which the optically definable structures are suspended. Observations on this by light microscopy after fixation and staining have described it as finely granular, fibrillar, or alveolar but at least a part of this morphology is referable to the coagulating action of the fixative used in preserving the cells. With the limited resolution provided by visible light the problem has been to determine the degree of artefact and beyond this the organization and structure at submicroscopic levels. Since most behavioral phenomena of cells are in the last analysis dependent on structure at these levels, the sources of interest in ultrastructure are both obvious and numerous.

That submicroscopic structures are in fact present is shown by a considerable number of observations.

Gaidukov, for example, as early as 1910 (20) in a darkfield, ultramicroscope study of cells, noted the presence of refractile bodies in parts of cells appearing empty under transmitted light. By similar devices, Bayliss (3) described Brownian motion of tiny particulates in clear protoplasm of the *Amoeba* pseudopodia. Observations on cultured tissue cells by darkfield microscopy disagree to some extent in what may be seen at submicroscopic levels. Strangeways and Canti (63) noted a "variable number of very small particulates" distinct from mitochondria in an otherwise clear ground substance. Lewis (29) and later Ludford (31), on the other hand, describe the ground substance as appearing dark and structureless except in malignant cells or in cells beginning degeneration (30). It is probable that the intensity of illumination used in these latter studies was not great enough to show up the small and weakly refractile bodies mentioned by Strangeways and Canti (63). In any case, with light of sufficient brightness, Brenner (7) observed in the hyaloplasm of living liver cells "shimmering points" which he identified as components of the basophilic material.

Through its ability to define anisotropic structures, polarized light has been useful in revealing additional features of the cytoplasmic fine structure. The majority of studies with this form of illumination have described the hyaloplasm as isotropic except in the cortical regions of the cell. Thus the ectoplasm of *Amoeba verrucosa* has been reported to show weak birefringence (57) as has also the cortex of the eggs of

several marine invertebrates (56). Evidence of orientation has also been noted in osteoblasts grown in culture (27) and here it appears that the responsible elements bear some relation to the migrational activities of the cell and probably are to be identified with stress fibers or fibroglial fibers (32) common in such cells.

Certain of the anisotropic elements of cytoplasm, especially in egg cells, are made more evident by centrifuging the cell before or during study. Thus Pfeiffer (46) defined birefringent components in the eggs of *Rana temporaria*, and Monné (37) has shown bundles of cytoplasmic fibrils in the eggs of *Psammechinus* and *Mytilus edulis*. Using similar procedures plus electron microscopy, McCulloch (35) has identified in *Arbacia punctulata* eggs a fine, diffusely birefringent component which stratifies in the clear zone at the centripetal pole, and a "coarse fibrous component" which shows a birefringence negative with respect to its long axis. Certain features of these latter, to be mentioned in the discussion, relate them to the submicroscopic component that is the main interest of this report.

Another component of cytoplasm that may be classed as "submicroscopic" is characterized by its affinity for basic dyes, and is variously referred to as basophilic material, chromophilic substance, ergastoplasm, and Nissl's substance. (See Garnier (21) and Prenant (55) for original observations and early reviews of the literature on this component.) This basophilic component is not defined in living cells by conventional light microscopy[1] and even in stained preparations it is in most cases poorly resolved. In so far as it is visible in the light microscope it is known to vary in quantity, form, and distribution in different kinds of cells and even among cells of the same type. Thus in some preparations of liver cells it has the appearance of a fine stippling (42) whereas in other preparations it is described as occurring in clumps or strands (18). The inference is that the morphology, optically defined, is a reflection of different organizations or aggregations of submicroscopic units. It is now well established that nucleic acid of the ribose type is localized in this chromophilic part of the cytoplasm (5, 8, 22).

Evidence that this component is made up of units which differ in density from the rest of the cytoplasm is provided by the observation of Claude (12) that it will under centrifugation separate out as a fairly distinct stratum in the living cell. It seemed "probable" to Claude that this basophilic component was the intracellular equivalent of the "small particle" or "microsomal" fraction isolated by centrifugation from ground-up liver cells and this correlation has since been more definitely established by Brenner (7).

From such observations as the above, it is evident that the hyaloplasm of some cells at least contains submicroscopic particulates, anisotropic structures, and basophilic components. Aside from their existence, however, very little is known about them, and questions concerning their relation to one another and to the cell, and concerning their structure and function, remain to be answered.

It is to be expected that electron microscopy will contribute to a solution of

[1] However, ultraviolet studies of living neurons reveal absorbing structures similar in form to Nissl bodies (64) and it is probable that both the refractile bodies in liver cells, observed in dark field by Brenner (7), and the strongly birefringent fibers of McCulloch (35) in *Arbacia* eggs, represent basophilic inclusions.

these problems. The major requirements for efficient use of the greater resolution provided are that the cells or sections of cells be sufficiently thin to permit differential penetration of electrons and that the material be fixed in such a way as to represent faithfully the original living morphology. From fairly unpromising beginnings the techniques required for getting ultrathin sections of cells have recently developed to near perfection. Paraffin and celloidin as imbedding matrices have been supplanted by plastic (*n*-butyl methacrylate) (39) and steel knives have to some extent given way to glass fracture edges (28). Most recently the quality of fixation has been greatly improved by appropriate control of pH during osmium tetroxide penetration and other considerations (43). Obviously, this combination of technical developments permits the application of electron microscopy to all types of biological material and morphological problems and introduces a new, and what should prove to be very interesting, period for cytologists.

Another approach to the study of fine structure may be made through electron microscopy of cells grown in culture. It has previously been shown that under certain conditions these are suitably thin (50) and while they do not provide for the breadth of studies permitted by thin sectioning, they are uniquely valuable for observing fine structural organization in the *whole* cell and for experimental studies designed to examine structural-functional relationships through control of environment. In studies of such cells information has been gathered on the submicroscopic structures of the basophilic or chromophilic substance of the cytoplasm and on its relation to other cell components. The present report describes these observations in some detail.

Materials

All the observations to be reported have been made on cultured cells that have spread out thinly on the surface supporting the culture. Explants from several kinds of tissues have provided the cells, and the sources and types used for illustration are indicated in the figure legends. Besides these a large variety of cells have been examined and our experience to date indicates that if a cell can be cultured it can be studied by electron microscopy. For the present purpose, which is to gather information on certain of the common features of cytoplasmic fine structure, cell types have been selected which are most easily cultured and for other reasons best for microscopy. Thus most study has been given to fibrocytes, myoblasts, and mesothelial units. In addition to these, however, sarcoma cells, macrophages, lymphocytes, osteoblasts, and epithelial cells from kidney and mammary gland have been examined. The explants have been taken from very young and embryonic chickens, rats, rabbits, and mice.

Methods

The procedures outlined in the preliminary report are still followed (50). Cells for study are grown in culture on a coverglass that has been previously coated with a thin film of poly-

vinyl resin (formvar). When suitably thin units have spread out on this surface, they are fixed and small portions of the formvar film to which they are attached are stripped from the glass, transferred to the usual disc of wire mesh, drained of water, and dried. They are then ready for microscopy.

Since several details of the procedure are important in determining the characteristics and quality of the preparations, the following, more complete description is provided (see also 33).

Preparation of Coated Coverglass.—It is important for the resin membranes supporting the cells to be of a suitable and controllable thickness; *i.e.*, of the order of 20 to 30 mμ. Like the cells or any other matter in the path of the electron beam, the membrane scatters electrons, and if it is excessively thick, there is some loss of resolution and image contrast. On the other hand, if the membrane is too thin, it will neither withstand the manipulation involved in peeling it away from the glass slide nor resist the tensions that develop when the specimen is exposed to the electron beam. The following procedure has been found to give reliable and reproduceable films.

The coverglasses to be coated are first cleaned with the aid of soap or a detergent, thoroughly rinsed, and towel-dried. They are then fastened to regular 1 by 3 inch glass microscope slides by any device that will permit free drainage of fluid from their upper edges. A useful type of support is shown in Text-fig. 1. The whole assembly is dipped in a solution of the resin[2] (0.25–0.3 per cent) in ethylene dichloride[3] to a level above the edge of the coverglass. When removed, the solution drains smoothly and quickly evaporates. Obviously, the film of resin at the lower edge of the coverglass will be thicker than that formed at the upper edge. However, the cell colony is usually grown near the center and so from preparation to preparation the film beneath it is of approximately the same thickness. The thicker glass backing for the coverglass is important because it provides heat for the evaporation of the solvent. If it is not used, the temperature of the coverglass is so reduced that atmospheric moisture condenses out on it and the tiny droplets of water formed are incorporated in the film. As a consequence it then appears milky and shows perforations under the electron beam.

After the coated coverglasses have thoroughly dried they are sterilized by dipping in two baths of 70 per cent alcohol[4] and thence placed in a sterile glass box or Petri dish where they dry and remain until used. In order that all the ethylene dichloride will have left the films before the cultures are set up on them, the slides are coated 24 hours in advance of use. Slide inserts (11 by 22 mm.) sometimes used in flask cultures, are prepared by first scoring larger coverglasses (*e.g.* 22 by 22 mm.) with a diamond pencil, then coating, and finally breaking into the required 11 mm. strips.

Culturing.—The cells for these studies have been grown by a variety of procedures, all of which might be called standard for tissue culturing. The chief purpose has been to obtain populations of thinly spread cells as free as possible of extraneous material.

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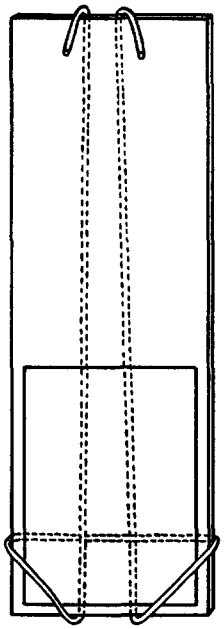
[2] For this purpose a resin known as formvar has been found satisfactory. The Shawinigan Products Corp., New York, which produces it, describes it as a polyvinyl formol resin. It is available as a dry powder and the percentages given are for dry weight of this in solvent. The solution is filtered before use. There is no evidence that it is toxic and it may be supposed that even if it were, the albumins and globulins of the media would be effective as detoxicants.

[3] During a period of storage, especially if not protected from light, ethylene dichloride decomposes in part to HCl. If such a solvent is used for formvar, the films produced tend to break and develop holes under the stresses of use. For this reason only freshly distilled ethylene dichloride should be employed and new formvar solutions should be made every few weeks.

[4] It is advisable to use reagent quality 100 per cent ethyl alcohol for making this. Lesser grades may leave a residue on the formvar surface.

A few trials have been made with liquid drop cultures. These provided suitable material in some cases, and possibly with some attention to content of media, etc., they could be made the source of most cells for microscopy. It has been observed that it is best to keep such cultures as hanging rather than lying drops after the first 24 to 48 hours of incubation so that particulate material in the culture medium will drop away from the cells and the resin-coated surface of the slide.

In most of the experiments providing material for these reports, however, the cells have been grown under fibrin clots. These have been made from one part of chicken plasma collected



TEXT-FIG. 1. Outline drawing (actual size) of the support used for coating coverglasses with thin film of formvar. It consists of an ordinary glass microscope slide (1 inch by 3 inches) wound with 0.06 inch silver wire (probably desirable to avoid copper) as indicated. The wires running diagonally across the lower corners of the slide hold the coverglass against the slide. In applying the film, the whole assembly is dipped in the formvar solution to a level just above the upper edge of the coverglass and then allowed to drain and dry.

from young cockerels and two parts of nutrient. The nutrient has, in the majority of cases, consisted of five parts Tyrode's solution (19), three parts human placental cord serum, and two parts embryo extract. This medium has been used with satisfactory results in both slide cultures and roller flask cultures.[5]

In such cultures many of the cells which grow or migrate from the tissue explant come into contact with the plastic coated surface of the coverglass and spread out under the clot. If the

[5] Maximow slides (1¾ by 3 inches) are commonly used and the cultures are set up on floating coverglasses (38). The roller flask and technique for using it have been described earlier (50). The flask neck, however, need not be more than half as long as originally shown.

culture is correctly prepared, they remain attached to this surface and are not disturbed when the clot is later removed. This "correct" preparation procedure is as follows:—

The explant is placed on the coverglass (or slide insert) first in a drop of Tyrode's solution, which is then used to wet the resin-coated surface around the tissue. If the Tyrode's solution fails to spread smoothly, adding an extremely small proportion of serum will make it do so. The excess Tyrode's solution is next withdrawn and replaced by a plasma-nutrient mixture in sufficient quantity to provide only a shallow drop which will hold the explant close to the slide. Clotting should be permitted before the culture is again disturbed. It is assumed, in explanation of the efficacy of this procedure, that wetting the formvar film first with Tyrode's solution prevents the fibrin strands of the clot from attaching to the resin surface, except around the edges where the film was dry. An extremely shallow fibrin-free pocket is thus formed under the clot. Whether or not this is the correct explanation, it is known that in cultures prepared otherwise, the clots are difficult to remove without destroying the underlying cells.

Culture conditions in flasks appear to be better than in the well-slide preparations, and cultures prepared in flasks normally yield larger populations of usable cells. For this reason and because of the greater facility with which such cultures can be fed, most of the preparations have been grown on slide inserts within roller flasks.

Fixation.—When cells of a suitable character have spread out on the coverglass, it is removed from the culture flask or well-slide and immersed in Tyrode's solution (Earle's formula regulated with CO_2 to a pH of between 7.2 and 7.8). The clot, if present, is peeled away and the underlying cells are washed briefly in a slow stream of Tyrode's solution produced with a pipette.

A variety of agents and procedures have been employed to fix the material for microscopy and the effects of some of these have been described elsewhere (49, 52). Osmium tetroxide has been by far the most generally used and the technique for it only, will be described in detail here.

The coverglass supporting the cells is removed from the Tyrode's solution in which it was washed and is placed over OsO_4 solution in a Maximow well-slide. It is customary to use 0.2 cc. of a 2 per cent solution of OsO_4 in H_2O.[6] Thus, while being fixed in osmium vapors, the cells are bathed in a hanging drop of Tyrode's solution. The duration of fixation, which is carried out at room temperature, has been varied from 5 minutes to 24 hours, with consequent variations in the electron microscope image of the cells (see below).

Preparation of Screens.—Following fixation, the cells, still attached to the coverglass, are transferred from Tyrode-OsO_4 solution to water. They are washed in distilled water for periods varying from 15 to 30 minutes. The effects of this variable on the final form of the cell have not been observed to be as pronounced as those arising from variations in the fixation time.

The procedure for transferring the fixed cells from the coverglass to the disc of wire mesh,[7]

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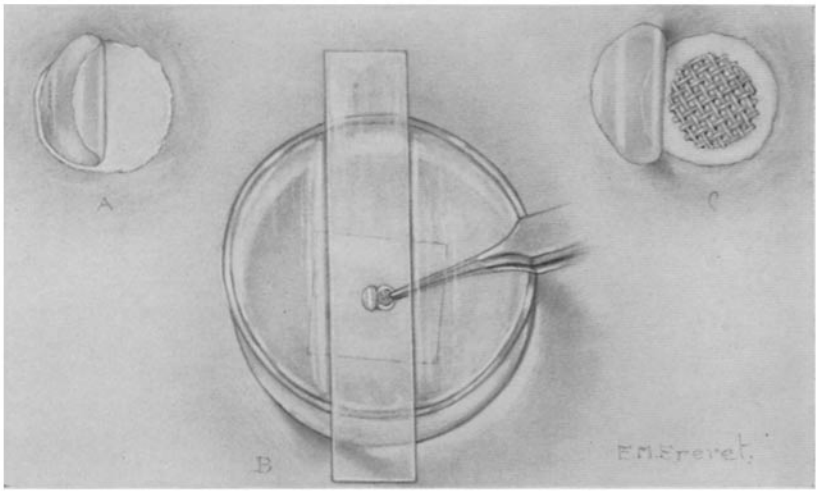
[6] The concentration of OsO_4 in a solution gradually decreases during storage, even when kept in the dark at icebox temperatures of 6–8°C. As a result of experiences in which the character of the fixation has been noticeably influenced through the use of old solutions, it has become a rule to avoid the use of preparations stored longer than 2 months. Palmer (45) has described a colorimetric test for OsO_4 that can be used in checking the concentration in stored solutions.

[7] Stainless steel wire cloth, 150 mesh to the inch, has been found satisfactory for this type of material (available from the Newark Wire Cloth Co., Newark, New Jersey). A single hole is large enough (*ca.* 75 μ on a side) so that many complete cells can be contained within its margins. Unfortunately the wire strands, which are 60 μ in diameter, block out a fair percentage of the culture area. The cloth should be rolled before using, to flatten the surface.

the object holder for the electron microscope, is illustrated in Text-figs. 2 and 3 and described in the figure legends. A few other details should be mentioned. (*a*) For example, it has been found desirable to use filtered distilled water for washing, thereby reducing particulate contamination of the screens. This clarification may be done with a porcelain filter (Selas 01 or 02 porosity). (*b*) The manipulations involved in transferring the resin film from coverglass to screen are rendered less difficult by placing a small glass strip over the field of operations and in contact with the water. This prevents any vibrational motion of the water surface and



TEXT-FIG. 2. This depicts the first steps in the procedure by which cells after fixation and washing are transferred from coated coverslips on which they have been grown, to the small wire grid used for electron microscope specimens. All manipulations are performed with watchmaker's forceps[8] under a dissecting microscope, at a magnification of about 12 diameters. In *A*, the continuity of the film around the area supporting the cells has been broken and the resulting small disc of film has been partly peeled away from the surface of the coverglass. *B* is an unenlarged image of the arrangement of dish, coverglass, and narrow slide which is placed over the assembly to eliminate image aberrations resulting from wave motion on water surface. As indicated in *B*, the transfer is made under water. In *C*, the flap of film has been peeled back further and the small electron microscope grid has been put in place under it.

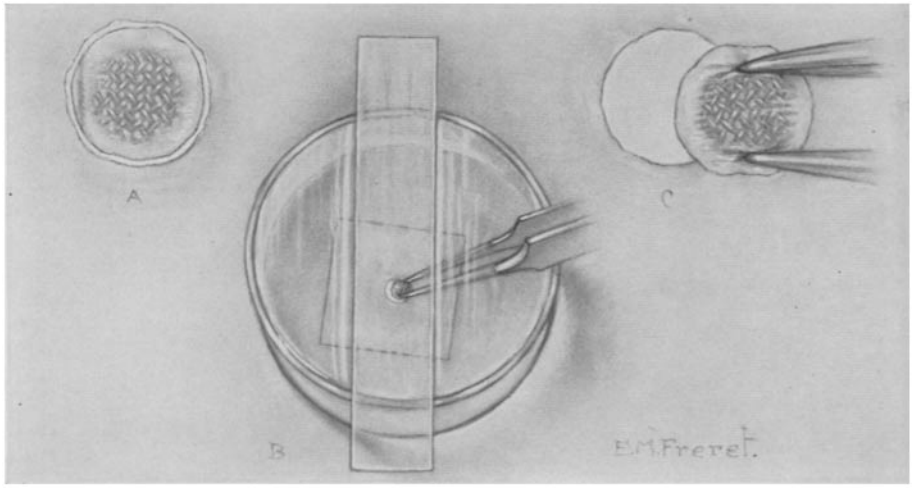
consequent image distortion. (*c*) After removing the coated screens from the water, they are drained on filter paper, which of course facilitates more rapid drying of the specimens. For thorough drying they are placed over P_2O_5 and kept there until examined. They are finally stored in gelatin capsules in a dry atmosphere. It has frequently been necessary in our ex-

Copper electromesh (available from the C. O. Jelliff Mfg. Co., Southport, Connecticut), which may also be used, has slightly less open area for viewing, and over long periods of storage the copper or some compound of copper diffuses into the film. It has the advantage, however, that under the electron beam it displays less thermal drift.

[8] Size No. 3 or 4 forceps, available from Krieger and Dranoff, New York, are most satisfactory for this operation.

perience to examine a screen a second or third time for additional micrographs, and in the majority of cases, the prolonged period of storage has not been observed to result in any pronounced change in the material.

Other Fixations.—A fairly good preservation of these thinly spread cells may be obtained by simply drying them. Apparently the cell is so thin and so adherent to the supporting film that drying as a rule effects little or no shrinkage of the whole unit. At any rate, the shrinkage under these conditions is nothing compared to that which takes place when the cell margins are free.



TEXT-FIG. 3. In *A*, the formvar flap, with cultured cells (not shown) on the surface, has been brought back over the grid, and with forceps placed as in *B* and *C*; the grid and the film it now supports are being removed from the water. While still held in the forceps, the coated grid is touched to filter paper to draw off the water and is thereafter dried.

The cells, in preparation for drying, are washed in balanced salt solution to remove the culture medium and while still in the saline are transferred in the usual way to the wire grid. This must be done quickly because the cells tend to react to the manipulation and pull up. In so doing they produce puckers in the supporting plastic film. The salt solution is then drained off by placing the screen on filter paper and the preparation is dried in room air. When the salt solution is thoroughly drained away, only minute areas of the cell's surface show crystalline material.

It has been found that *formaldehyde* may also be used in a manner to give a nearly faithful fixation. For this, at least two conditions should be controlled. The formalin must be neutral and it should be applied to the cell in isotonic saline. In our fairly limited experience the best results in terms of preservation of cell form have been obtained with a neutral solution of 8 per cent formaldehyde containing 0.85 per cent NaCl. This was allowed to act for 4 to 6 hours. The cells were then washed in water for 20 minutes and transferred to screens as described above.

After such fixation the cells are remarkably smooth, show little evidence of coagulation, and retain, after drying, a greater proportion of their native thickness than after either simply drying or fixing with osmium. As is well known, tissues fixed with formaldehyde harden with-

out shrinkage and some of this rigidity of form is retained through the surface tensions which develop during drying. Only lipid granules or vesicles are unfixed, as shown by their tendency to vaporize within the vacuum of the microscope and leave a crater in the cell.

Microscopy.—The electron microscopy involved was done with either an RCA model (EMU-2B) or a Philips model (EM-100). It was found desirable to examine the material initially at relatively low magnifications (between 2000 and 3000 diameters) so that a greater portion of a single cell could be seen on the viewing screen at one time and the image more easily related to that familiar from light microscopy. In the case of the RCA microscope (EMU-2B), this range was obtained by shortening a specimen holder about 1.5 mm. and making appropriate adjustments in lens current for the increase in the focal length.[9] Micrographs taken at the lower magnifications thus obtained have shown resolutions between 50 and 100 A.

It has been found important also in electron microscopy of cells to use an objective aperture. The thickness of many specimens as well as that of the membrane required to support them leads to considerable electron scattering and consequent lack of contrast in the image. This is very largely corrected with small apertures of 20 to 30 μ diameter. Smaller openings than these are not feasible because the margins will cut into the field at low magnifications and also quickly contaminate, with consequent loss of resolution.

With the RCA instrument and a self-biasing electron gun, it has also been found important for the study of this material to use a condenser aperture about ½ the standard size (0.5 instead of 0.8 mm.). This reduces the beam intensity, which otherwise destroys too many specimens, and in the opinion of Hillier (24) improves the resolution by reducing space charge effects on the image.

OBSERVATIONS

The Submicroscopic Basophilic Component Defined.—As indicated above, the observations to be reported will be concerned very largely with "submicroscopic" entities of the cytoplasm; i.e., with components which in size lie at or beyond the limits of resolution of the light microscope. This obviously excludes such relatively macroscopic bodies as mitochondria and lipid granules, but includes structures of the cytoplasmic ground substance or hyaloplasm. The distinction is readily made by reference to Fig. 1, which shows a photomicrograph of three thin cells grown from an explant of rat heart. They are mesothelial units from the pericardium and are typical of the kind of thinly spread cultured cell that is particularly good for electron microscopy. For this preparation they were fixed in vapors of osmium tetroxide and stained with iron-hematoxylin.

The "microscopic" components are easily identified. Apart from these, however (and a number of well known special inclusions which characterize differentiated cells) such structural components of the cytoplasm as exist in the rest

[9] Later models of RCA electron microscopes are supplied with an intermediate lens which provides for continuous variation of magnification over a wide range without changing spacers in lens pole pieces. The lower part of the range includes the magnification mentioned above, so that shortening of specimen holder would not be necessary. The Philips EM-100 Model provides a continuous range of magnifications from 500 to 15,000 on the photographic film without lens alterations.

of the cytoplasm are at best poorly resolved and may be classed as "submicroscopic." In this category might be placed then such ill defined components as microsomes and chromidia (see Wilson (65) p. 32) and other basophilic material (ergastoplasm) as well as all the smaller, entirely invisible granules, fibrils, and macromolecules.

In the ground substance of these particular fixed and stained cells some sort of reticular structure can be made out (*er*, Fig. 1) but the dimensions of its divisions are so close to the limits of light resolution that little can be learned about its actual form. Indeed, without better evidence of its nature than that provided by this photomicrograph it would probably be considered an artefact of fixation or staining.

Fig. 2 is electron microscope image (low power) of a cell of the same type as the cells shown in Fig. 1. This cell was fixed for 10 minutes in vapors of OsO_4 and prepared for microscopy by the procedures described above. The "microscopic" components, nucleus, lipid granules, mitochondria, etc., differ from the light micrograph equivalent only in being more sharply defined. Similarly, the ground substance in the electron micrograph shows a pattern of densities in places constituting a reticulum (*er*) which apparently corresponds to the hematoxylin-staining reticulum faintly depicted in the photomicrograph. This component is absent from the thinner (ectoplasmic) margins of the cell and appears instead to occupy the central or endoplasmic portions of the cytoplasm. From its location and form it has come to be referred to as the endoplasmic reticulum and by this name it has been noted in previous reports on other elements of tissue cell fine structure (48, 50, 54). From its affinity for hematoxylin and from evidence of its stainability with toluidine blue (presented below) this component appears to represent a basophilic component of the cytoplasm.

The Electron Microscope Image

It has been found that the image of this reticular system of the cytoplasm may be clarified in the case of most cells by prolonging the period of fixation in vapors of OsO_4 (51). This treatment (which combines the oxidative effects of OsO_4 and the extractive properties of Tyrode's solution) removes differentially the continuous and more or less homogeneous matrix in which the formed elements of the cytoplasm are normally suspended and leaves what may be thought of as a membrane skeleton.

The electron microscope image typical of a cell thus prepared for microscopy is shown in Fig. 3. The central or perinuclear part of this endothelial cell is too thick and dense to provide much information, but the thinner margins show great numbers of mitochondria, and, in the background, a finely divided network or lace-work of strands. These strands are obviously irregular in outline and varied in diameter. In some places they are not more than 25 mμ wide where-

as in others they are 250 mμ or more. A reticular system such as this or some reasonable variant or derivative of it has been found in all cultured cells examined with the possible exception of chicken lymphocytes. It appears therefore to be a constant element of the fine structure of animal cell cytoplasm.

In any micrograph of a whole or part of a cell the distribution of this reticular component is seldom uniform, but varies with the over-all quantity of cytoplasm. Thus in the thinner margins there is so little as to give in the picture the impression of a two dimensional dispersal and a clear image of the reticular character of the system. Around the cytocentrum, on the other hand, where the cell is considerably thicker, the three dimensional distribution confuses the image. In some instances it appears to be absent entirely from the outermost margin of the cell (Fig. 5). The reason for this is not known but conceivably it results from the fusion in this region of the upper and lower ectoplasmic layers (plasma gel layers) which, because of their gelatinous nature, might exclude the particulate elements of the endoplasm. It should be noted that the strands of the reticulum are often oriented along directions taken by the mitochondria, as though some organization of the imbedding matrix of the ground substance obliged them to adopt this orientation (see also lower left of Fig. 3). In epithelioid macrophages this orientation is frequently along radii from the cytocentrum and is occasionally quite striking (Fig. 4).

The irregularities in size and form that the component strands of the system may show in a single cell are depicted in Figs. 3 and 4 and even greater variations than these are evident in a comparison of very different cells. For instance, in the cells of a certain rat sarcoma the elements of the system appeared as strings of vesicles varying considerably in size (Fig. 5). Contrasting with this, the picture in cells from a mammary carcinoma of the mouse was that of a complex meshwork of relatively uniform, smoothly contoured strands or canaliculi (Fig. 6). In yet other cells that have come under observation the reticular character of the system was entirely lacking and the corresponding material was present in the form of unconnected vesicles. Variation seems then to be the rule and while a certain form or configuration may be fairly common for a certain cell type, these studies of cultured cells have not established any such relationships. It has been noted rather that cells of widely different types may show similar reticular systems and that conversely, cells of identical types may differ strikingly in this regard. This suggests that variations in form accompanying changes in physiological activity or state of cells *in vitro* are more profound than variations associated with different cell types.

This is illustrated by observations made on a small group of endothelial cells all contiguous in a single sheet. Because of their close association they received identical preparation treatments and such variations as they show are not, therefore, referable to differences in fixation procedures. It seems rather, as just noted, that they must reflect differences in the physiological state of the

cell at the time of fixation. The various forms found in different cells of this single sheet are shown in Figs. 7 to 11.

In the first micrograph of the series (Fig. 7), which shows only a small portion of one cell, the system is finely divided into strands which are about 100 mμ in diameter and fairly uniform. As a general rule this form of a more finely divided reticulum appears to be characteristic of cells from young, actively growing cultures. It is to be noted that the total area of the interphaseal surface between the system and the surrounding matrix of the cytoplasm would be large considered in relation to the size of the cell and would, of course, increase or decrease as the system became more or less finely divided.

A variant of this in which the strands are vesiculated was the state of the system in an adjacent endothelial cell (Fig. 8). A few of the vesicles are only about 100 mμ in diameter, but the majority measure between 200 and 300 mμ. If such a pattern of structure is evident in one part of the cell, it is the same all over. This provides an additional reason for considering the form a reflection of physical and chemical conditions in the cell previous to fixation.

The form the system has adopted in a third cell of this endothelial sheet is shown in Fig. 9. Here the vesicular appearing components are much larger, ranging up to 600 or 700 mμ. They are, however, still associated to form a coarse reticulum. In terms of size they are obviously sufficiently large to be visible in ordinary transmitted light microscopy. That they are not generally apparent in the unstained cell implies that their refractive index is so close to that of the surrounding matrix that they fail to produce a visible shadow.

A fourth cell in this group showed the structure pictured in Fig. 10. Here the vesicles of the reticulum are widely separated but still attached to each other by slender strands. These are about 40 mμ in diameter and the vesicles range between 150 and 300 mμ. The mitochondria, it can be noted, are also partly vesiculated and this is recognized as a form they assume in cells subjected to unfavorable environmental conditions. From their appearance it would seem that the strands of the endoplasmic reticulum are likewise in the process of fragmenting as though responding in a fashion similar to that of the mitochondria.

A later stage in this progression to cytolysis, as defined by the form of the mitochondria, is depicted in Fig. 11, which is a micrograph of a portion of yet a fifth cell in this endothelial sheet. Here the mitochondria are almost completely vesiculated. The smaller discoidal bodies between them are recognized as remnants of the reticulum now completely detached from one another, and the vesicular character of these is evident from the presence of folds which develop in their surface as they collapse in drying. The shadow length determined by the thickness of the flattened vesicles is not as great as that from a mitochondrion, but is about the same as that cast by a fold in the cell membrane. This indicates that the membrane constituting the wall of these vesicles

has a thickness similar to that of the plasma membrane surrounding the cell. Calculations of the thickness of the fixed and dried membrane which may be made from measurements of the shadow length and a knowledge of the shadowing angle (in this case 12°) give values of the order of 8 mμ or 80 A.

The Light Microscope Image

A. In Living Cells.—The electron micrographs show that in many cells, parts of the reticulum are large enough to be resolved by the light microscope. That they are not very evident must mean that they have refractile properties similar to those of the cytoplasmic matrix in which they are suspended. This obstacle notwithstanding, an attempt has been made to obtain a light microscope image of it in unfixed cells, since it is important to establish the reticulum as a structural entity of living protoplasm as opposed to an artefact of fixation.

A careful *bright field* microscopic examination of cultured cells of several types which are favorably spread on a coverglass surface reveals, as a rule, the existence of tiny particulates just at the limits of light resolution. These are extraordinarily difficult to see with this type of illumination and their visualization does not seriously conflict with the observation that the ground substance of the cytoplasm is optically empty. If, however, *phase contrast* illumination is used, a somewhat better picture of them may be obtained, presumably because this device is particularly useful in detecting slight refractile differences. Such a phase contrast image of an epithelial cell cultured from an explant of rat kidney is shown in Fig. 12. Besides the mitochondria there are evident in the ground substance numerous small particulates and occasional strands (arrows). It can be noted that they reside in the same focal plane as the mitochondria and so represent intracellular structures. Their size and general distribution lead one to conclude that they are parts of the endoplasmic reticulum, which in electron micrographs of these kidney epithelial cells shows essentially the same form observed in other cell types (Fig. 13). If under these conditions of microscopy this image is watched carefully, it is possible to detect a motion which might be interpreted as a migration of nodosities along the strands.

Refractile particulates and strands of similar size are likewise apparent in the dark field picture of the same type of epithelial cells (Fig. 14). They are especially apparent out in the thinner margins where the more refractile mitochondria are not present to interfere with their visualization. The same methods of observation have been applied to a few additional cell types and in each case "submicroscopic" elements of a similar nature could be seen. That these are the light image of parts of the endoplasmic reticulum seems beyond question, because in these thin cell margins the reticulum is the only structure revealed by electron microscopy that could produce such tiny shadows and refractilities.

B. In Fixed and Stained Cells.—Considerable interest obviously centers around the identification of this cytoplasmic component with structures of the cytoplasm described by light microscopy of fixed and stained cells. It was noted in reference to Fig. 1 that the hematoxylin-staining material of the cytoplasm is finely divided and has the general form of the reticulum of the electron microscope image. This indicates that the reticular system corresponds to the basophilic substance of the cytoplasm. To provide further evidence for this, thinly spread mesothelial cells from explants of day old rat heart have been fixed with formalin (neutral 8 per cent) or with vapors of OsO_4 and thereafter stained with toluidine blue at pH 6.0 and pH 8.0. After all combinations of fixation and staining used, an intracellular basophilic component of reticular character was apparent (Fig. 15) and after fixation with OsO_4 its form and character were especially reminiscent of the electron microscope image. Though more study of this is clearly required, it is reasonable on the basis of present evidence to conclude that the reticular system of the electron micrographs is represented by the basophilic or chromophilic component of the light microscopic image.

This conclusion finds further support in an earlier and preliminary study made in collaboration with G. H. Hogeboom. In this, the microsome fraction prepared in sucrose from liver cells was fixed with OsO_4 while still in sucrose and thereafter washed and examined with the electron microscope (26). The image (Fig. 16) shows a highly variable, partly amorphous component which here and there, however (arrows), displays enough similarity to vesiculated strands to convince one that it is made up, in part at least, of elements of the reticulum. More recently, Slautterback (62) has described micrographs of "microsome" fractions showing similar structures. Since Claude (12), Brenner (7), and others have identified the microsomal fraction with the basophilic component, this morphological similarity between the fraction and the reticulum again relates the latter to the basophilic component.

Relation to Rest of Ground Substance

A. The Matrix.—When cells are fixed for only a few minutes in vapors of OsO_4, the endoplasmic reticulum is apparent but not as sharply defined as in cells exposed to the fixative for several hours (compare Figs. 2 and 17 with Figs. 8 to 11). This appearance describes the presence of a matrix or continuum which remains after a short exposure of OsO_4 and serves to scatter electrons, hide embedded components, and limit contrast in the thinner parts of the cell. It also smooths over the pronounced shadow-casting edges and elevations apparent around mitochondria and reticular elements after prolonged fixation (compare shadows in Fig. 17 with those in Figs. 9 and 10). It may be divided into ectoplasmic and endoplasmic portions on the basis of degree of gelation (17). As indicated in Fig. 18 and noted earlier (2, 48) the material of this matrix

may be condensed into fibers of greatly variable dimensions. Certain of these are variable in phase contrast images of living fibrocytes and other cell types and under such observation can be seen to form and disappear as the cell changes shape in its slow motions over the substrate (47). They may also be identified with the birefringent elements noted by Hughes and Swann (27) in osteoblasts. Some evidence of a periodicity of structure similar to that in collagen and fibrin has been noted in other micrographs of these fibrils but it is not considered conclusive. Though partially hidden by this matrix, the reticulum is shown where the fiber arrays are not too dense (*er*, Fig. 18).

The matrix is left apparently intact by other methods of fixation besides brief fixation with OsO_4 and, as a result, the reticulum is largely hidden from view after such procedures. Nonetheless, in especially thin and otherwise favorable areas, portions of the reticulum can be distinguished in cells fixed with neutral formalin (8 per cent), though its density is not so great as after osmium. This is the only other fixing reagent to give a near-to-faithful preservation of the native morphology. However, in cells fixed merely by drying from balanced salt solution there are small densities which in size and distribution resemble parts of the system.

It might be expected that some functional relationships would call for a close association between the reticulum and the mitochondria, but no direct continuity or other obvious association has thus far been noted.

B. Certain Dense Particulates and Filaments.—It is important by way of segregating the endoplasmic reticulum from other submicroscopic components of the cytoplasm to refer briefly to the structural units comprising the third category of components. These are the dense particulates and strands of varying sizes (diameters range from 25 mμ to 250 mμ) which are especially abundant in growing cells, both normal and malignant (Figs. 5 and 19). They have been described and referred to as growth granules in previous reports from this laboratory (51, 53) and noted by others (40, 60). The distinction to be made between these and the elements of the endoplasmic reticulum is chiefly one of density since in some other respects they are similar. This density to the electron beam is not dependent solely on osmiophilia but is inherent, for it is evident after formalin fixation. Furthermore, in the dried and shadowed preparation these dense particulates retain an elevation considerably in excess of that shown by the reticulum. It follows then that in the living cell they are less hydrated than the elements of the reticulum.

The characteristics in which these inclusions of the ground substance are similar to elements of the endoplasmic reticulum provide some reasons to relate the two. For example, the dense strands, such as those present in Fig. 19, sometimes show vesiculation at scattered points along their lengths and in this regard they resemble a common form of the reticulum strands. Then too it is not unusual to find the particulates of this class of inclusion occurring in large

numbers at the ends of pseudopodia of tumor cells. These are regions of intense basophilia in stained cells so that in staining properties also these dense granules (growth granules) appear to be similar to the endoplasmic reticulum (51, 53).

DISCUSSION

The possibilities for artefact production in electron microscopy are unfortunately very real and deserve first consideration in any discussion of the above observations. The question is whether the electron microscope image of the OsO_4-fixed cell closely describes the morphology of the living unit or whether the structures defined are largely the product of preparation procedures.

Of the various treatments given the cells after they are taken from the culture medium, the initial rinsing in balanced salt solution, the fixation with OsO_4 vapors, and the subsequent washing and drying might be implicated in artefact production. The first of these is of no consequence in this regard, since essentially the same image of the endoplasmic reticulum, for example, is obtained whether it is included in the preparation procedure or not. Furthermore, cells cultured in balanced salt solution for 72 hours are morphologically similar to those grown in media considered more natural.

Likewise, it is improbable that the postfixation washing (which is brief) and drying could operate in producing a system of this nature. When, for example, the drying procedure is varied by replacing the H_2O with alcohol and this latter with liquid CO_2, as in Anderson's (1) procedure designed to avoid the surface tension effects of phase boundaries, elements of the reticulum are apparent in the ultimately dry cell. It is reasonable also, in this connection, to point to the light microscope evidence (Fig. 1) of the reticulum in stained cells that at no time were dried in air.

The OsO_4 fixation then is left as the procedure most to be suspected of artefact production, but even in the case of this the observations support the opposite view. Although several arguments could be presented to describe the faithfulness of such fixation in its preservation of cell form, none would be as convincing as a demonstration of it by light microscopy; *e.g.*, the endoplasmic reticulum in living cells. Since evidence of this latter type is included above, it seems sufficient to mention only a few of the less conclusive arguments in favor of the faithfulness achieved.

1. It can be readily observed (as was done some years ago by Strangeways and Canti (63)) that the structure of living cells, even under darkfield observation, is not greatly altered upon contact with OsO_4. The nucleus gains in refractility but otherwise there is little to note at the time of contact with the reagent except a cessation of Brownian motion.

2. If it is assumed that OsO_4 artifically brings about the development of the endoplasmic reticulum, its effect in this regard might be expected to become more pronounced with time. Actually, however, the reticulum in its outline and structure appears to be the same after short as after long fixation.

3. The endoplasmic reticulum or a structure of similar form may be discerned in cells after fixation with formaldehyde or after simple drying. If then the system represents an artefact it must be one that results from these very dissimilar agents of fixation, which seems very unlikely.

Other arguments supporting the faithfulness of the fixation could be presented but none would diminish the inconclusiveness of this indirect type of evidence. More significant and convincing evidence comes from light microscopy of living cells. As pointed out above, the system as defined by the electron microscope frequently shows component parts that are large enough to bring them within range of light resolution, and when phase contrast illumination is used, there appears, in the ground substance of the living cell, a component having the size, distribution, and form of the system as pictured by electron microscopy. Its three dimensional distribution in the living cell and the shallow depth of focus of phase contrast, plus the weak refractility of the system, would account for the discontinuity and feebleness of the image. The worth of these observations is enhanced by the presence of similar, weakly refractile structures in the darkfield picture of these cells. With little doubt these correspond to the "very small particulates" of Strangeways and Canti (63) and it is very probable that the same component of the ground substance accounts for the "shimmering bodies" and particulates observed in quite different materials with darkfield illumination (3, 7).

Evidence, such as this, and the above considerations taken together justify the conclusion that OsO_4 has provided a near to faithful fixation of a submicroscopic component of the cytoplasm. On the strength of this conclusion, it becomes reasonable to consider several of its aspects.

The electron micrographs describe the system as extremely complex and labile. Considered as a unit system, whether continuous or discontinuous, it becomes a form or kind of cytoplasm (subsequently referred to as basophilic or chromophilic component or system, or as ergastoplasm) set off from the matrix of the ground substance by a membrane. This membrane makes vesicles of the rounded elements and canaliculi of the strands, but nothing is indicated concerning the nature or state of the content before fixation. After prolonged action of OsO_4 and simultaneous extraction with Tyrode's solution, the content must be very hydrated, as evidenced by the extreme flattening upon drying.

When the system is a continuum it may be viewed as a finely reticulated ameboid body which in these thinly spread cultured units sends out slender filapodia into the thinner margins of the cell. It is assumed that with changing physiological conditions the finer degree of reticulation gives way to a coarser one or *vice versa* by a blending or division of strands. By such changes the surface area of the system in contact with the matrix is, of course, greatly altered. If, as may be supposed, large surfaces or phase boundaries are important to the functioning of intracellular enzymes and enzyme systems, the endoplasmic reticulum with its large surface may be regarded as important and highly adaptive to varying functional states.

The extreme variation in form of the system, *i.e.* from a reticulum of slender strands to an emulsoidal dispersal of vesicles, seems to reflect among other conditions a concomitant variation in the physical character (viscosity) of the surrounding matrix. The observations thus far made show that in general the

system is finely reticulated in cells from young cultures; *i.e.*, in cells which under light microscope observation are normal and in which there are a minimum of Brownian motion and a greater degree of gelation. It is equally clear (supported by phase contrast observations and parallel changes in the form of the mitochondria) that the vacuolated and dispersed form of the system accompanies cytolysis, under which conditions the matrix is highly solated. Presumably a gelled or possibly paracrystallin state in the matrix may, through its own organization, influence the form of the reticulum and its distribution. Perhaps only when the fibrous elements of the reticulum are randomly oriented, as in solation, would the membrane tensions of the system be equivalent in all directions and result in the production of separate vesicles.

The electron micrographs by themselves provide little information on the composition of the system. The presence of a membrane similar in thickness and density (after fixation with OsO_4) to that of the plasma membrane might lead one to expect a relatively large lipid component. But, regarding the content of the strands or vesicles, no real information is provided by the micrographs. Such information must be drawn instead from the correlation which has been made between the reticulum and the basophilic or chromophilic component of the cytoplasm.

This identification of the reticulum with the basophilic material is based on the similarity of form noted above between the hematoxylin- and toluidine blue-staining component just visible optically, and the electron microscope image of the reticulum. Added reason for relating the two can be found in the fact that there is no other element of fine structure that could reasonably provide a basis for the stained image and so be confused with the reticulum. Even the older literature on the basophilic material, known variously as "cytomitoplasm" in the exocrine pancreas cells (34) and the ergastoplasm of salivary gland cells (21) described the basophilic material as having a fibrillar or reticular structure easily related to the form of the endoplasmic reticulum. There appears then to be adequate reason for the correlation and it seems important to take brief notice of the information on composition and function which may thus be applied to the reticulum.

As is now well known from studies with the ultraviolet absorption method (9, 10, 22) and with the ribonuclease test (5, 22) ribonucleic acid is localized in the basophilic parts of the cytoplasm. Since their original description, these methods have been fairly widely employed, with generally confirmatory results. At about the same time it was shown by Claude (12) that this chromophilic or basophilic material of the cytoplasm of liver cells is sedimentable within the cell by centrifugation. This component has since been identified with the small particle or microsomal fraction which may be segregated centrifugally from other constituents of ground-up cells (7, 12, 14) and which is now known to be rich in phospholipids and ribonucleic acid (11, 13, 14, 26). The small particles of this fraction, it was noted above, are morphologically similar

to fragments of the endoplasmic reticulum. Even without this latter evidence of identicalness, however, the fact that both the reticulum and the small particle fraction represent the basophilic material of the cytoplasm makes it reasonable to transfer the properties of the basophilic component or the "microsomal" fraction to the endoplasmic reticulum of the electron microscope image. Thus the reticulum would appear to contain approximately 50 per cent of the ribonucleic acid of the cell and an abundance of phospholipids possibly derived from the large proportion of it that is membrane. Its content of enzymes thus far discovered is neither as impressive as that of the mitochondria nor as easily correlated with a single metabolic system. The list to date includes DPN cytochrome C reductase (25), certain esterases (41), a glucose-6-phosphatase (23), and a lactonase (36) (see also Schneider and Hogeboom (59)). Of more than usual interest in this regard, however, is a recent report showing that small particles and mitochondria react synergistically to incorporate alanine into tissue protein (61). This apparent involvement of the basophilic component in protein synthesis was previously suggested by the relatively intense basophilia and high RNA content of cells in stages of rapid growth or repair or active in the production of protein secretions (58). It appears then that the endoplasmic reticulum is in some manner involved in or represents a morphologic stage in the synthesis of new protoplasm or cell secretions. The sequences in this process, or the physical means by which the reticulum makes this contribution, remain to be discovered.

It is to be noted that the information presently available on this basophilic system comes largely from a convergence on the problem of the histochemical techniques of Caspersson and Brachet, the fractionation procedures of Claude, and now electron microscopy. Since the possibilities of these are not nearly exhausted there is reason to think that their further application will soon provide a better understanding of this fundamental component of the cell.

While the present report provides the first detailed description of this cytoplasmic component based on electron microscopic evidence, it is not the first time that attention has been directed to the system nor does all the evidence for its presence come from observations on cultured cells.

In the earliest electron micrographs of cultured units there was noted a "lace-like reticulum" (50) and subsequent reports on similar material provided improved images of the same system and made incidental reference to it as the endoplasmic reticulum (51, 53, 54). These observations were soon confirmed in studies from other laboratories (33, 40, 60).

Some indication of the widespread occurrence of submicroscopic strands in the ground substance of tissue cell cytoplasm was provided by Dalton *et al.* (15, 16) in electron microscope studies of thin sections of cells of the mouse liver, exocrine pancreas, and zymogenic cells of the stomach. These observers interpreted the structures as representing sectioned lamellae and as possible artefacts of fixation but felt that they were "in some way related" to the basophilic material of the cytoplasm. Very similar images of fine structure in liver, pancreas, and salivary gland cells have been published more recently by

Bernhard *et al.* (4). In this latter report, the observed structures were interpreted as filaments and more reason was provided to correlate them with the basophilic material of these cells; *i.e.*, to the ergastoplasm of Garnier.

It was our thought, on the basis of structure, size, and apparent staining properties, that these formations observed in thin sections might be the equivalent of the endoplasmic reticulum, or the basophilic system of the cultured cells, and that the interpretation of the structures as parts of lamellae or as solid filaments was in part a result of poor fixation. This proved to be correct, for after the improved fixation attained with buffered OsO_4 (43) the filaments (of Dalton and Bernhard) appeared as canaliculi and vesiculated strands, and were easily identified with the endoplasmic reticulum of cultured cells (44). It was furthermore evident from thin sections that the strands or canaliculi are part of a complex reticular system and not largely separate entitites as supposed by Bernhard *et al.*, and implied in their adoption of the name chromidia for the filaments.

For this and reasons which follow, the adoption of this terminology seems ill advised, especially at a stage when so much uncertainty exists on every hand. For instance, as pointed out by Wilson (65), the term chromidia was originally used by Hertwig for intensely basophilic bodies, apparently of nuclear origin, in certain protozoa. Similar structures in vertebrate cells classed by some as chromidia were subsequently shown to be mitochondria and their origin in the nucleus was repeatedly questioned. Since, therefore, there is no reason from observations thus far made, to believe the endoplasmic reticulum is derived from the nucleus, and since the term chromidia implies particulate entities, whereas the component under discussion is usually organized in a system, it seems best to refer to it as the basophilic component or material or to call it ergastoplasm as originally suggested by Garnier.

A further indication of the fairly universal occurrence of the endoplasmic reticulum as a component of cytoplasm is supplied by some findings of McCulloch (35) in *Arbacia* eggs. With polarized light he was able to observe in centrifuged cells some birefringent fibrils which he calls the "coarse fibrous component." They are about 100 mμ in diameter, show a sign of birefringence negative with respect to the long axis of the fiber, and in thin sections of fixed material they appear similar to the elements described by Dalton and others in vertebrate tissue cells. McCulloch relates them to certain similar basophilic fibers described by Monné in *Psammechinus* eggs. It may be further noted that the birefringence could result from the presence of a membrane around the "fibers" and that such a membrane is readily demonstrable as a structural feature of the strands of the endoplasmic reticulum. There seems, therefore, sufficient reason to consider McCulloch's "coarse fibrous component" as the *Arbacia* egg equivalent of the endoplasmic reticulum.

It is appropriate while pointing out the similarities which can be noted between this basophilic component and certain filamentous structures of various tissue cells, to direct attention again to the not dissimilar morphology of the dense strands characteristic of growing cells (Figs. 5 and 19). This similarity and the abundance of them in cells producing new cytoplasm support the proposal that some of these dense strands are the precursors of the less dense elements of the endoplasmic reticulum. It is conceivable also that structures such as those described by McCulloch and observed in sections are more properly related to these growth granules and filaments than to elements of the reticulum. Many of the dense submicroscopic granules of young cells are not, however, appropriately sized to be related to the reticulum but more nearly resemble small mitochondria. Possibly, as has been suggested elsewhere (51) there are various species of particulates in this category of submicroscopic components and each may have a distinct and separate destiny—some to form mitochondria; others, elements of the reticulum; and still others, additional formed structures of the cytoplasm.

SUMMARY

The cytoplasmic ground substance of animal tissue cells grown *in vitro* has been found by electron microscopy to contain, as a part of its submicroscopic structure, a complex reticulum of strands, to be referred to as the endoplasmic reticulum. It has been found in all types of cells extensively studied.

The components of this reticular system vary considerably in size and form, apparently in some relation to physiological changes in the cell. Thus in one cell of a culture colony it may be finely divided into strands or canaliculi, 50 to 100 mμ in diameter, whereas in an adjacent cell of the same type the components of the reticulum may be relatively coarse, 600 mμ in diameter, and vesiculated. The membrane, which can be shown to limit the system and separate it from the rest of the ground substance, is similar in thickness to the plasma membrane surrounding the cell.

Photomicrographs of living cells taken by phase contrast and dark field microscopy define a structure of similar form and indicate that the reticulum of the electron microscope image has its equivalent in the living unit. Where its component units are sufficiently large, a structure of identical form can be resolved by light microscopy in cells stained with hematoxylin or with toluidine blue. This indicated that the endoplasmic reticulum is to be identified with the basophilic or chromophilic component (the ergastoplasm) of the cytoplasm and that such properties of this component as have been determined by cytochemical methods, such as a high RNA content, may be assigned to this "submicroscopic" system.

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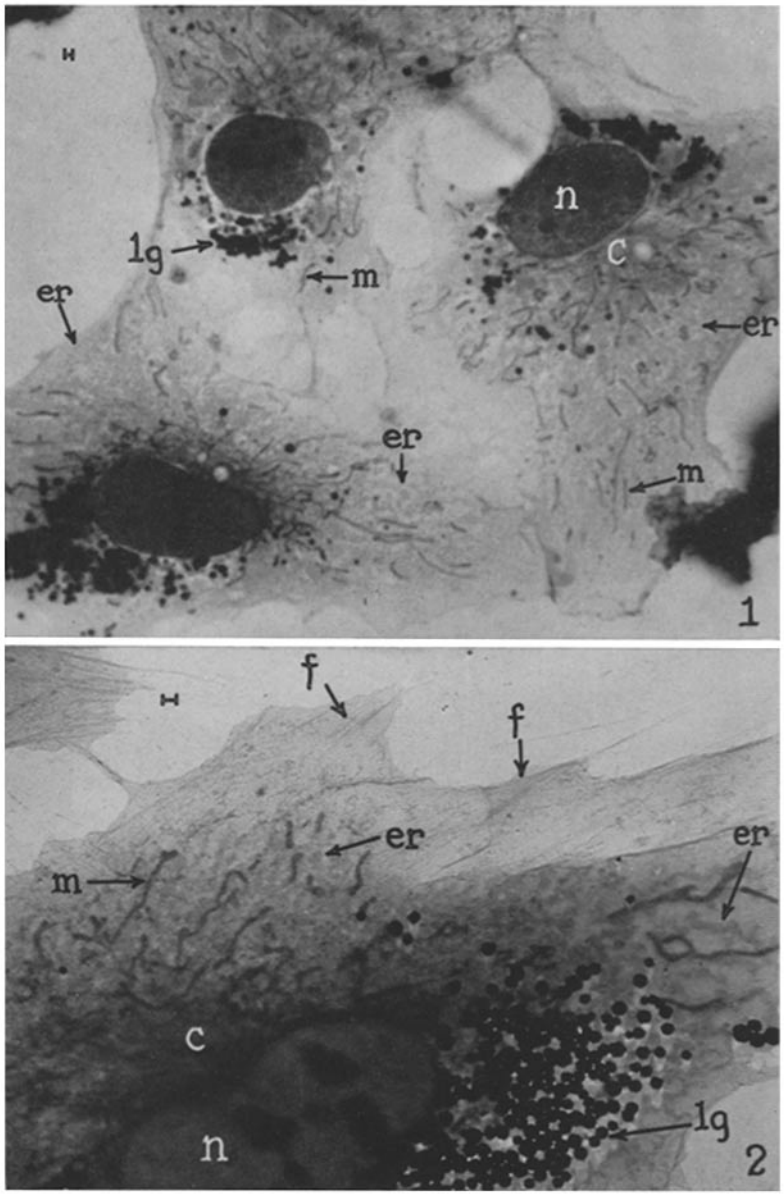
EXPLANATION OF PLATES

PLATE 44

Reference lines, |——|, represent 1 micron.

FIG. 1. Photomicrograph of mesothelial cells from explant of 2 day old rat heart. The nuclei (*n*) and filamentous mitochondria (*m*) are readily identified. The dense particles, collected in clumps, are lipid granules (*lg*) or fat bodies. The centrosome (*c*) displaces the nucleus from the center in each cell. Apart from these inclusions, the cytoplasm shows as a component of its ground substance or hyaloplasm a faintly stained, reticular pattern to be designated the endoplasmic reticulum (*er*). Cells were fixed briefly in vapors of OsO_4 and stained with iron-hematoxylin. × 1100.

FIG. 2. Electron micrograph of part of a cell from the same tissue and apparently of the same type as the cells shown in Fig. 1. The "microscopic" components are similarly designated (*n*) nucleus, (*m*) mitochondria, (*lg*) lipid granules, and (*c*) centrosome. The ground substance shows a pattern of densities which roughly constitute a reticulum corresponding to the endoplasmic reticulum (*er*) of the photomicrographs. There is also in the ground substance evidence of fine fibrillar elements (*f*). As mentioned in the text, these are condensations of the ground substance matrix which along with the rest of the matrix are removed by prolonged fixation with OsO_4. This cell was fixed for 5 minutes over vapors of OsO_4 (2 per cent). × 2100.



(Porter: Submicroscopic basophilic component of cytoplasm)

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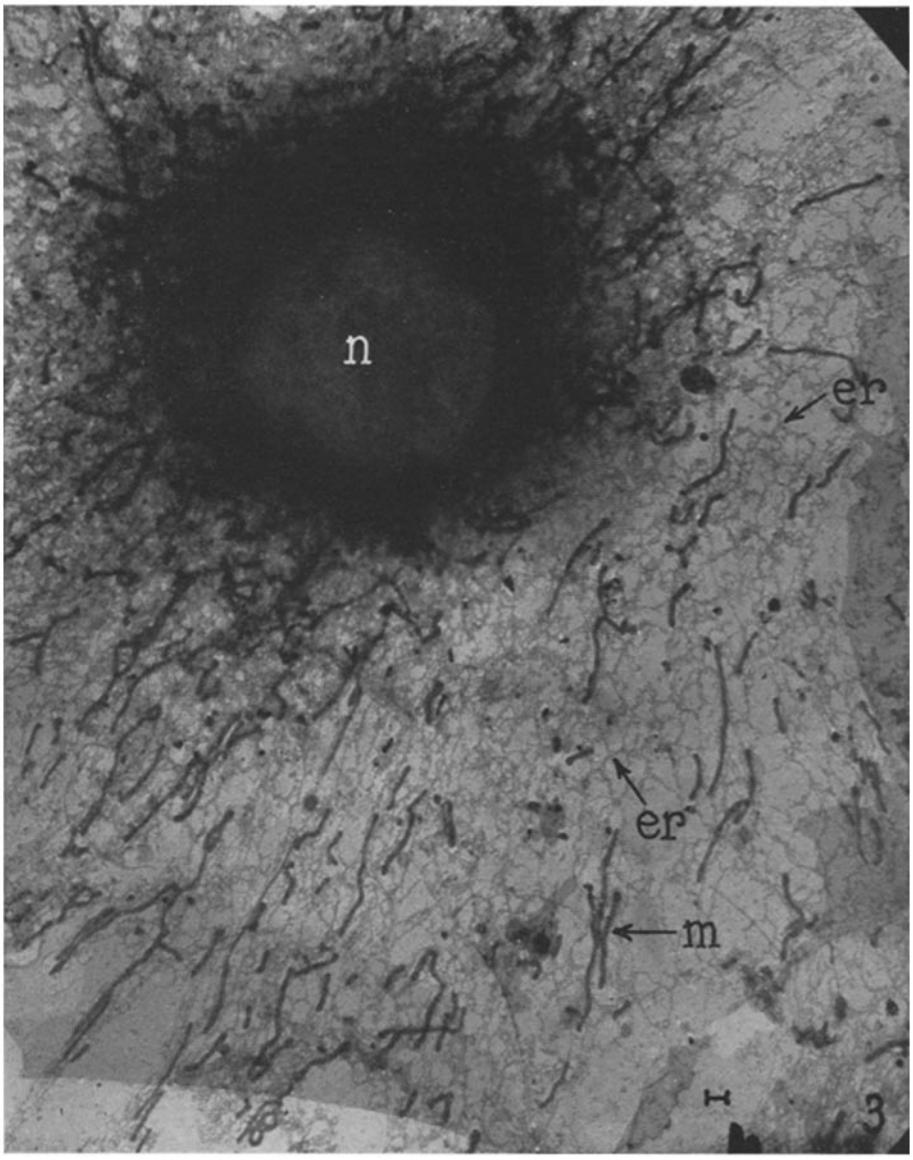
FIG. 3. Electron micrograph of an endothelial cell cultured from umbilical cord of the rabbit. Nearly the whole cell is shown. One margin, in contact with an adjacent cell, lies along the right-hand edge of the picture. Another margin passes out of the micrograph at the bottom but the upper membrane of the cell is folded back at this point. The center of the cell with nucleus (*n*) is too dense for much electron penetration. Thinner parts of cytoplasm show numerous mitochondria (*m*) and a lace-like pattern of fine strands, the endoplasmic reticulum (*er*). This system is present in all parts of the cell but appears more abundant in the center where distribution is three dimensional. It may be noted that mitochondria and strands of the reticulum are oriented parallel to each other in the lower half of the cell. The mitochondria are remarkably uniform in diameter (300 mμ), whereas strands of the reticulum vary enormously, from about 25 mμ up to 250 mμ in occasional vesiculated strands. This cell was one of a small group fixed for 16 hours over vapors of OsO_4. $\times$ 2800.



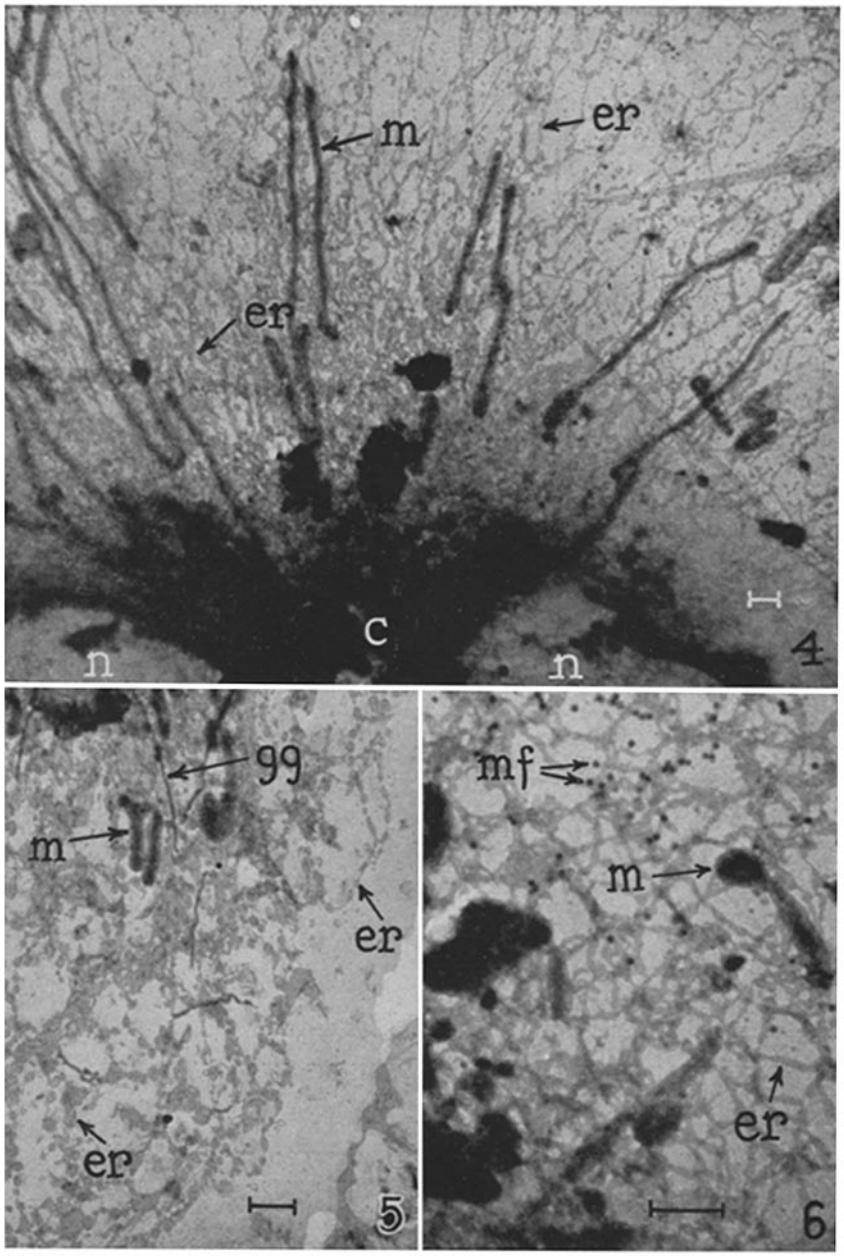
(Porter: Submicroscopic basophilic component of cytoplasm)

PLATE 46

FIG. 4. Electron micrograph of a central portion of a chick macrophage. The cell margins are not included. The cytocentrum (*c*) is in the lower center of the micrograph with parts of two nuclei (*n*) on either side. Long mitochondria (*m*) radiate from the cell center paralleled by strands of the endoplasmic reticulum (*er*). It appears that some organization external to both of these elements acts to orient them in this manner. Cell cultured 96 hours from buffy coat of adult chicken blood. Fixed over vapors of OsO_4 for 24 hours. × 4000.

FIG. 5. Electron micrograph of a small marginal portion of a rat sarcoma cell. Strands of the endoplasmic reticulum (*er*) or chromophil substance are here largely vesiculated and the vesicles show considerable size variation. The margin of the cell passes across the lower right, and along part of its visible length is in contact with the edge of an adjacent cell. There is a zone of this margin from which elements of the reticulum are excluded. This is presumed to mark the extent of the gelled ectoplasm of this thinly spread cell. A few mitochondria (*m*) are shown at the upper left of the micrograph and the slender, dense filaments (*gg*) are submicroscopic elements common in tumor cells and rapidly growing normal cells. They are shown again in Fig. 16. Cell fixed with vapors of OsO_4 (2 per cent) for 16 hours. × 6300.

FIG. 6. Electron micrograph of a small part of a mouse epithelial cell cultured from a mammary carcinoma. The strands of the reticulum (*er*) are possibly more abundant per unit volume of cytoplasm and show somewhat less variation in diameter than in adjacent figures. Otherwise, however, they are similar. Mitochondria are indicated at *m*. The small dense particles (*mf*) were described in an earlier report as possibly the milk factor involved in the genesis of this tumor (54). Cell one of epithelial sheet. Fixed over vapors of OsO_4 (2 per cent) 20 hours. × 9500.



(Porter: Submicroscopic basophilic component of cytoplasm)

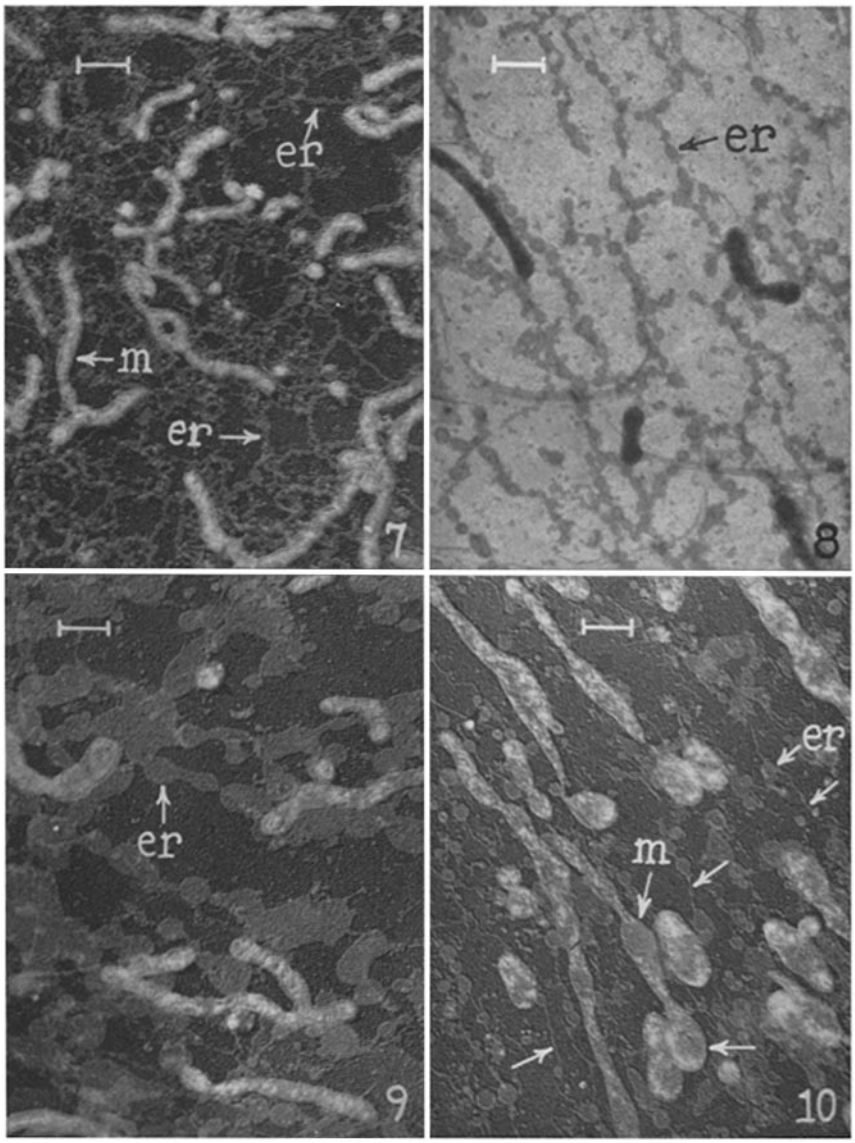
PLATE 47

FIG. 7. Electron micrograph showing a small part of an endothelial cell of the rabbit. This was one from a sheet of several cells others of which are shown in Figs. 3, 8 to 11. The micrograph in Fig. 8 was taken before the preparation was shadowed; the others, Figs. 7, 9 to 11, were taken after coating with chromium. Strands of the endoplasmic reticulum (*er*) here are relatively uniform in size and generally slender, for the most part less than 100 mμ in diameter. This represents the more finely divided form of the system and obviously provides a large interphaseal area between the content of the reticulum and the surrounding matrix. × 6800.

FIG. 8. Portion of adjacent cell of endothelial sheet. Strands of the reticular system (*er*) are less numerous per unit area and are uniformly vesiculated. The majority of vesicles are between 200 and 300 mμ in diameter. If this is the form of the reticulum in one part of the cell, it is the form all over. × 6800.

FIG. 9. Portion of another cell of the group. Here the elements of the reticulum (*er*) are much larger. The majority of the vesicles shown have diameters which in this dry, collapsed state exceed those of the mitochondria and bring them well within the range of the light microscope. × 6800.

FIG. 10. Portion of yet another cell of this group, in which the strands of the reticulum appear to be breaking up. They are at any rate composed of widely separated vesicles (*er*) connected only by long tenuous strands (arrows). The mitochondria (*m*) are also vesiculating (arrow) as they are known to do in the early stages of cytolysis. The two systems, mitochondrial and reticular, appear then to be adopting similar forms in response to the same environment. × 6800.



(Porter: Submicroscopic basophilic component of cytoplasm)

PLATE 48

FIG. 11. Electron micrograph of a portion of a fifth cell from the same group of rabbit endothelial cells shown in Figs. 7 to 10. Here the strands of the reticulum have completely broken up into separate vesicles (*er*, arrows). The mitochondria likewise occur as distinct vesicles which are connected only by slender strands if connected at all (*m*, arrows). Folds may be discerned in the surface membranes of some of the collapsed vesicles of the reticular system and also in the cell membrane (*cm*). × 6800.

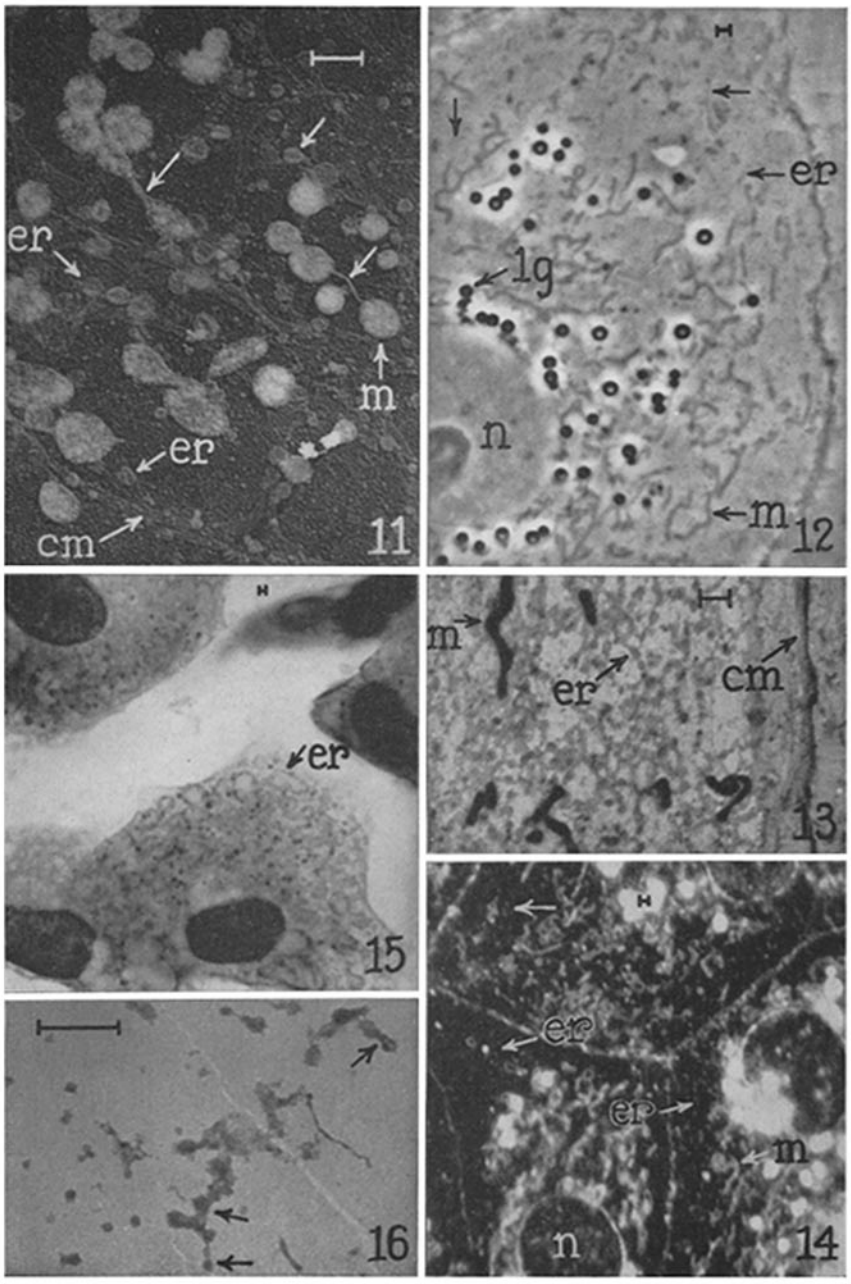
FIG. 12. Photomicrograph with phase contrast microscope of a part of a living kidney epithelial cell. Contiguous cell margins are shown along the right-hand edge. The cell nucleus (*n*), very refractile lipid granules (*lg*), and mitochondria (*m*) are easily identified. Faintly shown in the ground substance are small granules and strands (*er* and arrows) representing elements of the reticulum occurring in the shallow, in-focus, optical section photographed. × 1800.

FIG. 13. Electron micrograph of a small part of margin of kidney epithelial cell from same culturing as that which provided the cell for Fig. 12. Here it was fixed with vapors of OsO_4 for 15 hours before examination. The cell margin (*cm*) is at the right, and mitochondria at (*m*). The ground substance is composed in part of the typical vesiculated strands of the reticulum (*er*). Their size and form relative to other components of the cytoplasm identify them with the fine granules and strands shown in the photomicrographs in Figs. 12 and 14. × 4100.

FIG. 14. Photomicrograph of living kidney epithelial cells taken with dark field illumination. Here, as in the preceding figures, the ground substance shows (at *er* and arrow) fine granules and filamentous elements which appear to represent portions of the reticulum in the same plane of focus as mitochondria (*m*), nucleus (*n*), etc. × 1300.

FIG. 15. Photomicrograph of mesothelial cells from culture of newborn rat heart. The cells were fixed for 10 minutes over vapors of OsO_4 and thereafter stained with toluidine blue to demonstrate the basophilic components of the cytoplasm. These are seen in the thinner cell margins to form a reticular pattern (*er*) seemingly identical with that in similar cells in Figs. 1 and 2 and in other cells in the other micrographs. × 1050.

FIG. 16. Electron micrograph of the so called microsomal fraction isolated from liver cells in sucrose medium (procedure described by Hogeboom, Schneider, and Palade (26)). In fixation, equal parts of the sucrose suspension and 1 per cent OsO_4 in H_2O were mixed and allowed to stand for 30 minutes. The blackened suspension was then centrifuged and the sediment was resuspended and washed twice with H_2O to rid it of sucrose. A small sample of the final suspension was placed on a coated electron microscope grid and provided this image. Particulate and apparently vesiculated elements of the fraction (arrows) have the approximate size, density, and sometimes strand-like organization that would be expected of fragments of the endoplasmic reticulum. × 11,100.



(Porter: Submicroscopic basophilic component of cytoplasm)

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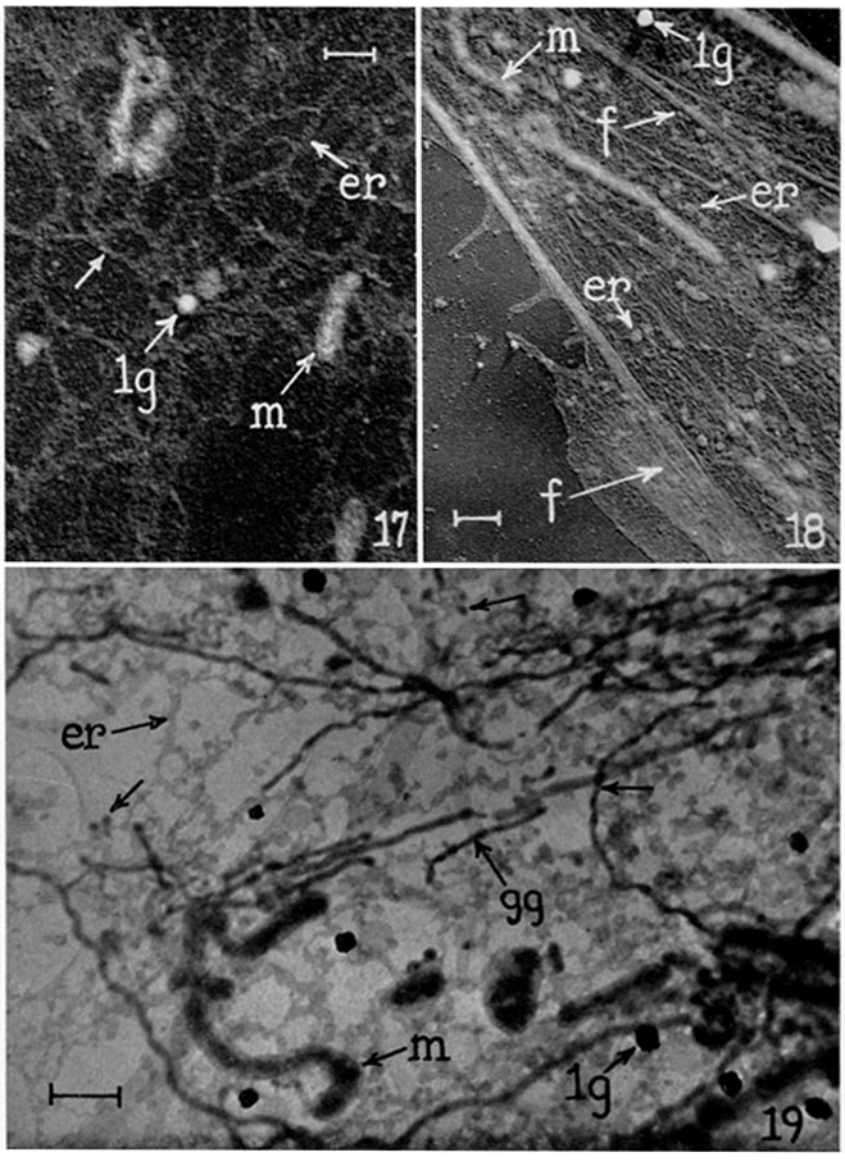
FIG. 17. Portion of a rat mesothelial cell fixed only 10 minutes over vapors of OsO_4 (2 per cent in H_2O) and thereafter washed 20 minutes in H_2O. Preparation shadowed with chromium. Components of the reticulum (*er*) are evident all through the area shown. Neither these, nor the mitochondria (*m*), nor lipid granules (*lg*) have the sharply defined edges depicted in Figs. 7 to 11 and others. There is clearly an amorphous material over and around these formed elements which acts to scatter electrons and blur margins. This matrix is removed by the longer (16 to 25 hour) action of OsO_4 and extracted as the cell is bathed in balanced salt solution (51). × 6800.

FIG. 18. Small portion of rat fibroblast, likewise fixed only briefly in vapors of OsO_4 and shadowed with chromium. Here the matrix of the ground substance is similarly present and in places is organized into intracellular fibers (*f*), which in this cell are taken to represent stress or fibroglial fibers (32). Elements of the chromidial system are also shown at (*er*), mitochondria at (*m*), and lipid granules at (*lg*). × 6000.

FIG. 19. Electron micrograph of portion of a rat myoblast cultured from a partly developed (9 mm.) rat embryo. Mitochondria (*m*), lipid granules (*lg*), and components of the reticulum (*er*) are easily identified. The micrograph shows as well several dense strands and a few granules that are commonly encountered in rapidly growing cells (*gg* and arrows). In this smooth contoured, dense form they are easily segregated from the strands, etc., of the reticulum. Occasionally, however, they are somewhat vesiculated and less dense and in this state they resemble closely the elements of the reticulum into which, in fact, they may transform. × 9250.



(Porter: Submicroscopic basophilic component of cytoplasm)