THE ENDOPLASMIC RETICULUM*

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PLATES 31 TO 38

History

Original Findings on Cultured Material.—The relatively short history of the endoplasmic reticulum started 10 years ago, in 1945, when Porter, Claude, and Fullam (1), noted the presence of a "lace-like" reticulum in the ground substance of cells grown in tissue culture and examined *in toto* in the electron microscope. In describing their findings, these authors mentioned that "at higher magnifications vesicle-like bodies, that is elements presenting a center of less density and ranging in size from 100 to 150 mμ, appear arranged along the strands of the reticulum." In retrospect, it can be said that two of the main features of this new cytoplasmic system, namely (*a*) its reticular disposition and (*b*) the vesicular character of its component elements, were already clearly noted in its first description. In subsequent papers, Porter and his collaborators described the preferential concentration of the vesicular elements of the reticulum in the endoplasm, and their scarcity or absence in the supposedly exoplasmic periphery of the cytoplasm (2, 3), a finding which eventually led to the selection of "endoplasmic reticulum" as a name for the system. The term was first cautiously tried in a caption in 1948 (3) and finally used in an article published by Porter and Kallman in 1952 (4). It appears that, at that time, our group was not yet engaged in large scale production of new cytological terms with a heavy Latin flavor, and was still proceeding with cautious restraint in matters of nomenclature. Besides the reticular disposition and the endoplasmic location implied in the name, Porter's studies established a number of other important features for the new cytoplasmic component, namely the usual continuity of the system throughout the endoplasm of normal cells, the remarkable polymorphism of its component elements, and the breaking down of the entire system in cytolysis into a collection of isolated vesicles.

The Shift from Spread to Sectioned Cytological Specimens.—The information obtained from the study of cultured cells was presented by Porter in a final article in 1953 (5), at a time when new embedding and microtomy procedures

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(6) were rapidly replacing spread cells by sectioned cells as cytological specimens in electron microscopy. Already Dalton (7, 8) and Bernhard (9, 10) and their respective collaborators had described in sectioned cells various filamentous and laminated structures in the dimensional range of the elements of the endoplasmic reticulum, but no clear correlation with the latter was established. Further improvements in fixation and microtomy procedures (11–13) rendered possible the study of sectioned cells at a level of resolution equal to, or better than, that previously obtained with cultured specimens. As a result, it became feasible and profitable to examine in the electron microscope cells *in situ*; *i.e.*, cells in their usual relationship within tissues, organs, and organisms. Evidently the development had considerable importance and far flung implications. It opened for investigation a "wide" range (from 2000 A down to 20 A) in the "submicroscopic" realm of biological organization, a virgin territory, extremely rich and diverse, which promises to yield in time a disturbingly abundant crop of morphological literature. There was nothing revolutionary in the technical improvements mentioned, yet they caused a real and spectacular upheaval in the then dormant field of biological morphology which since then has traversed a period of intense activity, reminiscent of a gold rush in its general conditions and atmosphere. Only filaments, membranes, and particles have taken the place of more conventional nuggets. The great rush is still on, judging by the number of papers presented at this conference and by the multitude of membranes and particles to which they refer.

Besides these consequences of rather general import, the shift from spread to sectioned cytological specimens had other results of a more technical and practical nature. For instance, it changed entirely the geometrical conditions under which observations are made. The angle of cutting, the direction of cutting, and the relation between the size of the structure under study and the thickness of the sections, all became important factors in the interpretation of the results (*cf.* reference 14).

Consequences of the Shift for the Study of the Endoplasmic Reticulum.— In the case of the endoplasmic reticulum, the most important geometrical factor proved to be the relative thickness of the sections used for electron microscopy. The usual thickness of such sections, *i.e.* 20 to 40 mμ, being much smaller than the mesh size of the reticulum, and even smaller than the diameter of the vesicles and tubules that form its trabeculae, it follows that in sections only slices of these trabeculae or "profiles" can be found. Fragments of meshes, or more or less complete meshes are only occasionally encountered and the continuity of the system throughout the cytoplasm is never apparent; it has been lost by microtomy and can be regained only by the difficult and tedious operation of tridemensional reconstruction (12, 15; *cf.* references 14, 16). In this respect, one may comment that the use of spread cells as initial specimens for the study of the endoplasmic reticulum was a fortunate coincidence. The

main feature of the system, *i.e.* its disposition in a continuous reticulum that permeates the entire cytoplasm, would have been extremely difficult if not impossible to grasp from an exclusive study of sections. It is then easy to understand why, at the beginning at least, the shift from spread to sectioned specimens caused so much confusion about the endoplasmic reticulum. In the few years which have elapsed, a number of conflicting descriptions and interpretations have been advanced and a corresponding number of names proposed for the structures belonging to the system under consideration. A vacuum in terminology, be it only apparent, seems effectively to lead many a biologist into philological temptation.

Since one of the characteristic features of the system, *i.e.* its reticular disposition, is lost by sectioning, we are faced with a difficult problem, namely that of the criteria to be used for recognizing the endoplasmic reticulum in sections. The comparative study of cultured cells examined *in toto* and in sections (15) has suggested the use of the following features as identification criteria: (*a*) the size of the profiles—expected to vary from 50 to 300 mμ; (*b*) their composite structure, consisting of a relatively light content surrounded by a dense, thin membrane; (*c*) finally their apparent lack of internal structure: the content appears homogeneous at the present level of resolution. It is admitted that these criteria are entirely morphological in nature and, moreover, do not possess a narrow specificity. We equate all the profiles that have the features mentioned and consider them as parts of a common, cell-wide system because of our previous knowledge of the situation found in spread specimens and because we assume that structural elements which are similar in morphology are also similar in other respects. Actually the basic assumption for this broad generalization can easily be questioned. Biochemical and functional differentiation may well exist among morphologically similar structures. But at the present moment in the development of our knowledge, the morphological criteria mentioned, imperfect as they are, are the only ones available. We feel therefore justified in using them, although we understand that our interpretations remain subject to revision by further studies in cytochemistry and cell physiology.

Description of the Endoplasmic Reticulum in Various Cell Types

The difficulties introduced in the study of the endoplasmic reticulum by the use of sectioned specimens have been well compensated for by the wealth of new information obtained from sections. It became possible, for instance, to carry through a broad survey of different cell types from various sources and the survey showed that the system is present in all the cells thus far examined with the exception of the mature erythrocyte (17). It can be assumed, therefore, that an endoplasmic reticulum is present in all animal cells and as such represents an important piece of equipment in the organization of such cells.

The survey also showed that the system varies considerably in total volume, general disposition, and detailed structure from one cell type to another, and that these variations are not haphazard; on the contrary, a certain type of reticulum was found to characterize a certain cell type or a group of cell types. There are, for instance, cell types endowed with a reticulum which shows a limited amount of organization (17); cell types which always exhibit a highly organized reticulum (18–21); and, between these two extremes, a number of characteristic, intermediate situations. These findings are taken to indicate that the endoplasmic reticulum is greatly affected by, or involved in, the process of cell differentiation. The results of our survey are illustrated by the following series of examples presented according to the increasing degree of organization shown by the corresponding endoplasmic reticula.

Fig. 1 shows the situation encountered in seminal epithelia; namely, in a rat spermatocyte. The reticulum is mostly represented by circular and oval profiles. Elongated profiles are few and short. Most of these profiles appear to be scattered individually and at random throughout the cytoplasm from the cell membrane to the nuclear envelope. There are a few rows of profiles which occasionally outline mesh fragments. The examination of a large number of sections of various incidences, as well as the examination of serial sections indicates that in three dimensions these profiles correspond to vesicles and short tubules disposed in strings interconnected in a randomly disposed reticulum. A point which deserves comment concerns the limiting membrane of these vesicular elements. Most of them are bounded by a smooth membrane, and only occasionally vesicles are encountered which bear a few dense particles attached to their outer surface (17). In summary, seminal epithelia appear to be characterized by a randomly disposed, predominantly smooth surfaced reticulum, made up primarily of interconnected vesicles and tubules. A similar type of reticulum is encountered in other cell types, for example in mature leucocytes, in mast cells, and in the oxyntic cells of the gastric mucosa (22). It may be of interest to note that a similar type of reticulum is also present in cells specialized in one form or another of lipide metabolism such as adipose cells, cells of the brown fat, and cells of the adrenal cortex (17).

Fig. 2 shows, as another example in the survey mentioned, a macrophage forming the lining of a splenic sinus (rat). The profiles ascribable to the endoplasmic reticulum fall clearly into two categories: (*a*) smooth surfaced profiles, concentrated at the periphery of the cell, and (*b*) rough surfaced profiles, present in the central region of the cytoplasm. These latter bear small attached particles on the outer surface of their limiting membrane. Besides this regional distribution, one can point to a number of other new features, such as the predominantly elongated shape of the rough surfaced elements and the fact that they are disposed in more or less parallel rows.

Some of these features are more striking in the next example which shows a

parenchymal cell of the liver (Fig. 3). Smooth surfaced profiles appear in agglomerations usually located at the periphery of the cell. In three dimensions they correspond to tightly packed, randomly disposed networks (20). As in the case of the spleen macrophage, the rough surfaced profiles have frequently a regional distribution, are predominantly of elongated shape, and show preferred orientation; *i.e.*, they are disposed parallel to one another at more or less regular intervals in arrays or skeins (23, 20). Theoretically these elongated profiles could represent longitudinal sections through tubules, but they are more frequently encountered than reasonably expected (15, 24). Accordingly, it may be inferred that in this case, the endoplasmic reticulum includes not only vesicles and tubules, as in the first example, but also some other structural elements. The examination of serial sections and of sections of various incidences indicates that the elements in question are relatively large, flattened vesicles of irregular outline, but of shallow and relatively constant depth for which the name of *cisternae* has been recently proposed (15). Because such elements were not originally described in cultured cells examined *in toto* (5), spread specimens were reexamined (15) and in many cases the endoplasmic reticulum was found to consist mainly of large, flat cisternae of irregular outline with only a few tubular and vesicular elements present. Even in such instances, however, the continuity of the system throughout the cytoplasm and, to a certain extent, its reticular disposition were found to be maintained. A whole spectrum of intermediary forms exists between such cases and the usual reticular appearance of the system originally described by Porter (5). These findings suggest that the endoplasmic reticulum can change from a relatively bulky, predominantly cisternal form to a finely divided condition in response to still obscure causes. In the light of these observations, it can be concluded that the elongated profiles found in sections of parenchymal hepatic cells represent normal, or nearly normal sections through cisternal elements. As already mentioned, these elongated profiles are frequently disposed parallel to one another in more or less extensive arrays (23, 20). The latter correspond in three dimensions to stacks of cisternae which represent preferentially oriented portions of the endoplasmic reticulum. The factors responsible for this orderly disposition are unknown. The arrangement may be the result of an interplay of repulsion and attraction forces, because the arrayed cisternal elements respect a minimal spacing of $\sim$150 mμ and appear in stacks even when there is no crowding or packing.

One may wonder if in such cases we can still speak of a reticulum. We believe we can, because these layered cisternae appear to be frequently connected by anastomoses and branchings. Notwithstanding the preferred orientation of some of its segments, the system has not broken down into isolated sacs, it is still continuous and, when considered in its entirety, still reticular.

The stacked cisternae are sometimes secondarily oriented parallel to the nu-

clear surface, the cell walls, or even the surface of a mitochondrion, but these dispositions are quite variable and as such do not appear to have functional significance. In muscle fibers, however, the network is regularly oriented around other cell components, *i.e.* the myofibrils, and this orientation probably has functional implications, as suggested by Porter (25) and Bennett (26) during this conference.

A point which deserves particular attention is the existence of two types of profiles; *i.e.*, smooth surfaced and rough surfaced ones, in the last examples. In this relation, one may question whether or not we are justified in considering them as belonging to the same system. It may be argued that morphologically they are distinct enough to be considered as two different types of structures, possibly two different cell organs. It should be pointed out, however, that continuity of membrane and content is relatively frequently encountered between rough surfaced profiles and smooth surfaced ones in sectioned cells of various types (15, 17, 19–21). Reverting to spread specimens one can find similar and equally convincing examples (15). In our belief, such cases of continuity between the two types of elements are convincing and frequent enough to support the conclusion that they represent two local differentiations within a common system, rather than two different, unrelated cytoplasmic structures.

An endoplasmic reticulum, similar in its general disposition to that described in parenchymal hepatic cells, is encountered in perikarya where the so called Nissl bodies represent agglomerations of rough surfaced profiles (19).

The next example in the series is represented by the acinar cell of the pancreas (Fig. 4). In this case the smooth surfaced part of the endoplasmic reticulum is reduced to a minimum, whereas the rough surfaced part is highly developed and actually takes up most of the cytoplasmic volume. The reticulum shows preferred orientation in the basal half of the cells and appears to be distributed at random in the apical pole (21).

Finally the last example is provided by a plasma cell (Fig. 5) in which, as in the acinar cells of the pancreas, there are very few smooth surfaced elements in the reticulum. In this case, however, the network usually shows preferred orientation throughout the entire cytoplasm. As a result of the appreciable increase in volume of the endoplasmic reticulum, the cytoplasmic matrix of plasma cells and pancreatic exocrine cells occupies a relatively small space and appears to be disposed in a network complementary to that of the system under study.

The preceding examples indicate that the differentiation of the endoplasmic reticulum from one cell type to another is obtained by varying (*a*) the total and relative volume of the system, (*b*) the extent of its preferred orientation, and finally (*c*) the relative importance of the two local differentiations mentioned. Rough surfaced elements, for instance, are scarce in seminal epithelia and become preponderant in acinar cells and in plasma cells. It should be

added that the particles attached to the rough surfaced elements of the endoplasmic reticulum represent a distinct cytoplasmic component, recently described in some detail, which can occur freely distributed in the cytoplasmic matrix (27). Consequently the rough surfaced segments of the endoplasmic reticulum appear to be the result of the association of two basically distinct components: the membrane (or the vesicles) of the reticulum and the dense, small particles of the matrix.

A Critique of the Literature Bearing on the Endoplasmic Reticulum

After describing the endoplasmic reticulum along its general lines as they have gradually evolved from studies carried through by our group, I shall try to integrate this presentation with other contemporary descriptions of the same structure. Because of their unusual appearance, the oriented parts of the reticulum were the first to attract the attention of electron microscopists. They have been described in the past as filaments (7) or lamellae (8), sometimes as ergastoplasmic filamentous or lamellar structures (9, 10, 28). In many cases these "lamellae" were probably the layers of cytoplasmic matrix compressed between the distended cisternae of the reticulum. I believe that at present there is general agreement in considering such filaments and lamellae as preliminary descriptions affected to a variable extent by artifact production.

Another concept bearing on the subject is that of "cytoplasmic double membranes," or "double-edged membranes" of Sjöstrand and his collaborators (29–32). Some of these double membranes correspond to infoldings or interdigitations of the cell membrane and were recently recognized as such by Rhodin (31) and Pease (33). Some others, like those studied in the pancreas by Sjöstrand and Hanzon (32) represent the cisternae of the endoplasmic reticulum. "Double membrane" is a relatively ambiguous term; it may apply equally well to a folded membrane, to two apposed membranes, to a flattened vesicle, *i.e.* cisterna according to our dictionary, and finally to a solid lamella made up of layers of different densities. The last interpretation is favored by Sjöstrand (29) but I believe that the first three interpretations come closer to reality, each of them for a certain number of cases.

Finally there is the concept of "ergastoplasmic sacs" advanced by Weiss (34) and the related concept of "intracytoplasmic sacs" proposed by Watanabe (35). These sacs correspond to the rough surfaced elements of our description. Here the point in dispute is the continuity of the system. In Weiss's and Watanabe's interpretation the system is considered as a collection of isolated sacs not as a continuous network of cavities.

In these various disputes we have the advantage of a favorable background represented by the information collected on spread cells examined *in toto*, and by the information obtained in surveying a large number of different cell types. In cultured cells examined *in toto*, the continuity of the system is obvious.

From the survey, we learned that ergastoplasm and endoplasmic reticulum are not synonymous and also that the cisternae, one possible type of "double membranes," are not the only elements of the endoplasmic reticulum. The "intracellular cytoplasmic membranes" of Sjöstrand and Hanzon (32), the "ergastoplasmic sacs" of Weiss, and the "intracytoplasmic sacs" of Watanabe represent only a differentiated part of the system.

This review of the literature may sound to you like the refutation ritual at the presentation of a thesis in an old university. After disposing of the arguments of my opponents, I should, according to the ritual, proclaim the endoplasmic reticulum as a piece of lasting and immutable truth. Actually I shall confess that the original concept has already mutated a number of times during the last few years; for instance the common sense of the word "reticulum" was stretched a little to accommodate preferentially oriented dispositions of the system, whereas the qualification introduced by the adjective "endoplasmic" was frequently found to be too narrow. In many cells *in situ* the elements of the reticulum are found more or less evenly distributed throughout the cytoplasm from the nucleus to immediately below the cell membrane (17). Only in certain cell types distinguished by a relatively dense, fibrillar, cortical layer, like the cells of the intestinal epithelium, are there fewer vesicles in this "exoplasmic" layer than in the endoplasm. It is possible that the situation encountered in tissue culture specimens is due either to excessive spreading or to a displacement taking place while the specimens are drying. It is evident therefore that the name "endoplasmic reticulum" has a number of admitted shortcomings. We retain it because at present we do not have a better one, but we consider it as a temporary label to be used until a more appropriate name descriptive of the morphology or preferably the physiology of the system is found. There are some hopes in this respect so that people displeased with the present label may not have long to wait.

More Recent Information on the Endoplasmic Reticulum

This presentation has dealt thus far with those features of the endoplasmic reticulum which at present appear to be satisfactorily established. Recent work has added to this body of knowledge new information which will be presented briefly in what follows. The existence of two membranes around the nucleus has been known since Hartmann's work (36). Also known was the distinctive presence of attached particles on the outer nuclear membrane (27). During the last year, Watson (37) showed that in certain cells of the spleen, tentatively identified as reticulocytes, the outer nuclear membrane is continuous with the membrane of the endoplasmic reticulum. In such cases he found that the space in between the two nuclear membranes was in continuity with the cavities of the reticulum. Similar appearances were recently encountered in lymphocytes, granulocytes, macrophages (17), parenchymatous liver cells, and acinar cells

of the pancreas. They suggest that the "nuclear envelope" is another local differentiation of the endoplasmic reticulum: a perinuclear cisterna.

Dalton and Felix (38) and following them numerous other workers (39, 31, 40–42) have identified an agglomeration of membranous structures in the centrosphere region of the cell with the Golgi apparatus of classical cytology and have described in these agglomerations three different components; *i.e.*, lamellae, vacuoles, and granules. The profiles found in such an agglomeration are similar to the smooth surfaced profiles of the endoplasmic reticulum from which they are distinguished by smaller diameters, tight packing, and preferred orientation. As indicated by the situation found in many cell types, all possible intermediates are encountered between the supposedly typical profiles of the Golgi complex and the usual profiles of the endoplasmic reticulum (Fig. 6). In cell types with a predominantly smooth surfaced reticulum, such as seminal epithelia, it is difficult to ascertain whether or not continuity is established between the two structures, because we do not have definite criteria for distinguishing the profiles of the centrosphere region from the smooth surfaced profiles of the endoplasmic reticulum (17). Occasionally, however, continuity of content and limiting membranes is found established between typical Golgi elements and rough surfaced profiles which can be safely recognized as elements of the endoplasmic reticulum on account of the particles attached to their outer surface. Such appearances have been described in seminal epithelia (17), in perikarya (19), and have been noted in the olfactory epithelium and in the acinar cells of the pancreas.

At a particular stage in the evolution of rat spermatids, namely after the formation of the acrosome, and after the caudal migration of the Golgi apparatus, rows of profiles appear disposed parallel to one another in the immediate vicinity of the Golgi complex. They have the large spacing characteristic of the endoplasmic reticulum, but do not have attached particles; instead there is a noticeable condensation of amorphous material around these profiles. Occasionally the arrangement is further complicated by the existence of radially disposed profiles at the end of this stack of fenestrated cisternae (Fig. 6). Apparently we are dealing in this case with still another local differentiation of the endoplasmic reticulum, namely with a temporary differentiation, clearly connected with the evolution of the spermatid (17). The stacked, fenestrated cisternae of the spermatid are apparently similar to structures recently described as "annular lamellae" by Rebhun (43) and Swift (44).

Finally smooth surfaced profiles, similar to those belonging to the endoplasmic reticulum, are found in close contact with the cell membrane in numerous cell types. Some of these profiles are closed, whereas others appear to be partially or completely open at the level of the cell membrane. They can represent vesicles of the endoplasmic reticulum establishing contact with the cell surface, or small invaginations of the cell membrane forming cytoplasmic vac-

uoles or vesicles. Such appearances have been noted in the epithelium of the yolk salk by Dempsey (45), in the endothelium of blood capillaries (46) (Fig. 9), in the nephron epithelium by Rhodin (31) and by Pease (33), in the epithelium of the gall bladder by Yamada (47), in smooth muscle fibers (Fig. 8), and recently have been described as being particularly frequent in macrophages (48). In these latter, the periphery of the cell is occupied by large flat pseudopodia or "ruffles" separated by narrow infoldings of the cell membrane. The direction of many of these infoldings is continued inside the cytoplasm by rows of smooth surfaced profiles entirely similar to those of the endoplasmic reticulum (Fig. 7). Favorably oriented sections show that in three dimensions the rows of profiles correspond to reticular sheets opened in the bottom of an infolding of the cell membrane. Direct connections between these rows and the rough surfaced profiles of the central region have not been encountered, but connections between isolated smooth surfaced profiles and rough surfaced ones are frequent. The electron microscope findings in macrophages acquire particular significance when correlated with the intensive membrane activity exhibited by these cells *in vivo* and *in vitro*; *i.e.*, in tissue culture. As is known, the continuous movement of the "ruffles" results in the incorporation of droplets of fluid from the surrounding medium into the cytoplasm. The phenomenon described as "pinocytosis" (49) has been repeatedly demonstrated by cinematographic recordings in living cells (49–51). The appearances described in the electron micrographs of macrophages suggest that pinocytosis extends down to the dimensional level explored at present by the electron microscope and that the incorporated cell membrane is fed into the smooth surfaced parts of the endoplasmic reticulum. The quantities involved in pinocytosis in macrophages (49) as well as the abundance of "submicroscopic" pockets and vesicles associated with the cell membrane in many other cell types (46) render unlikely a uni-directional flow of membrane material from the cell surface towards the interior of the cytoplasm. It is more probable that the membrane is repeatedly circulated between the two locations mentioned. In this general circulation the centrosphere region might play the role, among others, of a temporary depot of membranous material. Information thus far available suggests that pinocytosis at the "submicroscopic" level is a phenomenon of widespread cccurrence among animal cells. It follows that such cells in general may be capable of incorporating matter in "bulk" from the surrounding medium not only molecule by molecule or ion by ion as is usually assumed at present. Such findings will affect in time our ideas on the permeability of the cell membrane, although it remains to be found how much of the total exchange is dependent on pinocytosis.

The various connections described between the endoplasmic reticulum and other cell structures have been demonstrated thus far in a relatively limited number of cases. Undoubtedly more observations are required before accepting them as general features of the system. If repeatedly demonstrated they may throw some light on the functional role of the entire network.

With all these observations and findings taken into account, it is evident that the endoplasmic reticulum should not be considered a simple cell organ, but rather a complex system in which various organs and apparatus are integrated (52). Unfortunately we have thus far relatively little information on the biochemistry and the physiology of the system. What is available comes from cytochemical studies on the microsome fraction which, as indicated by recent work on liver (20) and pancreas (21), is mainly composed of fragments of the rough surfaced portion of the reticulum. It is still in doubt whether such activities as shown by the microsomes *in vitro* (incorporation of labelled amino acids into proteins and, by implication, protein synthesis)[1] should be ascribed to the entire structure or only to its associated ribonucleoprotein particles (53, *cf.* reference 27). Under these circumstances, the role played by the endoplasmic reticulum in the physiology of the cell is still a matter of speculation based primarily on the multifarious morphological findings already described. We believe that the most important feature of the system thus far uncovered is the existence of its apparently continuous membrane which separates two main phases in the cytoplasm facing each other, in many cases, over an unexpectedly large area. Such a device obviously provides the cell with a relatively large interior surface for various metabolic reactions. It may also function as an intracellular conductor (*cf.* references 25, 26), as an apparatus for directing and facilitating intracellular diffusion, or as a segregation apparatus (54). Its connections with the cell membrane may enable the system to function as an apparatus for the exchange of matter between the cell and the surrounding medium. In other words we may have in the endoplasmic reticulum a system involved in the import (52, *cf.* references 55, 56), export, and intracellular circulation of various substances.

In summary, it appears that the endoplasmic reticulum is a continuous network of membrane-bound cavities permeating the entire cytoplasm from the cell membrane to the nucleus. Within this network, there are a number of local differentiations some of them clearly established, others to be confirmed by further observations. The system is apparently in continuity with the cell membrane, at least intermittently, and extends down to the nucleus which it surrounds with a discontinuous, tridimensional moat.

It is realized that some of the features described for the endoplasmic reticulum contradict many concepts now prevailing in cell physiology. For instance the conflict with present ideas on the permeability of the cell membrane is evident. The situation amounts to a challenge for all interested in cell biology and as such it should not be deplored. Our function is not to provide confirmatory morphological evidence for old concepts, but to define a new morphological background against which old and new physiological data will be interpreted in the future.

[1] A short review of the literature on amino acid incorporation into microsomal proteins can be found in the Introduction of reference 22.

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EXPLANATION OF PLATES

PLATE 31

FIG. 1. Rat spermatocyte.

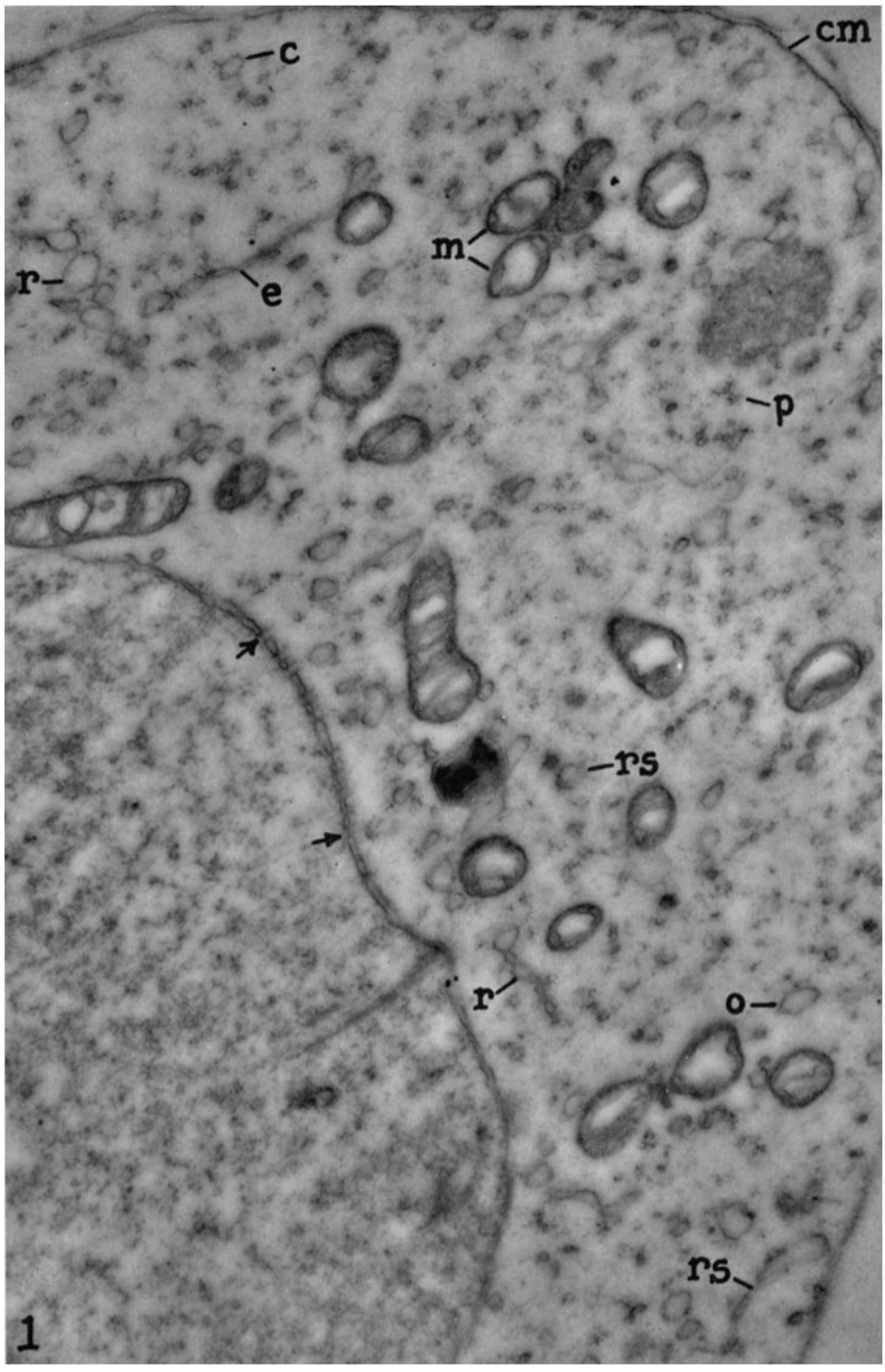
In this micrograph the endoplasmic reticulum (ER) is represented by numerous circular (*c*) and oval (*o*) profiles, isolated or in rows (*r*), and by a few elongated elements (*e*).

Note that most of the ER profiles belong to the smooth surfaced variety; *i.e.*, are limited by a smooth surfaced membrane. Profiles with a few dense particles attached to the outer surface of their membrane are relatively rare (*rs*).

In three dimensions, these various profiles correspond to vesicles and tubules interconnected in a randomly disposed reticulum. This is the simplest form the system takes in animal cells.

Numerous mitochondria (*m*) and clusters of small, dense particles (*p*) can be recognized in the cytoplasm. The cell membrane appears at *cm*.

Part of the nucleus is seen in the lower left corner of the figure, surrounded by a nuclear envelope provided with relatively numerous "pores" (arrows). × 26,000.



(Palade: Endoplasmic reticulum)

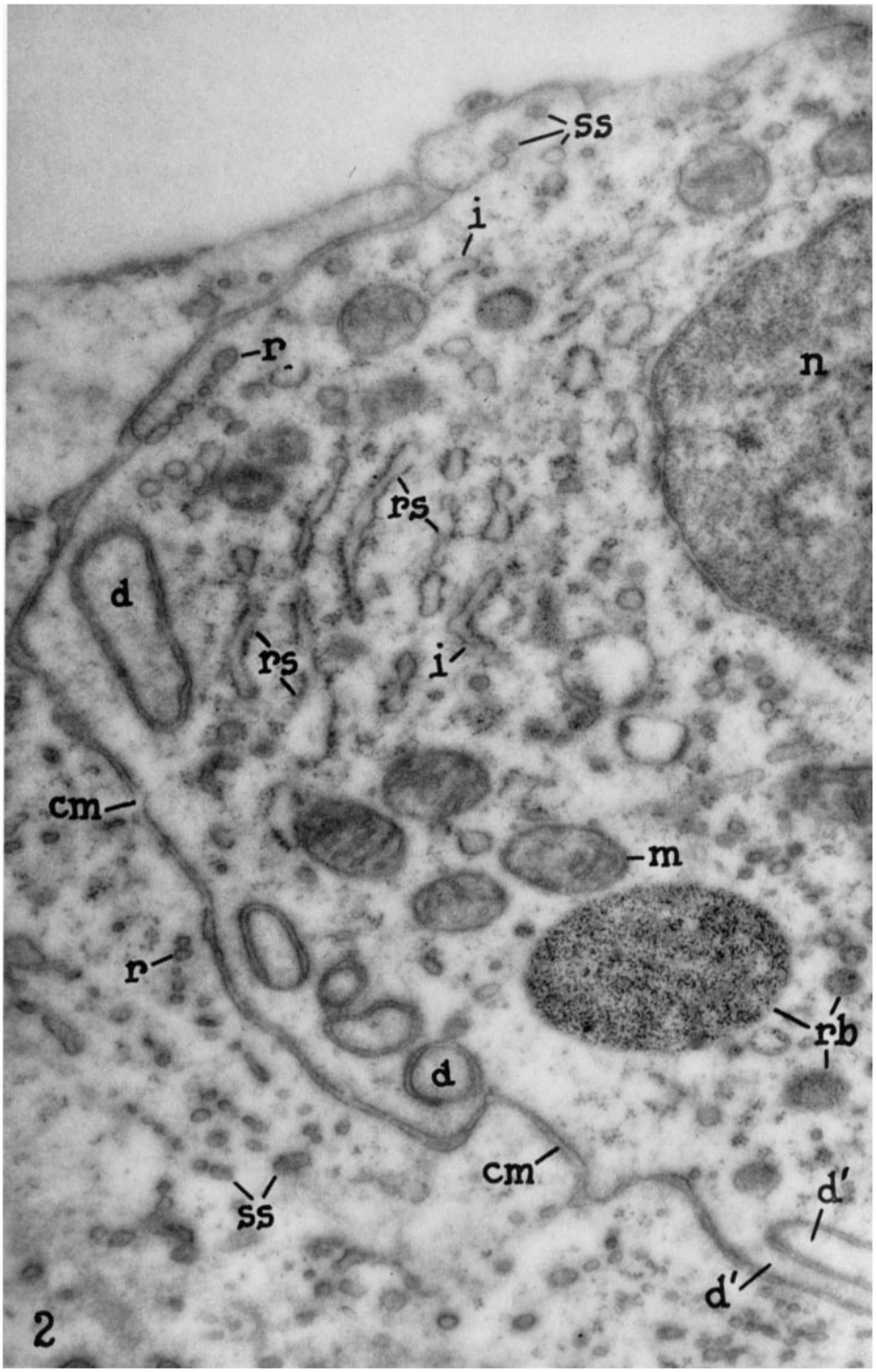
PLATE 32

FIG. 2. Macrophages lining a splenic sinus (rat).

The lumen of the sinus can be seen in the upper left corner of the figure. The nucleus of the cell to the right appears at *n*, the closely apposed cell membranes at *cm*, and a number of cross-sectioned and lengthwise sectioned interdigitations at *d* and *d'* respectively.

The endoplasmic reticulum is represented by smooth surfaced profiles (*ss*), more numerous at the periphery of the cells, and by rough surfaced elements (*rs*), usually more centrally located in the cytoplasm. The smooth surfaced profiles are of predominantly circular shape and occasionally occur in rows (*r*), sometimes in connection with small invaginations of the cell membrane. The rough surfaced elements, mostly of elongated form, are frequently disposed parallel to one another in small arrays. Profiles of intermediary appearance; *i.e.*, partly free of, and partly covered with dense particles, can be seen at *i*.

In addition to mitochondria (*m*) and clusters of small, dense particles, the cytoplasm contains round bodies (*rb*) of various sizes and polymorphic structure. They characteristically contain a fine granular material of unusually high density. It is assumed that these bodies represent terminal appearances of phagocytic vacuoles and that the dense granular material they contain is a metal-organic compound (hemosiderin? ferratin?). × 38,000.



(Palade: Endoplasmic reticulum)

PLATE 33

FIG. 3. Parenchymal cell of the liver (rat).

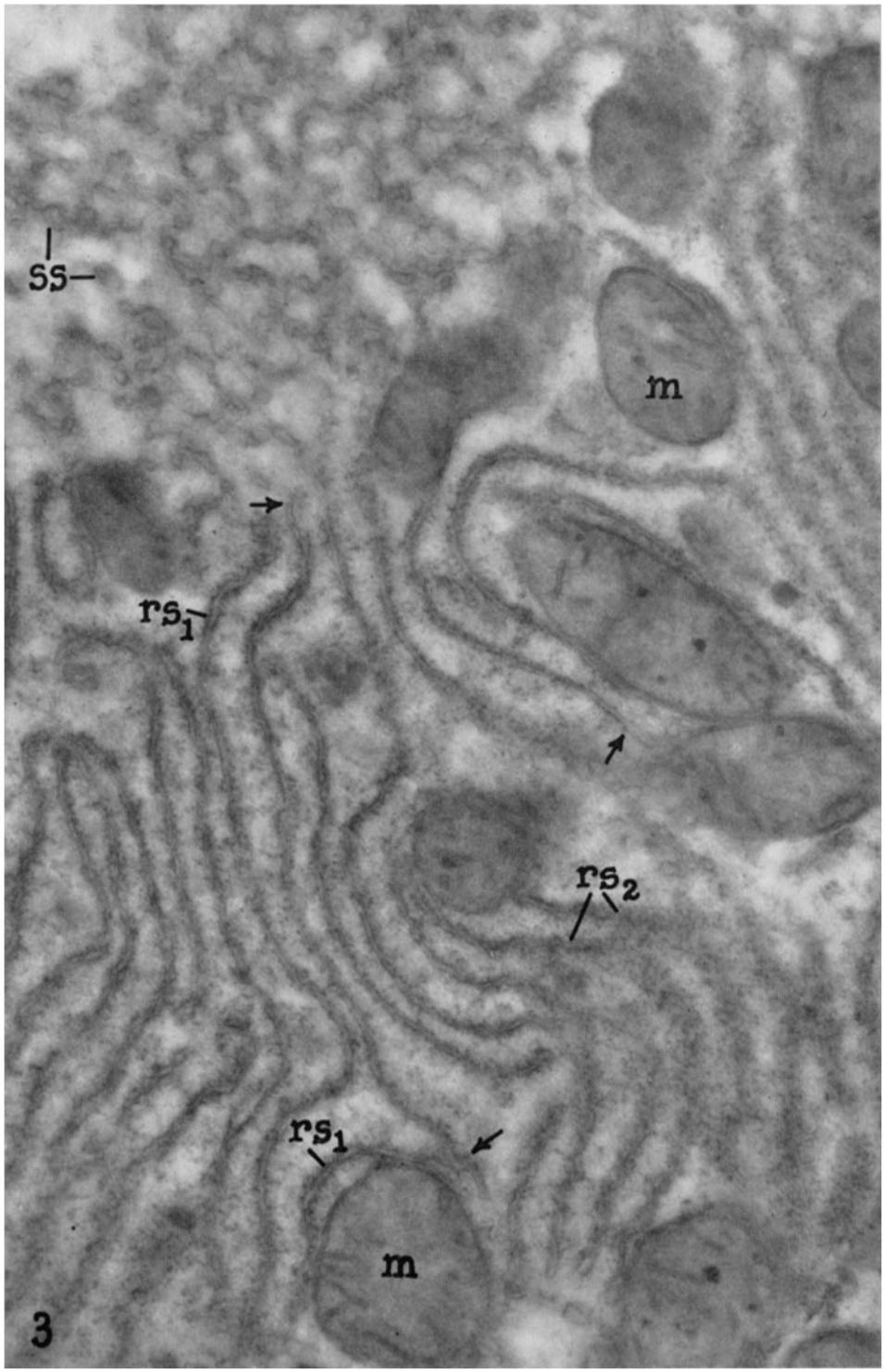
In this cell, the endoplasmic reticulum has a greater relative volume than in the preceding examples. The local differentiation into smooth and rough surfaced portions is also more evident.

The smooth surfaced elements (*ss*) are vesicles and tubules agglomerated in discontinuous masses, mostly at the periphery of the cell. In three dimensions they form tightly meshed, randomly disposed networks, a disposition which is clearly visible in the upper left corner of the figure.

The rough surfaced profiles (rs_1, rs_2) belong to cisternal elements and are disposed more or less parallel to one another in relatively large arrays. In this figure the cisternal elements appear either normally (rs_1), or obliquely (rs_2) sectioned.

Continuity between the two types of profiles is indicated by arrows.

In addition to elements of the endoplasmic reticulum, the field contains a number of mitochondria (*m*). × 44,000.



(Palade: Endoplasmic reticulum)

PLATE 34

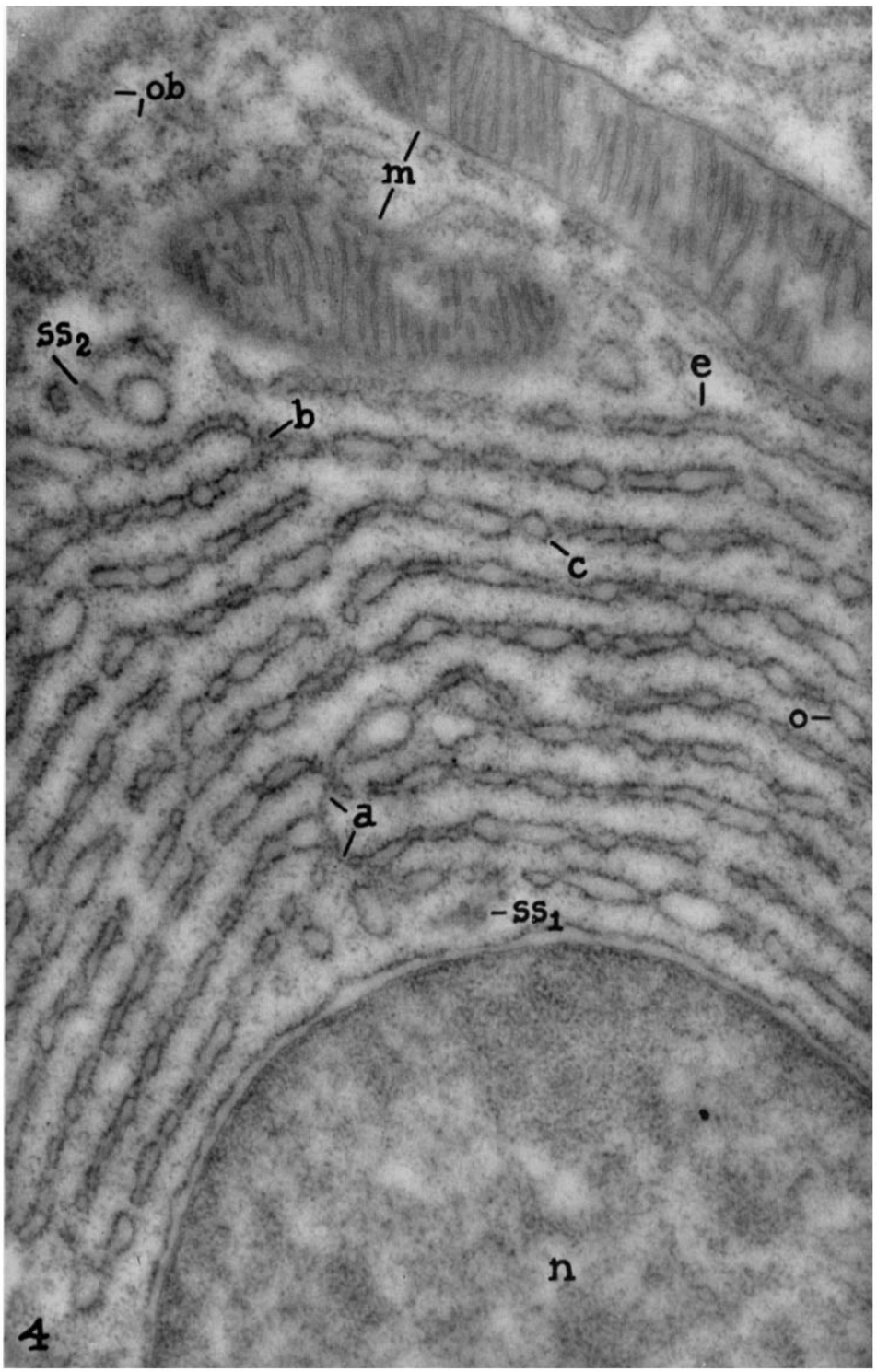
FIG. 4. Pancreatic exocrine cell (guinea pig).

The figure shows a relatively large field in the basal region of an acinar cell. The nucleus appears at *n* and two mitochondrial profiles at *m*. The rest of the field is occupied by numerous profiles belonging to the endoplasmic reticulum (ER). Almost all of them are of rough surfaced variety and appear aligned in rows which are disposed parallel to one another at more or less regular intervals. In this case the entire arrangement is concentric with the nucleus. Note that this orderly disposition is disturbed in a few places by branching rows (*b*) and by anastomoses (*a*) between adjacent rows.

Note that although elongated profiles (*e*) predominate, circular (*c*) and oval (*o*) profiles are also present in many rows. In three dimensions these rows correspond to fenestrated cisternae or to reticular sheets.

In the middle region of the figure, the ER elements are normally sectioned and show clearly their lumen, limiting membrane, and attached granules. In the upper left corner similar elements appear in oblique section (*ob*).

Smooth surfaced elements (ss_1, ss_2) are very rare in pancreatic acinar cells. When present they frequently occur in small clusters (ss_1). × 40,000.



(Palade: Endoplasmic reticulum)

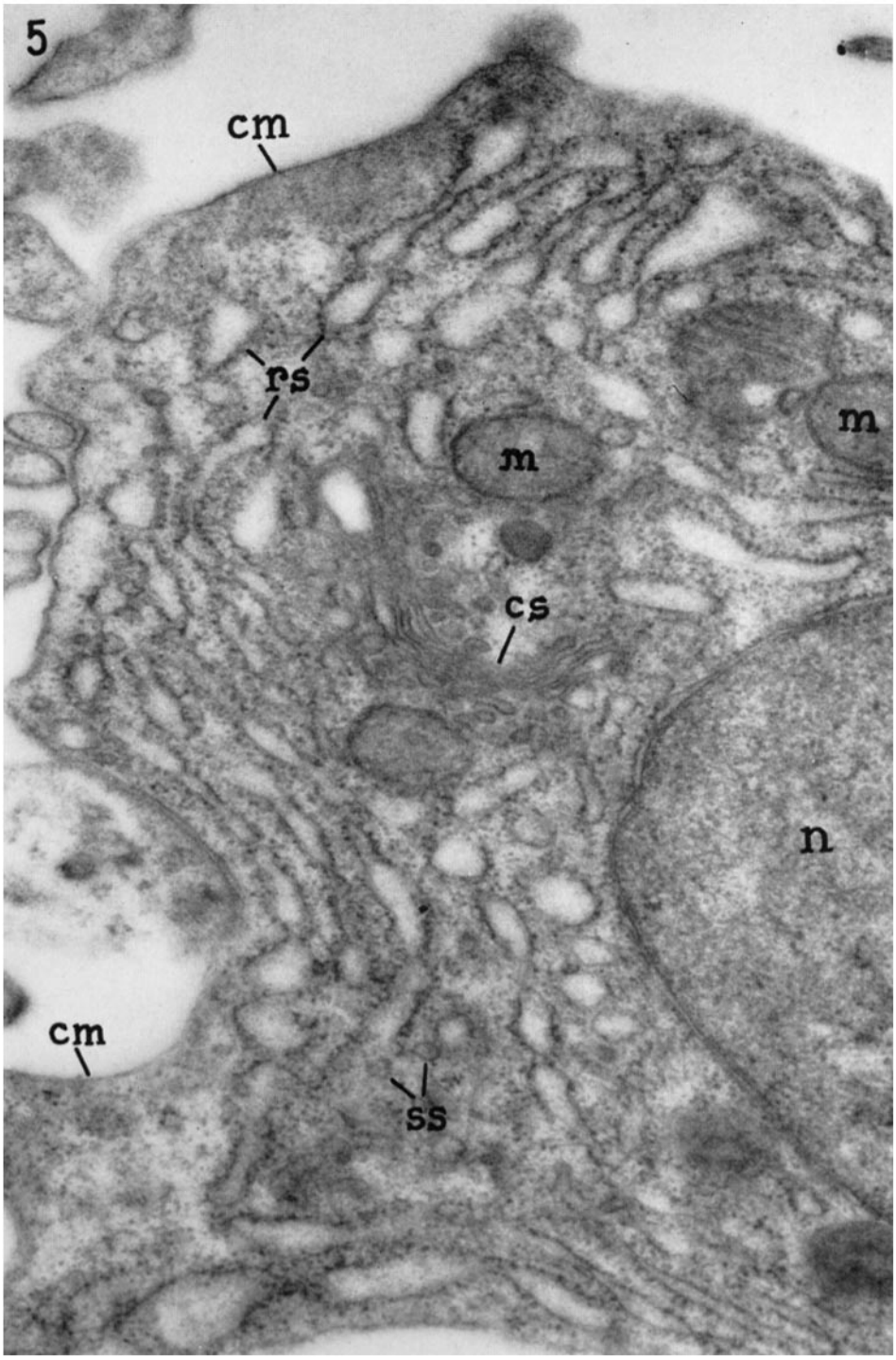
PLATE 35

FIG. 5. Plasma cell. Chorion of the nasal mucosa (cat).

Part of the nucleus can be seen at *n*, mitochondrial profiles at *m*, the membranous system of the centrosphere region ("Golgi complex") at *cs*, and the cell membrane at *cm*.

The cytoplasm is occupied by numerous profiles of the endoplasmic reticulum which belong predominantly to the rough surfaced variety (*rs*) and are disposed parallel to one another in relatively large arrays. There are only a few smooth surfaced profiles (*ss*) scattered among these rough surfaced elements.

Note that the cytoplasmic matrix appears disposed in narrow bands in between and around the profiles of the endoplasmic reticulum. × 60,000.



(Palade: Endoplasmic reticulum)

PLATE 36

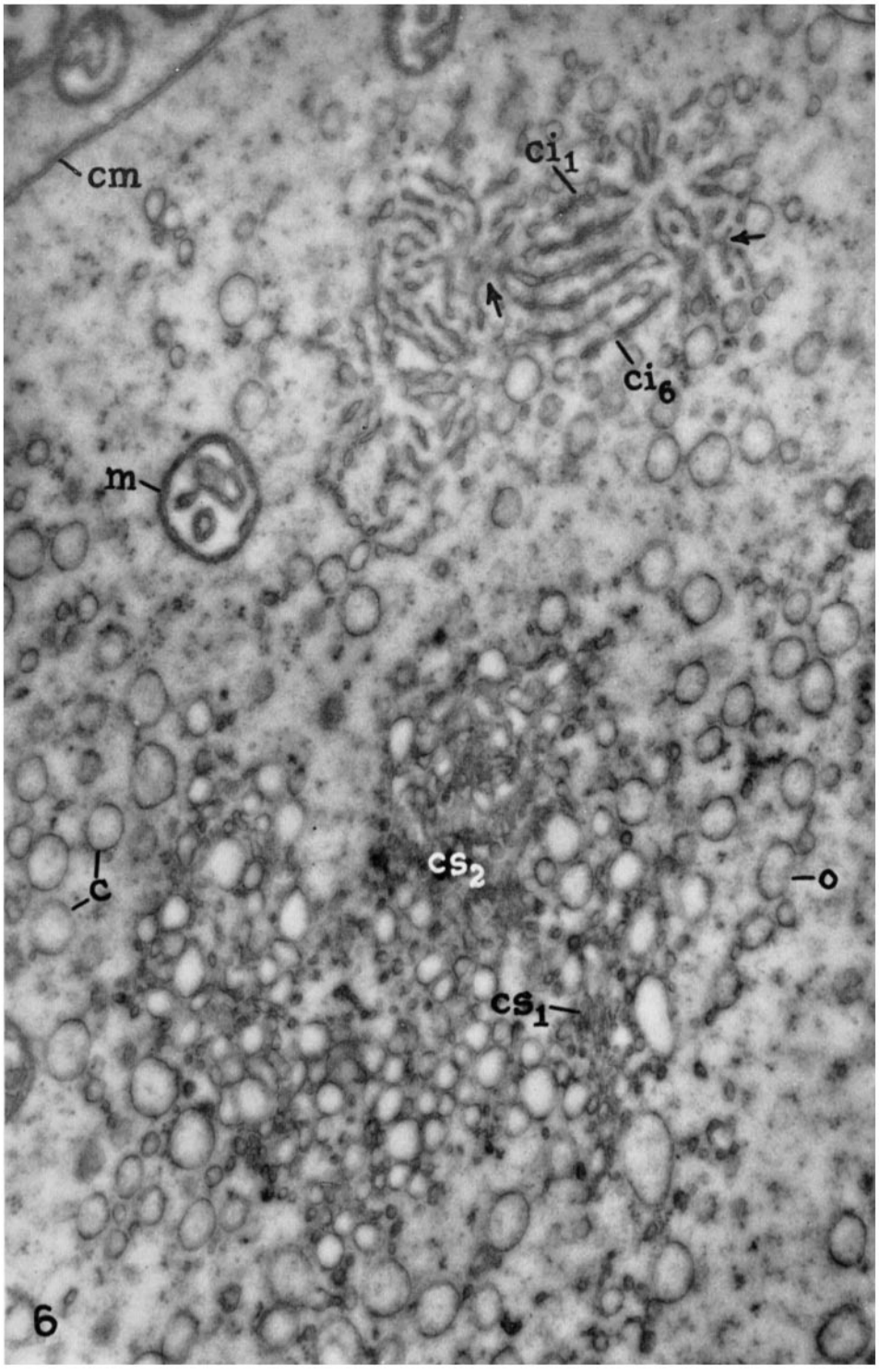
FIG. 6. Rat spermatid.

The section cuts through the Golgi complex and the adjacent stack of smooth surfaced cisternae (ci_1, ci_6). As described in reference 17 these profiles belong to fenestrated cisternae and are characteristically surrounded by a condensation of apparently amorphous material. At the two ends of the stack, similar profiles appear disposed radially or in star figures (arrows).

The elements of the Golgi region appear in normal section at cs_1 and in oblique section at cs_2 where a reticular sheet can be seen in full faced view.

Circular (*c*) and oval (*o*) profiles of the endoplasmic reticulum are scattered throughout the cytoplasm. Note that similar profiles are agglomerated in the Golgi zone among and around the smaller and more tightly packed elements which characterize this region.

A mitochondrial profile is marked *m*. The cell membrane appears at *cm*. × 38,000.



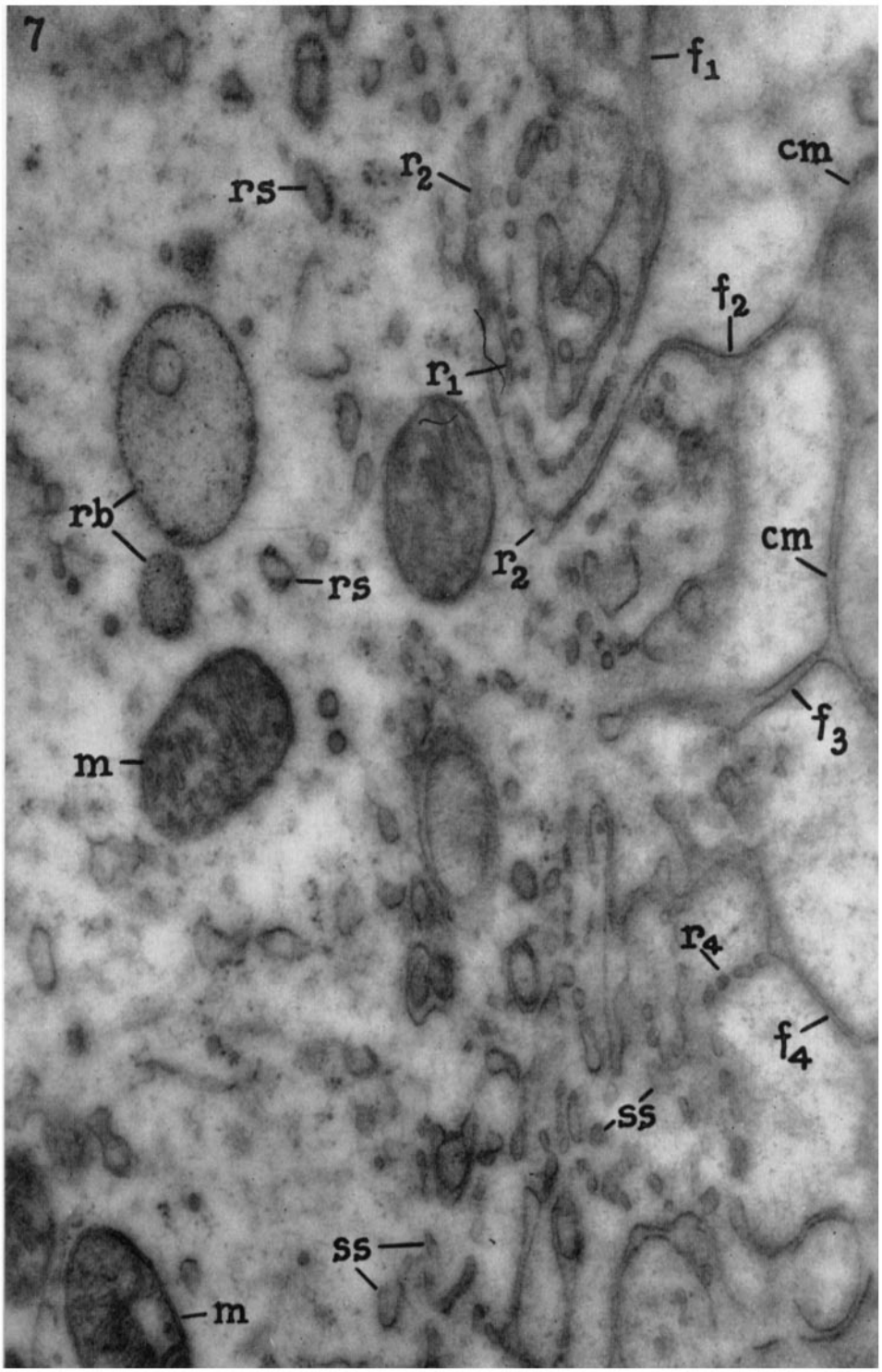
(Palade: Endoplasmic reticulum)

PLATE 37

FIG. 7. Splenic macrophage (rat).

The micrograph shows a relatively small field at the periphery of the cell. The plasma membrane (*cm*) presents a number of deep infoldings (f_1 to f_4) some of which (f_1, f_2, f_4) show complicated branchings. Note that the direction of certain folds is continued inside the cytoplasm by rows of smooth surfaced profiles (r_1, r_2, r_4). Similar profiles presumably belonging to the endoplasmic reticulum (*ss*) are found agglomerated in the vicinity of the infoldings. Profiles of intermediate appearance, partly free and partly covered with dense particles, can be seen at *rs*.

Mitochondria are marked *m* and residual bodies *rb*. (For the latter see the legend of Fig. 2.) × 44,000.



(Palade: Endoplasmic reticulum)

PLATE 38

FIG. 8. Parts of two smooth muscle fibers in the tunica media of an arteriole (rat).

The respective plasma membranes marked cm_1 and cm_2, are closely apposed on most of their course and diverge only in a few places (is_1, is_3) thus limiting small intercellular spaces.

Note the presence of numerous small vesicles in the immediate vicinity of the cell membranes. Some of these vesicles are completely closed and well separated from the membrane (v_1), others are contiguous with the latter (v_2), while still others (v_3) appear to open at the surface of the cell and as such can be considered as small invaginations of the plasma membrane.

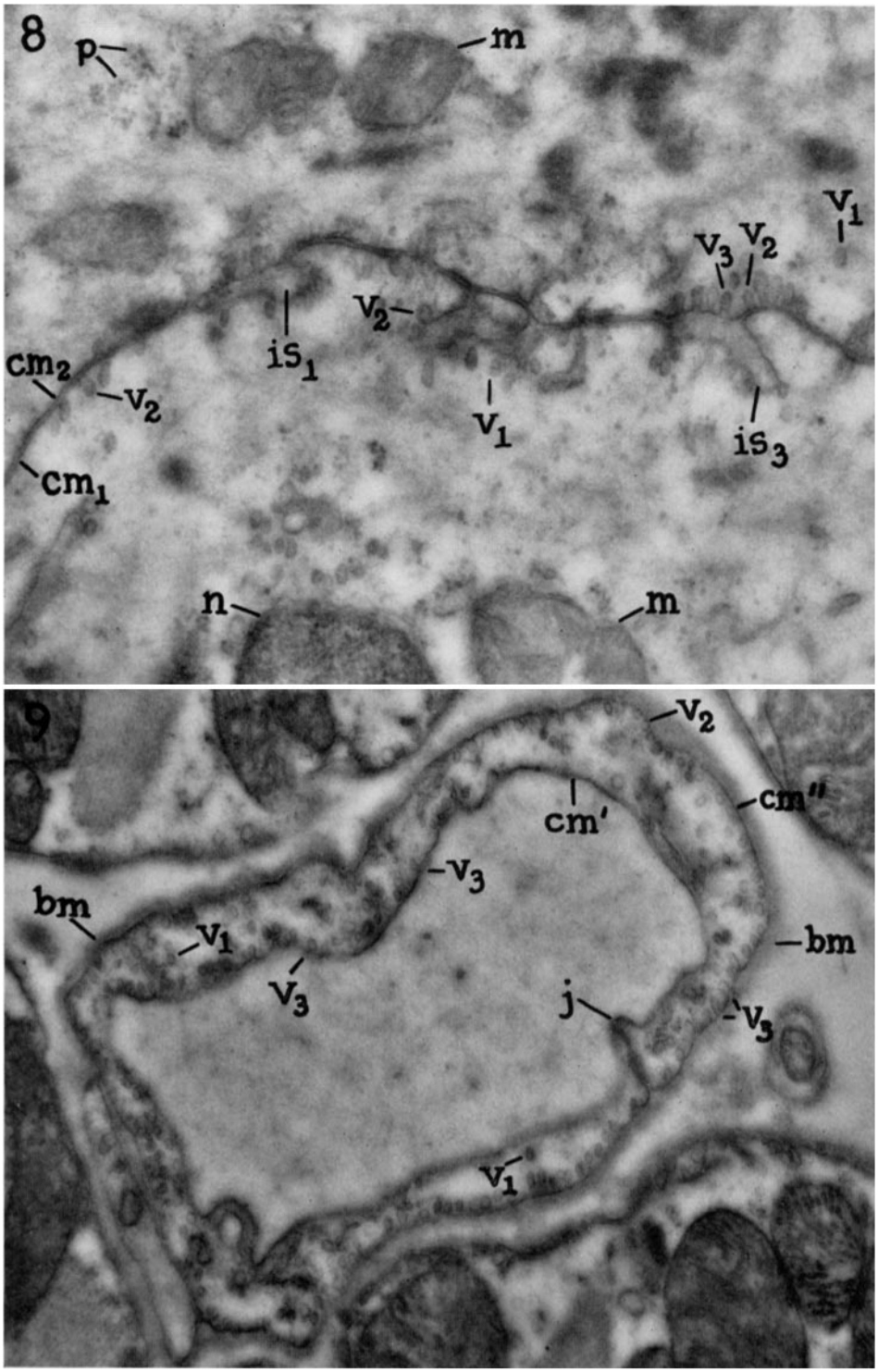
Mitochondria are marked *m*, the nucleus *n*, and clusters of small dense particles *p*. × 44,000.

FIG. 9. Cross-section through a blood capillary in the myocardium of the right ventricle (rat).

Parts of striated muscle fibers can be seen in each of the four corners of the figure.

A single endothelial cell lines the capillary; its margins are joined at *j*. Numerous vesicles are present in the cytoplasm either randomly distributed throughout the entire depth of the cell (as in the upper left sector of the capillary), or preferentially concentrated immediately below the cell membrane (as in the right half of the capillary). In this particular case, such vesicles are more numerous below the membrane facing the peri-capillary spaces (*cm''*) than below the membrane facing the lumen (*cm'*). Some of these vesicular profiles are completely outlined (v_1) and well separated from the cell membrane, others (v_2) are contiguous with it, while still others are more or less widely open (v_3) at the surface of the cell membrane and as such could be considered as invaginations of the latter.

The layer of relatively dense, apparently amorphous material (*bm*) which covers the outside surface of the endothelium represents the basement membrane of the capillary. × 27,000.



(Palade: Endoplasmic reticulum)