STUDIES ON THE ENDOPLASMIC RETICULUM*

I. Its Identification in Cells in Situ

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(Received for publication, July 26, 1954)

In 1945, Porter, Claude, and Fullam (1) noticed the presence of a "lace-like reticulum" in the cytoplasm of avian cells cultured in vitro and examined in the electron microscope after appropriate fixation and mounting. The reticulum, which appeared to be part of the ground substance of the cytoplasm, was found to consist usually of interconnected "strands and vesicles" of small dimensions (100 to 150 mÅ) and relatively low density. The new cytoplasmic component was later studied in more detail, in various avian and mammalian cell types by Porter and Thompson (2, 3), Porter and Kallman (4), and Porter (5), who described it under the name "endoplasmic reticulum" because of its general morphology and intracellular location. Comparable findings were reported by Oberling et al. (6), Martin and Tomlin (7), and by Selby and Berger (8) using similar materials and methods.

While observations on the endoplasmic reticulum in cells cultured in vitro continued to accumulate, information concerning corresponding structures in cells in situ remained sparse and unsatisfactory, mainly because, in their case, preparatory procedures were too crude to permit examination at the level of resolution attained with cultured material. In the last few years, however, this impediment has been removed, at least in part, by a general improvement in the techniques for fixation (9), imbedding (10), and microtomy (10, 11). As a result, satisfactory resolution can be obtained consistently at present on sectioned material as demonstrated by a number of recent electron microscope studies. Moreover, in some of these studies, structures in the range of dimensions of the endoplasmic reticulum were actually resolved in the cyto-

* The substance of the articles in this series was presented in 1952 at the Meeting of the American Association of Anatomists held in Providence, and has already been published in abstract form (Palade, G. E., and Porter, K. R., Anat. Rec., 1952, 112, 370).

1 The expression is used to designate cells found in tissue blocks excised for fixation directly from living animals. The morphology of such cells, which have retained until excision their normal connections within their respective tissues and organs, provides in electron microscopy the best possible approximation of the morphology possessed by cells in their normal surroundings; i.e., in situ.
plasm and described under various names (9, 11-14). In the majority of cases, however, no correlation was established between the appearances recently found in sections and those already described in cultured material.

Such a correlation is attempted in the present work. To this intent a few representative cell types were studied in parallel both in vitro and in situ. A comparison of appearances encountered in cells of the same type when examined in these two different situations led to the identification of structural elements belonging to the endoplasmic reticulum in cells in situ; in addition it helped in understanding the modifications introduced in the appearance of this reticular system by the necessarily different procedures required for the preparation of cultured and sectioned specimens.

Materials

Observations were restricted to a few cell types known to grow satisfactorily in vitro and to spread thinly enough to provide convenient specimens for electron microscopy. They included:

(a) monocytes and derived macrophages from the buffy coat of adult chicken blood as well as macrophages from chicken spleen; (b) mesothelial cells from the peritoneum of newborn rats; (c) nephron epithelia from newborn rabbits; (d) endothelial cells from the blood vessels of newborn rabbits; (e) fibroblasts from the skin and heart of chick embryos; and (f) glandular epithelia (acinar cells) from the parotid of newborn rats.

In a number of experiments, explants for tissue culture and small blocks of tissue for the fixation of cells in situ were secured at the same time from the same animal; they were processed separately thereafter, as indicated under Methods, and the results obtained in the two different types of preparations were finally compared. Such results were supplemented with numerous observations made on the cell types listed, which were repeatedly encountered both in cultured and sectioned specimens prepared for other purposes.

Methods

1. Usual Techniques. (a) For Cells in Vitro.—The tissue explants were cultured on formvar-coated coverslips inserted in roller flasks as described in a previous work (5). They were planted in a 1:2 chicken plasma-nutrient medium, the nutrient being a 5:3:2 mixture of balanced salt solution (Tyrode), human placental cord serum, and embryo extract. After 2 to 4 days, cultures showing large growth zones were fixed over osmium tetroxide vapors for relatively long periods (18 to 24 hours), a procedure known to give better images of the endoplasmic reticulum (15) by differential extraction of the cytoplasm. Following washing in H2O, selected groups of cultured cells were removed from the coverslip while still attached to the plastic film and mounted on electron microscope screens. After drying in the air, the specimens were ready for examination.

(b) For Cells in Situ.—Small fragments of tissue excised from living, anesthetized animals were fixed in buffered OsO4 solutions, dehydrated, and imbedded according to techniques outlined in a previous work (9). Embryonic tissues, presumably because of their higher water content, proved more difficult to fix and process in this way than did adult specimens and usually showed more precipitation artifacts and extensive extraction effects. It was found that the former could be noticeably reduced by buffering the fixative at a more alkaline pH, e.g. 7.8 to 8.0 instead of 7.3, whereas extraction could be slowed down to an appreciable extent by shortening the time for fixation and dehydration. The specimens were fixed for 30
to 120 minutes and dehydrated completely within 3 hours. They were imbedded thereafter in n-butyl methacrylate and sectioned with one or the other of the two microtomes described by Porter and Blum (11) at an estimated thickness of 300 to 600 A.

2. Special Techniques. (a) Buffy Coat Preparations.—White blood cells were separated from adult chicken blood by the albumin flotation procedure of Fawcett and Vallee (16). Small aliquots of the cell suspension were used respectively for tissue cultures (17) and direct fixation in buffered (pH 7.30) OsO₄ solutions. At each step during the washing, dehydration, and plastic impregnation procedure, the cells were centrifuged down at low speed from the medium in which they were suspended, and resuspended thereafter in that required by the subsequent step. The cells were finally placed in a gelatin capsule containing n-butyl methacrylate with added catalyst and, after they had settled down at the bottom, the mixture was allowed to polymerize under the usual conditions.

(b) Sectioning of Tissue Culture Material.—For a direct demonstration of the appearance taken by the endoplasmic reticulum in thin sections, an attempt was made to obtain such sections of cells cultured in vitro. Tissue explants were cultured in a plasma clot on a narrow glass strip cut out of a coverslip and coated with a thick film of formvar. Specimens with a large “growth zone” were selected and fixed in OsO₄ vapors according to the techniques commonly used for cultured specimens. They were thereafter washed, dehydrated, impregnated with methacrylate and imbedded by polymerization in the latter while still in contact with their glass support. The latter was chipped off only before cutting, the thick layer of formvar serving as a protective coat for the imbedded cells and as a marker for finding the cells more easily in the sections. This procedure rendered possible the sectioning of the thinly spread cells of the “growth zone”—which in the past have been the preferred object for observations on the endoplasmic reticulum. Sections through the thicker zone of the cultures, i.e. the zone immediately around the explants, were obtained either in such preparations or in cultures which, after fixation, were detached from their glass support and processed thereafter in the manner used for small blocks of tissue.

3. Electron Microscopy.—The cultured specimens and the sections, the latter with the imbedding plastic left in, were examined either in an RCA (model EMU-2B) or in a Philips (model EM-100) electron microscope. For each microscope the contrast in the image was increased by using small (20 to 30 μ) objective apertures. The electron micrographs were taken at an initial magnification of 700 to 8000 and enlarged photographically thereafter as desired.

OBSERVATIONS AND IMAGE INTERPRETATION

For a better interpretation of the comparative observations which follow, a few general facts are worth mentioning at the beginning.

1. In tissue culture, cells have the tendency to spread thinly when provided with a firm and flat support. Within a relatively wide margin, e.g. 2 to 10 μ, at the periphery of the cell, the living cytoplasm may be less than 1 μ in thickness and become even thinner after fixation and drying. Within this peripheral zone, the cytoplasmic components, endoplasmic reticulum included, can be examined in toto and appear much like the various geographical details in an aerial photograph: their general shape, intracellular distribution, and connections are self-evident or easy to visualize. It has to be pointed out, however, that as the cytoplasm thins out by spreading, an increasing amount of orientation is imposed on the various cell components. In the very thin, peripheral regions, best suited for electron microscopy, the endoplasmic reticulum, for instance, appears as a network disposed predominantly or exclusively
in two dimensions. By virtue of simplification this orientation facilitates the interpretation of electron microscope images, but, to a certain extent, it represents a distorted picture. There are good indications (1, 5) that in the thicker, perinuclear regions of cultured cells the endoplasmic reticulum is disposed as a tridimensional network, an arrangement that may be considered more general in character and, as such, more likely to be encountered in cells in situ.

2. Because electron microscope resolution improves in general with the thinness of the specimen, considerable effort has been applied recently toward reducing the thickness of tissue sections below 0.1 μ and preferably below 0.05 μ (~ 50 m = 500 Å). The estimated thickness of sections used in the present work, for instance, is between 30 and 60 μ. This is less than the diameter of most “vesicles and strands” of the endoplasmic reticulum and represents only a small fraction of the usual mesh size of the network. Under such circumstances the endoplasmic reticulum, if present in cells in situ, cannot be expected to appear in sections as a network. Occasionally, in favorably oriented sections, fragments of meshes or whole meshes of the reticulum could be included in the thickness of a given section, but in the vast majority of sections only profiles of “vesicles and strands” will be encountered and these will appear as independent structures because their original connections have been severed by the microtome. For this reason, cell sections constitute a much less favorable material than whole mounted, cultured cells for studying the general morphology and intracellular disposition of a highly dispersed and complicated component such as the endoplasmic reticulum.

These points are clearly illustrated by the electron micrographs shown in Figs. 1 to 3. Fig. 1 represents a small field in the cytoplasm of a chicken macrophage cultured in vitro from a buffy coat explant. Such cells are identified as macrophages because of the characteristic motility, affinity for vital dyes, and phagocytic activity they show while in culture. The cell, of which a limited field is shown in Fig. 1, is usually described as an “epithelioid macrophage” and represents a relatively late appearance in a series of transformations incurred by buffy coat monocytes when cultured in vitro. These mononucleated cells of the blood increase rapidly in size after explantation and for 2 to 3 days exhibit intense ameboid movements while actively phagocytizing other blood cells, e.g., granulocytes and thrombocytes. During this period the active cells are identical in form and function with tissue macrophages and consequently are considered to result from an in vitro transformation of monocytes into macrophages (18, 19). After 3 to 4 days in culture, with their movements reduced to a peripheral “undulating membrane,” the macrophages spread, flatten down to a considerable extent, and sometimes may form sheets of cells of epithelial appearance. The flattened cells are characterized in addition by an enlarged centrosphere, around which a “rosette” of small vacuoles usually appears after vital staining. It is in this later state that the cells are described as “epithelioid macrophages” (19, 20). The small cytoplasmic field shown in Fig. 1 contains a lipid inclusion (l), a branching mitochondrion (m), and part of the endoplasmic reticulum (n, s, f) of the epithelioid macrophage, the nucleus and margins of which are out of the field. The trabeculae of the endoplasmic reticulum are made up of strings of round elements connected in a few places by thinner “strands.” The round elements measure from 100 to 300 μ and apparently represent flattened vesicles. Their density is lower than that of mitochondria and is closely approached by that of the folds (f) in the upper membrane of the cell. The density of the latter results from the superimposition of four membrane layers. A few vesicles, similar in dimensions and density to those lined up in strings, appear isolated
in the cytoplasm. The appearance just described for the endoplasmic reticulum of this macrophage is actually one of those most frequently encountered in cells *in vitro* (5) and illustrates both the type of "bird's-eye view" and the predominantly two-dimensional orientation of the reticulum usually obtained in cultured specimens.

Fig. 2 shows at the same magnification a comparable cytoplasmic region in a sectioned macrophage; the cell comes from a buffy coat culture prepared at the same time and with the same material as the culture which supplied the macrophage in Fig. 1. After fixation the culture corresponding to Fig. 1 was dried and mounted for examining cells *in toto*, whereas the culture corresponding to Fig. 2 was imbedded and sectioned as described under Special Techniques. The section shown in Fig. 2 had an estimated thickness of ~50 m, and in cutting it the knife was oriented more or less normally in respect to the flattened cell; thus it would correspond to a section of a-a' thickness cutting perpendicularly along ab, in Fig. 1.

In addition to a few readily identifiable mitochondrial profiles (m), a number of circular, oval, and elongated formations (c, o, e) are present in the cytoplasm. Comparison with Fig. 1 shows that they have the same location, size, and general density as the trabeculae of the endoplasmic reticulum. For this reason they are identified as profiles of the latter, and the identification appears reasonably certain because usually no other structures that could interfere with it are present in this peripheral zone of the cytoplasm. Oval and elongated profiles appear to be, respectively, the results of oblique and longitudinal sections through oblong vesicles or short tubules, whereas circular profiles may correspond to transverse sections of such structures or to sections of any incidence through spherical vesicles. All these profiles appear as independent structures and so would the elements of the endoplasmic reticulum contained in the thickness of the hypothetical section ab in Fig. 1. It is evident that the linkage of these elements in a reticular system can be completely lost by sectioning and this illustrates what was said at the beginning of these Observations, namely, that sectioned material is not particularly suitable for studying the interconnections of a dispersed cytoplasmic system such as the endoplasmic reticulum. In sectioned material a difficult and time-consuming tridimensional reconstruction from serial sections would be necessary to bring out the fact that the apparently independent elements present in Fig. 2 are actually part of a more or less continuous reticular system, a feature which is self-evident in cultured cells examined *in toto* as illustrated by Fig. 1.

Sectioned material, however, seems to be better suited for studying fine structural details. In Fig. 2 for instance, most of the profiles show a dense, continuous outline that can be interpreted as a limiting membrane surrounding a content which, for some of them, is as light as the matrix of the ground substance of the cytoplasm. The corresponding structures appear therefore to be cavitary in nature, *i.e.* to be vesicles and tubules, a feature previously suggested by the fact that in cultured cells, examined *in toto* after drying, the elements of the endoplasmic reticulum were usually found to be completely collapsed with occasional folds at their surface (5). The limiting membrane of this network of cavities varies considerably in apparent thickness, *i.e.* from 6 to 12 m, but this wide variation can be satisfactorily explained by differences in the angle of cutting: a membrane of a given thickness is expected to appear in sections as a broader band when cut obliquely and as a narrower one when perpendicularly sectioned. For this reason it is assumed that the limiting membrane of the endoplasmic reticulum is of rather stable thickness and that the latter is most closely approximated by the lower figure mentioned, *i.e.* 6 m, which apparently corresponds to normal sections. This figure is in satisfactory agreement with the one previously calculated (5) from the length of the shadow cast by collapsed vesicles in shadowed preparations. As is

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*The expression is used to describe a section for which the plane of cutting was normal to the sectioned structure, in this case the membrane of the endoplasmic reticulum.*
known, in cultured material the limiting membrane of the endoplasmic reticulum was found to be comparable in thickness to the cell membrane (5), a finding which is now substantiated by direct measurements in sectioned material. In this respect it may be pointed out that in electron micrographs of whole cell preparations the density of folds in the cell membrane is similar to that shown by the elements of the endoplasmic reticulum (Fig. 1). In each case the density is given by four superimposed membrane layers: in the first case all layers belong to the cell membrane, whereas in the second only two are contributed by it, the remaining two being supplied by the membranes (upper and lower) of the endoplasmic reticulum.

In sections passing through the perinuclear region of cultured macrophages (Fig. 3) the situation is more complex than in the marginal zones. The profiles are more numerous, more diversified in size, and appear to be distributed at random throughout the cytoplasm, a disposition compatible with a tridimensional reticulum. In the centrosphere zone, tight clusters of small, circular, or elongated profiles are frequently encountered; they may represent either a differentiated part of the reticulum or a different, unrelated structure.

Fig. 4 shows at the same magnification as the preceding ones a monocyte examined in a suspension of buffy coat cells fixed, imbedded, and sectioned as indicated under Special Techniques. The buffy coat used for this preparation was actually an aliquot of the material employed for the tissue cultures that supplied the specimens for Figs. 1 to 3. The cell in Fig. 4 has been identified as a monocyte because of its size, nuclear shape, and cytoplasmic components, all differing in number or shape from those of granulocytes and lymphocytes, the other cell types present in the buffy coat. As mentioned before, monocytes survive in vitro and transform into macrophages of the type shown in the previous figures, while granulocytes disappear by disintegration or phagocytosis; the fate of lymphocytes is not entirely clear; according to some observers they disappear rapidly by disintegration (18, 19), while others claim that they also transform into macrophages although less actively than the monocytes (21, 22). The monocyte shown in Fig. 4 can therefore be considered as representing among the buffy coat cells the same cell type as the cultured macrophage, or at least its parental type. Besides a mitochondrion and a few dense granules, the sectioned monocyte exhibits a number of "hollow" profiles of circular, oval, and elongated shape scattered throughout the cytoplasm. In size and shape they are similar to those found in Figs. 2 and 3 and consequently are considered to be profiles of vesicles and tubules belonging to the endoplasmic reticulum. Their linkage in a tridimensional network is suggested by the fact that in some instances the profiles occur in rows (r). For some of these profiles the limiting membrane appears to be smooth, while for others it seems to have small thickenings on its outside surface which look like attached small granules of a type recently described (23). For reasons that will become clear in the following pages, these rough surfaced elements are considered as part of the same system as the smooth surfaced ones.

Finally, Fig. 5 shows, at the same magnification, a cytoplasmic field in a sectioned macrophage from the spleen of an adult chicken. Besides a number of mitochondrial profiles, easily identifiable because of their cristae (24, 25), the cytoplasm contains numerous "hollow" profiles of circular, oval, and elongated shape which, again, are taken to represent sections through the elements of the endoplasmic reticulum. A cluster of smaller circular profiles and a tight skein of elongated ones are present in the centrosphere zone and appear to be similar to those found in Fig. 3. The examination of various tissue specimens (liver, lung, skin, intestinal mucosa) showed that macrophages in general have the same cytoplasmic appearance as the cell in Fig. 5, irrespective of the tissue where found.

In this survey, a cell of a particular type, namely a macrophage, derived from various tissues of mesenchymal origin, has been examined in different situations, e.g. in vitro and in situ, by using various preparatory procedures.
e.g. whole mounting and sectioning. As a result, it appears that an endoplasmic reticulum is present not only in cultured macrophages, as previously described (5), but also in monocytes (i.e. “circulating macrophages”) and in “fixed” macrophages or histiocytes examined in situ in various tissues. In cells in situ it does not appear as a continuous reticular system (as it usually does in cultured cells examined in toto) but as a scattering of independent elements. The same “broken down” appearance is encountered, however, in cultured cells when examined in sections, and this finding is taken as good evidence that the appearance mentioned is the result of sectioning. The trabeculae of a given reticulum are expected to appear as independent structures when, as in the present case, the network is cut, and subsequently examined, in sections much thinner than the size of its meshes. It seems, therefore, that in situ as in vitro, the vesicular and tubular elements of the endoplasmic reticulum are linked together in a network but it remains obviously difficult to ascertain whether or not this applies to all the elements found in sections. Considering the situation known to exist in cultured macrophages it may be assumed that in the whole cell in situ, as well as in vitro, most of the vesicular and tubular elements are aggregated in a continuous network.

By using the comparative approach outlined for macrophages, similar results were obtained for all the other cell types investigated.

Fig. 6 for instance, illustrates the appearance of the endoplasmic reticulum in a cultured mesothelial cell examined in toto. The corresponding culture was obtained from an explant taken from the margo acuta of the liver of a newborn rat and the cell was identified as mesothelial because of its morphology and its location: it was part of a sheet of cells which had the appearance of a simple, squamous epithelium. At variance with the situation found in Fig. 1, the trabeculae of the reticulum appear to be more slender and more uniform in diameter, although in a few places they show successive dilatations or strings of round elements. Such a reticulum is apparently formed mostly by interconnected tubules with only occasional strings of vesicles present. The tubules show in some places ampullar dilatations which may be considered as transition forms towards the vesicles aligned in rows. A few large and irregular formations appear at the nodal points of the network and occasional, isolated vesicles are scattered in the cytoplasm. The companion image in Fig. 7 shows at the same magnification cytoplasmic fields in two adjacent mesothelial cells sectioned in situ in the visceral peritoneum of a rat. Their appearance is similar in all respects to that encountered in the mesothelia of other serosa examined; i.e., the pericardium and the pleura. A comparison with Fig. 6 indicates that the numerous circular, oval, and elongated profiles represent sections of the reticulum; their linkage in a network is indicated in a few places by short rows of circular profiles apparently corresponding to strings of vesicles.

Figs. 8, 9, and 10 illustrate a similar experiment, the object of which was to compare epithelial cells derived from the same source, namely from the nephron epithelium of a newborn rabbit. Fig. 8 shows such a cell cultured and examined in toto. The endoplasmic reticulum appears as a network of rounded and elongated elements imbedded in a rather dense matrix which has been only partially extracted during a prolonged fixation. Fig. 9 represents small cytoplasmic fields in two epithelial cells encountered in a sectioned kidney culture, prepared at the same time and with the same material as the culture that supplied the specimen for Fig. 8. The cells in Fig. 9 were found close to the explant and were part of a well preserved
tubule. Their appearance is very similar to that shown in Fig. 10 by cells of the same type fixed in situ. The corresponding kidney block was obtained at the same time and from the same animal as the explants for the cultures which supplied the cells for Figs. 8 and 9. A comparison of Figs. 9 and 10 indicates that no perceptible modification was introduced in the morphology of the compared cells by culturing them in vitro for 48 hours. It should be noted, however, that this does not apply for the epithelia of all nephron segments; those of the first convoluted tubules (portio principalis) were found to degenerate rapidly in culture, the types usually surviving being the epithelia of undifferentiated tubules together with those of partially differentiated ones represented in Figs. 9 and 10; the latter are similar in appearance to the epithelia of the thin segment of Henle's loop (portio conducens) in the adult nephron. As in the previous examples, the apparently independent structures found in sectioned specimens can be identified as profiles of the endoplasmic reticulum and their shape, which varies from circular to elongated, can be correlated with the angle at which each element of the reticulum was sectioned.

A more complex situation is found in endothelial cells of newborn rabbits, as illustrated by the series of electron micrographs shown in Figs. 13 to 17. Fig. 13 represents a relatively large field in the cytoplasm of a cultured, endothelial cell grown from an explant that contained the blood vessels of the umbilical cord. The cell was identified as endothelial by its general morphology and its inclusion in a large cellular sheet which had the appearance of a simple, squamous epithelium. In the case of the endothelial cell shown in Fig. 13, the appearance of the endoplasmic reticulum is to a certain extent different from the appearances of reticula illustrated in Figs. 1, 6, and 8. Strings of vesicles and elongated, tubule-like formations are still present but in many places they seem either to be confluent in or to branch from broad structures of irregular outline for which the name cisternae is proposed. The term is already used in morphology at a much higher scale for designating large, reservoir-like formations in a system of communicating cavities or vessels. The examination of a large number of specimens indicated considerable variation in the extent and outline of such cisternae. For instance, in a given preparation that had a homogeneous cell population, as far as the cell type was concerned, cells were encountered in which (a) a single cistern spread without interruption throughout a considerable expanse in the cytoplasm of the flattened cells; (b) large cisternae appeared "fenestrated"; (c) smaller cisternae occurred, as in Fig. 13, in continuity with tubules and strings of vesicles; and finally (d) the endoplasmic reticulum displayed its usual reticular appearance with no cisternae present. As the preparatory procedure had been the same for all cells involved, it was assumed that such appearances represented intrinsic cellular variations which might be related to different phases of activity or growth. In the past (5) two different appearances of the endoplasmic reticulum were assumed to have functional implications. One, encountered in normal cells from young, actively growing cultures, was a finely divided form in which the reticulum had tight meshes formed by "strands" of small dimensions (100 mµ); the other, characteristic for cells undergoing cytolysis, was a disintegration form in which the reticulum was replaced by a scattering of independent vesicles. The new form, i.e. a reticulum provided with cisternae, was encountered more frequently in the endothelia of embryos and newborn animals, but it was found to be absent from other embryonic or rapidly proliferating cell types. Its possible functional significance will be discussed later in this paper.

Fig. 14 represents part of an endothelial cell fixed in situ in the wall of a blood capillary found in a small block of myocardium taken from a newborn rabbit. Endothelial cells as a rule are thinly spread in situ, approaching to a certain extent the condition shown by the cells of the "growth zone" in tissue cultures. Such was the case, for instance, with the cell in Fig. 14, which was sectioned almost normally in relation to its flat surface. Fig. 15 shows part of the same endothelial cell at a higher magnification to facilitate the identification of the various cytoplasmic components. In addition to a few mitochondrial profiles (m), a num-
ber of elements of circular (c) and elongated (e) shape can be seen in the section and it may safely be assumed that they represent profiles of the endoplasmic reticulum. In the endothelia of embryos and newborn animals elongated profiles are, as a rule, more frequently encountered than circular ones, and this is considered to be a noteworthy finding because it is not compatible with the existence of a usual type of reticulum which when sectioned is expected to yield predominantly circular profiles. Figs. 16 and 17 represent two serial sections through an endothelial cell in the heart of the newborn rabbit which supplied the material for Figs. 14 and 15. The elongated profiles are predominant and can be followed from one section to the other; there are places in which elements that appear to be independent in Fig. 16 run together in a common stem in Fig. 17, thus demonstrating their linkage in a common tridimensional system. The system, however, does not seem to be a simple reticulum of the type illustrated in Fig. 1 but one provided with cisternae of the type shown in Fig. 13. The left ends of the profiles marked ci and ci measure ~70 μm in diameter, show a continuous lumen, and can be superimposed along most of their length. The thickness of each section being evaluated at approximately 50 to 60 μm, it follows that the structure to which profiles ci and ci belong has been reconstructed on a 100 to 120 μm depth. This is much more than the expected 70 μm diameter of a tubular structure and, as in these sections there is no indication of narrowing or closing of the lumen, it may be safely assumed that these elongated profiles correspond to normal sections through cisternae.

Two additional findings may be pointed out in relation to these observations on endothelial cells. The first is the possibility of encountering in them, in situ, a predominantly two-dimensional orientation of the reticulum comparable to the one so frequently found in the marginal cytoplasm of cultured cells. The second is the particular structure of the cisternae. They seem to be actually flat vesicles with a narrow though relatively vast cavity, as indicated by the fact that the top and bottom membranes remain parallel and close together (~50 μm apart) over wide expanses. This is suggested by the essential absence of folds at the surface of the cisternae in cultured cells examined in toto (Fig. 13) and is substantiated by findings in sectioned material, particularly in serial sections (Figs. 16 and 17).

A situation similar to that described in endothelial cells was encountered in fibroblasts of chick embryos derived from the heart and the skin and examined both in vitro and in situ. Here again the endoplasmic reticulum appeared to be provided with numerous and relatively large cisternae.

Figs. 18 and 19 illustrate in an epithelial cell a similar though more complex situation than the one encountered in endothelia and in fibroblasts. Fig. 18 represents a small cytoplasmic field in an epithelial cell grown in vitro from a parotid explant taken from a newborn rat. The endoplasmic reticulum shows the usual network appearance in the upper left corner and the presence of relatively large cisternae in the lower right one. Some of the elements of the reticulum, cisternae included, show a relatively homogeneous, low density, whereas others have a higher, unhomogeneous density, their surface being apparently dotted with small granules of a type recently described (23) in association with the endoplasmic reticulum. The electron micrograph indicates that elements with and without attached granules are linked together in a continuous reticulum and thus appear as differentiated segments in a common system. It is for this reason that apparently independent profiles with a uniformly smooth or rough surface found in the sectioned material of the preceding examples were considered to belong to a common system, notwithstanding the obviously different appearance of their membranes.

Continuity between similarly differentiated segments of the endoplasmic reticulum was also encountered in sections of various cell types as illustrated by Fig. 11, which shows part of an endothelial cell in a blood capillary (rabbit).

Fig. 19 represents a small cytoplasmic field in an acinar cell fixed in situ in a small block of glandular tissue taken from the parotid of the same newborn rat that supplied the material.
for Fig. 18. The profiles of the endoplasmic reticulum show their cavity, their limiting membrane, and the small granules covering the outside surface of the latter, the granules appearing more clearly whenever the section, by its obliqueness, exposes the limiting membrane over a wider expanse. All these features can easily be correlated with appearances found in Fig. 18, but this does not apply for the general disposition of the profiles, which \textit{in situ} appear to be arranged in successive and more or less parallel layers. Such an appearance could be explained by assuming that in this case numerous two dimensional reticula of the type encountered in endothelial cells are piled up more or less parallel to one another at a relatively regular spacing inside the cytoplasm. The disposition undoubtedly deserves further study. For the moment the predominance of elongated profiles and the relative scarcity of circular ones suggest that these reticular sheets contain large and numerous cisternae.

A comparable layering of reticular sheets has not been noted in previous studies on cultured material. It may be assumed that such an arrangement is eliminated by the extremely thin spreading of the cytoplasm in the cultured cells usually selected for electron microscopy. The layering may be present in the thicker regions of the cytoplasm or in unflattened cells which, being poor electron microscope specimens, have been infrequently examined and when examined have given images of uncertain interpretation. Some suggestion of layering can be found, however, in cells of intermediate thickness, especially when the layering occurs at the level of large cisternae. Such a case is illustrated by Fig. 12, which shows this particular disposition in a mesothelial cell of the rat.

\section*{Discussion}

In the preceding pages the morphology of a few cell types was presented comparatively in two different situations, namely \textit{in vitro} and \textit{in situ}, with the aim of finding out whether cells \textit{in situ} possess an endoplasmic reticulum. As already indicated, such a reticulum was originally found and thereafter studied almost exclusively in cultured cells examined \textit{in toto}, \textit{i.e.} in whole amounts, for which reason similar preparations were used as controls in the present study. The situation \textit{in situ} was examined in fixed and sectioned specimens, this being for the moment the only technically possible approach to the problem.

It was found that in all cells thus examined there are structures which have the location, diameter, and density expected of the trabeculae of the endoplasmic reticulum. Similar structures were found in sections of cultured cells known to possess an endoplasmic reticulum and this finding was taken to indicate that the formations mentioned were actually profiles; \textit{i.e.}, sections through the trabeculae of the network. The identification seems well established for elements found in the peripheral zone of cultured cells; \textit{i.e.}, the zone for which cells examined \textit{in toto} had already provided satisfactory information. In such regions, practically all profiles found in sectioned material could be correlated with structures seen in specimens examined \textit{in toto}. The situation was less satisfactory in the central zone of the cells, especially in the centrosphere region, which is poorly resolved in whole mounts of cells, and which in sections usually showed agglomerations of profiles smaller than the ones found in the rest of the cytoplasm. These latter may represent a differentiated part of the reticulum or a completely unrelated system. In sections the profiles
of the endoplasmic reticulum were usually found as isolated elements with no connection between them. This was to be expected in view of the fact that the sections used in this study were much thinner than the usual mesh size of the reticulum. However, indications of the linkage of the sectioned elements in a network were occasionally found in the form of profiles showing branchings or occurring in rows. Moreover, in the case of endothelia, it was possible to show by actual reconstruction from serial sections, that apparently independent elements were part of a continuous, tridimensional reticulum. Similar results were published by Porter and Blum (11) who reconstructed on a greater depth part of the reticulum in an acinar cell of the parotid.

It appears therefore from the preceding observations that cells fixed in situ, i.e. in their normal environment, possess an endoplasmic reticulum which in its general morphology is similar to that previously described in cultured cells. This finding rules out the possible role of an artificial environment, i.e. that of tissue cultures, in the formation of the reticulum; moreover the latter appears to be a significant cell structure as indicated by the fact that it has been found to be a regular part of the cytoplasmic organization for all cell types examined in situ as well as in vitro.

The observations reported deal only with fixed material and accordingly do not have any direct bearing on the problem of the existence and morphology of the endoplasmic reticulum in vivo, for which evidence has been brought forward and discussed by Porter (5). It is believed, though, that the demonstration of the reticulum in cells fixed under widely different conditions (individually as in buffy coat preparations, in layers as in tissue culture, and in bulk as in tissue blocks) could be taken as additional evidence towards establishing the endoplasmic reticulum as a component of the living animal cell.

Most of the profiles of the endoplasmic reticulum displayed a dense, continuous outline surrounding a content of light density, an appearance suggesting that they were produced by the sectioning of cavitory structures such as tubules and vesicles. A cavitory nature for the elements of the endoplasmic reticulum was previously suggested by observations in cultured cells. It appears therefore that the reticulum does not have solid trabeculae of the kind usually described as strands, filaments, or fibers, but should be considered rather as a network of cavities bounded by a limiting membrane which encloses a content of variable, usually low density. These features describe it as a finely divided vacuolar system of the cytoplasm (5).

Considerable variation was found in the morphology of the profiles of the endoplasmic reticulum: most of them were of circular, oval, or elongated shape; few irregularly shaped or branching profiles were encountered. For simple geometrical reasons it was concluded that: (1) circular profiles were produced either by sections of any incidence through spherical vesicles or by transverse sections through tubules; (2) oval profiles were the result of oblique sections...
through tubules or corresponded to deformed vesicles; whereas (3) elongated profiles represented very oblique or longitudinal sections through tubular formations; such profiles may in addition be the result of normal sections through formations described in this work as cisternae. The term is used to designate a flat element of large size and irregular outline which appears to communicate freely with the tubules and vesicles of the endoplasmic reticulum because both their membranes and contents are usually continuous. Although such cisternae may assume considerable breadth they seem to retain, in general, a depth of ~50 μm, thus assuming the form of large flattened vesicles. Comparable formations have been described in the past as “sinusoids” (11); the new term is proposed because it has been used in morphology for large, reservoir-like formations in a system of cavities and because sinusoid in its present usage, at a higher scale, implies a vessel or cavity imperfectly separated from its surroundings by a discontinuous wall. This, as a rule, does not seem to apply to the formations under discussion, which, at the present level of resolution, appear to be bounded by a continuous membrane.

A point of particular significance seems to be the considerable variation in the extent of cisternae versus meshes in the endoplasmic reticulum of certain cell types such as endothelia and glandular epithelia. In cultured specimens of such cells practically all intermediates can be encountered between a reticulum without cisternae and one in which the latter are the most conspicuous components of the system, with only a few connecting meshes left between them. As a rule, the appearance of the endoplasmic reticulum was found to be the same throughout the cytoplasm of a given cell, but different appearances could be encountered in neighboring cells in a specimen with an apparently homogeneous cell population in which all cells evidently have been subjected to the same preparatory procedure. As such the varied forms found for the endoplasmic reticulum do not appear to be connected with variations in preparatory techniques; it seems more likely that they reflect, as already suggested (5), actual transformations in the endoplasmic reticulum, which may behave like a labile structure, changing with function. It is conceivable that during such changes the fenestration of broad cisternae may end in the formation of a network; and conversely that the broadening of the tubules and vesicles of a usual reticulum may lead to their fusion into broad cisternae. It has to be pointed out, however, that for the moment there are only suggestions, no definite knowledge, about the functional significance of the various appearances taken by the endoplasmic reticulum. The present observations and especially those to be reported in the subsequent articles of this series, suggest, for instance, that a reticulum provided with cisternae occurs more frequently in cells actively engaged in secretory processes in which a protein-rich product is involved. This seems to be the case with the acinar cells of the parotid and other glands of the digestive tract; with the cells of some endocrine glands;
and with the skin fibroblasts in the period in which collagen is actively produced for the formation of the dermis. Embryonic cells and rapidly proliferating cells in the adult frequently have a poorly developed reticulum in which the cisternae are rare or absent. The case of endothelia in the embryo and young animal is admittedly more difficult to interpret along such lines, although it may be remembered that in the embryo at least, the vascular endothelium is supposed to participate in the formation of blood plasma.

In most of the cell types observed the elements of the endoplasmic reticulum appeared to be disposed at random in a tridimensional reticulum, whereas in some others a certain amount of orientation occurred which in its simplest form amounted to a predominantly two dimensional arrangement resulting in a "reticular sheet." Such two dimensional reticula were usually encountered in thinly spread cells in tissue culture and a similar though less rigorous orientation was found in flat endothelial and mesothelial cells in situ. In such cases the orientation seemed to be imposed by simple mechanical factors resulting from the flattening or spreading of the cytoplasmic body. A much more complex situation occurred in acinar, parotid cells in situ in which the cytoplasm appeared to be packed with reticular sheets oriented parallel to one another and more or less regularly spaced.

The factors responsible for the orderly layering of these reticular sheets are unknown, but it is conceivable that the molecular organization of the cytoplasmic matrix may play some role in this respect, as already speculated upon by Porter (5) in respect to the variations in appearance shown by the endoplasmic reticulum in cultured cells.

Notwithstanding the individual variations mentioned, some features appeared to recur persistently in the morphology of the endoplasmic reticulum of certain cell types. Such was the case, for instance, with the predominance of vesicular elements in the reticulum of macrophages, and the layered disposition of the system in the acinar cells of the parotid. Although these differences were more quantitative than qualitative in nature, they seemed to be consistent enough to suggest that in addition to functional variations within a given cell type, the endoplasmic reticulum may show variations which could be regarded as an expression of the process of cell differentiation.

In the present study an attempt was made to demonstrate the presence of the endoplasmic reticulum in cells in situ in a representative series of specimens which actually included avian and mammalian cells belonging to connective as well as to epithelial tissues, the latter being of both ectodermal and mesodermal origin. The results suggest a widespread occurrence of the endoplasmic reticulum in animal cells but the series is undoubtedly too small to permit any generalization. A survey of the reticulum in a larger series of cell types examined in situ would be desirable not only for determining how

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*It is possible that similar factors are sometimes at work in the fenestration of cisternae.*
widespread this cytoplasmic component actually is, but also for finding out whether and to what extent it becomes an expression of the process of cell differentiation as suggested in this discussion.

Problems related to the staining affinities of the endoplasmic reticulum, its biochemical composition, and its possible role in the general physiology of the cell have been discussed in a previous paper (5). The present observations do not add significant information thereto because of their limited scope. For this reason it is believed that a rediscussion of the problems mentioned should be postponed until more information is available about the general occurrence, intracellular location, variation according to cell type, and functional variation of this new cytoplasmic component. Such information is expected to result from a general survey of the endoplasmic reticulum, now in the course of being completed, the results of which will be published in the following articles of this series.

SUMMARY

A series of representative cell types including avian fibroblasts, and macrophages; rabbit mesothelia, endothelia, and nephron epithelia; and rat glandular epithelia (parotid) were studied comparatively in vitro and in situ with the electron microscope. Cells in vitro were examined in whole mounts and in sections whereas cells in situ were observed exclusively in sections.

It was found that an endoplasmic reticulum similar to that previously described in cultured material is present in situ in all cell types examined.

Modifications in its appearance introduced by the sectioning technique were discussed and explained. The observations showed in addition that the endoplasmic reticulum is a network of cavities which may enlarge into relatively vast, flattened vesicles here described as cisternae.

BIBLIOGRAPHY

EXPLANATION OF PLATES

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Figs. 1 to 4 illustrate an experiment in which cytoplasmic appearances encountered in chicken monocytes and macrophages were compared. The monocytes were examined in sections of buffy coat preparations (Fig. 4); the macrophages, resulting from the transformation in vitro of buffy coat monocytes, were studied in parallel in whole mounts (Fig. 1) and in sections (Figs. 2 and 3). The four electron micrographs represent cells found in, or derived from, the same buffy coat preparation; all are reproduced at the same magnification, namely 23,800.

Fig. 1. The electron micrograph represents a small field in the peripheral cytoplasm of a cultured macrophage examined in toto after light shadowing with chromium at an angle of 12°.

The endoplasmic reticulum appears as a network made up of strings of round (n) and oval (s) elements which measure from 100 to 300 μ in diameter and are connected by thinner (30 to 40 μ) trabeculae (t). As indicated by the short shadow they cast, all these elements are flat. It has been assumed that in vitro they correspond to vesicles with a very watery content which evaporates when the specimen is dried thus allowing a nearly complete collapse of the vesicular membrane. Only a few isolated vesicles (n) can be seen in the cytoplasm; all the others are linked in an apparently continuous reticulum, together with vesicular elements of irregular shape and elongated elements (t) which in vivo probably correspond to short tubules.

A lipid inclusion can be seen at l, and at m there is a branching mitochondrion which covers in part the endoplasmic reticulum.

The fine grain shown by the electron micrograph does not describe the texture of the matrix of the cytoplasm, but the fine structure of the cell membrane brought out by the shadowing procedure. It is through this covering membrane that all the various cytoplasmic components are seen. At f the cell membrane is folded; it should be noted that the density of this fold is similar to that of the elements of the endoplasmic reticulum. X 23,800.

Fig. 2. Electron micrograph of a normal section, approximately 50 μ thick, through the peripheral cytoplasm of a thinly spread macrophage grown in vitro. As such it would correspond to a section cutting normally through a and b in Fig. 1 and having the thickness a-a'.

The cell membrane is clearly visible at the free surface of the cell (cm); its "twisted ribbon" appearance indicates that, because of its waviness it has been cut at various angles. The
membrane appears less distinct at the cell surface which was attached in culture to the formvar film; part of the latter is still visible at p; the rest has peeled away.

The formations marked m can be identified as mitochondrial profiles because of their internal structure. It should be noted that their average diameter is smaller than that of the branched mitochondrion in Fig. 1; their ratio is 1:1.8. This discrepancy, largely due to the flattening of mitochondria in air-dried specimens (Fig. 1), is actually greater than expected. A complete flattening, which should have been prevented in this case by the internal structure, would result in a 1:1.57 ratio. Possible factors enlarging the discrepancy are the compression of mitochondrial profiles in Fig. 2 by the microtome knife and the probability that the profiles correspond to lateral rather than medial sections of mitochondria.

The remaining circular (c), oval (o), and elongated (e) elements found in the cytoplasm can be identified as profiles of vesicles of the endoplasmic reticulum because no other components are usually present in such regions. Owing to knife compression most of these elements are more (e) or less (c) flattened in the vertical direction. In some they most probably correspond to spherical or oval vesicles. The elongated profile marked e may represent a short tubule. If all factors involved are taken into consideratión (i.e., complete flattening by air-drying in Fig. 1, partial flattening by knife compression in Fig. 2, and variation in profile size due to the fact that in Fig. 2 some profiles result from lateral sections and some from medial sections of vesicles), there is satisfactory agreement in the size of the elements concerned in Figs. 1 and 2.

Note that the profiles of the endoplasmic reticulum appear "hollow"; i.e., they show a limiting membrane and a light content. Their membrane is cut at various angles and varies accordingly in density and apparent thickness. Some smaller profiles (o, left) have a relatively high density; they probably correspond to tops of vesicles included in the thickness of the section. × 23,800.

Fig. 3. This electron micrograph represents a section cutting through the central region of a macrophage grown in vitro. The nucleus does not appear in the field; the cell membrane, obliquely cut, is visible in the upper right corner. A mitochondrial profile appears at m and part of a large lipid inclusion at the figure number 3. Note around the latter a few concentric, dense layers and a vacuole which developed between them and the homogeneous core of the lipid droplet. The layers probably correspond to successive bimolecular films of phospholipid disposed, as expected, at the oil-water interface.

Most of the numerous "hollow" profiles (c, i, b) scattered in the cytoplasm correspond to elements of the endoplasmic reticulum. The tight bundles of elongated profiles at c and the clusters of irregular profiles at c correspond to membranous structures in the centrosphere region; their relation to the endoplasmic reticulum is uncertain. The very dense profiles (l) are not satisfactorily identified—they may represent residues of phagocytosis. The considerable variation in density and apparent thickness shown by the membrane limiting the profiles of the endoplasmic reticulum can be correlated with variations in the angle of cutting. The variation is particularly noticeable for the larger profiles (c and l). Some vesicles (c) contain granular material while others (b) appear to be broken. × 23,800.
(Palade and Porter: Endoplasmic reticulum. I)
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Fig. 4. Electron micrograph of a section through a monocyte in a buffy coat preparation (chicken). The slightly indented nucleus of the cell is visible at n; its membrane appears double wherever normally sectioned. At m is a mitochondrial profile and at g profiles of dense structures, apparently granules or granules enclosed in a vacuole, which may have some relation to the azurophil granules described by light microscopists in this type of cell. The numerous “hollow” profiles (c, o, e) found in the cytoplasm are taken to represent the elements of the endoplasmic reticulum. A circular profile like c may correspond to a vesicle or a transversely cut tubule; an oval profile like o may represent a normal section through a flattened vesicle or an oblique section through a tubule; an elongated profile like e most probably corresponds to a tubule sectioned longitudinally or very obliquely. The majority of these elements appear scattered in the cytoplasm as independent structures; the occurrence of a row of circular and irregular profiles at r suggests, however, that in three dimensions many of these apparently independent elements were linked in a continuous network of cavities like the endoplasmic reticulum of the macrophage in Fig. 1 and that most of their connections have been severed by sectioning. × 23,800.

Fig. 5. The electron micrograph shows part of a chicken macrophage in a spleen section. The cell limits are visible at cm in close apposition to the membranes of neighboring cells. The dense, homogeneous material between the cells is probably blood plasma. The field contains part of a deeply indented nucleus (n) and numerous mitochondrial profiles, one of them marked m. Hollow profiles of circular (c), oval (o), elongated (e), and irregular (i) shape, scattered throughout the cytoplasm, are taken to represent sections through the vesicles and tubules of the endoplasmic reticulum. A few elongated profiles show some evidence of branching. The skin of elongated profiles at cs represents the same type of membranous structure as that encountered in the centrosphere region in Fig. 3. × 23,800.
In Figs. 6 and 7 a comparison is made between the cytoplasmic appearance of rat mesothelial cells in vitro and in situ.

**FIG. 6.** Electron micrograph of a small field in the peripheral cytoplasm of a mesothelial cell grown in vitro and examined in toto. The culture developed out of an explant taken from the margo acuta of the liver of a newborn rat; the cell belongs, therefore, to the peritoneal mesothelium.

The cell margin appears at mg, a lipid inclusion at l, and a few mitochondria at m. The trabeculae of the endoplasmic reticulum are in general slender structures more or less uniform in diameter (t); as such they most probably correspond to tubular structures. In some places, however, they show successive dilatations (d) which may be interpreted as intermediary forms between tubules and strings of vesicles (v). At some of the nodal points of the network larger elements of irregular shape (ci) are present. The upper membrane of the cell has a number of foci (f) that obscure in part the disposition of the reticulum because they have the same density as its trabeculae. × 19,000.

**FIG. 7.** The electron micrograph shows cytoplasmic fields in two adjacent mesothelial cells fixed in situ and examined in a section. They were part of the mesothelial layer of the peritoneum covering the intestine of a young rat.

The cell membrane (cm1) facing the peritoneal cavity bears fine finger-like projections (mn1) or “microvilli” (26); some are in continuity with the cell surface while others have been cut nearer their free ends and appear as independent, circular, or oval profiles in the peritoneal cavity (mn2). The membrane itself has been cut very obliquely and consequently shows up as a broad, poorly outlined band. The membrane facing the connective tissue of the serosa can be seen at cm2. At cm3 appear the apposed membranes of the two adjacent mesothelial cells, which show successive thickenings and densifications along the line of cell junction. At their level (ap) the two membranes are maintained in close parallelism, whereas between them they may pull apart, leaving behind intercellular spaces (is). The membrane thickenings along the junction line (adhesion plates) are very similar to those described by Porter (27) between epidermal cells at the level of the so called intercellular bridges.

A few mitochondrial profiles can be seen at m; the numerous “hollow” profiles encountered in the cytoplasm are taken to result from the sectioning of the endoplasmic reticulum. Note that profiles of circular (c) or oval (o) shape predominate and that most of them appear as independent structures as a result of the sectioning of the cell; a few short strings of vesicles (r1, r2) happened to be included in the thickness of the section. Note also that the profiles have a homogeneous and relatively dense content.

Note finally the numerous small vesicles found immediately below the cell membranes facing the peritoneal cavity and the connective tissue; they are reminiscent of the vesicles described recently in endothelia (28). × 19,000.
(Palade and Porter: Endoplasmic reticulum. I)
Figs. 8, 9, and 10 illustrate an experiment made for comparing cytoplasmic structures found in nephron epithelia in vitro and in situ. Epithelia grown in vitro were examined in parallel in whole mounts (Fig. 8) and in sections (Fig. 9), while epithelia in situ were studied exclusively in sections (Fig. 10). All three electron micrographs represent epithelia taken from the same animal; all are reproduced at the same magnification, namely 23,800.

Fig. 8. Electron micrograph of a small field in the cytoplasm of an epithelial cell grown in vitro from a kidney explant taken from a newborn rabbit. The cell was examined in a whole mount, without shadowing.

The nucleus and the cell margins lie outside this field, which contains only a few filamentous or rod-like mitochondria (m) and part of the endoplasmic reticulum of the cell. The reticulum consists of interconnected, round (v1), oval (v2), and elongated (v3) elements linked in some places by thinner trabeculae (s). Few isolated elements of similar shape and electron density are present in the cytoplasm.

The cytoplasmic matrix is relatively dense and has a mottled appearance. This is taken to indicate that in kidney epithelia the cytoplasmic matrix is more difficult to extract by a long fixation than in the cell types previously shown (Figs. 1, 2, 3, and 6). X 23,800.

Fig. 9. The electron micrograph shows a small field in the cytoplasm of two adjacent epithelial cells which were part of a well formed tubule found close to the explant in a sectioned kidney culture. In its general morphology the tubule was similar to the portion condensens (thin limb of Henle's loop) of the adult nephron. The membrane limiting the cell against the lumen appears at cml; the apposed membranes of the adjacent cells at cm2. Their continuity is interrupted in a few places and this probably represents an artifact developed during or after fixation.

A mitochondrial profile appears at m. The “hollow” profiles of circular (c), oval (o), elongated (e), or irregular shape are taken to represent the endoplasmic reticulum. Their linkage in a threedimensional network is suggested by the presence of a branching (b) and of a row of profiles (r). Note the roughness of the outside surface of the membrane limiting the vesicular and tubular elements of the reticulum and the agglomeration of small dense granules around these elements and in contact with their membrane. In a few places, where the section apparently exposes the limiting membrane over a relatively large surface (g), the small granules appear to be disposed in linear series.

At cs, in the centrosphere region, note the presence of tightly packed clusters of circular and elongated profiles similar to those found in Figs. 3 and 5. X 23,800.
Fig. 10. Electron micrograph showing a small field in the cytoplasm of two nephron epithelia fixed in situ in the kidney of a newborn rabbit and examined in a section. The cells belonged to a nephron segment which had the general morphology of the thin limb of Henle's loop (portio conducens); they are therefore of the same type as the cells shown in Fig. 9.

The cell membrane (cm) bears microvilli (mv) where it faces the lumen, is coated with a basement membrane (bm) where it forms the outside surface of the tubule, and shows an adhesion plate (ap) and interdigitations along the cell junction.

In addition to a few mitochondrial profiles (m), numerous “hollow” profiles of circular (c), oval (o), and elongated (e) shape can be seen in the picture. They are taken to represent the vesicles and tubules of the endoplasmic reticulum; their linkage in a continuous network is suggested by the presence of a few short rows of profiles (c, o). As in Fig. 9, the outside surface of many of these profiles is covered with small dense granules, and similar granules appear scattered in the matrix of the cytoplasm. X 23,800.

Fig. 11. The electron micrograph shows part of an endothelial cell in a section through the wall of a blood capillary in the myocardium of a newborn rabbit. The nucleus of the cell, visible at n, is limited by a membrane which appears distinctly double whenever normally sectioned. The cell membrane facing the pericapillary spaces can be seen at om; it is covered by a layer of relatively dense material (bm) which is poorly outlined towards the pericapillary spaces and corresponds, most probably, to the basement membrane of the capillary.

Numerous “hollow” profiles of circular (c), oval (o), and elongated (e) form are present in the cytoplasm and are separated from the matrix by a limiting membrane the outside surface of which is smooth in some cases (c, o) and heavily dotted with small dense granules in others (e). The two kinds of profiles do not necessarily represent two different structures; they are actually differentiated segments of a single structure, the endoplasmic reticulum, as indicated by the occasional continuity between segments with and without granules. Such a continuity is clearly visible in this figure at 11 and 12.

Dense bands of granular appearance (g) link some of the profiles in the picture; at their level the knife apparently cut tangential to the membrane of the endoplasmic reticulum, exposing its grain-dotted surface over relatively large areas. Besides showing the abundance of attached granules, these dense bands help in understanding the linkage of the variously shaped profiles found in this section in a common tridimensional reticulum. X 26,600.

Fig. 12. The electron micrograph shows part of a mesothelial cell grown in vitro from an explant containing peritoneal mesothelium (newborn rat). It was less flattened than tissue culture cells usually selected for electron microscopy and for this reason its cytoplasm was disposed in a relatively thick layer.

The nucleus of the cell is out of the field; the cell margin, poorly outlined, appears at cm, and a few mitochondria at m. The endoplasmic reticulum in this part of the cytoplasm consists of two superimposed reticular sheets: the first one, which spreads over the entire field, exhibits the usual reticular appearance in the upper right corner of the picture (er) and has a number of large cisternae in the rest. The cisterna marked ci is particularly broad as it extends over 7 μ. Projected on this cisterna as a background, appears the second reticular sheet which spreads through only the central zone of the field and contains a number of smaller cisternae (ci).

The density of the second reticular sheet is noticeably greater than that of the first; this is not due to its intrinsic high density, but to its almost complete superimposition on a large cisterna belonging to the first reticular sheet. The density at the level of ci is due to four superimposed membrane layers (two belonging to the first reticular sheet and the other two contributed by the cell membrane), whereas at the level of ci the density results from the superimposition of six membrane layers (the four already listed and two belonging to the second reticular sheet). X 9,050.
Figs. 13 to 17 compare the cytoplasmic appearance of endothelial cells grown in vitro with that of endothelial cells examined in situ. All cells shown in these electron micrographs are blood vessel endothelia obtained from newborn rabbits.

Fig. 13. Electron micrograph of a relatively large field in the cytoplasm of an endothelial cell grown in vitro from an explant containing umbilical vessels and examined in toto after light shadowing with chromium. A few lipid inclusions (l) and numerous mitochondria (m) appear in this field which contains in addition elements comparable in electron density to the vesicles and tubules of the endoplasmic reticulum shown in the previous examples. Some of these elements are also of comparable size and shape (e.g. circular at v₁, oval at v₂, and elongated at t) to appearances found in Figs. 1, 6, and 9 and may similarly correspond to vesicles and tubules in the living cell. But other elements (c₁, c₂, c₃) are of much larger size, and irregular outline. They represent relatively vast, flattened vesicles described in this paper as cisternae. A few of them are fenestrated (fₕ) and all of them appear to be part of a common reticulum together with the vesicles (v₁, v₂) and tubules (t) already mentioned.

The upper membrane of the cell, through which the various cytoplasmic components are seen, has a number of fine converging folds (m). × 6,450.

Figs. 14 and 15. Fig. 14 shows at the same magnification (× 6,450) part of an endothelial cell in situ in the wall of a blood capillary of the myocardium. The cell membrane faces the lumen at im and the pericapillary spaces at om; a cell junction appears at j and part of an erythrocyte at ec. To facilitate the identification of the formations present in the cytoplasm, part of the same endothelial cell is shown at a higher magnification, i.e. × 24,300, in Fig. 15. The section contains three mitochondrial profiles, one of them marked with m, and a number of “hollow” profiles of circular (c) and elongated (e) form. The latter may represent longitudinal sections of tubules, but also normal sections through cisternae. Note the presence of a few small vesicular profiles (v) immediately below the cell membrane, as described in a recent paper (28).
FIGS. 16 and 17. The electron micrographs represent two serial sections through an endothelial cell in the wall of a blood capillary of the myocardium. The nucleus, sectioned close to its surface, appears at \( n \) and a mitochondrial profile at \( m \). The cell membrane faces the lumen at \( im \) and the pericapillary spaces at \( om \). Note the layer of relatively dense material coating the outer membrane; it corresponds, in all probability, to the basement membrane of the capillary. The junction of two adjacent endothelial cells can be seen at \( j \); along it, at \( ap \), the apposed membranes are thicker and denser than in the rest. The thickening may represent an “adhesion plate.”

The profiles marked \( o, ci_1, ci_2, ci_3 \) are taken to represent the endoplasmic reticulum. \( o \) shows little change from one section to the next and hence it can correspond either to a tubule sectioned obliquely or to a narrow cisterna. \( ci_1 \) appears as an elongated profile in Fig. 16 forming a row with \( ci_1' \) and \( ci_1'' \); in Fig. 17 the three of them run together in a common elongated profile (\( ci_{1i} \)). On the other hand the elongated profile \( ci_2 \) of Fig. 16 breaks down into two separate profiles, \( ci_3 \) and \( ci_2' \), the latter but slightly touched by the section in Fig. 17. A nearby confluence between \( ci_3 \) and \( ci_2 \) is suggested in Fig. 16 by the band of dense material that links these profiles.

The analysis of the two successive sections indicates clearly, therefore, that most of the profiles mentioned are part of a common system.

The thickness of the sections being evaluated at approximately 50 to 60 \( \mu \), it follows that this system, i.e. the endoplasmic reticulum, has been reconstructed through a depth of 100 to 120 \( \mu \). The diameter of \( ci_1 \) in Fig. 16 is \( \approx 70 \mu \); if \( ci_1 \) were a tubule, its cavity would show signs of closing from one section to the next, or at least its lumen would appear narrower. The fact that this is not so indicates that the profile corresponds to a cisterna, as do \( ci_2 \) and \( ci_3 \). The occurrence of large cisternae in the endoplasmic reticulum, similar to the cisternae present in Fig. 13, explains the frequency of elongated profiles and the scarcity of circular and oval ones in the sections of this cell; the reverse situation would be expected in the case of a reticulum made up of tubes and strings of vesicles. The interruptions shown by \( ci_1 \) in Fig. 16 may correspond to a zone of confluence of smaller elements in a cisterna or to the presence of fenestrac interrupting its continuity.

Note that the cisternae appear to have a rather constant “depth” and that in this case their homogeneous content is more dense than the matrix of the cytoplasm. \( \times 29,600. \)
Figs. 18 and 19 illustrate an experiment in which cytoplasmic structures in glandular epithelia of the parotid were studied in parallel in \textit{vitro} (Fig. 18) and in \textit{situ} (Fig. 19). The respective specimens came from the same animal, namely a newborn rat.

Fig. 18. Electron micrograph of a small field in the cytoplasm of an epithelial cell grown \textit{in vitro} and examined \textit{in toto}, without shadowing.

In addition to a few lipid inclusions (l) and mitochondria (m), the electron micrograph shows part of the endoplasmic reticulum of the cell, which appears to be made up of more or less even trabeculae (t1, t2) with large, irregular elements (c1, c2, c3) at its nodal points. In three dimensions the strands correspond most probably to tubules and the irregular elements to small cisternae. Larger cisternae, approaching in size those present in Fig. 13, were present in the rest of the cell.

Most of the trabeculae have a more or less homogeneous and rather light electron density which can be contrasted with the higher and unhomogeneous density of the cisternae. The surface of the latter appears to be dotted with small, dense granules that cover them in part (c1, c2) or in entirety (c3). The figure shows clearly that elements with and without granules are in continuity throughout the entire network, a finding taken to indicate that they are differentiated segments of a single structure, the endoplasmic reticulum. The visualization of the small granules covering the membrane of the endoplasmic reticulum has been helped in this instance by the fact that the cell membrane was accidentally stripped away, leaving the cytoplasmic structures directly exposed. × 24,100.

Fig. 19. The electron micrograph shows parts of two adjacent cells in an acinus of the parotid gland fixed \textit{in situ} and examined in a section.

The cell membranes appear at cm, the nucleus of one of the cells at n, and a mitochondrial profile at m. The cytoplasm is occupied by a large number of profiles which are taken to result from sections through the endoplasmic reticula of the two cells. Among these profiles, those of elongated shape (e) are clearly predominant, whereas those of circular (c) and oval (o) form are in the minority. This finding strongly suggests that the reticula contain many cisternae similar to those found in Figs. 13, 16, 17, and 18. Interruptions like those labeled b indicate either the presence of fenestrae in these cisternae or their branching into smaller elements. What complicates the situation in this case, is the fact that the profiles are oriented more or less parallel to one another and appear relatively regularly spaced. In three dimensions this would correspond to a layering of reticular sheets, each containing numerous and relatively vast cisternae.

Note that nearly all the elements of the endoplasmic reticulum have the outside surface of their limiting membrane covered with numerous small granules which appear in profile at p, where the membrane was normally sectioned, and in full-face view at f, where it was cut obliquely. × 24,100.
(Palade and Porter: Endoplasmic reticulum. 1)