STUDIES ON THE ENDOPLASMIC RETICULUM

II. Simple Dispositions in Cells in Situ

By GEORGE E. PALADE, M.D.

(From The Rockefeller Institute for Medical Research)

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In the first article of this series (1) it was demonstrated that the endoplasmic reticulum, a cytoplasmic component originally described in cells cultured *in vitro* (2, 3), is also present in cells *in situ*, *i.e.*, in cells that retain until fixation their usual connections within the tissues and organs of a living animal. Indeed the reticulum was shown to be present *in situ* in six different cell types belonging to connective and epithelial tissues of both mammalian and avian origin, and this finding was taken to suggest that the new component is widespread in its occurrence among animal cells. The study mentioned (1) showed, in addition, that the system exhibits, from one cell type to another, a certain amount of characteristic variation suggestive of its involvement in the process of cell differentiation. The number of cell types examined was considered, however, to be too small to permit any generalization, so that a wider survey of the endoplasmic reticulum in cells *in situ* was deemed necessary before arriving at any conclusion concerning the generality of its occurrence among animal cells and the extent to which it is affected by cell differentiating processes.

The results of such a survey, to be presented in this article and in subsequent ones, show that the endoplasmic reticulum is a regular cytoplasmic component in more than 40 different mammalian and avian cell types which have been studied *in situ*. With the exception of the adult red blood cell, the new component was encountered in the cytoplasm of all cell types thus far examined. This finding is taken to indicate, at least tentatively, that the endoplasmic reticulum is a ubiquitous cytoplasmic component and, as such, a part of the fundamental organization of the animal cell. In addition, the survey has revealed that this system varies in volume, form, and intracellular disposition from one cell type to another in a characteristic way and to such an extent that it can be considered as one of the cell components most responsive to the process of cell differentiation.

Because of the extent of these observations, it appears desirable to present them in a series of successive articles. The present article contains general indications on Materials and Methods together with observations on the simplest form assumed by the endoplasmic reticulum in the cell types surveyed;

the following articles will comprise observations on more complex dispositions and will include a general discussion of the subject.

Materials and Methods

The observations reported in this paper were carried out on tissues and organs obtained mostly from adult, newborn, and embryonic albino rats. Cells belonging to the following tissues were examined:

(*a*) *Epithelial Tissues:*—Skin epidermis; epithelium of the oral mucosa; epithelium of the gastric and intestinal mucosa; epithelium of the nasal mucosa; epithelium of the bronchi and alveoli of the lung; epithelium of the various segments of the nephron; epithelium of the kidney pelvis and the bladder; glandular epithelium of the mammary gland; glandular epithelium of the stomach and intestine; parenchymatous cells of the liver; glandular cells of the pituitary (pars anterior), thyroid, adrenal, and pancreatic islets; epithelium of the seminiferous tubules, including Sertoli cells; epithelium of the epididymis, prostate, and seminal vesicles; epithelium of the oviduct and the uterus; vascular endothelia; mesothelia of the pericardium, pleura, and peritoneum.

(*b*) *Nervous Tissues:*—Perikarya, dendrites, and axons of nerve cells in the central and peripheral nervous system; Schwann cells.

(*c*) *Mesenchymal Tissues:*—Fibroblasts; chondrocytes; adipose cells, including cells of the brown fat; macrophages (histiocytes); mast cells; various cellular elements of the spleen, lymph nodes, and thymus; blood cells.

(*d*) *Muscular Tissues:*—Smooth muscle fibers from the tunica muscularis of the digestive tract and from the tunica media of various arteries; heart muscle fibers; skeletal muscle fibers.

In addition to mammalian (rat) cells of the types listed, numerous avian (chicken) cell types belonging to mesenchymal, muscular, and epithelial tissues were also examined.

Preparatory Techniques:—The techniques for collecting, fixing, embedding, and sectioning the material have been described in a previous article (4). For the present study most specimens were fixed in 1 per cent OsO_4 buffered at pH 7.2 to 7.5 with acetate-veronal buffer. The influence of more acid or alkaline OsO_4 solutions upon the appearance of the endoplasmic reticulum was also explored. In addition, other buffer systems, such as ammonium acetate (0.12 N), phosphate-phosphate (0.05 M $KH_2PO_4-K_2HPO_4$), and phosphate-bicarbonate, were tested.

The fixative used as a routine, namely 1 per cent OsO_4 solution buffered with 0.028 M (Na acetate + Na veronal), is hypotonic in relation to mammalian red blood cells (5-8). Attempts to compensate for this hypotonicity by adding sucrose or NaCl to the fixative solution (final concentration: 0.15 M and 0.10 M respectively) did not result in a noticeable difference in the appearance of the endoplasmic reticulum.

In order to minimize the extraction of the specimen by the fixative (9, 6), the fixation was shortened in many cases to 1 or 2 hours and carried out at 0°C. (8).

The tissue blocks were rapidly (6) dehydrated either in ethanol or methanol and embedded subsequently in *n*-butyl methacrylate.

Sections were cut at 20 to 50 mμ with a microtome provided with a mechanical advance designed by Porter and Blum (10).

Electron Microscopy.—The sections were examined without removing the plastic, either with an RCA model EMU-2b or with a Philips electron microscope. The contrast in the image was increased by introducing small apertures both in the condenser (250 μ) and objective ($\sim$ 25 μ) lenses of the RCA microscope. In the Philips instrument the electrons were accelerated by 60 or 80 kv. The original electron micrographs were taken at magnifications of from 4000 to 13,000 and enlarged photographically thereafter as desired.

OBSERVATIONS AND INTERPRETATION OF THE IMAGES

In thin sections, the cytoplasm of all cell types thus far examined contains a certain class of structural elements characterized by: (*a*) a shape that varies from circular to oblong, (*b*) smallest diameter ranging from 50 to 250 mμ, (*c*) a composite structure consisting of a dense limiting membrane surrounding a light content, and (*d*) an apparent lack of internal structure. The only exception is the adult erythrocyte, the cytoplasm of which appears to be free of such elements. The structures concerned account for a variable part of the ground-substance of the cytoplasm, the rest consisting of a continuous matrix which, at the resolution power available, appears homogeneous in some cases and finely granular or fibrillar in others.

Elements morphologically identical with the ones described in the present paper were recognized as profiles of the endoplasmic reticulum in a previous article (1) in which a few cell types were examined comparatively in whole mounts and in sections. It has been found that such elements were actually produced by sectioning the vesicles, tubules, and cisternae that compose the reticulum and it has been demonstrated that variations in the shape of these profiles can be satisfactorily correlated with the inherent form of the components of the reticulum as well as with variations in the angle at which they were sectioned. It is on the strength of these findings that the elements concerned in the present observations are assumed to be profiles of the endoplasmic reticulum and are referred to as such in the following description.

Seminal Epithelium[1]

General Disposition.—In sections of certain cell types, as for instance the cells of the seminal epithelium, circular and oval profiles are found to be predominant with very few or no elongated elements present. Fig. 1 shows part of a spermatocyte of the second order in which such elements are more or less evenly scattered throughout the cytoplasm. Some of them appear as relatively dense bodies and, because of this appearance, might be taken for sectioned granules; on closer examination, however, most of them show a dense, continuous outline surrounding a lighter, usually homogeneous content. Such features would describe the profiles as belonging to "hollow" rather than solid structures. In thick sections, the "granular" appearance is predominant, whereas in thin ones the hollow profiles are more numerous, an indication that probably all of these elements are provided with a central cavity which shows clearly in thin sections, and is obscured to a varied extent in thick preparations by excessive, non-specific electron scattering. The dense, narrow band that outlines the profiles is assumed to correspond in three dimensions to a thin, limiting membrane. This assumption is consistent with the continuity of the

[1] The term is used to designate the epithelium of the seminiferous tubules of the testis.

band, its high and apparently homogeneous density, and with the fact that, in sections, it is similar in appearance to other recognized membranous structures, *e.g.*, the cell membrane. Such variations as are encountered in the width and density of this band can be satisfactorily explained by variations in the angle at which a membrane of relatively constant thickness and density is sectioned. Indeed a membrane is bound to appear as a narrow, dense, sharply outlined band when perpendicularly sectioned, and as a band of increasing width, decreasing density, and decreasing sharpness of outline when sectioned at increasing obliquity. The actual variations found in the appearance of the band in sections are in good agreement with this expected pattern of variability and accordingly it can be safely concluded that the band outlining the elements of the endoplasmic reticulum represents a limiting membrane of relatively constant thickness and density. When normally sectioned, this limiting membrane measures 5 to 6 mμ in thickness. No particular significance is attached to these figures because they are not greater than twice the best resolution obtained at present in electron micrographs of sectioned material.

Usually, in seminal epithelia, both surfaces of the membrane limiting the endoplasmic reticulum are smooth. Occasionally, however, the outside surface, *i.e.*, the surface in contact with the cytoplasmic matrix is covered by small, dense particles of a type recently described (11). Even in such cases, the corresponding profiles bear attached particles only on part of their perimeter (Fig. 4) while the rest of their surface remains smooth. Few particles of the same size and density occur freely scattered, individually or in clusters, throughout the cytoplasmic matrix. The scarcity of elements with attached particles and the absence of elements heavily loaded with such particles constitute one of the characteristic features of the endoplasmic reticulum in seminal epithelia.

The content of the profiles appears homogeneous at the present level of resolution, but varies noticeably in density, being lighter than the cytoplasmic matrix in some specimens, and denser in others. The content of the smaller profiles is frequently denser than that of the larger ones. The difference may reflect various degrees in the concentration of the content, but, in some cases at least, it is due to other factors. Some of the smaller profiles represent tops of vesicles entirely included in the thickness of the sections and, in their case, the density of the curving membrane is superimposed upon the density of the content.

The profiles described are scattered throughout the entire cytoplasmic body from the vicinity of the nuclear membranes to immediately below the cell membrane (Fig. 2). In this respect the situation is different from that described in cultured cells examined *in toto* (3) in which the elements of the reticulum appeared restricted to the endoplasm. The distribution of the profiles does not seem to be entirely at random; relatively frequently they occur in rows of variable length in which they appear usually as isolated (Fig. 1), but occa-

sionally as interconnected units (Fig. 3). On rare occasions these rows of profiles are found to branch or anastomose.

By examining and comparing a large number of electron micrographs of seminal epithelia, it is possible to obtain information about the form of the individual elements of the reticulum and about the arrangement of the entire system in three dimensions, *i.e.*, in the whole cell. The circular profiles could represent either (*a*) vesicles cut at various angles or (*b*) tubules sectioned transversely. In the second alternative, the electron micrographs should show not only circular profiles but also, and in decreasing frequency, oval and oblong profiles corresponding respectively to oblique and longitudinal sections through the hypothetical tubules. The absence or extreme scarcity of oblong profiles in any cell section, irrespective of its incidence, renders very unlikely the existence of tubules. Oval profiles are frequent, but, in the absence of oblong ones, they are taken to represent flattened vesicles. In some cases the flattening might be genuine; in many others, however, it appears to be the result of the general compression of the specimen by the microtome knife. These findings and considerations indicate that in seminal epithelia most of the elements of the endoplasmic reticulum are vesicles of more or less spherical shape.

An appearance such as the one illustrated in Fig. 1 can be obtained by cutting thin sections through (*a*) an agglomeration of isolated vesicles; (*b*) a reticulum made up of strings of vesicles; or (*c*) a combination of both. In sections, the isolated elements are predominant, whereas simple and branching rows of profiles are of less common occurrence. The presence of the latter indicates, however, that at least some of the vesicular elements have been integrated in the whole cell into a reticulum formed by strings of vesicles. It was shown previously (1) that the vesicles and tubules of the endoplasmic reticulum can appear as independent structures in thin sections, even when all, or nearly all, of them were integrated into a continuous network in the whole unsectioned cell. On this basis it is assumed that the vesicles encountered in sectioned seminal epithelia are part of a continuous reticulum spread throughout the whole cytoplasm, but it remains obviously impossible to ascertain whether this applies for all the vesicles present or only for part of them.

In the cells sectioned at various incidences, the isolated profiles as well as the rows of profiles belonging to the endoplasmic reticulum appear to be distributed in the cytoplasm without any preferential orientation. Such an appearance can be obtained by sectioning through a reticulum with trabeculae distributed at random in three dimensions. It can be concluded, therefore, that the cells of the seminal epithelium possess an endoplasmic reticulum composed primarily of strings of vesicles interconnected in such a way as to form a randomly disposed, tridimensional network.

Variations Connected with Differentiation within the Seminal Epithelium.—The size of the vesicular elements appears to be constant, within certain limits, for semi-

nal cells of the same generation, but is usually found to be greater in cells more advanced in their evolution towards adult spermia. For instance, in spermatogonia, and spermatocytes of the first order the vesicles are as numerous as in spermatocytes of the second order, but they are usually smaller in size and, in addition, frequently have a dense, homogeneous content. Some of these features are illustrated in Fig. 6 which shows a rat spermatogonium. In spermatids, on the other hand, the vesicles are less numerous but noticeably larger in size (Fig. 5); their diameter frequently reaches 200 to 300 mμ, thus approaching or even surpassing the diameter of mitochondria. The content of the vesicles, usually lighter in density than in the less differentiated cells of the seminal series, is less dense than the matrix of the ground-substance of the cytoplasm, a feature that appears with particular clarity in thick sections. As in the other cells of the germinal epithelium, the vesicles described in spermatids frequently occur in rows of various length. Such rows can be seen in Figs. 7 and 8 which represent two consecutive sections through the same spermatid. The profiles of the row marked o_1 appear as independent units in Fig. 7, but the following section (Fig. 8) reveals that they are actually connected by thin tubules. In three dimensions the appearance corresponds to a string of intercommunicating vesicles. An additional connection with a profile belonging to another row is indicated by an arrow. It can be assumed that a complete reconstruction of this cell would show that most of the vesicular elements found in the two sections are linked together in a continuous network of cavities.

In contradistinction to the usually random disposition of the endoplasmic reticulum in seminal epithelia, a small part of the reticulum appears to be preferentially oriented in spermatids. Elongated profiles or rows of circular and oval profiles are found disposed parallel to one another at more or less regular intervals in a certain region of the cytoplasm, namely in the vicinity of the idiosome (Figs. 9 and 10). In the evolution of the spermatid of the rat, this structural peculiarity is contemporary with the margination of mitochondria (5, 12, 13) and appears after the differentiation of the acrosome, when the idiosome has already migrated towards the posterior pole of the cell. The preferentially oriented appearance exhibited by part of the endoplasmic reticulum in spermatids is reminiscent of the situation described in parotid cells (1) and in Nissl bodies (14) and, as in these cases, corresponds in all probability to a layering of reticular sheets. These reticular sheets apparently contain relatively large cisternae, in addition to the usual vesicular components of the reticulum, as indicated by the frequency of elongated profiles at their level (Fig. 10). At variance with the situation found in other cell types (1, 11, 14), the elements of these regularly disposed reticular sheets are free of associated small particles. However, the cytoplasmic matrix around them appears diffusely denser than in the rest of the cell (Figs. 10 and 11).

In the sustentacular cells of the seminal epithelium (Sertoli cells), the elements of the reticulum are still predominantly vesicular and generally free of associated particles, but considerable variation prevails in the size of the vesicles and in the density of their content (Fig. 5). Cisternal elements are occasionally encountered along the cell margins.

Relation to Other Cell Structures.—As already mentioned, the profiles of the endoplasmic reticulum are found scattered throughout the entire cytoplasm from the

nuclear envelope to the plasma membrane of the cell. Certain profiles are in close contact with the cell membrane and other profiles appear to be partially or entirely open towards the exterior. The membrane of these latter profiles is continuous with the cell membrane and their content seems to communicate freely with the pericellular medium. Similar appearances occur at the level of the second nuclear membrane, *i.e.*, the membrane which apparently limits the cytoplasm towards the nucleus, but vesicles opened towards the space separating the two nuclear membranes are, as a rule, of a less frequent occurrence than vesicles opened toward the extracellular spaces.

In the various cells of the seminal series, a region of considerable concentration of membranous elements is regularly encountered close to the nucleus. This region contains tight skeins of elongated profiles surrounded by circular and oval profiles of various sizes, which, together, correspond in location to appearances described in light microscopy as idiosomes, idiozomes, acroblasts, dictyosomes, lepidosomes, Golgi apparatus, or Golgi bodies (15, 16). In electron microscopy, structures of similar appearance, but not necessarily of similar location, have been observed in many cell types. They are usually found in the centrosphere region (1) but, in certain cell types, *e.g.*, in nerve cells, they occur in apparently isolated masses scattered throughout the cytoplasm. In this latter situation they were recently described as the "agranular reticulum" (14). In other cell types, similar tight bundles of predominantly elongated profiles have been interpreted as piles of lamellae (17) or "double membranes" (8, 18) and have been identified as the main or unique component of the Golgi apparatus. In the cells of the seminal epithelium, the circular and oval profiles found at the periphery of the membranous system described, are similar in shape, size, and density to the profiles scattered throughout the rest of the cytoplasm and described in this study as representing the endoplasmic reticulum. This general similarity, illustrated in Figs. 6 and 12, may suggest that the membranous structures concerned are interrelated. The suggestion derives additional support from micrographs showing that the smaller, circular or elongated profiles of the idiosome are occasionally continuous with the larger profiles of the endoplasmic reticulum (Figs. 13 and 14).

Leucocytes

Except for minor differences, the disposition of the endoplasmic reticulum in various leucocytes is similar to that already described in the cells of the seminal epithelium. For instance, in the cytoplasm of granulocytes, as in that of seminal epithelia, profiles of circular and oval shape are predominant, whereas elongated profiles are either absent or rare. The vesicles corresponding to these profiles are limited by a smooth membrane and have in general a light content. Usually they appear individually scattered throughout the cytoplasm and only occasionally are disposed in rows. These general features are illustrated by Figs. 15 and 16 which represent respectively a basophil and an eosinophil granulocyte. In basophil, eosinophil, and especially in neutrophil or heterophil granulocytes (Figs. 17, 27 and 28), however, profiles with small particles attached to the outside surface of their membrane are more frequently encountered than

in seminal epithelia. Such profiles are found irregularly scattered among the more numerous profiles of the usual, smooth surfaced variety from which they also differ by being more varied in shape. (Elongated and irregular forms are relatively frequent among the profiles of this rough surfaced variety.) The two types of profiles do not seem to represent different, unrelated structures, but appear to correspond to differentiated portions of a common system, as indicated by the occasional continuity of profiles of different type and by the occurrence of profiles of mixed appearance, *i.e.*, profiles partially covered with, and partially free of, small dense particles (Figs. 27 and 28). The larger variety of shapes encountered among rough surfaced profiles is taken to indicate that in such differentiated parts of the endoplasmic reticulum tubular and cisternal elements occur more frequently than in the parts of the network not associated with small particles.

As in seminal epithelia, the profiles of the endoplasmic reticulum are scattered throughout the entire cytoplasm of granulocytes from immediately below the plasma membrane to the second (outer) nuclear membrane. At the level of both these membranes, circular, or elongated profiles are found partially or widely open either in the extracellular space or in the perinuclear space, *i.e.*, the space in between the two nuclear membranes (Fig. 26). Relations similar to those described in seminal epithelia are found in granulocytes between the profiles of the endoplasmic reticulum and the tightly clustered profiles of the centrosphere region.[2]

In neutrophil granulocytes the identification of the structural elements belonging to the endoplasmic reticulum is rendered more difficult than in the other cell types described because of the presence of two new types of profiles of partially similar morphology (Figs. 17 to 20). The first type is represented by circular, oval, and, more rarely, elongated profiles with the same size distribution as the profiles of the endoplasmic reticulum. They are limited by an equally thin membrane (5 to 6 mμ) but their content, although homogeneous at the present level of resolution, is noticeably denser. These profiles which correspond in all probability to the neutrophil or heterophil granules of the cell are therefore similar in their general morphology to the profiles of the endoplasmic reticulum, the only distinct feature in electron micrographs being their denser content. Their differentiation, by this criterion, is rendered uncertain by the existence of profiles with contents of intermediate density (Fig. 17) and of profiles partly filled with homogeneous material of the usually high density (Fig. 20). It is also noteworthy that in sections of neutrophil granulocytes the profiles of the endoplasmic reticulum are, as a rule, fewer in number per unit area of cytoplasm than in the previous examples. Moreover, the respective numbers of specific granules and endoplasmic reticulum profiles

[2] The centrosphere region of granulocytes has been the object of a recent study by Policard and Bessis (32).

seem to vary in inverse proportion: cells with many granules have few profiles ascribable to the endoplasmic reticulum and cells with many hollow profiles have few neutrophil granules.

The other type of profile which must be considered in making a proper identification of the elements of the endoplasmic reticulum is represented by formations of usually larger size. They are of circular or irregular shape and measure 150 to 300 mμ in diameter thus overlapping at least in part the size range of the profiles of the endoplasmic reticulum (50 to 250 mμ). They are limited by an equally thin membrane (5 to 6 mμ) and usually have a light content in which dense irregular bodies are encountered (Figs. 17 to 19). Such formations represent, in all probability, digestive vacuoles formed as a consequence of the phagocytic activity of granulocytes. Similar vacuoles are of a less frequent occurrence in eosinophiles. Although such a complex heterogeneous content seems to be more or less characteristic for the large digestive vacuoles, it has to be noted that in granulocytes even smaller profiles, which in all other respects are similar to the profiles of the endoplasmic reticulum, frequently show a complex content consisting of a small dense granule or a dense rod-like body surrounded by a light, more or less homogeneous matrix (Fig. 16).

In the lymphocytes of lymph nodes, spleen (Fig. 21), and thymus the profiles of the endoplasmic reticulum are similar in form and disposition to those found in granulocytes except that in lymphocytes the profiles are usually less numerous per unit cytoplasmic area, a finding suggesting that such cells possess a reticulum of small total volume provided with widely spaced trabeculae. Circular profiles differentiated by a dense, homogeneous content, but otherwise similar in size and general morphology to the profiles of the endoplasmic reticulum, are frequently encountered in the cytoplasm of sectioned lymphocytes. It is assumed that such profiles correspond to the azurophil granules described in light microscopy (Fig. 24).

The situation is different in monocytes in which the profiles are more numerous and more varied in shape than in other leucocytes (Figs. 23). In the central zone of the cytoplasm of these cells, most profiles belong to the rough surfaced variety and occasionally exhibit a certain degree of preferential orientation: thus, they occur in arrays consisting of a few profiles disposed parallel to one another and spaced at more or less regular intervals (80 to 200 mμ). At the periphery of the cell, smooth surfaced profiles are more frequent. Occasionally rows of such profiles appear to continue inside the cytoplasmic body infoldings of the cell membrane (Fig. 25), an appearance which is currently encountered in macrophages (19). As in the case of neutrophil granulocytes, the identification of some of the profiles found in the cytoplasm of monocytes is rendered difficult by the existence of profiles of comparable size possessed of a dense, homogeneous content and by the occurrence of hollow profiles which contain

dense polymorphic bodies within a light matrix. Such formations may correspond respectively to the azurophil granules described in light microscopy and to digestive vacuoles resulting from phagocytosis.

The adult erythrocytes are distinguished by a complete lack of profiles ascribable to the endoplasmic reticulum. Their dense cytoplasm appears to be also deprived of other cellular organelles or components such as mitochondria and small particles. The younger elements of the erythrocytic series, however, possess an endoplasmic reticulum, as indicated by the fact that erythroblasts and normoblasts occasionally encountered in rat spleen have profiles of appropriate size and morphology scattered throughout a granular cytoplasm. These profiles are even less numerous than in lymphocytes, an indication of small original volume or of involution of the endoplasmic reticulum.

A situation comparable to that described in leucocytes is encountered in mast cells, in adipose cells, in the cells of brown fat, in those of the adrenal cortex, and in the parietal cells of gastric glands. In all these examples the profiles ascribable to the endoplasmic reticulum are mostly of circular shape and smooth surfaced variety. Profiles with attached particles appear to be relatively rare. The findings on the parietal cells of the gastric glands are in agreement with those already reported by Sedar (20). It is of interest to note that three of the cell types listed are particularly active in lipide metabolism.

DISCUSSION

Criteria for Identification.—The general survey outlined in the introduction and presented, in part, in detail in the preceding observations demonstrates that structural elements having the general morphology expected from profiles of the endoplasmic reticulum are encountered in the cytoplasm of all cell types thus far examined *in situ* with the exception of the mature erythrocyte. For the purpose of this survey, a given formation was identified as a profile of the endoplasmic reticulum when it possessed (*a*) a small diameter ranging from 50 to 300 mμ; (*b*) a composite structure including a thin limiting membrane and, usually, a light and homogeneous content. An intracellular distribution which, in sections, was compatible with a reticular disposition in three dimensions and the actual demonstration of interconnections between the structural elements involved were also used as additional criteria. Admittedly these criteria are of an entirely morphological nature. It follows that the structures concerned are equated to one another and considered as parts of a common, cell wide system, *i.e.* the endoplasmic reticulum, on the basic assumption that what is similar in appearance is also similar or related in other respects, such as function and chemical composition. Actually there is no proof for so general a similarity and, moreover, it is conceivable that chemical and functional diversity may exist among structures of identical morphological appearance. It has to be pointed out, however, that present information on the function and

chemistry of cytoplasmic components in the dimensional range concerned is still rudimentary and subject to the limitations of statistical methods. Under such conditions and at this particular time in the development of our knowledge, the use of purely morphological criteria for equating these elements and grouping them in a common system appears to be permissible. It remains, of course, subject to future confirmation or revision by physiological and biochemical investigations. In the present stage, the demonstration of actual continuity among these profiles and between them and other cell structures acquires particular significance, as representing thus far the only indication of identity or functional relationship among the elements concerned.

Difficulties in Identification.—The difficulties inherent in the exclusive use of morphological criteria become particularly disturbing when the position of elements of partly different appearance has to be ascertained. Profiles similar in appearance to those identified as belonging to the endoplasmic reticulum are found in the centrosphere region of the cells (idiosome or acroblast region for seminal epithelia) where they occur in swarms in close association with skeins of tightly packed, elongated elements. Usually the two forms of profiles, *i.e.*, those of the endoplasmic reticulum and those of the centrosphere region, are different in size, packing, and location but all possible intermediate forms between these two types can be encountered. This similarity may suggest that the two systems are related; moreover, the actual demonstration of continuity between them in seminal epithelia and in other cell types (14) supports such an hypothesis.

In granulocytes and in monocytes the situation is further complicated by the existence of profiles of digestive vacuoles, a consequence of the phagocytic activities of these cells. Such profiles differ from those of the endoplasmic reticulum by having a larger diameter and an unhomogeneous content. Finally in neutrophil granulocytes the profiles of the heterophil granules are similar in size and general morphology to those of the endoplasmic reticulum, the only morphological difference being the higher density of their homogeneous content. There again the situation is complicated by the existence of profiles partly filled with dense material and of profiles with a content of intermediate density, *i.e.*, elements having an appearance one would expect to find in "transition forms." A comparable situation is encountered in relation to the profiles of the azurophil granules of lymphocytes and monocytes.

With the presently available information it is impossible to decide whether the morphological similarities mentioned are simply coincidental or are indicative of actual, functional relationship among the structures involved. It is conceivable, for instance, that an exchange of vesicular elements may take place between the membranous system of the centrosphere region and the endoplasmic reticulum and it is also possible that "granules" may form by an accumulation and condensation of material inside the vesicles of the endoplasmic reticulum. Solutions to such problems may come from future experimental work.

Relations with Other Cell Structures.—Another relationship suggested by the preceding observations is that of the elements of the endoplasmic reticulum

with the cell membrane and with the nuclear envelope. As already mentioned, vesicular elements of the reticulum are found in contact with the cell membrane. Moreover, the latter occasionally shows limited invaginations which are similar in size and form to the vesicles of the endoplasmic reticulum. Between the simple, widely open invaginations of the cell membrane and the intracytoplasmic vesicles showing a continuous outline, all possible intermediary forms are encountered. This finding suggests that the membrane of the endoplasmic reticulum is continuous, at least intermittently, with the cell membrane and that equally intermittent continuity may be established between the content of the endoplasmic reticulum and the extracellular medium (Fig. 25).

A comparable spectrum of appearances, ranging from simple invaginations to completely closed vesicles, is found at the level of the outer nuclear membrane, *i.e.*, the membrane that apparently limits the cytoplasm towards the nucleus (Figs. 22 and 26). Here again the membrane of the endoplasmic reticulum appears to be continuous, at least intermittently, with the outer nuclear membrane. The latter, as is already known (21, 22), is in turn continuous with the inner nuclear membrane at the periphery of the numerous pores of the nuclear envelope. Continuity is also established between the content of the endoplasmic reticulum and that of the perinuclear space. In view of these findings, which confirm and extend to other cells Watson's observations (21) on splenic reticulocytes, the nuclear envelope, with its two membranes and the space bounded by them, appears to be an extension of the endoplasmic reticulum which could be described as a perinuclear cisterna. The pores of the nuclear envelope are reminiscent of the fenestrae described within the usual intracytoplasmic cisternae of the reticulum.

If generally and repeatedly demonstrated, the connections described may throw some light on the role played by the endoplasmic reticulum in the general economy of the cell and at the same time explain the similarity mentioned between the vesicles of the reticulum and the digestive vacuoles which are known to be formed by an invagination process of the cell membrane. Numerous, clear cut connections of the endoplasmic reticulum with the cell membrane have been noted in tissue macrophages (19) a material which appears to be particularly favorable for studying the relations between the two cellular structures concerned.

Tridimensional Disposition.—The appearances encountered in sectioned seminal epithelia and in leucocytes can be satisfactorily explained by assuming that in these cells (*a*) the constituent elements of the reticulum are predominantly of vesicular form, (*b*) they are connected in strings of vesicles, and (*c*) these strings are disposed at random in three dimensions and form by their linkage a network that, in general, shows no preferential orientation. This general representation of the system is in agreement with all appearances thus far encountered in sections and derives additional support from observations made on serial sections as well as from observations of cells of various

types grown in tissue culture and examined *in toto* (3). As indicated in a previous article (1), cultured cells are particularly favorable for studying the tridimensional aspects of the endoplasmic reticulum, *i.e.*, the form of its composing elements, their interconnections, and their intracellular disposition. It appears therefore that the vesicular elements found in cells *in situ* are usually integrated in a more or less continuous network of cavities, but obviously it remains very difficult to ascertain whether the integration affects all or only part of these vesicles. Studies on cultured cells have indicated that a few vesicles are actually isolated in the cytoplasm and moreover that under certain conditions, such as approaching cytolysis, the reticular disposition can be entirely lost, the system becoming a collection of independent vesicles (3). Similar situations probably occur in cells *in situ* where the degree of integration or disintegration of the system may conceivably be influenced by the physiological condition of the cell.

Local Differentiations within the Reticulum.—The preceding observations indicate that within the endoplasmic reticulum of a given cell type, there are circumscribed modifications which could be interpreted as the result of a local process of differentiation. The most obvious of these local differentiations results from the association of the membranes of certain elements of the endoplasmic reticulum with small, dense particles of a type recently described (11). In sections the profiles of such elements have a characteristic appearance conveniently described as "profiles of rough surfaced variety." Continuity between such profiles and profiles of the usual smooth surfaced type has been convincingly demonstrated both in sectioned material and in cultured cells examined *in toto* (1), so that the conclusion that the two types of profiles represent local differentiations in a common, continuous system appears to be well founded. Another local differentiation of thus far unique morphology is represented by the small pile of preferentially oriented cisternae found in spermatids in the vicinity of the idiosome. The nuclear envelope may be considered as yet another example of local adaptation of the system. Finally, connections described in this paper and in other studies (14) suggest that the membranous system present in the centrosphere region may also represent a local differentiation of the endoplasmic reticulum. The functional basis of these local differentiations is unknown. In this respect the only lead is supplied by the finding that profiles of the rough surfaced variety predominate in secretory cells engaged in active protein synthesis (11, 23) and that the associated particles responsible for the appearance of the profiles consist mainly of ribonucleoprotein (24), a compound generally considered to be related to the process of protein synthesis. As noted in the preceding observations, profiles of the smooth surfaced variety are found to predominate in cells active in lipide metabolism.

Differentiation According to Cell Type.—The type of endoplasmic reticulum found *in situ* in seminal epithelia and in leucocytes, *i.e.*, a reticulum formed by connected strings of vesicles belonging mainly to the smooth surfaced variety,

is the type most frequently encountered in cells cultured *in vitro*. In such cells, the type mentioned has been interpreted (3) as characterizing a physiologically normal condition of the cell, independent of the cellular type observed. More recent studies (1) however, have indicated that *in situ* the form and disposition of the elements of the endoplasmic reticulum varies noticeably and consistently from one cell type to another, suggesting that the system is responsive to cell differentiation processes. The survey mentioned in the introduction confirmed this hypothesis and showed that the endoplasmic reticulum of seminal epithelia and leucocytes is noticeably different from, and in general simpler than, the reticulum found in the other cell types examined in which the total volume of the system is usually larger, the proportion of elements with associated particles higher, and the occurrence of zones of preferred orientation more frequent. It appears therefore that in cells *in situ* a reticulum formed mainly by strings of vesicles characterizes certain lines of cell differentiation rather than the general physiological condition of the cell.

The preceding observations show that even within the small group of cell types described in this paper there are differences in the appearance of the endoplasmic reticulum. Seminal epithelia, for instance, have a randomly disposed reticulum composed almost exclusively of vesicular elements of the smooth surfaced variety. In leucocytes the tridimensional disposition of the system appears to be similar, but tubular and cisternal elements, some of them belonging to the rough surfaced variety, often participate in the formation of the network. In terms of differences encountered among other cell types, these differences are minor, but they appear to be constant and characteristic.

Although the endoplasmic reticulum displays a certain amount of differentiation clearly related to the cell type, its elements still show noticeable variation in size, shape, distribution, and density of content when individual cells of the same type are considered at various stages in their development. The finding is taken to suggest that the endoplasmic reticulum is a dynamic structure undergoing appreciable and probably continuous changes throughout the life of a given cell. The development of a zone of preferred orientation in the reticulum of spermatids at a certain time in their evolution is a good example.

Integration with Observations Already Published.—In recent years electron microscope studies of the seminal epithelium were published by Watson (12, 13), Challice (25), and Burgos and Fawcett (26). Challice did not mention the presence of structural elements which could be homologized with the endoplasmic reticulum. Watson, however, described such elements under the name of "microsomes" (12, 13), a term which in view of more recent findings (24) appears to be partly justified. He noted that these microsomes frequently varied in size with the degree of cell differentiation but, at variance with the view expressed in this paper, concluded that the microsomes were not membranous structures. Burgos and Fawcett (26) described the presence of a reticulum in spermatids and Sertoli cells and noted that

its elements do not have attached particles "as seems to be the rule with the endoplasmic reticulum of other cells."

Studies of normal and pathological leucocytes have been more numerous in the electron microscope literature (27–32). Grey and Biesele (27) did not mention the existence of elements ascribable to the endoplasmic reticulum. Kautz and de Marsh (28) described small vacuoles in the cytoplasm of normal neutrophils and monocytes and interpreted them as spaces left behind by dissolved lipide inclusions or by granules which "have fallen out of the section." Watanabe (29) did not find an endoplasmic reticulum in adult cells, but noticed the presence of "filaments" with "a trilamellated structure" in promyelocytes and myeloblasts. These filaments were found to disappear completely when the immature cells transformed into adult granulocytes. His description clearly indicates that the structures concerned are elongated profiles of the rough surfaced variety. More recently Bessis and Breton-Gorius (30) observed leucemic granulocytes and noted in the cytoplasm both vesicles and "ergastoplasmic sacs or structures." It is evident that all these elements correspond to the different forms and varieties of profiles described in this paper as belonging to the endoplasmic reticulum. Such differences as appear in the description of these structures can be easily traced to variations in the quality of the preparations and micrographs. Differences in interpretation and nomenclature are to be expected at this time when the available information has not yet been satisfactorily integrated.

SUMMARY

A survey of a large number of different cell types has indicated the presence of a network of membrane-bound cavities (the endoplasmic reticulum) in the cytoplasm of all cell types examined, with the exception of the mature erythrocyte.

In its simplest form, encountered in seminal epithelia and in leucocytes, the reticulum consists mainly of interconnected strings of vesicles and appears to be randomly disposed in three dimensions.

Local differentiations occur within the endoplasmic reticulum of all the cell types studied.

The membrane limiting the cavities of the endoplasmic reticulum appears to be continuous with the cell membrane and the nuclear membranes.

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EXPLANATION OF PLATES

PLATE 141

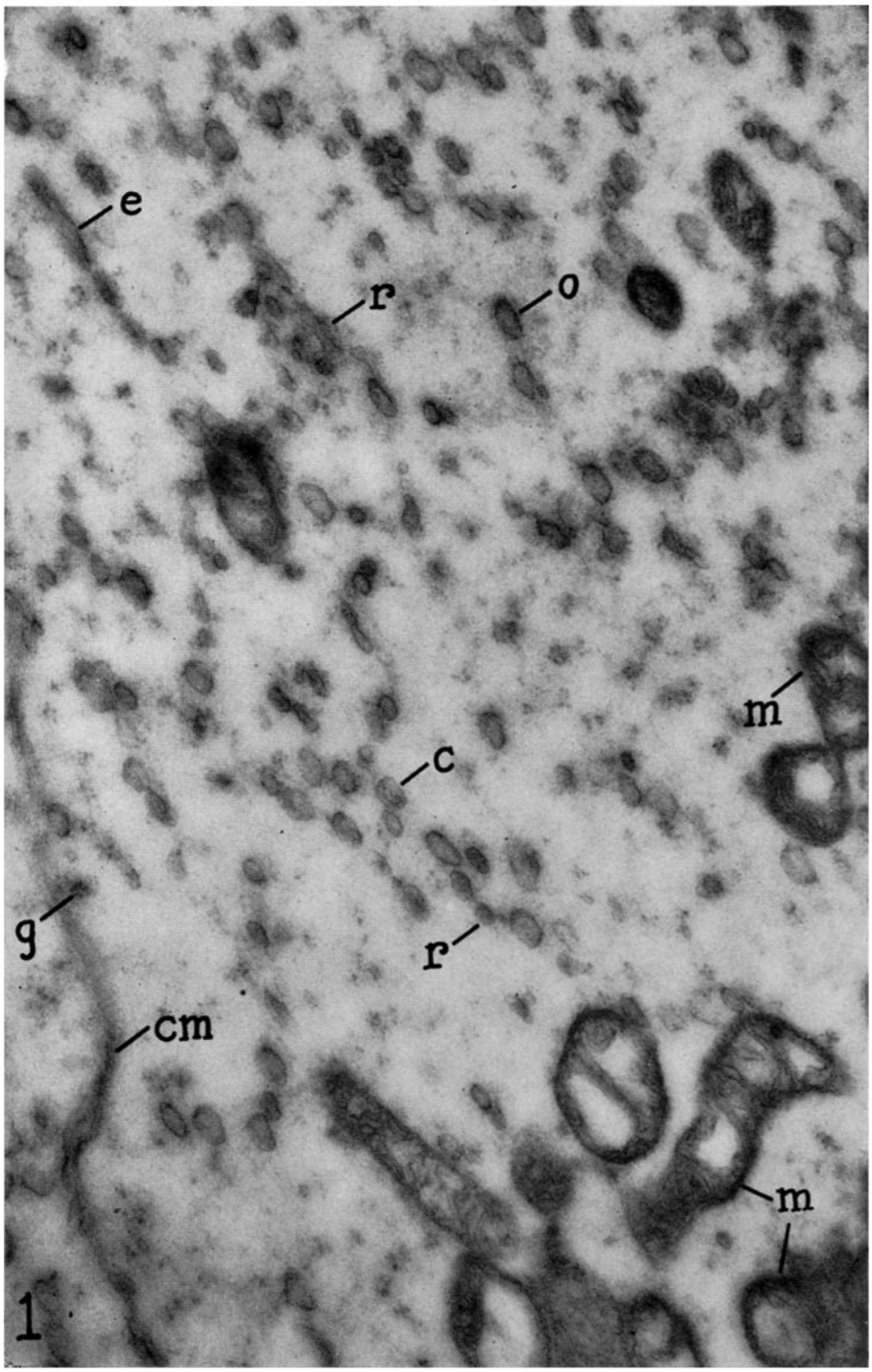
FIG. 1. Electron micrograph of a spermatocyte of the second order (rat).

At *cm* appears part of the cell membrane and at *m* profiles of mitochondria scattered indivudally or in clusters throughout the cytoplasm. The latter contains, in addition, numerous circular (*c*) and oval (*o*) profiles which measure 50 to 150 mμ in diameter, and represent the vesicular elements of the endoplasmic reticulum (ER). They are limited by a thin, dense membrane and have an apparently homogeneous content which, in this case, is higher in density than the cytoplasmic matrix. Note the scarcity of elongated profiles (*e*) and the lack of attached particles on the outer aspect of the membrane limiting the elements of the endoplasmic reticulum; in this case, practically all the profiles belong, therefore, to the smooth surfaced variety.

A few small, dense particles (*g*) appear freely scattered individually or in clusters in the cytoplasm.

In general the ER profiles occur evenly distributed throughout the cytoplasm; in a few places they appear to be disposed in rows (*r*).

Magnification 42,500.



(Palade: Endoplasmic reticulum. II)

PLATE 142

FIG. 2. Spermatocyte of the second order (rat).

The nucleus appears at *n*. The two membranes (nm_1, nm_2) limiting the perinuclear cisterna are not parallel to one another; the outer membrane exhibits a number of invaginations towards the cytoplasm. The cell membrane (*cm*) can be seen in close apposition to the membrane of neighbouring Sertoli cells on most of its course.

Note that the circular (*c*) and oval (*o*) profiles of the endoplasmic reticulum are more or less evenly distributed throughout the cytoplasm, from the vicinity of the nuclear envelope until immediately below the cell membrane. The arrows point to vesicles in close contact with the cell membrane or the membrane limiting the perinuclear cisterna.

Magnification 34,500.

FIG. 3. Small field in the cytoplasm of a spermatocyte (rat).

A row of circular profiles appears at *r* and an elongated profile at *e*. In three dimensions the first (*r*) correspond to a string of communicating vesicles, whereas the second (*e*) to a short tubule.

Magnification 61,500.

FIG. 4. Small field in the cytoplasm of a spermatocyte (rat), showing the profile of two interconnected vesicles. Note that their limiting membrane bears attached particles (*g*) on its outer aspect. The particles do not cover the entire membrane surface; they occur in small groups separated by relatively large expanses of particle-free membrane.

Magnification 86,500.

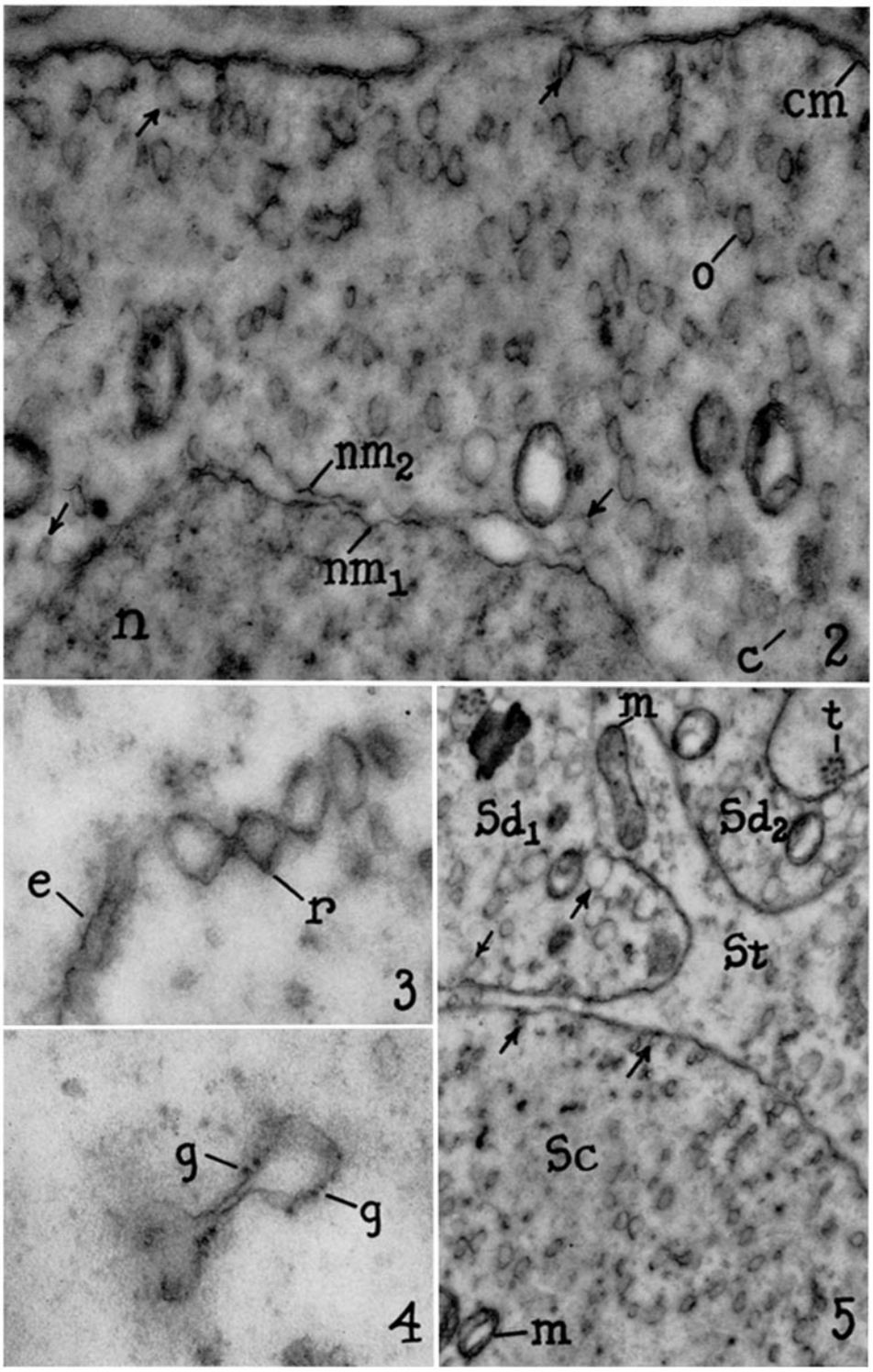
FIG. 5. The electron micrograph shows a relatively large field in the seminal epithelium of a rat.

At *Sc* is a spermatocyte of the second order; at Sd_1 and Sd_2 two spermatids and at *St* a Sertoli cell sending its protoplasmic expansions in between the germinal elements of the epithelium.

Note that the profiles of the endoplasmic reticulum differ in size and number in the various cell types represented in the figure. Note also that many vesicular elements of the endoplasmic reticulum are in close contact (arrows) with the cell membranes.

A few mitochondrial profiles are indicated by *m* and the developing tail of a spermatid by *t*.

Magnification 20,000



(Palade: Endoplasmic reticulum. II)

PLATE 143

FIG. 6. The electron micrograph shows part of a spermatogonium (rat).

The basement membrane of the seminal tubule appears at *bm*, the cell membrane of the spermatogonium at *cm*, and part of its nucleus at *n*. A mitochondrial profile is marked *m*.

Circular (*c*) and oval (*o*) profiles representing the vesicular elements of the endoplasmic reticulum occur more or less evenly scattered throughout the cytoplasm. Elongated profiles (*e*) are scarce. Most of the ER profiles belong to the smooth surfaced variety.

The array of elongated profiles at *cs* is similar in appearance to structures encountered in the centrosphere region of other cells and described by many workers (17, 18) as the Golgi apparatus. Note that circular and oval profiles clustered around the array are morphologically similar to the profiles of the endoplasmic reticulum.

Magnification 30,000.

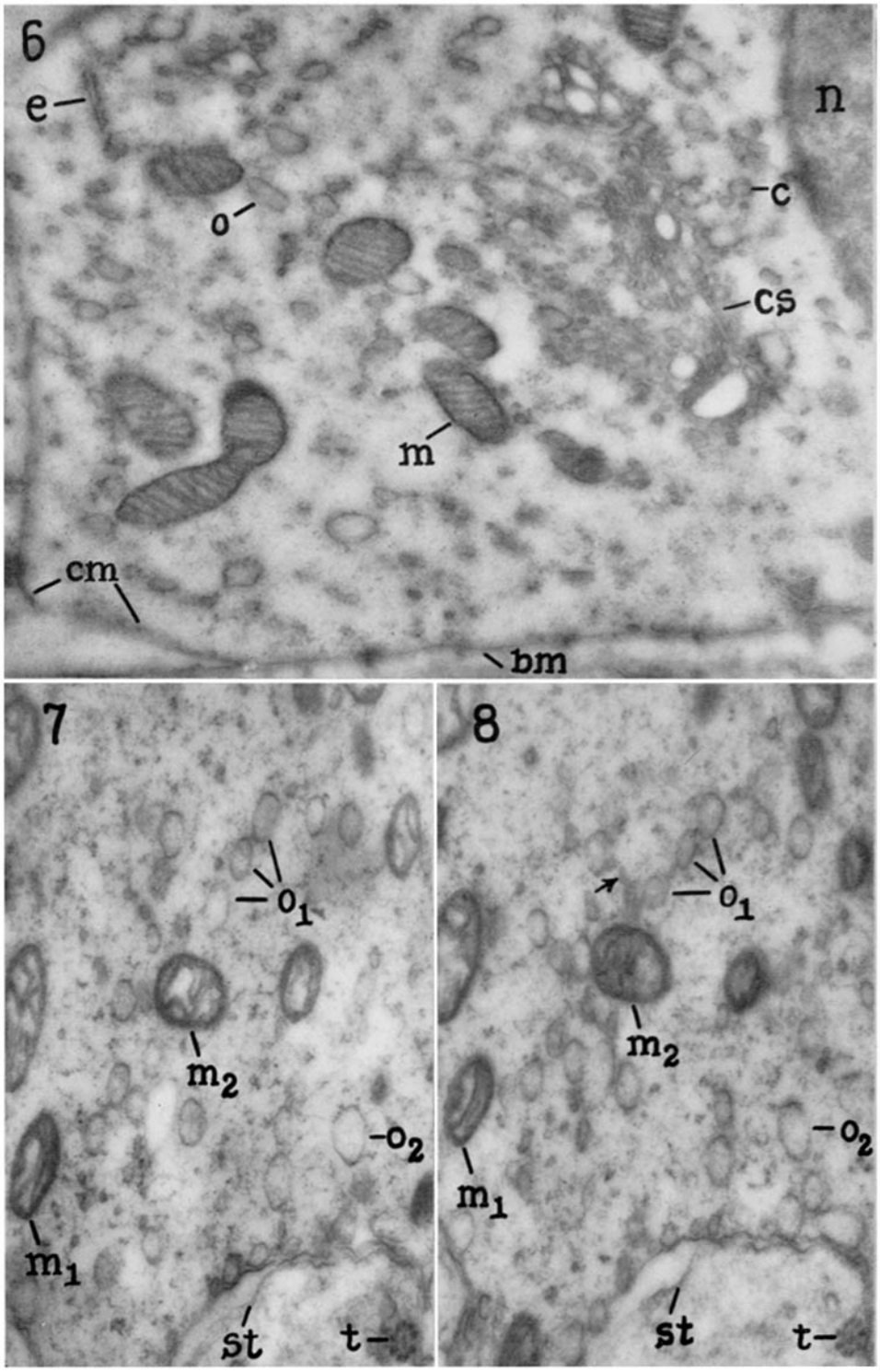
FIGS. 7 and 8. These micrographs represent two consecutive sections through the same spermatid (rat).

At *st* appears the cytoplasmic sheath in which takes place the development of the tail. The filaments of the latter can be seen at *t*. Mitochondrial profiles can be recognized at m_1 and m_2, and ER profiles (100 to 250 $m\mu$ in diameter) at o_1 and o_2.

The three profiles marked o_1, appear as independent units aligned in a row in Fig. 7 whereas in Fig. 8 they are linked together by narrow, apparently tubular connections. Another connection with an adjacent, though differently oriented row of profiles is indicated by an arrow in Fig. 8.

The profile marked o_2 bears a few attached particles on its outer surface.

Magnification 34,000.



(Palade: Endoplasmic reticulum. II)

FIG. 9. A relatively large field in the cytoplasm of a rat spermatid.

In the upper half of the field the section cut through the idiosome (*id*) which appears as an agglomeration of variously shaped and sized profiles. In this case, small circular profiles are predominant.

An array of six elongated profiles, the first one marked e_1 and the last e_6, can be seen covering the opening of the horseshoe formed by the idiosome. Note that these profiles are parallel to one another and are spaced at wider intervals than the characteristic elongated profiles of the idiosome (Golgi apparatus). Note also that the cytoplasmic matrix appears to be condensed around each of these profiles. Profiles 1, 4, 5, and 6 seem to be continuous with circular (*c*) or oval (*o*) profiles belonging to the endoplasmic reticulum.

Magnification 26,000.

FIG. 10. Small field in the cytoplasm of a rat spermatid showing, at a higher magnification, a structure similar to the one in Fig. 9.

The first and the last of the elongated profiles are marked e_1, and e_6 respectively. As in the previous figure they are disposed parallel to one another at relatively large and nearly uniform intervals ($\sim$ 150 mμ). Closer examination shows that almost all of these profiles are interrupted (arrows). Each is actually a row of elongated, circular or oval profiles. In three dimensions, the whole structure corresponds most probably to a pile of fenestrated cisternae. Note around each cisternal profile the condensation of dense, apparently amorphous material that fades gradually into the rest of the cytoplasmic matrix.

Circular ER profiles of various sizes (*c*) can be seen clustered close to the ends of the piled cisternae.

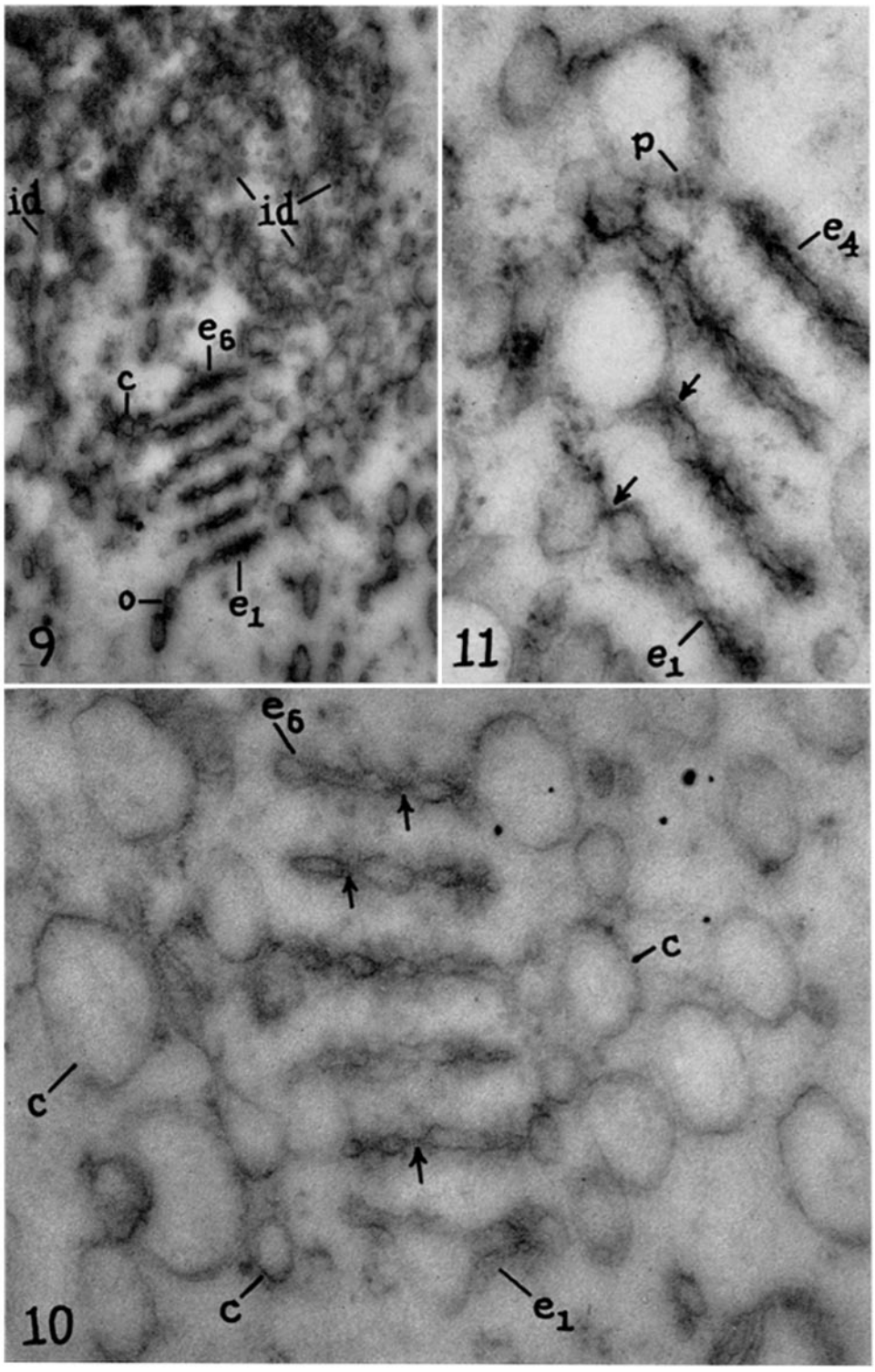
Magnification 72,000.

FIG. 11. Rat spermatid. The electron micrograph shows, at high magnification, one end of a pile of cisternae similar to the ones in Figs. 9 and 10.

The profiles e_1 and e_2 are in continuity (arrows) with large circular profiles of the endoplasmic reticulum. The pile appears therefore to represent a preferentially oriented part of this network reminiscent of the piles of fenestrated cisternae found in Nissl bodies (14). In contradistinction with the latter, the piled cisternae of the spermatids do not bear attached particles on the outside surface of their membrane, but are covered with a dense, apparently amorphous material.

A few particles (*p*) appear at the surface of a circular profile of the endoplasmic reticulum.

Magnification 74,500.



(Palade: Endoplasmic reticulum. II)

FIG. 12. Rat spermatid.

The cell membrane and profiles of marginated mitochondria can be seen at *cm* and *m* respectively. A number of circular (*c*) and oval (*o*) profiles belonging to the endoplasmic reticulum occur scattered throughout the cytoplasm either individually or in rows.

In the center of the field, the section cut through the idiosome (Golgi apparatus, acroblast) which appears as a large agglomeration of membrane-bound profiles of various shapes and sizes. Characteristic of this structure are the arrays of elongated profiles disposed parallel to one another at small, more or less regular intervals (a_1, a_2) and the swarms of small circular profiles (*s*) which accompany these arrays. In the section, five arrays of elongated profiles appear disposed discontinuously along an oval contour. Numerous circular and oval profiles ranging in size from the small, characteristic profiles of the idiosome (d = 30 to 50 mμ) up to the usual, relatively large profiles of the endoplasmic reticulum (d = 150 to 250 mμ) can be seen clustered in the concavity and along the convexity of the contour. Note that the larger profiles of the idiosome are morphologically similar to the profiles of the endoplasmic reticulum. The content of the largest elements of the idiosome appears, however, lighter.

Magnification 26,000.

FIG. 13. The electron micrograph shows at a higher magnification the array of elongated profiles marked a_1 in Fig. 12.

The characteristic elongated and small circular profiles of the idiosome are marked *e* and *s* respectively. A few small circular profiles appear to be in contact (arrows) with a large circular profile (*c*) of the type ascribed to the endoplasmic reticulum of the spermatid.

Magnification 66,500.

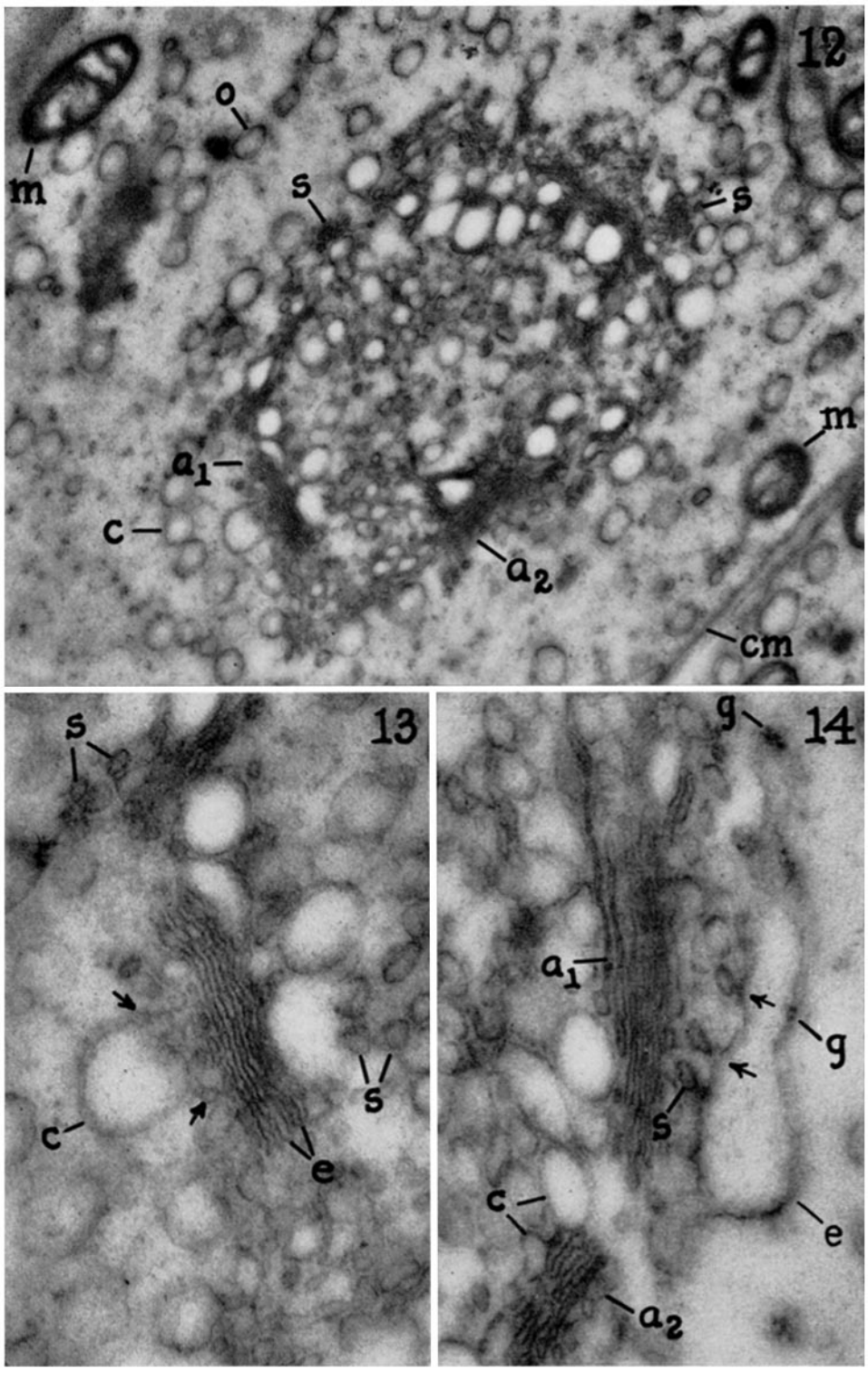
FIG. 14. Part of the idiosome of a rat spermatid.

Two arrays of elongated profiles appear at a_1 and a_2 with a number of small circular profiles (*s*) around them. At *e* can be seen the elongated, irregular profile of a relatively large element of the endoplasmic reticulum. The section cuts tangentially through its extremities and opens its cavity in between. The few small dense particles (*g*), attached to the outer surface of the membrane limiting this profile, indicate that the latter belongs to the endoplasmic reticulum. As is known the profiles of the idiosome are free of attached particles.

Note that three small, circular profiles of the idiosome are in close contact with the ER element described and that two of these profiles appear to open (arrows) in it. Such appearances indicate that continuity of membrane and content exists between the two systems at least intermittently. In the light of such findings, the idiosome (Golgi apparatus) and the endoplasmic reticulum may appear as local differentiations within a continuous system of intracytoplasmic, membrane-bound spaces.

The innermost profile of the array a_2 shows a number of interruptions and local swellings (*c*) which suggest that the vesicles of the idiosome are derived by fragmentation and swelling from the piled cisternae of the arrays.

Magnification 74,500.



(Palade: Endoplasmic reticulum. II)

PLATE 146

FIG. 15. Part of a basophil granulocyte found in the spleen of a rat.

The profile of a nuclear lobe can be seen at *n* showing a finely granular, apparently ordered structure. The cell membrane is visible in the lower left corner of the figure.

In the cytoplasm appear: a mitochondrial profile (*m*); numerous profiles of the basophil granules (*bg*) characteristic of this type of cell; clusters of small, dense particles (*p*); and numerous profiles of the endoplasmic reticulum. Most of the ER profiles are circular (*c*) or oval (*o*) in shape, measure 50 to 150 mμ in diameter, and belong to the smooth surfaced variety; only a few elongated profiles (*e*) are present and very few profiles bear attached particles on the outer aspect of their limiting membrane.

At *v* appears the profile of a large, vesicular structure that contains smaller vesicles and seems to be related to the membranous structures of the centrosphere region. A few such formations seem to be present in every cell. Recent observations (33) indicate that they occur in large numbers in the cytoplasm of ovocytes.

At this level of resolution, the basophil granules (*bg*) appear to be spherical bodies of high density and homogeneous texture. In their general appearance they are reminiscent of the zymogen granules of the acinary cells of the pancreas (11).

Magnification 30,000.

FIG. 16. Small field in the cytoplasm of an eosinophil granulocyte (submucosa of the jejunum; rat).

The cell membrane, sectioned very obliquely, appears in the upper corners of the figure. The cytoplasm contains two mitochondrial profiles (*m*); numerous profiles of eosinophil granules (*eg*) cut at various angles;[3] small, dense particles isolated or in clusters (*p*); and numerous profiles ascribable to the endoplasmic reticulum. The latter are mainly circular (*c*) or oval (*o*) in shape, with only a few elongated profiles present, measure 50 to 150 mμ in diameter, and belong mostly to the smooth surfaced variety. A few rough surfaced profiles are present, one of them marked *op*.

Some circular profiles, otherwise comparable to the ER profiles, contain a small, dense, granular or rod-like body; one example appears at *x*; another one can be seen in Fig. 15 to the left of the mark *v*.

Magnification 47,000.

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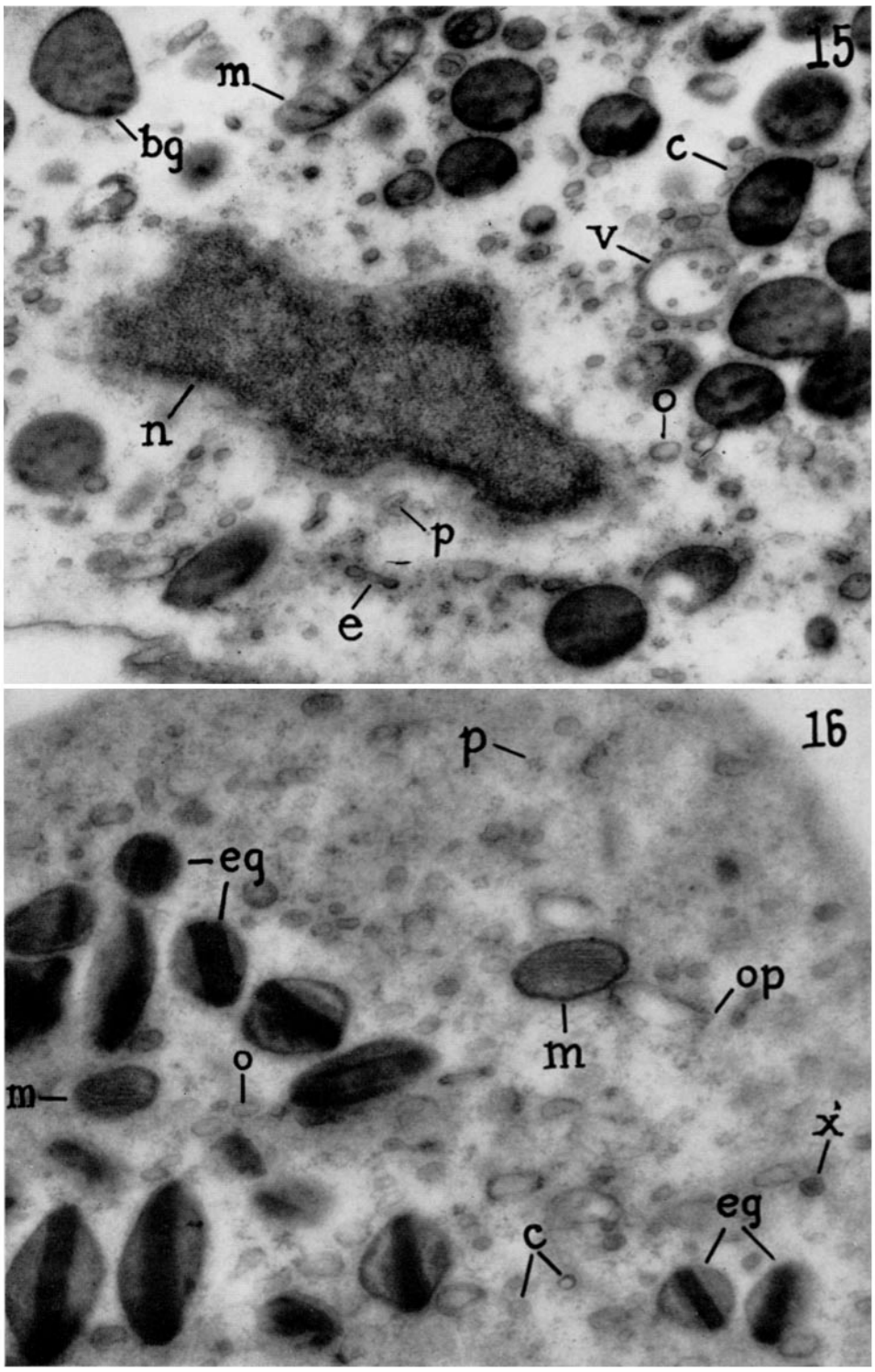
[3] Such granules have been studied in more detail by Watanabe (29) and more recently by Sheldon and Zetterquist (31). The latter however assumed that they were neutrophil granules.



(Palade: Endoplasmic reticulum. II)

PLATE 147

These four electron micrographs represent limited fields in the cytoplasm of neutrophil granulocytes (rat spleens).

The cell membrane is visible only in the upper right corner of Fig. 18; parts of the lobulated nuclei, distinguished by their fine, granular texture, are marked *n*; mitochondrial profiles are indicated by *m*; smooth surfaced profiles of the endoplasmic reticulum (50 to 150 mμ in diameter) by *c* and rough surfaced ones by *cp*. The dense formations marked *ng* (100 to 150 mμ in diameter) are profiles of the neutrophil granules characteristic of this cell type. The cytoplasmic matrix is relatively dense and contains small clusters of small, dense particles (*p*).

FIG. 17. The general similarity between the ER profiles of smooth surfaced variety and the profiles of neutrophil granules is illustrated. Both have comparable dimensions and are limited by membranes of similar thickness. Their differentiation on account of their respectively light and dense contents is rendered difficult or arbitrary because of the existence of profiles with content of intermediate density (*im*).

The slightly larger (250 to 300 mμ in diameter) profiles (*dv*), each similarly limited by a membrane but containing in its cavity a dense, apparently granular body, may represent digestive vacuoles. The large structure at *x* is unidentified.

Magnification 66,000.

FIG. 18. The figure shows that the neutrophil granules are relatively polymorphic bodies. Most of them are spherical, but profiles such as ng_1 and ng_2 indicate that some granules are respectively of biscuit-like or cylindrical shape. The structures marked *dv* are in many respects similar to neutrophil granules; in their dense content, however, irregular masses of higher density can be distinguished. The interpretation of such structures is uncertain: they may represent faultily sectioned granules, but it is more likely that they are profiles of digestive vacuoles.

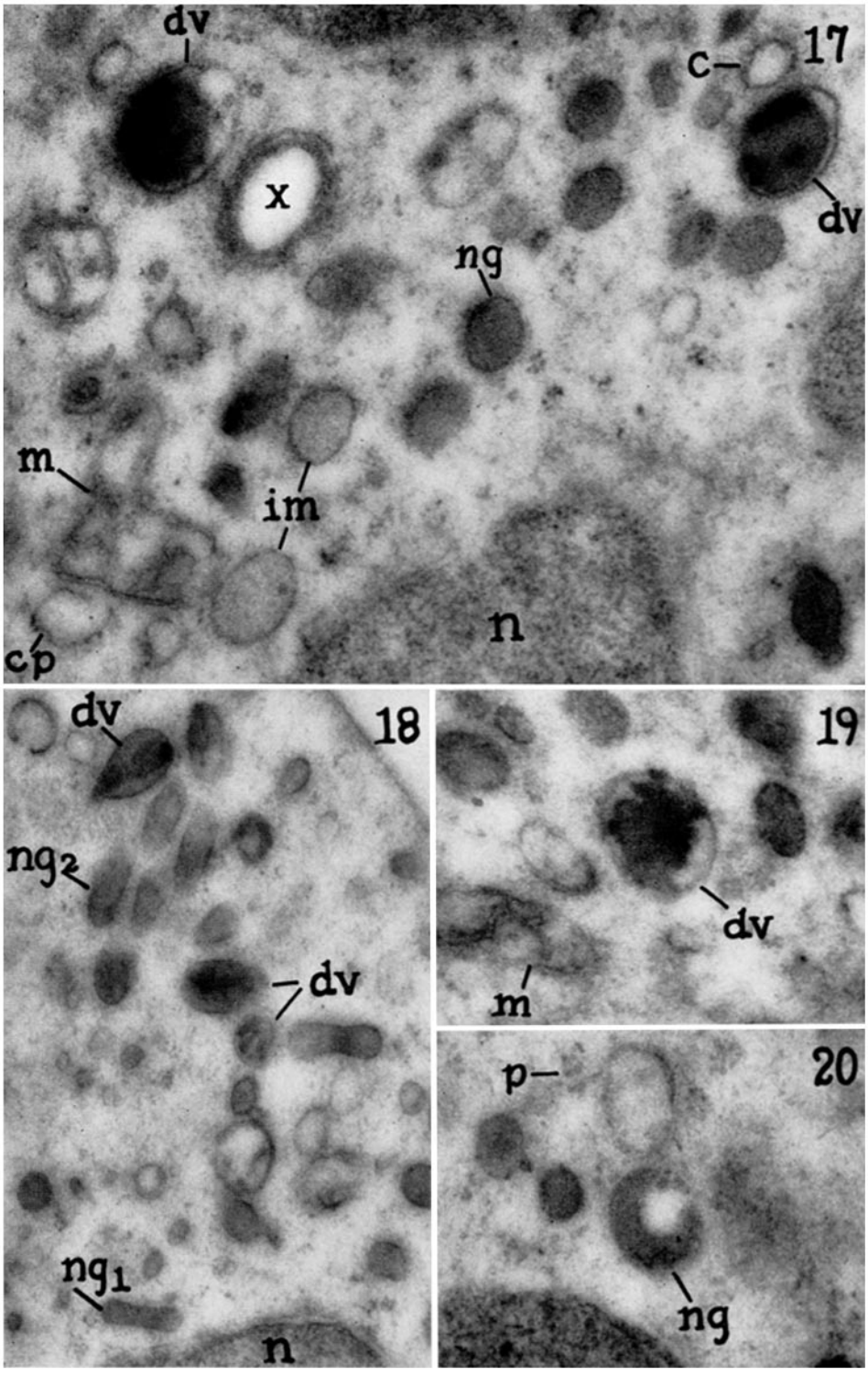
Magnification 46,500.

FIG. 19. The structure marked *dv* represents, in all probability, a digestive vacuole formed as a result of the phagocytic activity of the granulocyte. It is limited by a thin continuous membrane and has a complex content consisting of a dense, irregular, apparently shrunken body surrounded by a material of relatively light density. Digestive vacuoles of larger sizes and more diversified content are sometimes encountered.

Magnification 66,000.

FIG. 20. The electron micrograph illustrates a partly vacuolated neutrophil granule (*ng*). Such appearances, also encountered in basophil granules (see Fig. 15), may represent stages either in the development or involution of these specific granules.

Magnification 66,000.



(Palade: Endoplasmic reticulum. II)

PLATE 148

FIG. 21. Electron micrograph of a lymphocyte in the spleen of a young chicken.

The cell membrane (*cm*) appears as a single band varying in width and density with the angle at which it is sectioned. A "double membrane" is present only when the membrane of the lymphocyte is in close apposition to the plasma membrane of neighbouring cells.

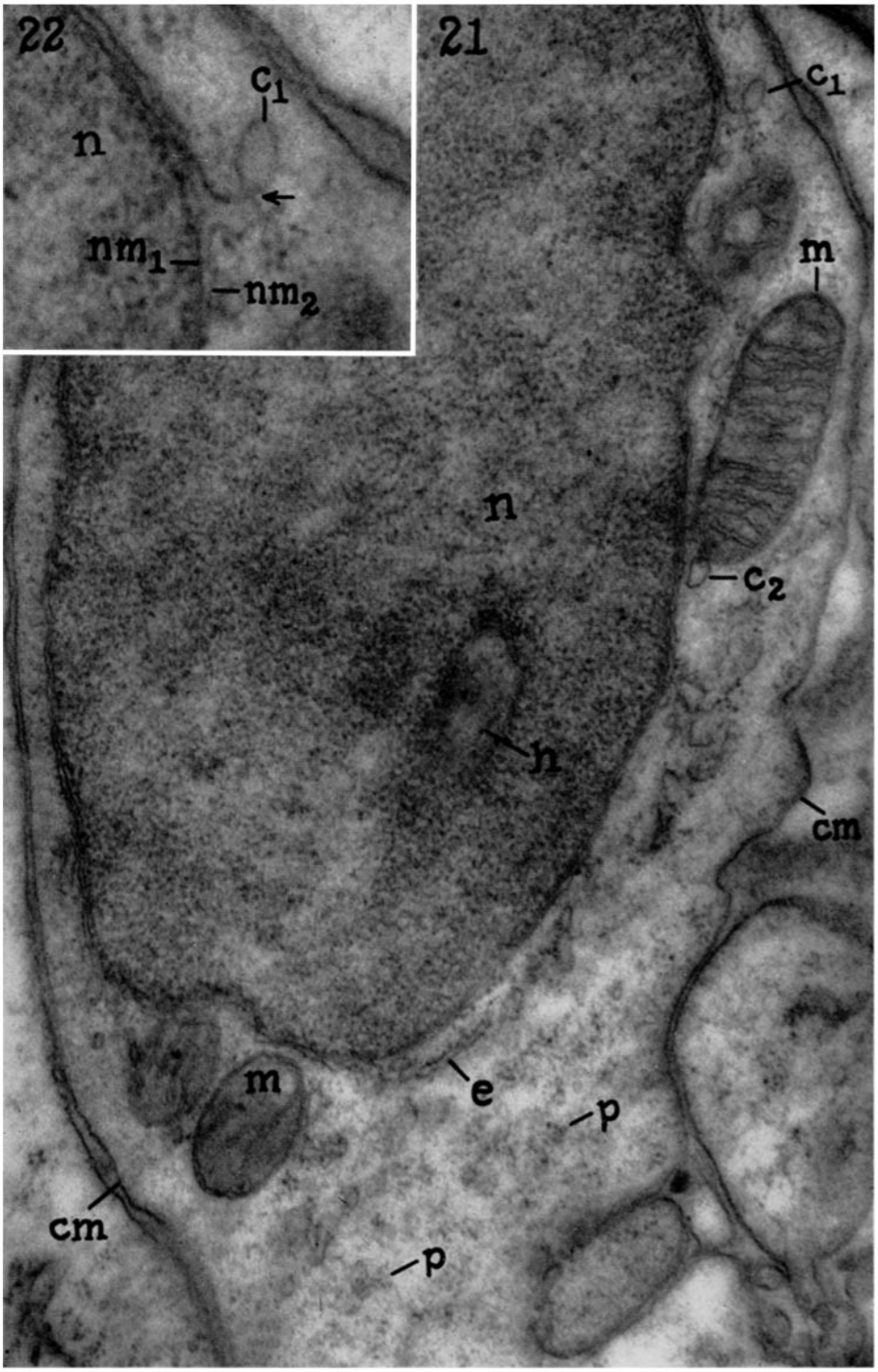
The cytoplasm, which is disposed in a relatively thin shell around the nucleus (*n*), contains a few mitochondrial profiles (*m*) and a few profiles that can be ascribed to the endoplasmic reticulum. These profiles vary in shape from circular (c_1, c_2) to elongated (*e*), measure 40 to 100 mμ in diameter, and belong to both the smooth (c_1, c_2) and the rough surfaced (*e*) variety. Numerous small dense particles, isolated or in clusters (*p*) can be seen freely scattered in the cytoplasmic matrix.

Note the relatively compact, granular structure of the nucleus. At *h* the section cuts tangentially to the bottom of a nuclear indentation.

Magnification 47,000.

FIG. 22. Higher magnification of a small field around c_1 in Fig. 21. The profile of the nucleus (*n*) is limited by two membranes (nm_1, nm_2) separated by a relatively light space. Note that the outer membrane has an invagination, which communicates through a narrow passage (arrow) with the vesicular element of the endoplasmic reticulum marked c_1. Such continuities of membrane and content indicate that the endoplasmic reticulum and the nuclear envelope are parts of a common system of membrane-bound spaces are recently advanced by Watson (21).

Magnification 100,000.



(Palade: Endoplasmic reticulum. II)

PLATE 149

FIG. 23. Part of a monocyte in the spleen of a young chicken.

The cell margins can be seen along the low and right sides of the micrograph. At *n* appears part of the nuclear profile and at *m* mitochondrial profiles. Note the lack of parallelism of the two nuclear membranes and the numerous small invaginations (one of them is indicated by an arrow) formed by the membrane limiting the cytoplasm towards the perinuclear space.

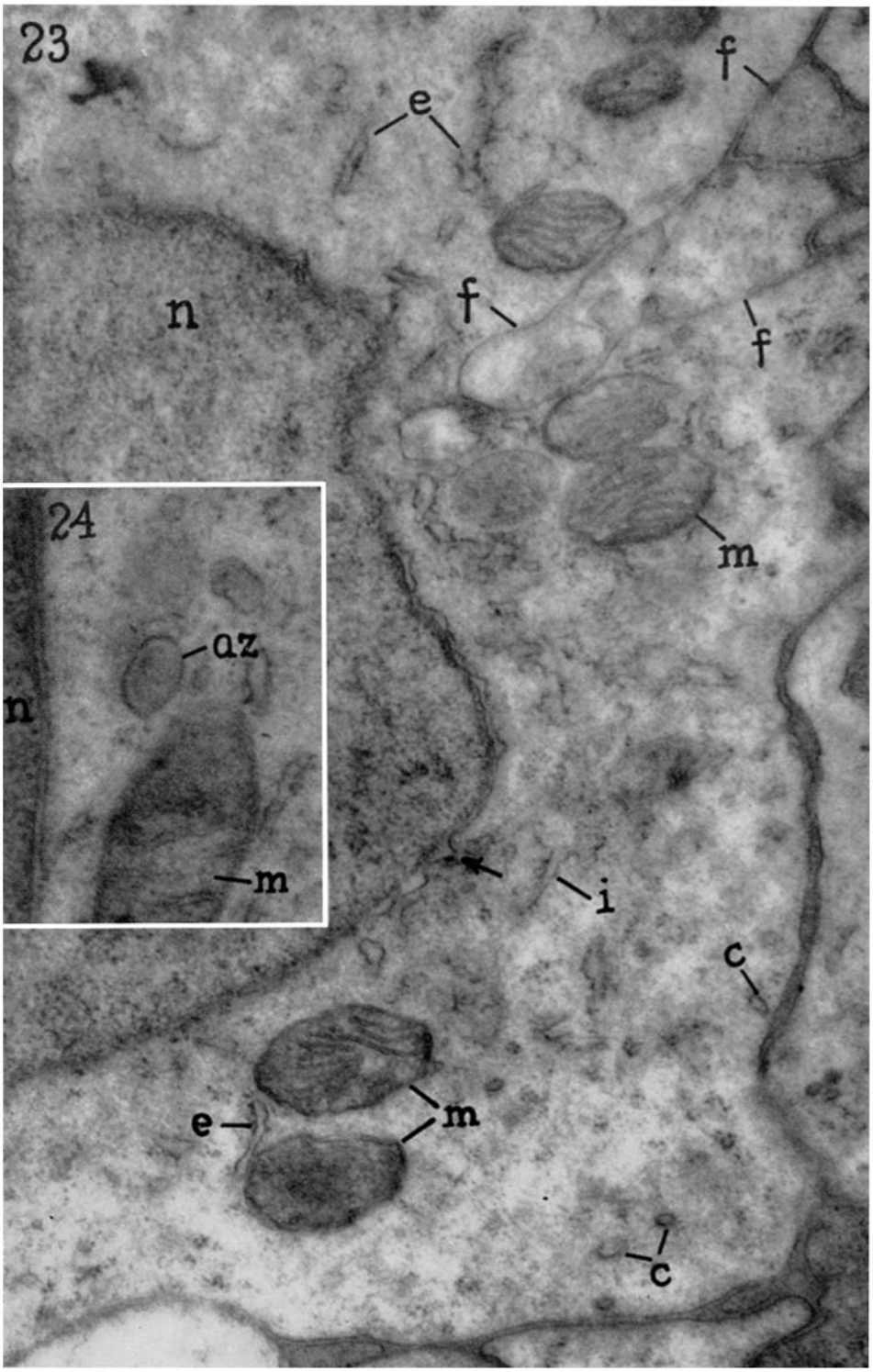
The cytoplasm contains a number of ER profiles which vary in shape from circular (*c*) and oval, to elongated (*e*) and irregular (*i*) and measure 40 to 80 mμ in diameter. At the periphery of the cell, most of the profiles are circular and belong to the smooth surfaced variety, whereas in more central regions elongated profiles and profiles belonging to the rough surfaced variety are predominant.

The cell membrane shows a deep infolding (*f*) filled in part by expansions of other cells. The examination of this infolding shows that a double membrane is present only where two cells are in close apposition. At the bottom of the fold which is not reached by any cell expansion, the limiting membrane of the monocyte appears simple. The profiles of two large vesicles with smaller vesicles inside can be seen close to the bottom of the fold marked *f*.

Magnification 43,000.

FIG. 24. Small field in a lymphocyte (chicken spleen). Part of the nucleus appears at *n* and part of a mitochondrial profile at *m*. The formation marked *az* is probably the profile of an azurophil granule.

Magnification 72,500.



(Palade: Endoplasmic reticulum. II)

PLATE 150

FIG. 25. The electron micrograph shows the periphery of a monocyte in the spleen of a rat.

The cell is in contact with two erythrocytes (et_1, et_2). The cell membrane forms a number of infoldings (f_1, f_2, f_3, f_4) and some of these are continued inside the cytoplasm by rows (*r*) of circular and oval profiles. Except for their alignment in rows, these profiles are entirely similar with the circular (*c*) and oval (*o*) profiles of the endoplasmic reticulum.

Such appearances suggest that part of the smooth surfaced profiles at the periphery of the cell may derive by invagination from the cell membrane.

Magnification 34,000.

FIG. 26. Small field in a basophil granulocyte (rat spleen). Part of the profile of a nuclear lobe appears at *n*, and basophil granules at *bg*.

The outer nuclear membrane (nm_2) forms two large pockets separated by a nuclear pore (arrow). The upper pocket is continuous with an elongated profile (*e*) of the endoplasmic reticulum. The second nuclear membrane as well as the membrane limiting the elongated *ER* profile bear attached particles on their outer surface.

The appearance suggests that the perinuclear space (perinuclear cisterna) represents a differentiated part of the endoplasmic reticulum.

Magnification 46,000.

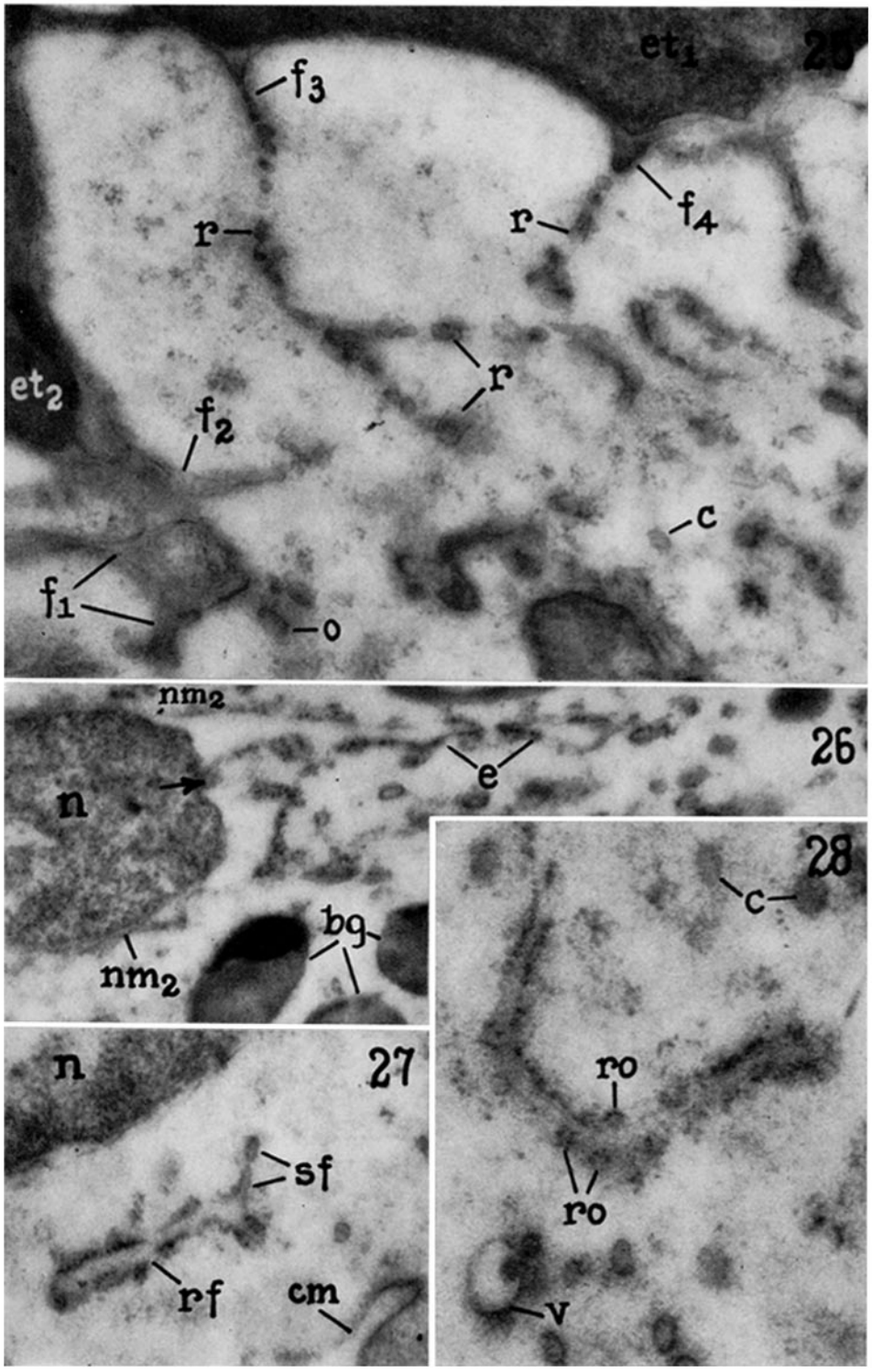
FIGS. 27 and 28. Small fields in the cytoplasm of a neutrophil granulocyte (rat spleen).

An example of continuity between rough surfaced (*rf*) and smooth surfaced (*sf*) profiles is seen in Fig. 27.

A cisternal profile of "mixed" appearance, *i.e.*, partly covered with and partly free of granules, appears in Fig. 28. Note that the particles seem to be disposed in small rosettes (*ro*) on the cisternal membrane.

Part of the nucleus can be seen at *n*; the cell membrane at *cm*; circular profiles of smooth surfaced variety at *c*; and a compound vesicle at *v*.

Magnification Fig. 27, 48,000; Fig. 28, 65,500.



(Palade: Endoplasmic reticulum. II)