A SMALL PARTICULATE COMPONENT OF THE CYTOPLASM*

BY GEORGE E. PALADE, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research*)

PLATES 11 TO 16

(Received for publication, October 15, 1954)

The morphological analysis of animal cells by electron microscopy has already revealed numerous structural elements of either fibrillar or membranous nature in the ground substance of the cytoplasm. This is the part of the cell's substance which usually appears as an amorphous, homogeneous matrix in the light microscope and which, because of this appearance and of various theoretical considerations, has been considered until recently to be devoid of structure at the supramolecular level of organization (1). In actual fact, some indirect evidence for the existence of certain, recently resolved, fibrillar structures was available from older studies with the polarizing microscope (2, 3); such was the case, for instance, for the elementary neurofibrils of the axoplasm (4); the elementary filaments of the myofibrils (5, 6); and the fine fibrils of the epidermal cells (7, 8). But for other components, *e.g.* the endoplasmic reticulum, the first evidence came from electron microscope studies. This reticulum, which appears to be a network of cavities separated from the rest of the cytoplasm by a membrane, was first described in electron microscope studies of cells cultured *in vitro* (9, 10) and was recently identified and surveyed in cells *in situ* (11, 12). The present paper introduces evidence for the existence of still another component of the ground substance of the cytoplasm which is particulate in nature and small in size. In many cells, though not in all, it appears to be in close association with the membrane bounding the cavities of the endoplasmic reticulum.

Materials and Methods

The small, granular component was studied in cells fixed *in situ* and examined in sections. The material, mostly rat and chicken tissues and organs, was fixed in OsO_4 buffered at a slightly alkaline pH (13) for periods of time ranging from 30 minutes to 24 hours. The specimens were subsequently embedded in *n*-butyl methacrylate (14) and sectioned at 0.05 μ or less with a microtome provided with a mechanical advance (15). The sections, with the embedding plastic left in, were examined in an RCA microscope, model EMU-2b. The micrographs were taken at an original magnification of 5,000 to 10,000 and thereafter enlarged photographically.

In a few cases, *i.e.* those of striated muscle and pancreas, tissue specimens were fixed not only in buffered OsO_4 but also in formaldehyde (4 and 10 per cent), buffered at the same pH (7.30) with acetate-veronal.

---

* The substance of this article was presented in 1953 at the eleventh annual meeting of the Electron Microscope Society of America in Pocono Manor, Pennsylvania, and has already been published in abstract form in the *Journal of Applied Physics*, 1953, **24,** 1419.

J. BIOPHYSIC. AND BIOCHEM. CYTOL., 1955, Vol. 1, No. 1

OBSERVATIONS

Form, Size, and Density.—The new cytoplasmic component usually appears in the form of small, rounded bodies. Their diameter being smaller than the thickness of the sections, it can be safely assumed that in three dimensions the bodies described are of more or less spherical shape. The form varies, however, to a certain extent as indicated by the occasional occurrence of rod-like, and polygonal particles of comparable dimensions.

Most of these bodies measure 100 to 150 A in diameter and thus fall in the range of dimensions usually ascribed to macromolecules. Few smaller (80 to 100 A), or larger (150 to 300 A) particles are encountered. In most specimens their presence can be explained, at least in part, by the sectioning of granules of usual size or by their conglomeration.

In electron micrographs, the small granules are distinguished by relatively high density which can indicate either an originally low water content or a particular affinity for osmium oxides. The particles show in better contrast after long OsO_4 fixation, a result that may be due to the extraction of surrounding materials (16). The fact, however, that the small granules appear dense also after short OsO_4 fixation, and even after formaldehyde fixation, is taken to indicate that they originally had a high density.

At the present level of resolution, most of these particles show as homogeneously dense bodies, but in some cases at least, there are suggestions of further organization in the form of a central, denser body or of an outer, denser shell. The latter form is particularly frequent in striated muscle fibers in which, in general, the particles are larger ($\sim$200 A) than in other cells (Fig. 8).

In view of their spherical shape, small size, high density, and usually massive structure, it is considered that the new particles can be conveniently described as small granules.

Distribution.—A survey covering more than 40 different cell types indicated that the small granules are present in all the types of cells examined, with the exception of the adult erythrocyte. Their number, however, varies considerably from one cell type to another, but seems relatively constant for any given type. The lowest concentration is found regularly in the cytoplasm of granulocytes and seminal epithelia, and the highest in embryonic cells, in rapidly proliferating cells in the adult (cells of the basal layer of the epidermis, epithelium of the intestinal crypts), and especially in certain glandular cells, such as those of the exocrine pancreas and of the salivary and mammary glands.

Relationship to Other Cell Components.—The small granular component seems to have a particular affinity for the membrane of the endoplasmic reticulum. This is indicated by the fact that in many cells the outside surface of this membrane, *i.e.* the surface in contact with the cytoplasmic matrix, is found to be covered by numerous granules of the size, form, and density described. Wherever this membrane is perpendicularly sectioned, the particles are seen in side view and appear to be in close contact with it, as if "attached" to its surface (Figs. 1 and 2). Usually they seem to be disposed without order, but occasionally short rows are encountered within which the granules occur at regular intervals. Additional significant information, concerning both the disposition and the number of "attached" granules, is obtained from sections passing very obliquely through the elements of the endoplasmic reticulum in such a manner as to show relatively large areas of their limiting membrane. Such favorably oriented sections offer almost full-faced views of this membrane and of the arrangement of the small granules upon its surface (Figs. 3 to 5). Electron micrographs of these sections show that the "attached" granules are frequently disposed in linear series and that in these series they are spaced at more or less regular intervals; *i.e.*, 80 to 150 A. The linear series in turn form consistent patterns, among which parallel double rows, loops, spirals, circles, and rosettes (Figs. 3 and 5) appear to be predominant. Although such patterns are of frequent occurrence, and, moreover, seem to recur preferentially in particular combinations for a given cell type, it should be noted that sometimes the particles are scattered at random (Fig. 4) and sometimes their disposition approaches a close packing.

In certain cell types known for the intense basophilia of their cytoplasm (17), such as the acinar cells of the pancreas (Figs. 1, 2, and 4), of the mammary and salivary glands (Fig. 3), certain cells of the hypophysis, and the plasma cells of the lymphatic tissue (Fig. 5), the majority of the numerous granules regularly found in their ground substance appear in close association with the membrane of the endoplasmic reticulum, and only a relatively few are freely scattered in the intervening matrix. It may be recalled that such cells are distinguished by a well developed and highly oriented endoplasmic reticulum and that the membrane bounding its tubules, vesicles, and cisternae offers a relatively large surface for the attachment of the granules. In the acinar cells mentioned, both the endoplasmic reticulum and the associated granules are preferentially concentrated in the basal half of the cell. When examined in the light microscope after appropriate staining, the cytoplasm at the basal pole of such cells is intensely basophilic and occasionally shows some indication of structure in the form of filaments, lamellae, and nebenkerne. This basal cytoplasm of acinar cells has, of course, been intensively studied in light microscopy since the original work of Garnier (18), who, recognizing its staining affinities and location, described it as "ergastoplasm" and considered it as a highly active form of cytoplasm.

In other cells, *e.g.* nerve cells of various kinds, the type of association found between the endoplasmic reticulum and the small granular component is slightly different (19); the two cytoplasmic components still occur in close association in the differentiated, intensely basophilic zones of the cytoplasm known as Nissl bodies, but in addition to numerous particles "attached" to the membrane of the endoplasmic reticulum, a considerable number of similar granules are found scattered in the matrix of the ground substance between and around the elements of the network. Similar but smaller masses of endoplasmic reticulum and associated particles are encountered in parenchymatous liver cells.

A disposition, fundamentally comparable to the one described in perikarya and characterized by the presence of a large number of small granules, some of them "attached" to the membrane of the endoplasmic reticulum and some free in the surrounding ground substance, occurs in other cell types; *e.g.*, fibroblasts and embryonic endothelial cells. In these cases, however, both the endoplasmic reticulum and the small particles are more evenly distributed throughout the cytoplasm. After appropriate staining, such cells appear diffusely basophil in the light microscope.

In certain other cells, namely in the epithelial cells of intestinal crypts (Figs. 6 and 9), in those of the basal layer of the epidermis, and in young lymphocytes in spleen (Fig. 7) and lymph nodes, the small granules still occur in considerable numbers, but the large majority of them are found to be more or less evenly distributed, individually or in small clusters, throughout the entire cytoplasm. Very few particles are associated with the endoplasmic reticulum, which is, in general, poorly developed in such cells, being represented by only a few profiles per cell section. The membrane limiting these profiles is actually free of, or only partially covered with, small granules (Figs. 6 and 7). It seems, therefore, that the presence of free granules in large numbers in the cytoplasmic matrix is not due to the lack of membrane surface available for their attachment. Histochemical studies indicate that the cells of this group also have an intensely basophilic cytoplasm (17).

An extreme condition is shown by the granulocytes of the blood and by certain elements of the seminal epithelium; namely, spermatocytes and spermatids. In these cell types, the elements of the endoplasmic reticulum are numerous, but their membrane is usually free of granules. There are only a few small particles present and these appear diffusely scattered throughout the ground substance (Fig. 10). Such cells have, in general, an acidophilic cytoplasm.

Finally, a special situation is encountered in striated muscle fibers (Fig. 8), in which the small granules, together with the endoplasmic reticulum, are found restricted to the thin layers of sarcoplasm that separate the myofibrils. Although close to the membrane of the reticulum, the granules do not appear to be attached to it. Besides this feature, they are also characterized by larger sizes, *i.e.* 200 to 300 A, and a more polymorphic appearance, rods and granules with a light core being a frequent occurrence.

In addition to the described affinity for the membrane of the endoplasmic reticulum, the small granules displayed in many cell types a similar affinity for the second nuclear membrane; *i.e.*, the membrane limiting the cytoplasm towards the nucleus (Figs. 2 and 6). The other membranes present in the cytoplasm, namely the plasma membrane (inner surface) (Figs. 1, 5, 6, 7, and 9), the outer membrane of the mitochondria (20, 21) (Figs. 1, 5, 6, 7, and 9), and the membranes of the centrosphere region[1] (11), did not share in this affinity and appeared conspicuously free of small granules.

The large majority of the observations reported in this study were made on material fixed with OsO_4, a reagent known to give satisfactory preservation of tissue specimens. In evaluating the structures found in such material, however, the possibility of osmium precipitation and osmium staining must be taken into account, especially when the structures involved could conceivably result from such processes. This may well apply for a granular material of high density as the one here reported. For this reason, the results obtained on OsO_4-fixed tissues were checked against formaldehyde-fixed muscle and pancreas. These two materials were chosen because of the characteristic and easily recognizable location of the small granules in striated muscle fibers and in pancreatic acinar cells. After formaldehyde fixation the cells appeared poorly preserved, but particles of appropriate size were found in the expected numbers and locations in the best fixed parts of the specimens. Although these particles were less dense than in OsO_4-fixed cells, they were found to be denser than the other components of the ground substance of the cytoplasm. The findings indicate that (*a*) the small granules represent native concentrations of cytoplasmic material and do not result from the precipitation of osmium oxides, (*b*) their high density is largely due to an originally low water content. Osmium staining, however, is not excluded as a factor enhancing this density.

DISCUSSION

The first point to be discussed in relation to these findings is the perennial and arduous question of artefact *versus* reality. The question is admittedly difficult to answer at the dimensional level examined, where direct comparison with the situation in the living cell is precluded by instrumental limitations. Only indirect evidence can be used, therefore, in support of the thesis that the small granules described are part of the cytoplasmic organization *in vivo*.

The granules concerned do not seem to be artefacts of precipitation introduced by the OsO_4 fixation of the specimens, because, as already mentioned, they are present as small, dense particles with the respective, characteristic distribution in the acinar cells of the pancreas and in striated muscle fibers after fixation with buffered formaldehyde. Moreover, a small particulate component of comparable size exists apparently in homogenates of fresh, un-

[1] In their general appearance these membranous formations are similar to structures recently described under the name of "Golgi apparatus" in epididymal epithelium (39), and under the name of "agranular reticulum" in various perikarya (19).

fixed hepatic and splenic cells from which it has been repeatedly isolated by means of differential centrifugation by Barnum and Huseby (22) and by Petermann, Mizen, and Hamilton (23–25). The former described these particles as "ultramicrosomes" and the latter as "macromolecules," and both groups stressed the finding that the particulates concerned contain a high amount of ribonucleic acid.

The relationship between the membrane of the endoplasmic reticulum and the small granules deserves special consideration. It is obviously unknown whether the association described pre-exists *in vivo* or is established only during fixation, and it is quite conceivable that during this process small granules originally free in the ground substance may be adsorbed out on available surfaces as a result of environmental changes induced by the fixative. Even if this were the case, however, the fact remains that the particles concerned are adsorbed not on all, but only on certain, membranes available in the cytoplasm and that on these latter they are disposed in recognizable and recurring patterns. This finding may indicate chemical differences among the various cytoplasmic membranes and may reveal in their texture an otherwise invisible pattern of chemical functions responsible for the adsorption. It should be noted, however, that in an electron microscope study of isolated microsomes, Slautterback (26) was able to demonstrate the presence of small particles, which he called "small microsomes," at the surface of larger vesicles, named "large microsomes," which, isolated or in strings, constituted the main component of the microsome fraction. The "small microsomes" are similar in size and density to the small granules described in this paper, whereas the "large microsomes" result in all probability from the fragmentation of endoplasmic reticula during homogenization. Slautterback's findings suggest therefore that the association of the small granules with the membrane of the endoplasmic reticulum is not necessarily induced by fixation; it may exist *in vivo*, and seems to be strong enough to resist the procedure for separating microsomes which involves tissue homogenization and long suspension in foreign media.

Another question which appears to have particular significance is that of the relationship of these particles to cytoplasmic basophilia, a property known to be due to a large extent to the ribonucleic acid (RNA) present in the cytoplasm. If the electron microscope analysis is limited to the acinar cells of various glands, *e.g.* pancreas, salivary, and mammary glands, then it appears that there are two different components in the intensely basophil cytoplasm, formerly called ergastoplasm, that occupies the basal regions of such cells. One is an essentially membranous component, represented by the membrane-bound vesicles, tubules, and cisternae of the endoplasmic reticulum; the other is the small granular component described in this paper. The same situation is encountered in the basophil cytoplasm of plasma cells, in the basophil masses found in parenchymatous liver cells, and in the Nissl bodies. It is obviously difficult to decide, in these cases, whether both components or only one of them is responsible for basophilia. But when the analysis is broadened to include a variety of cells known for their basophil cytoplasm, such as embryonic cells in general and rapidly proliferating cells in the adult, it becomes clear that the component which can be most satisfactorily correlated with basophilia is the small granular component. A good example in point is provided by the situation found in the epithelial cells of intestinal crypts. They have a poorly developed reticulum, a high concentration of small granules in their ground substance, and, when appropriately stained, an intensely basophil cytoplasm. The correlation suggested by this survey is supported by a number of cytochemical studies which have shown, as already mentioned, that small particles of comparable size isolated from liver or spleen homogenates contain a large proportion of RNA. In the case of the macromolecules separated by Petermann and her coworkers, the RNA may represent as much as 50 per cent of the total nucleoprotein content of the particles. To such data could be added the earlier results of Chantrenne (27), who found the greatest relative amount of RNA in the fraction of smallest cytoplasmic granules he was able to separate by differential centrifugation from liver homogenates. Final proof for this correlation can be expected in the future from an integrated electron microscopical and biochemical study of cytoplasmic fractions. Until such proof is available, it must be realized that the grouping of these particles under a common label, that of the small granular component, is based exclusively on morphological findings. This, naturally, does not exclude the possibility that they are chemically diverse; in fact, such a diversity is already suggested by small differences in size and morphology, as in the case of the granules found in striated muscle fibers, and by differences in the affinity shown for the membrane of the endoplasmic reticulum. It is to be noted, moreover, that such a diversity has been demonstrated in the case of the macromolecules isolated by Petermann. She succeeded, namely, in separating these macromolecules into six different categories according to their sedimentation constants and surface charges. In all probability, therefore, the small granules represent a class of cytoplasmic components rather than a single, chemically defined component. Available data suggest only that the majority of these components may be similar in having a high RNA content.

A further question that deserves discussion concerns some correlations recently established between electron microscope findings and earlier cytological observations. The term "ergastoplasm," originally coined by Garnier, has been revived in electron microscopy by Bernhard *et al.* (28) and more recently by Weiss (29) in their studies on the structure of the basophil cytoplasm in salivary and pancreatic acini. In these studies "ergastoplasm" was used as a synonym for either the endoplasmic reticulum or the basophil substance in general. It appears from the preceding observations and discussion that the term can properly be used only to designate the association of endoplasmic reticulum and small granules found in the basal cytoplasm of acinar cells. In this association the small granules seem to be responsible for the basophilia whereas the endoplasmic reticulum, by its high degree of orientation, may induce the laminated or "nebenkern" appearance. It does not appear advisable, however, to equate "ergastoplasm" with either the endoplasmic reticulum or the basophilic substance because the endoplasmic reticulum in itself does not seem to be basophil and because basophil cytoplasm may exist without "laminated" structure. Good examples in point are provided by the situation described in seminal epithelia and by that found in the epithelial cells of the intestinal crypts.

A similar situation is encountered in correlating the microsome fraction of the cytochemists with the present findings. Microsomes have been isolated mainly from liver cells (30–32), in which the two components, *i.e.* the endoplasmic reticulum and the small granules, are usually found in close association. The chemistry and activity of the fraction represent, therefore, the cumulative properties of the two components. It may be speculated that the presence of the membranous component is reflected in the high phospholipid content of the fraction, whereas the inclusion of the small granular component is responsible for its high RNA content. Here again the properties of the microsomes cannot be equated with those of the endoplasmic reticulum or those of the basophil substance in general.

It is hoped that a clearer understanding of the chemistry and function of the small granular component will be reached in the future, through more detailed cytochemical studies for which the present morphological findings may provide a basis.

Structural elements comparable in size and density to the small granules described in this paper have been noted in recent electron miscroscope studies. In 1952, Porter (33) described "macromolecular units" in the cytoplasm of myoblasts and epidermal cells in *Amblystoma* larvae, and in 1953, Sjöstrand and Rhodin (34) encountered "dense dots" in the ground substance of the nephron epithelium (proximal convoluted tubule) in the mouse. Since this study was published in abstract (35), similar granules have been described by Watson and Avery (36) in hamster odontoblasts and by Howatson and Ham (37) in parenchymatous cells of mouse liver and mouse liver tumors.

SUMMARY

A particulate component of small dimensions (100 to 150 A) and high density is described in the ground substance of the cytoplasm of mammalian and avian cells.

In many cell types that seem to have in common a high degree of differentiation, the new component is preferentially associated with the membrane of the endoplasmic reticulum; whereas in other cell types, characterized by rapid proliferation, it occurs more or less freely distributed in the ground substance of the cytoplasm.

In the Discussion an attempt is made to integrate the observations presented in this paper with the already available cytological, histochemical, and cytochemical information.

BIBLIOGRAPHY

1. Frey-Wyssling, A., Submicroscopic Morphology of Protoplasm and Its Derivatives, New York, The Elsevier Press, Inc., 1948.

2. Schmidt, W. J., Die Bausteine des Tierkörpers in polarisiertem Lichte, Bonn, Friedrich Cohen, 1924.
3. Schmidt, W. J., Die Doppelbrechung von Karyoplasma, Zytoplasma und Metaplasma. Protoplasma Monographien, Berlin, Borntraeger, 1937.
4. Schmitt, F. O., and Geren, B. B., *J. Exp. Med.*, 1950, **91,** 499.
5. Hall, C. E., Jakus, M. A., and Schmitt, F. O., *Biol. Bull.*, 1946, **90,** 32.
6. Bennett, S. H., and Porter, K. R., *Am. J. Anat.*, 1953, **93,** 61.
7. Porter, K. R., *J. Appl. Physics*, 1953, **24,** 1424.
8. Weiss, P., and Ferris, W., *Exp. Cell Research*, 1954, **6,** 546.
9. Porter, K. R., Claude, A., and Fullam, E. F., *J. Exp. Med.*, 1945, **81,** 233.
10. Porter, K. R., *J. Exp. Med.*, 1953, **97,** 727.
11. Palade, G. E., and Porter, K. R., *J. Exp. Med.*, 1954, **100,** 641.
12. Palade, G. E., *J. Biophysic. and Biochem. Cytol.*, 1955, **1,** in press.
13. Palade, G. E., *J. Exp. Med.*, 1952, **95,** 285.
14. Newman, S. B., Borysko, E., and Swerdlow, M., *J. Research Nat. Bureau Stand.*, 1949, **43,** 183.
15. Porter, K. R., and Blum, J., *Anat. Rec.*, 1953, **117,** 685.
16. Palade, G. E., *Quart. J. Micr. Sc.*, 1955, in press.
17. de Robertis, E. D. P., Nowinski, W. W., and Saez, F. Z., General Cytology, Philadelphia, W. B. Saunders Company, 2nd edition, 1954, 203.
18. Garnier, C., *J. anat. et physiol.*, Paris, 1900, **36,** 22.
19. Palay, S. L., and Palade, G. E., *J. Biophysic. and Biochem. Cytol.*, 1955, **1,** 69.
20. Palade, G. E., *J. Histochem. and Cytochem.*, 1953, **1,** 188.
21. Sjöstrand, F. S., *Nature*, 1953, **171,** 30.
22. Barnum, C. P., and Huseby, R. A., *Arch. Biochem.*, 1948, **19,** 17.
23. Petermann, M. L., and Hamilton, M. G., *Cancer Research*, 1952, **12,** 373.
24. Petermann, M. L., Mizen, N. A., and Hamilton, M. G., *Cancer Research*, 1953, **13,** 372.
25. Petermann, M. L., Hamilton, M. G., and Mizen, N. A., *Cancer Research*, 1954, **14,** 360.
26. Slautterback, D. B., *Exp. Cell Research*, 1953, **5,** 173.
27. Chantrenne, H., *Biochim. et Biophysic. Acta*, 1947, **1,** 437.
28. Bernhard, W., Haguenau, F., Gautier, A., and Oberling, C., *Z. Zellforsch. u. mikr. Anat.*, 1952, **37,** 281.
29. Weiss, J. M., *J. Exp. Med.*, 1953, **98,** 607.
30. Claude, A., *J. Exp. Med.*, 1946, **84,** 51, 61.
31. Hogeboom, G. H., Schneider, W. C., and Palade, G. E., *J. Biol. Chem.*, 1948, **172,** 619.
32. Schneider, W. C., and Hogeboom, G. H., *Cancer Research*, 1951, **11,** 1.
33. Porter, K. R., *in* Dynamics of Growth Processes, (E. J. Boell, editor), Princeton, Princeton University Press, 1954, 95.
34. Sjöstrand, F. S., and Rhodin, J., *Exp. Cell Research*, 1953, **4,** 426.
35. Palade, G. E., *J. Appl. Physics*, 1953, **24,** 1419.
36. Watson, M. L., and Avery, J. K., *Am. J. Anat.*, 1954, **95,** 109.
37. Howatson, A., and Ham, A. W., *Cancer Research*, 1954, in press.
38. Porter, K. R., *J. Histochem. and Cytochem.*, 1954, **2,** 346.
39. Dalton, A. J., and Felix, M. C., *Am. J. Anat.*, 1954, **94,** 171.

EXPLANANTION OF PLATES

PLATE 11

FIG. 1. Electron micrograph of a limited field in the basal region of an acinar cell of the pancreas (rat). The cell membrane (*cm*) is coated towards the exterior by a poorly defined layer of dense material (*bm*) that may be the equivalent of a basement membrane. Part of a mitochondrial profile appears at *m*.

The rest of the field is taken up by elongated (*e*), oval (*o*), and circular (*c*) profiles of the endoplasmic reticulum which, in this case, exhibits a certain degree of orientation as shown by the general parallelism of the profiles to the cell membrane. The cytoplasmic matrix, slightly denser than the homogeneous content of the endoplasmic reticulum, is disposed in bands in between the profiles.

Note that in the matrix there are numerous small and dense granules (*g*) which appear to have particular affinity for the membrane limiting the cavities of the endoplasmic reticulum. The outside surface of this membrane is actually covered by many such particles which in a few places (*r*) appear to be more or less regularly disposed in rows.

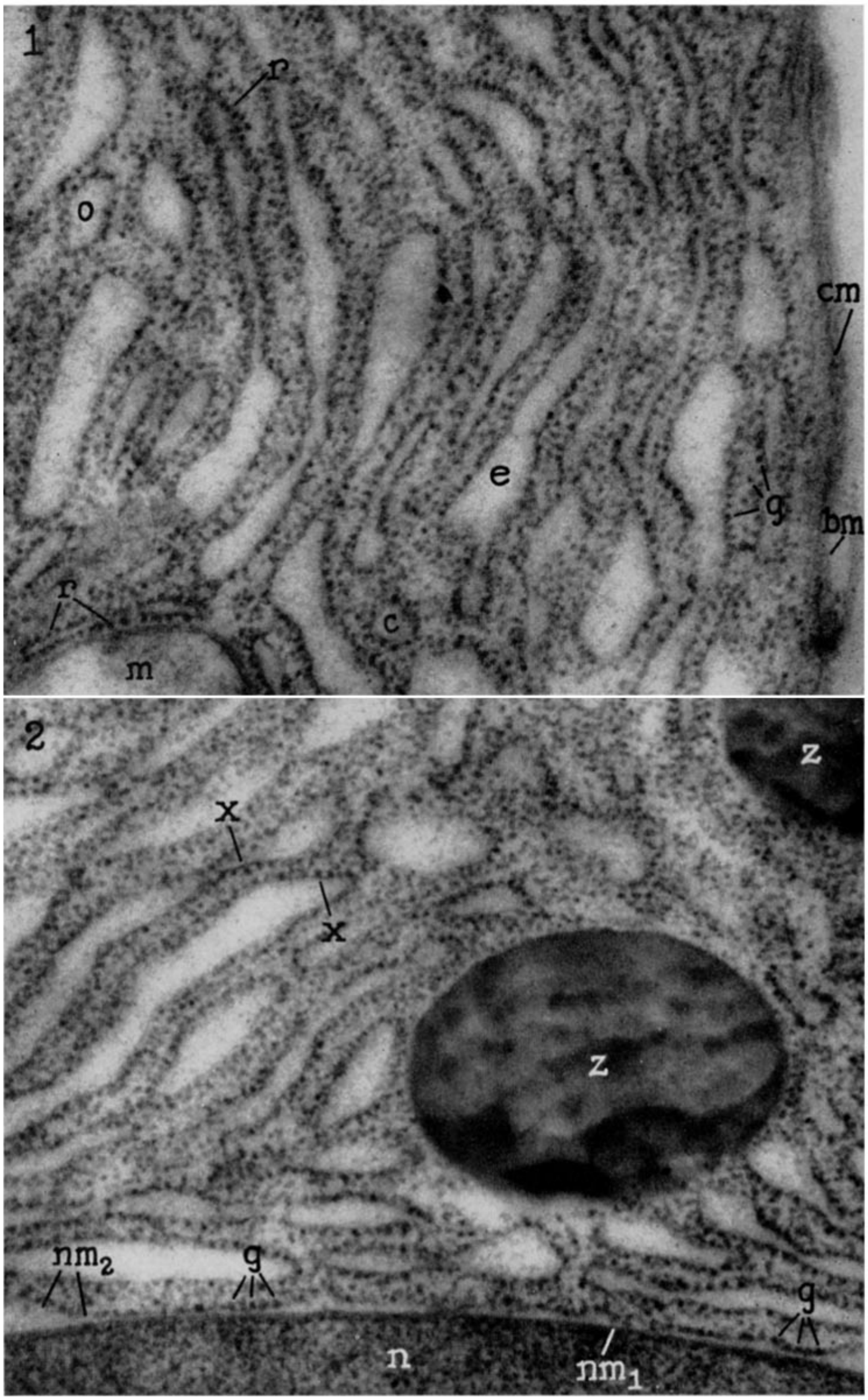
Note also that the granules do not show any affinity for the cell membrane (inner surface) and for the membrane limiting the mitochondrion. × 73,000.

FIG. 2. Electron micrograph of a limited field in the vicinity of the nucleus (*n*) in the same cell as in Fig. 1. The profiles of the endoplasmic reticulum are equally diversified in shape and in this field appear to be predominantly oriented around the nucleus. Small, dense particles are present in the cytoplasmic matrix.

At *x* the membranes limiting two adjacent profiles have been normally sectioned as indicated by their extreme thinness, high density, and sharp outline. Such sections show to advantage the relationship between the membrane and the small granules: the latter appear in close contact with, as if "attached" to, the outside surface of the membrane.

Note that the membrane limiting the cytoplasm towards the nucleus (nm_2) is not rigorously parallel to the nuclear membrane proper (nm_1), and that the former is dotted with small granules (*g*) like the membrane of the endoplasmic reticulum.

The large dense body marked *z* is a zymogen granule. × 73,000.



(Palade: Small particulate component of cytoplasm)

PLATE 12

FIG. 3. Acinar cell of the parotid (rat). This limited field in the basal cytoplasm of the cell contains only three profiles of the elements of the endoplasmic reticulum, the outside surfaces of which are covered by numerous, small and dense granules. The profile marked *ci* corresponds to a large and dilated cisterna of irregular outline. In a few places (*x*) its limiting membrane is covered by a single layer of granules and this is taken to indicate that in such places the undulating membrane of the cisterna has been sectioned normally, or nearly so. In other places, however, the granules are apparently plastered many layers thick at the periphery of the profile; in such places the section passed obliquely through the membrane, exposing it over relatively large areas and allowing a full-faced view of the granules disposed on its surface. Note that they seem to form certain patterns; *e.g.* rosettes (*ro*) and circles (*cr*). Note also that there are only a few particles scattered singly or in small clusters in the cytoplasmic matrix in between the profiles. $\times$ 74,000.

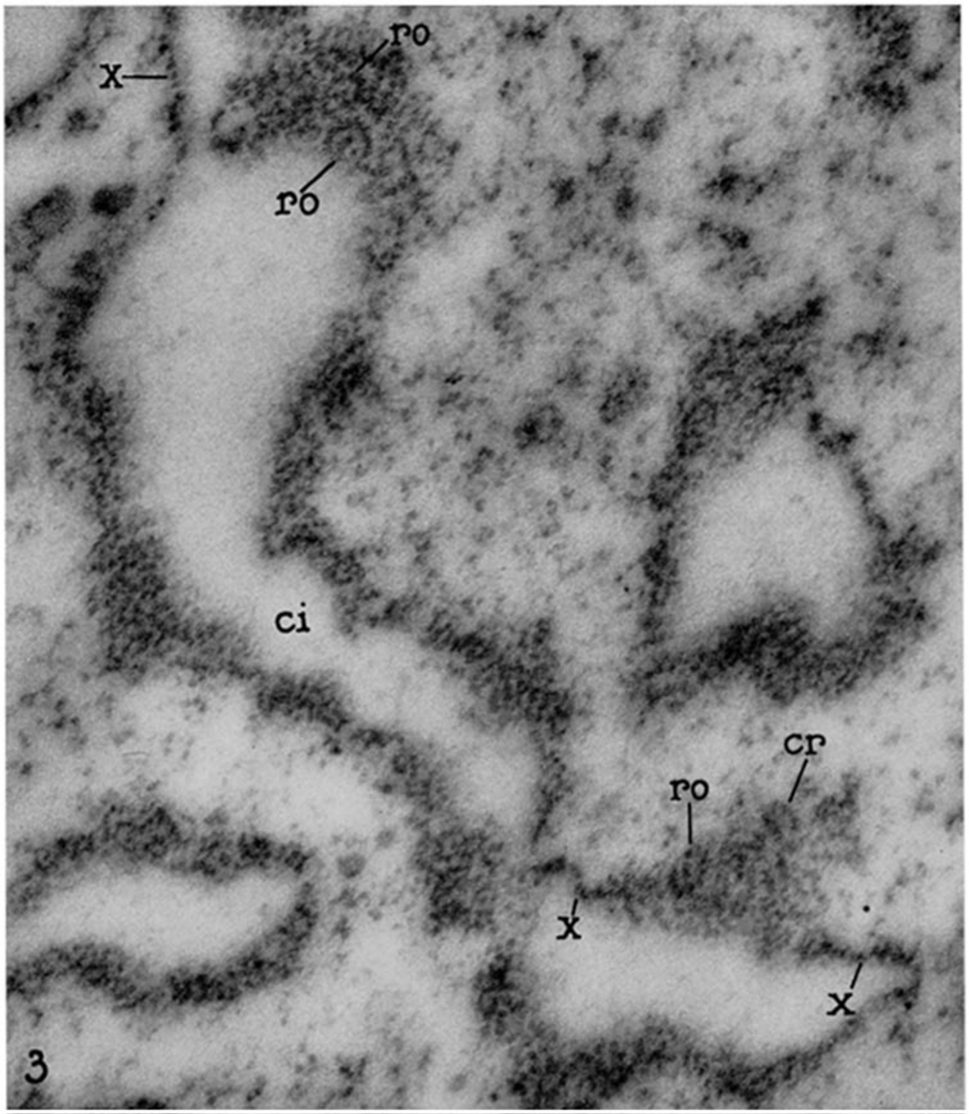
FIG. 4. Electron micrograph of a limited field in the basal cytoplasm of an acinar cell of the pancreas (rat).

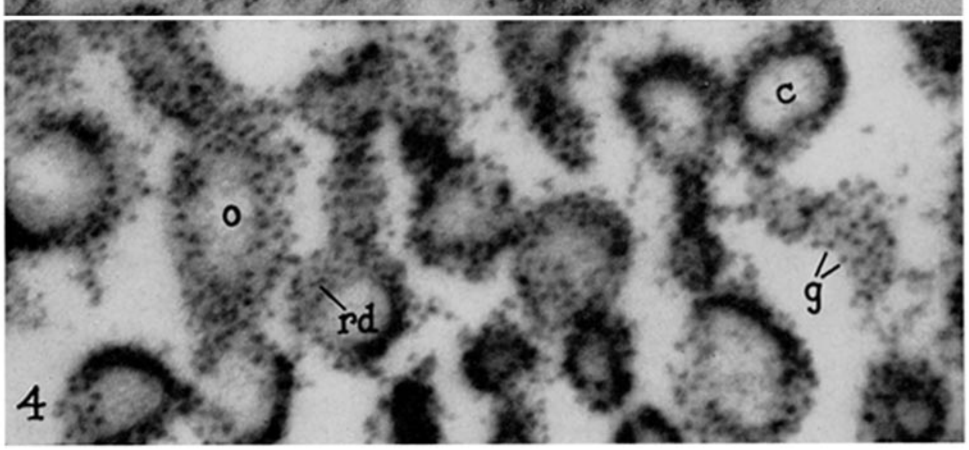
In contradistinction to the disposition encountered in Figs. 1 and 2, the profiles of the endoplasmic reticulum have smaller dimensions and are predominantly circular (*c*) and oval (*o*) in shape. The cytoplasmic matrix, however, is lighter and occupies relatively more space. The appearance probably indicates a more hydrated state of the matrix.

Note that the elements of the endoplasmic reticulum, mostly vesicles and tubules, have been sectioned obliquely or tangentially. Their limiting membrane is exposed over relatively large areas and consequently shows the disposition of its "attached" granules. The latter are very numerous, mostly of rounded appearance (*g*), rarely rod-like in shape (*rd*), and appear to be distributed at random on the membrane surface. Note that there are only a few granules scattered in the intervening matrix. $\times$ 91,000.

THE JOURNAL OF
BIOPHYSICAL AND BIOCHEMICAL
CYTOLOGY

PLATE 12
VOL. 1





(Palade: Small particulate component of cytoplasm)

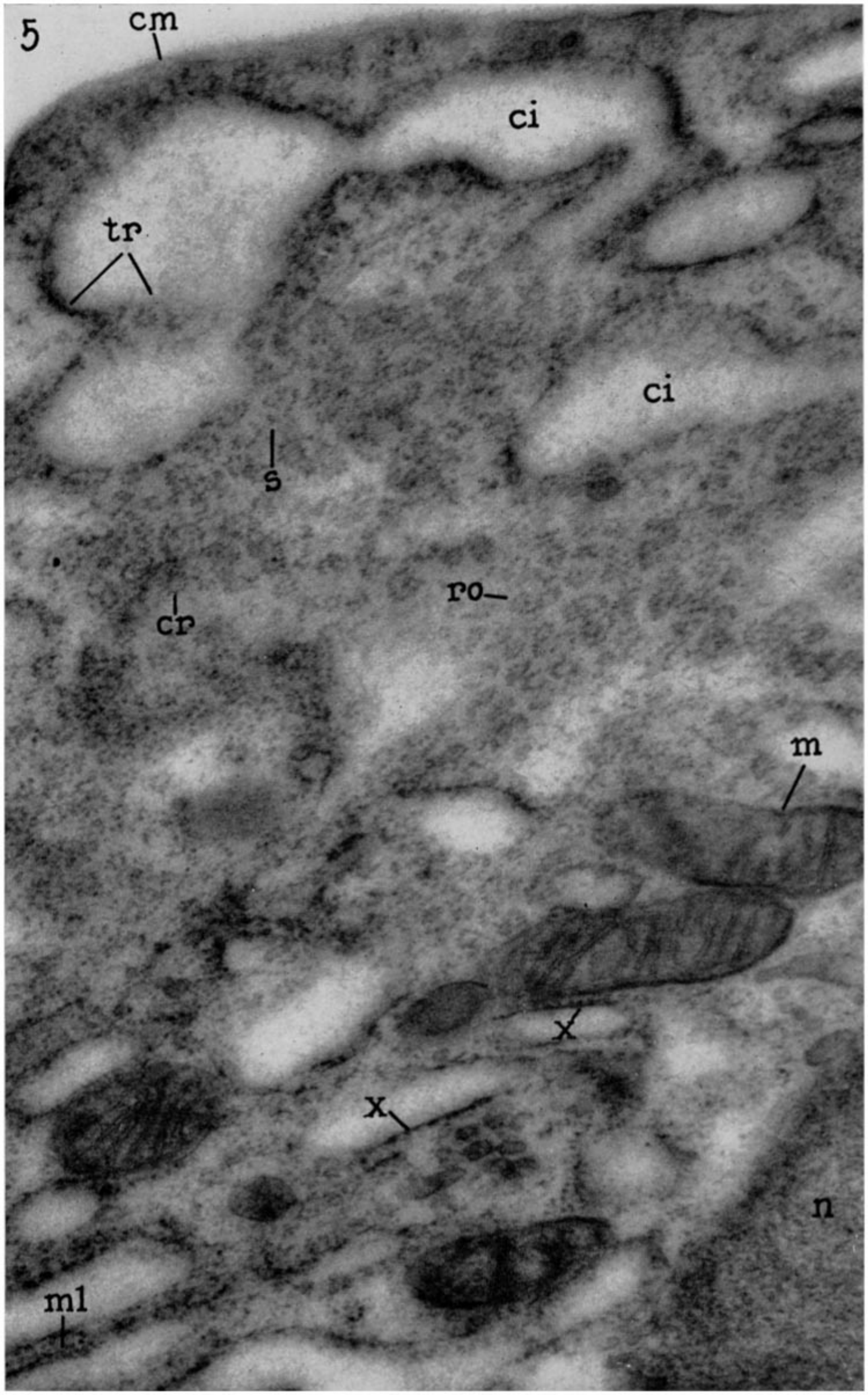
Plate 13

Fig. 5. Electron micrograph of a small field in the cytoplasm of a plasma cell found in the intestinal submucosa of a rat.

The cell membrane appears at *cm*, and part of the nucleus at *n*. Close to the latter are a few mitochondrial profiles, one of them marked *m*. The rest of the field is taken up by profiles of the endoplasmic reticulum, most of them representing large and dilated cisternae (*ci*). In a few places, the undulating membrane limiting these cisternae is sectioned normally (*x*) and appears covered towards the outside by a single layer of granules. In most places, however, the membrane is cut at various degrees of obliqueness and thus exposed over relatively large areas. A good image of a transition in the angle of membrane section is shown at *tr*. Note that at the surface of the exposed membranes the small, dense granules seem to form certain recurring patterns; *e.g.*, rosettes (*ro*), spirals (*s*), and circles (*cr*).

Note that the small granules show no affinity for the internal surface of the cell membrane and for the limiting membrane of the mitochondria.

The cytoplasmic matrix appears to be disposed in thin layers among the elements of the endoplasmic reticulum as indicated by its appearance at *ml* between two normally cut cisternae. $\times$ 54,300.



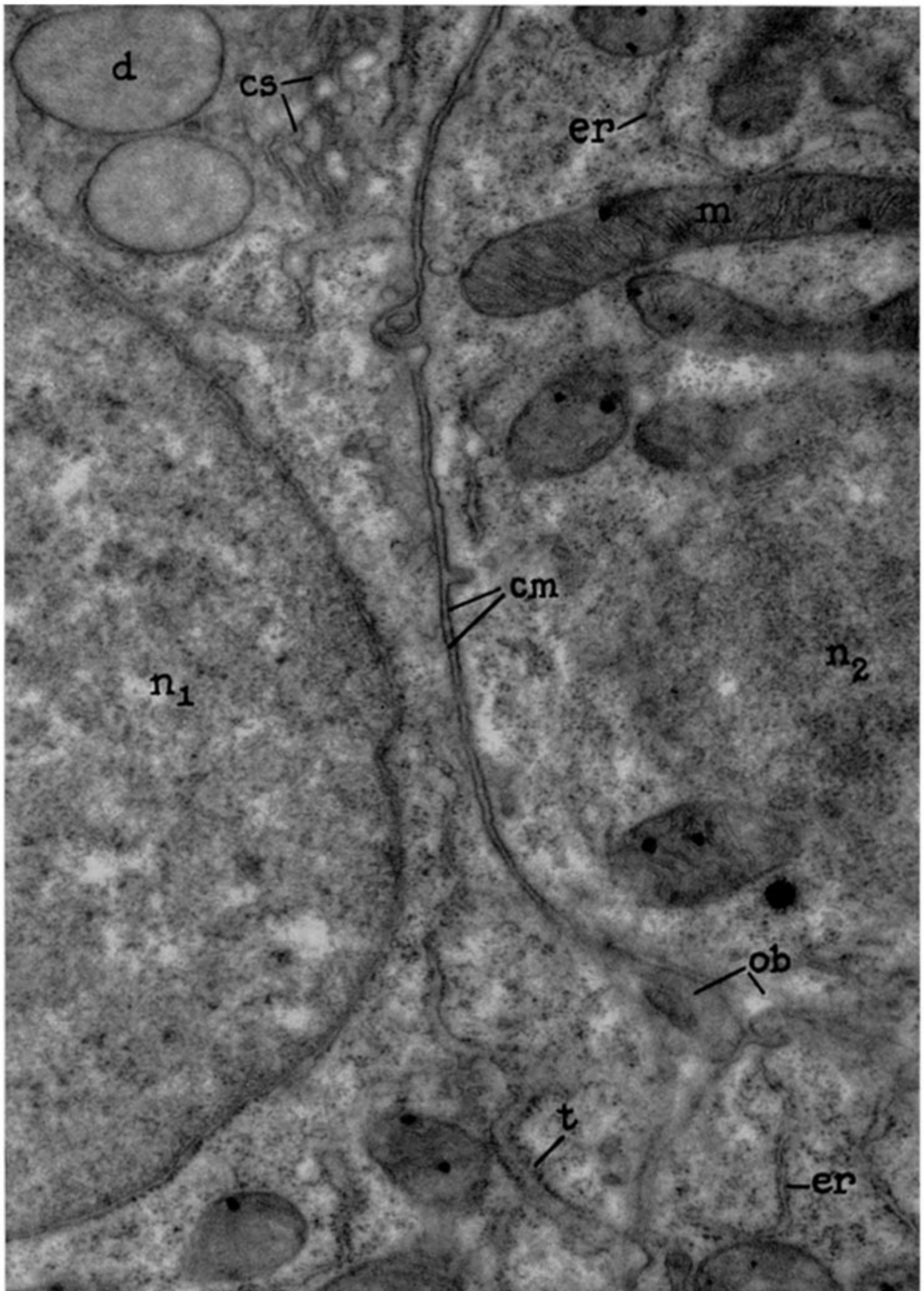
(Palade: Small particulate component of cytoplasm)

PLATE 14

FIG. 6. Electron micrograph of part of two adjacent cells in the epithelium of an intestinal crypt (rat jejunum). The basal pole of the cells is at the bottom of the figure. The cell to the left was differentiating into a goblet cell. Along most of the junction line, the contiguous membranes of the two cells (*cm*) have been perpendicularly sectioned and consequently appear sharply outlined. In the lower third of the picture the orientation changes and the membranes appear to be sectioned at various degrees of obliqueness (*ob*). Note along the junction line the presence of protrusions and invaginations which may help in maintaining the alignment of the cells.

The plane of the section was normal to the surface of the nucleus of the left cell (n_1) and consequently this nuclear profile shows clearly its two membranes. The nucleus of the right cell (n_2) is just slightly touched by the section. In the cytoplasm there are many mitochondrial profiles (*m*) showing both cristae and internal granules; a few mucus droplets (*d*); and a bundle of tightly packed, elongated profiles (*cs*) similar to the ones previously described in the centrosphere region of other cells (11) and in neurons (19). The endoplasmic reticulum is represented by a few elongated and sinuous profiles (*er*) which correspond in all probability to large, flat cisternae. Their limiting membrane is either partially (*p*) or totally (*t*) covered with small, dense granules. In addition to these "attached" elements, numerous particles of similar size and density appear freely scattered throughout the cytoplasm, either singly or in small clusters. As indicated by this electron micrograph, the epithelial cells of intestinal crypts possess a relatively poorly developed endoplasmic reticulum, but contain large amounts of small dense granules.

Note that the cell membranes, the limiting membranes of the mitochondria, and the membranes of the centrosphere region are free of small granules, whereas the membrane limiting the cytoplasm towards the nucleus, like the membrane of the endoplasmic reticulum, is covered by such particles. $\times$ 40,000.



(Palade: Small particulate component of cytoplasm)

PLATE 15

FIG. 7. Electron micrograph of part of a young lymphocyte in the spleen of a chicken.

The cell membrane appears at *cm*, a mitochondrial profile at *m*, and part of the nucleus at *n*. The two membranes of the latter are clearly visible in the upper part of the nuclear profile and cannot be resolved in the rest. The difference may be explained by a change in the orientation of the nuclear membrane from normal to oblique with respect to the plane of the section. Note a granular texture in some parts of the nucleus (*gt*); comparable granules have been described before in the nucleolus of other cells (38, 19).

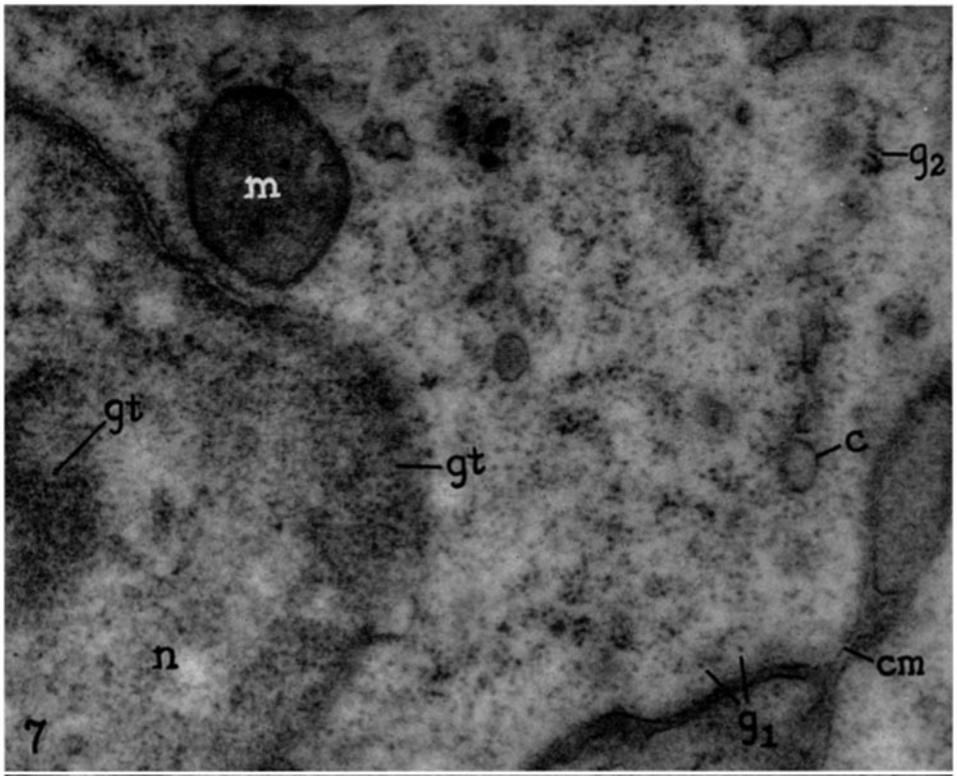
The cytoplasm contains a few small, mostly circular, profiles (*c*) which represent the endoplasmic reticulum. Most of them are limited by a membrane with smooth outside surface while numerous, small and dense granules appear scattered in the cytoplasm individually (g_1) or in small clusters (g_2). $\times$ 64,500.

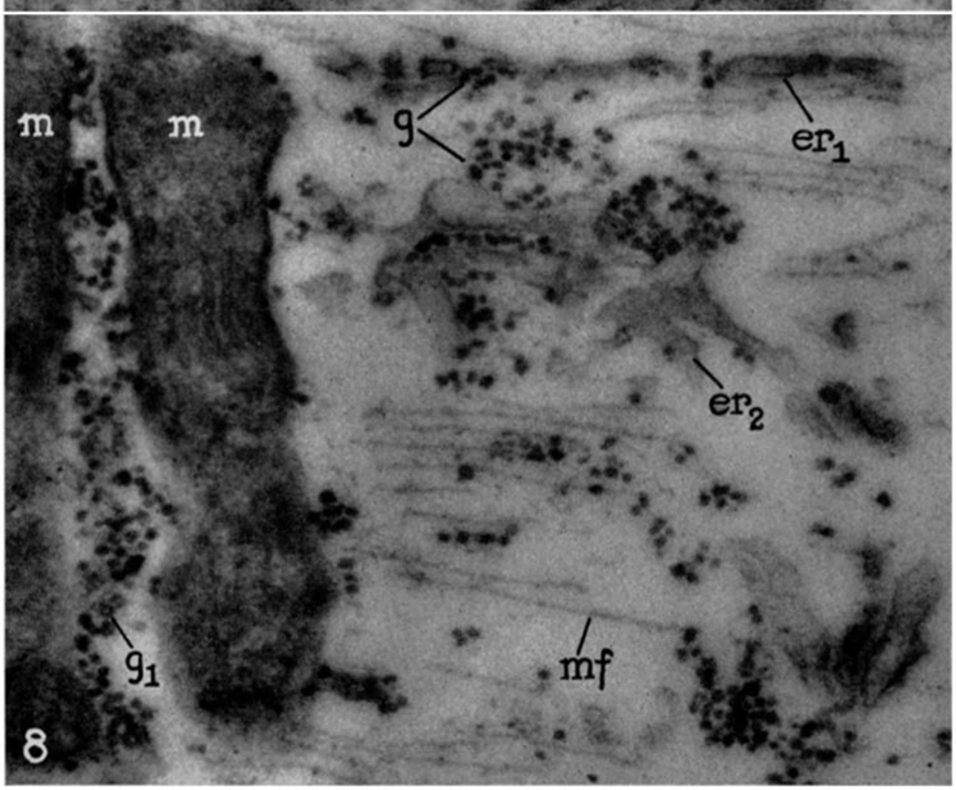
FIG. 8. The section presented in this figure was cut longitudinally through a striated muscle fiber (rat diaphragm) and contains part of the sarcoplasmic layer that separates two adjacent myofibrils. As such it shows the sarcoplasmic organelles located in the layer together with a few elementary filaments (*mf*) of the adjacent myofibrils.

Two mitochondrial profiles appear at *m* in an arrangement characteristic for this muscle (diaphragm): the mitochondria are disposed in pairs facing the I bands of the myofibrils with one mitochondrion on each side of a Z band.

The endoplasmic reticulum forms "reticular sheets" (11) around the myofibrils. Such a sheet appears as a row of profiles (er_1) when perpendicularly sectioned and as a more or less recognizable reticulum (er_2) when sectioned very obliquely.

Small, dense granules (*g*) are present in the sarcoplasm around the elements of the endoplasmic reticulum (but not "attached" to their membrane) and in between the mitochondrial profiles. Note that the particles are larger than in the previous examples and more varied in form; note also that some of them seem to have a light core (g_1). $\times$ 82,000.





(Palade: Small particulate component of cytoplasm)

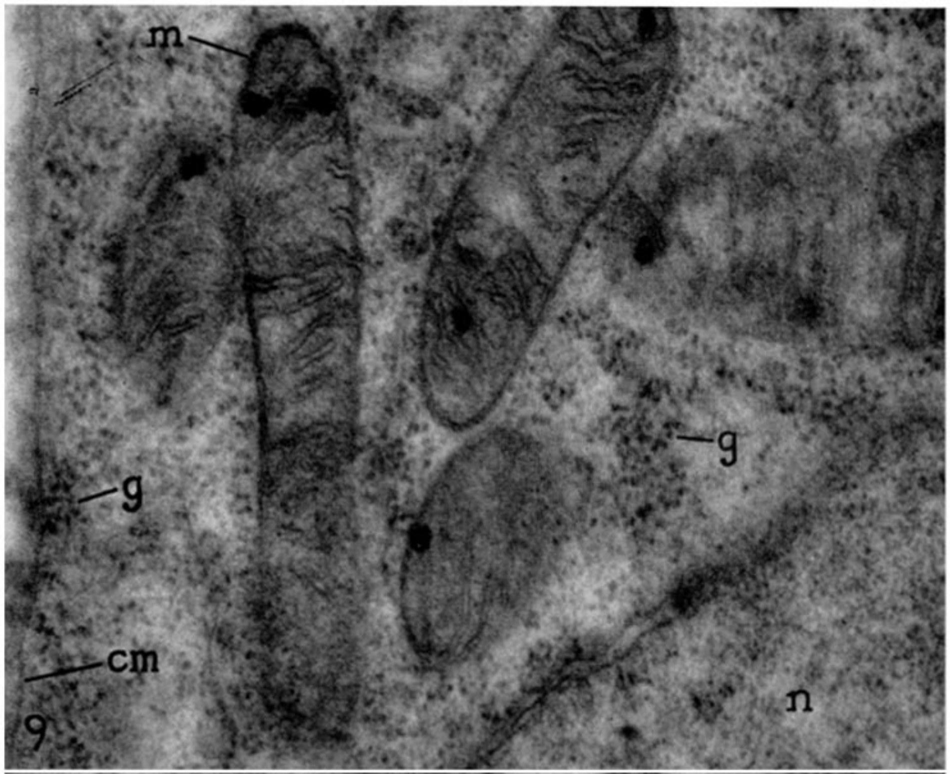
Plate 16

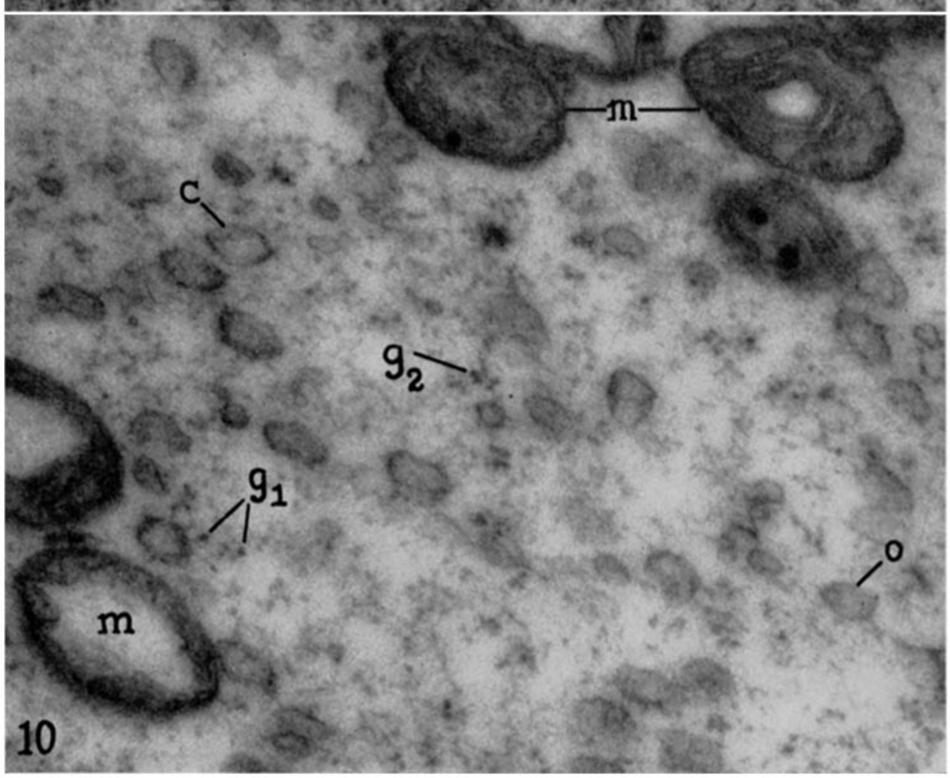
Figs. 9 and 10 are presented together in order to show in terms of fine structure the difference between basophil (Fig. 9) and acidophil (Fig. 10) cytoplasm. Their comparison is suggestive of the role played respectively by the endoplasmic reticulum and by the small granules in cytoplasmic basophilia.

Fig. 9. This is part of an epithelial cell from the zone of rapid cellular proliferation in an intestinal crypt (rat jejunum).

The cell membrane (basal pole) appears at *cm* and part of the nucleus at *n*. The cytoplasm contains five mitochondrial profiles, one of them marked *m*, and numerous small and dense granules (*g*) scattered freely in the cytoplasmic matrix either individually or in clusters. Note that there are no profiles of the endoplasmic reticulum in this field; there were only a few in the rest of the cell. The cytoplasm of such cells is intensely basophil. × 73,000.

Fig. 10. This small field in the cytoplasm of a spermatocyte (rat) contains a few mitochondrial profiles (*m*) and numerous profiles belonging to the endoplasmic reticulum. Most of them are of circular (*c*) or oval (*o*) shape and appear to be limited by a membrane with smooth outside surface. Only a few small, dense particles appear individually (g_1) or in small clusters (g_2) in the cytoplasmic matrix. The cytoplasm of this type of cell is known to be acidophil. × 73,000.





(Palade: Small particulate component of cytoplasm)