AN ELECTRON MICROSCOPE STUDY OF

MATURE AND DIFFERENTIATING PANETH CELLS

IN THE RAT, ESPECIALLY OF THEIR

ENDOPLASMIC RETICULUM AND LYSOSOMES

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ABSTRACT

In an electron microsope study, the morphology of mature Paneth cells from the small intestine of adult rats is compared with that of differentiating Paneth cells from young rats 2 to 4 weeks old. All mature cells exhibit a marked polarity similar to that of other exocrine gland cells and contain a well developed endoplasmic reticulum, an elaborate Golgi complex, and numerous large secretory granules; they also possess an abundance of lysosomes. The most conspicuous occurrence in the process of differentiation is the development of the endoplasmic reticulum. The most immature Paneth cells possess an endoplasmic reticulum of the vesicular type, which, during maturation, is replaced by the characteristic lamellated ergastoplasm of the mature cell. At a certain stage of differentiation the cavities of the developing cisternae show numerous communications with the perinuclear space, suggesting an outgrowth of the ergastoplasm from the nuclear envelope. Furthermore, the cavities and the perinuclear space at this particular stage contain a material which shows a remarkable intrinsic periodicity. An identical periodicity was exhibited by material contained in Golgi cisternae and secretory granules. Lysosomes are also present in the differentiating cells.

INTRODUCTION

The Paneth cells are located in the bottoms of the intestinal crypts. They are present in the small intestine in many higher vertebrates (for review, see reference 34) and are considered to be exocrine gland cells. Their secretory granules are said to contain acid mucopolysaccharides and protein (1, 2). Cytoplasmic bodies containing acid phosphatase have been observed in the Paneth cells (3), and Novikoff et al. (4) and Novikoff and Essner (5) have reported direct evidence of the identity of early secretory granules and granules rich in acid phosphatase in these cells. A high dipeptidase content has been demonstrated in the deep part of the intestinal mucosa, and this observation led to the hypothesis that Paneth cells secrete peptidase (6). In the ileum, however, where the number of Paneth cells is considerably greater than in the jejunum, the peptidase content is lower than it is in the jejunum. Actually, very little is known about the function of the Paneth cells or their secretory mechanism.

Mature Paneth cells from adult mice have been examined in the electron microscope by Hally (7), and Trier (32) has recently described the Paneth cells in humans. No description of the structural cytodifferentiation of these cells has

The legends of the illustrations indicate the embedding process and stain by the following abbreviations: E, Epon 812 embedding. GO, tissue fixed in glutaraldehyde followed by fixation in osmium tetroxide. K, lead staining, Karnovsky's method A. O, osmium tetroxide fixation. UA, uranyl acetate staining. W, Vestopal embedding.

FIGURE 1   Electron micrograph of the bottom of the intestinal crypt from the lower ileum of adult rat. Two mature Paneth cells containing many secretory granules are seen. Four nuclei of "undifferentiated" cells are marked U. SG: secretory granules. ER: rough-surfaced endoplasmic reticulum in lamellar array. SB: spherical nuclear body. L: lumen of the crypt. Capillaries in intimate relation to the bases of the Paneth cells are seen at C1 and C2. Unmarked arrows indicate lysosomes. O, W, UA. × 4,000.

been given. The present work concerns the fine structure of differentiating and fully developed Paneth cells in rats. Some observations on the endoplasmic reticulum of the differentiating cell will be discussed in detail.

MATERIAL AND METHODS

Mature Paneth cells were studied in 10 adult albino rats of both sexes. Differentiating Paneth cells were investigated in 11 young rats of varying ages: 15 (2 rats), 16, 17, 18 (2 rats), 19, 20, 21, 24, and 28 days old. The animals were kept under standard laboratory conditions and were examined either fasting (allowed water) or fed on standard diet *ad libitum*.

All the animals examined were anaesthetized with Nembutal, given intraperitoneally. For light microscope studies of acid phosphatase activity, tissue samples were taken from the duodenum (middle),

FIGURE 2 Apical part of a mature Paneth cell, showing microvilli, secretory granules (*SG*), and vesicles (*V*). Where the plasma membrane is sectioned favourably the asymmetry of the membrane is seen (arrows). *ZO*: zonula occludens; *D*: desmosomes. O, E, K. × 38,000.

and the upper, middle, and lower third of the jejunum and the ileum. The tissues were fixed for 3 to 12 hours in cold formol-calcium (8). Frozen sections were incubated for 15 to 30 minutes at 37°C in fresh Gomori medium (9) at pH 5 and subsequently treated with dilute ammonium sulfide. Controls were run either by omitting the substrate from the medium or by adding NaF in a concentration of 0.01 M to the incubation medium.

For electron microscopy, samples from the middle of the jejunum and from the lower ileum were fixed in buffered osmium tetroxide (10) as modified by Sjöstrand (11). Other samples were fixed for 2 hours in 6.5 per cent glutaraldehyde buffered to pH 7.2 with 0.1 M cacodylate buffer (12) and post-fixed for 1 hour in osmium tetroxide (11). Fixation was carried out at room temperature. Embedding was done in Vestopal W (13) and Epon 812 (14).

For the demonstration of acid phosphatase activity by electron microscopy, sections 50 microns thick were cut, on a freezing microtome, from glutaralde-

hyde-fixed tissue samples from the middle jejunum and the lower ileum. These sections were then incubated at 37°C at pH 5 for 15 to 30 minutes, according to the procedure given by Miller (15). After postfixation in osmium tetroxide for $\frac{1}{2}$ to 1 hour, the sections were embedded in Vestopal or Epon. Controls were runs as mentioned above.

Thin sections for electron microscopy were cut on an LKB Ultrotome and examined in a Siemens Elmiskop I at an accelerating voltage of 60 kv.

All sections were stained either with uranyl acetate (16) in a 2 per cent aqueous solution at 50°C for 15 to 45 minutes, or with lead by the "A method" of Karnovsky (17).

OBSERVATIONS

A. The Mature Paneth Cell

The Paneth cells were identified by their position in the crypts and by their large secretory

granules (Fig. 1). The cells are shaped like trun-
cated pyramids, with the base resting against the
basement membrane of the crypt, and show a
marked polarity as is commonly seen in cells with
exocrine secretion.

The plasma membrane is of the three-layered
asymmetrical type (18) (Fig. 2), and shows a
fairly straight course along the lateral cell sur-
faces (Fig. 1). Interdigitations with the plasma
membrane of neighbouring cells are infrequent
and not extensive. Microvilli project from the
apical surface into the crypt lumen (Figs. 1 and 2).
The lateral cell membranes exhibit contact zones
of the three types recently described by Farquhar
and Palade (19) (Fig. 2), and desmosomes are
found at various levels along the lateral surfaces
(Fig. 2). The basal plasma membrane is usually
smooth, but, in several instances, a cytoplasmic
projection equipped with microvillus-like struc-
tures penetrated the basement membrane (Fig.
5). Capillaries often show an intimate spatial
relationship with the bottoms of the crypts (Figs.
1, 12, 13), and sometimes form shallow impressions
in the bases of the Paneth cells (Fig. 1); a narrow
connective tissue space intervenes between the
basement membranes of the crypt and the capil-
laries. Pits with molecular spines similar to those
described by Roth and Porter (21) are sometimes
seen along the basal surfaces of the Paneth cells
(Fig. 3).

Basally in the cells a well developed, rough-
surfaced endoplasmic reticulum is seen (Figs. 1, 3,
4), which often extends into the para- and supra-
nuclear cytoplasm. The cisternae of the reticulum
are arranged in a lamellar array, although not so
regularly and tightly packed as, for example, in
the exocrine cells of the pancreas. The reticulum

in the most apical parts of the Paneth cells seems
to consist mainly of branching tubules. The cis-
ternae of the endoplasmic reticulum appears to be
empty in osmium tetroxide-fixed cells embedded
in Vestopal, and more or less empty when em-
bedded in Epon. In tissue fixed in glutaraldehyde,
postfixed in osmium tetroxide, and embedded
in Epon, the reticulum invariably contains a rather
electron-opaque material (Fig. 4).

The nuclei are round or oval and located in the
basal part of the cells. The nuclear envelope has
ribosomes attached to its outer surface (Figs. 3,
4) and is furnished with nuclear pores or fenestrae,
in which a so called diaphragm is seen in tissue
fixed in glutaraldehyde followed by osmium tetrox-
ide. Occasionally, communications are found
between the perinuclear space and the cisternae
of the endoplasmic reticulum (Fig. 5). According
to Hally (7), Paneth cells in mice have irregularly
shaped nuclei, due to infoldings of the nuclear
envelope. Such infoldings are infrequent in our
material. The nuclei contain one or more large
nucleoli (Fig. 1). They also present small spherical
bodies which are composed of an outer zone and an
inner zone or core (Figs. 1, 12, 14). The outer
zone encircles the core completely and consists
of a material of relatively high density. The core
is indistinguishable from the nuclear material
surrounding the bodies. The spherical bodies are
not simply parts of the large nucleoli, but appear
as morphologically independent structures.

The Golgi complex is located mainly in the
supranuclear region. In the sections, the complex
shows a number of subdivisions, each consisting
of about 3 to 5 closely packed, membrane-bounded
sacs or cisternae, more or less surrounded by
vesicles of varying sizes (Fig. 6). In cells studded

FIGURE 3 Basal part of a mature Paneth cell, showing the rough-surfaced endoplasmic
reticulum in lamellar array. The cisternae appear empty. *N:* nucleus; *NE:* nuclear envelope;
M: mitochondrion; *T:* tonofilaments; *BM:* basement membrane of crypt. At the basal
plasma membrane (*P*) two "pits with molecular spines" (21) are seen (arrows). O, W, UA.
× 33,000.

FIGURE 4 This micrograph shows the appearance of the endoplasmic reticulum after
glutaraldehyde-osmium fixation. In the cavities of the cisternae a homogeneous material
is seen. *M:* mitochondrion; *N:* nucleus; *T:* tonofilaments. E, K, × 24,000.

FIGURE 5 Base of Paneth cell, showing a cytoplasmic projection piercing the basement
membrane (arrows). The cytoplasm of the projection is dominated by tonofilaments (*T*).
At *C* a communication between the perinuclear space and the endoplasmic reticulum is
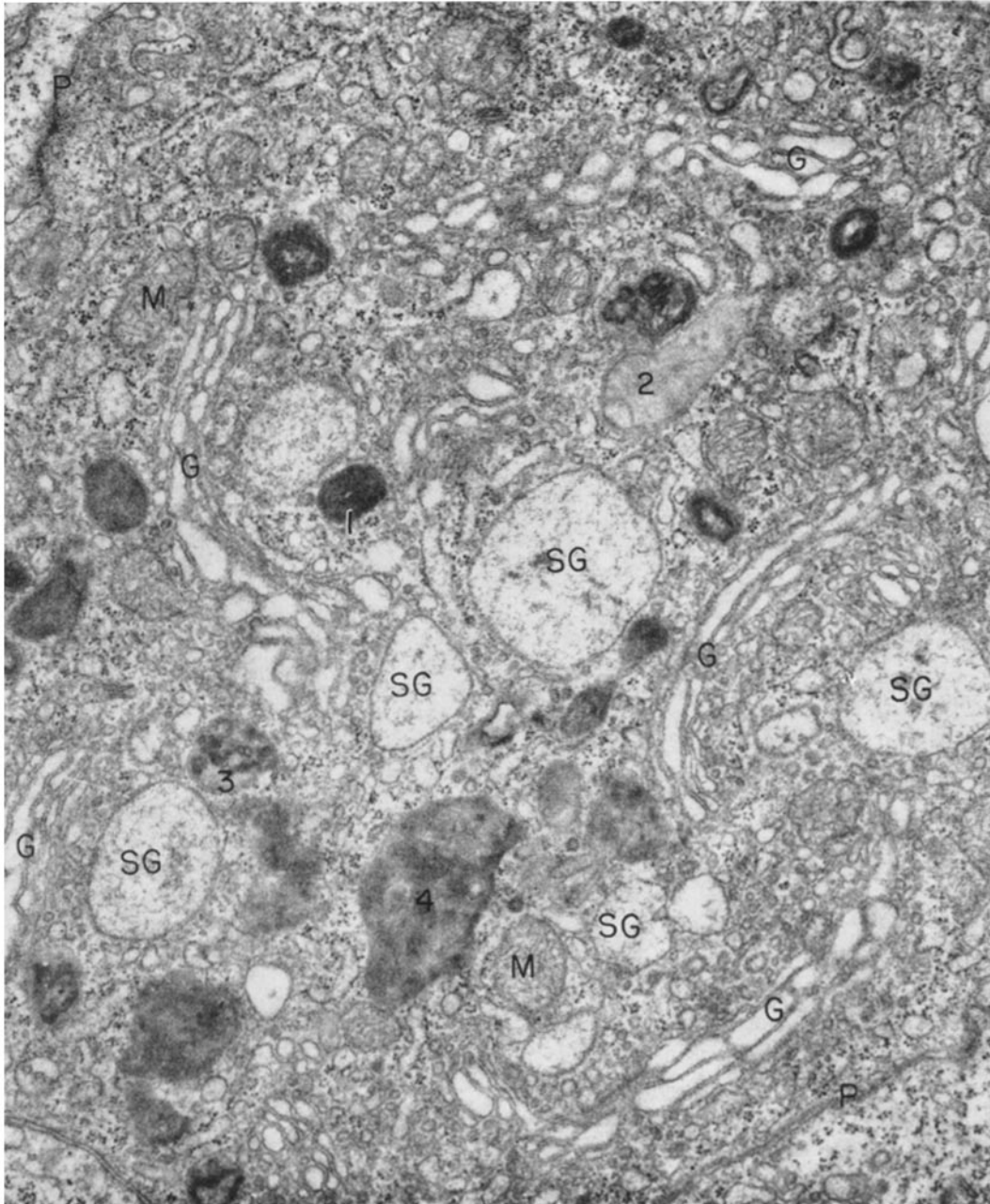seen. *N:* nucleus. O, W, UA. × 20,000.
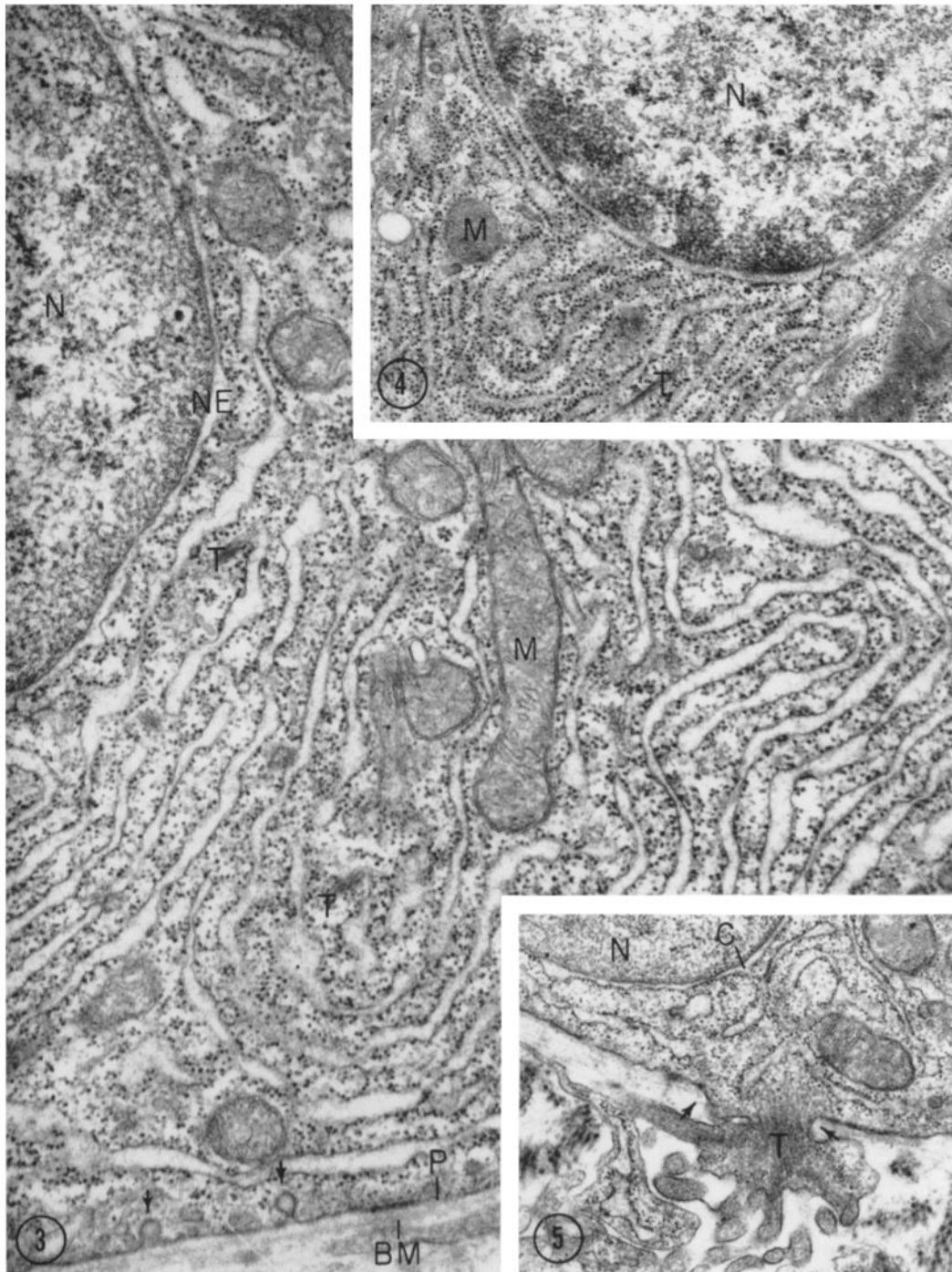
FIGURE 6 Supranuclear zone of Paneth cell in transverse section, showing several subunits of the Golgi complex (G) and early secretory granules (SG) of different sizes. Numerous lysosomes are seen (1–4), most of which contain myelin figures (1, 4). M: mitochondria, P: plasma membrane. O, E, K. × 31,000.

with secretory granules, parts of the complex are displaced to the lateral parts of the cell. The Golgi subdivisions are often seen in intimate relation to secretory granules, and sometimes the two oppos-

ing membranes of a Golgi cisterna are separated to enclose what seems to be secretory material.

The apical cytoplasm is more or less filled with large secretory granules. In favourable sections

the surrounding membrane shows a three-layered structure. The contents of the granules have a somewhat floccular appearance of low electron opacity in osmium tetroxide–fixed specimens embedded in Vestopal or Epon. Fixation in glutaraldehyde and osmium tetroxide reveals a wide variation in the density of the granules, some of them, probably mature ones, being very dense (Fig. 7).

Scattered among the secretory granules were seen a varying number of smaller vesicles, 500 to 2500 A in diameter (Fig. 2). Some appear empty, while others contain a small amount of a substance of low density. The relationship, if any, between the secretory granules and the vesicles is obscure. Coalescence of smaller vesicles to larger ones does not seem to take place, nor has any evidence been found that the small vesicles are pinched off from the secretory granules.

The mitochondria of the Paneth cells are chiefly confined to the medio-basal regions of the cells and are interspersed between the cisternae of the endoplasmic reticulum (Fig. 3). They were seldom found in the apical cytoplasm, which is dominated by the secretory granules. Most of the mitochondria contain in their matrix small granules, which are rather inconspicuous in sections stained with uranyl acetate but clearly seen when lead was employed as contrast medium. The matrix of the mitochondria is more dense than the cytoplasmic matrix, especially in specimens fixed in glutaraldehyde and postfixed in osmium tetroxide.

Tonofilaments were seen especially in the apical parts of the Paneth cells. From the microvilli they extend for a varying distance proximally between the secretory granules. In the basal parts of the cells, single filaments or bundles of filaments are oriented at random (Figs. 3 and 4). Not infrequently, centrioles were observed in the apical cytoplasm between the secretory granules. Multivesicular bodies were seldom observed.

All the Paneth cells studied contain lysosomes (20). Generally, they are located in the supra- and paranuclear regions (Fig. 6) and measure approximately 0.5 to 2 microns in diameter. As many as 15 of these bodies could be found in a single section through a Paneth cell. They are bordered by a single membrane and contain a homogeneous or finely granular substance, which, in many bodies, shows localized condensations of high electron opacity. Furthermore, the majority of the lysosomes contain complicated

membrane systems, often arranged concentrically or linearly in so called myelin figures (Fig. 10). Cytoplasmic components, such as mitochondria, endoplasmic reticulum, or ribosomes, or remnants of such components, are not observed within the lysosomes.

Examination in the electron microscope of sections incubated for the demonstration of acid phosphatase activity showed the reaction product to be localized both in the interior of the lysosomes and at their limiting membrane (Figs. 7 and 8). In our material no acid phosphatase activity was found in the early secretory granules (Fig. 7). Light microscope examination of incubated frozen sections from the duodenum, jejunum, and ileum revealed acid phosphatase activity in the Paneth cells to be present in granules which corresponded in distribution and number to the lysosomes seen in the electron microscope. The granule-bound acid phosphatase activity in the bottoms of the crypts corresponded to the number of Paneth cells, being lowest in the duodenum and increasing towards the lower ileum (Fig. 9).

B. The Differentiation of the Paneth Cells

Like mice (33), rats have no Paneth cells during the first 2 weeks of life (22). In 15-day-old rats they occur sporadically, increasing rapidly in number during the next 2 weeks. In rats 4 weeks old, the number of Paneth cells per crypt and their distribution in the small intestine are about the same as in adult rats. The following description of the process of differentiation is based on examinations of immature Paneth cells from rats aged 15 to 28 days.

The differentiative events in the individual cell seem to proceed rather rapidly. Thus, in the 18-day-old animals many mature Paneth cells were found, and at the same time all stages of the process of differentiation are represented, while nearly all the Paneth cells seen in the 15-day-old rats are immature. Secretory granules and spherical nuclear bodies were usually used as criteria for determining which cells were Paneth cells. The secretory granules of the Paneth cells were easily distinguishable from goblet cell granules and the granules of argentaffine and/or argyrophile cells. Even young Paneth cells contain a few secretory granules, and the spherical nuclear bodies were not observed in other crypt cells.

The most dramatic morphological changes during differentiation concern the endoplasmic

reticulum. In sections of very young Paneth cells this organelle consists of circular, or oblong, ribosome-covered vesicular profiles (Fig. 11). They occur in all parts of the cytoplasm except within the voluminous Golgi complex, and seem to make up a rather loose network of tubules and vesicles. In the basal parts of the cells, solitary and somewhat flattened cisternae occur. Thus, in its architecture the endoplasmic reticulum of the youngest Paneth cells is very different from that of the mature cell.

In more differentiated cells, this primitive endoplasmic reticulum is superseded by a predominantly lamellar reticulum. The first lamellae to appear possess some exceedingly characteristic features. When cut perpendicularly to their broad surfaces, they appear as long, regular, very flat cisternae (Fig. 12). Within the cisternal cavities a material was found that more or less fills out the cavity. This material is also present in the perinuclear space (Fig. 15) and shows a fine structure, which will be described later in this paper. The flat cisternae usually appear in groups, and each group is associated with the nuclear envelope (Figs. 12, 15). In a group, the cisternae are oriented roughly parallel to each other. Serial sections showed that the oriented cisternae intercommunicate at their margins, thus forming a continuum.

In some cells, besides the oriented cisternae, unoriented cisternae with more spacious cavities were found (Fig. 13). These unoriented cisternae are usually empty in osmium tetroxide–fixed material, and resemble the endoplasmic reticulum of the mature Paneth cells. Unoriented and oriented cisternae constitute a more or less communicating system. The unoriented cisternae are intercalated between oriented cisternae or they are located between oriented cisternae and the nuclear envelope. In addition, cells with peripheral, granular endoplasmic reticulum often show this component in continuity with the oriented cisternae. The amount of the two types of granular cisternal elements is quite variable. All intermediates were found between cells dominated by oriented cisternae (Fig. 12) and cells in which only one or two oriented lamellae are present as part of an elaborated ergostoplasm (Fig. 13).

The material in the cavities of the oriented cisternae and in the perinuclear space shows an intrinsic structure which varies with the plane of section. In cisternae cut perpendicularly to their broad surfaces, a fine continuous band about 100 A thick was seen. Two or even three such bands were sometimes seen side by side in one segment of a cisterna (Fig. 16). In some cisternae, the band (or bands) presents a regular cross-striation, with dense and less dense segments, each measuring approximately 50 A, alternating in complete

FIGURE 7   Electron micrograph of a Paneth cell fixed in glutaraldehyde and incubated for 20 minutes for the demonstration of acid phosphatase activity. The reaction product is seen to be localized mostly in the interior of the lysosomes, but is sometimes confined to the limiting membrane (arrow). The secretory granules (SG) show no activity. M: mitochondria; P: plasma membrane. Non-fasting rat. Postfixation in osmium tetroxide. W, UA. × 30,000.

FIGURE 8   Lysosomes with myelin figures, showing reaction product of acid phosphatase activity. GO, W, K. × 73,000.

FIGURE 9   Light micrograph of frozen section of intestinal crypt. Ileum of fasting rat. PA indicates region occupied by Paneth cells in the bottom of the crypt. The tissue was fixed in formol-calcium, and the section incubated for 15 minutes for the demonstration of acid phosphatase activity. Many lysosomes are seen in the Paneth area. The "large" lysosomes are due to overlapping caused by the thickness of the section (15 microns). Lysosomes are also present in the other crypt cells. mm: muscularis mucosae; g: cells in the submucosa, containing many lysosomes, presumably ganglionic cells. No counterstain. × 750.

FIGURE 10   Lysosomes in mature Paneth cell. Small arrows indicate the limiting membrane. At C, localized condensations are seen, which presumably are substances precursory to the myelin figures (MY). O, W, UA. × 47,000.
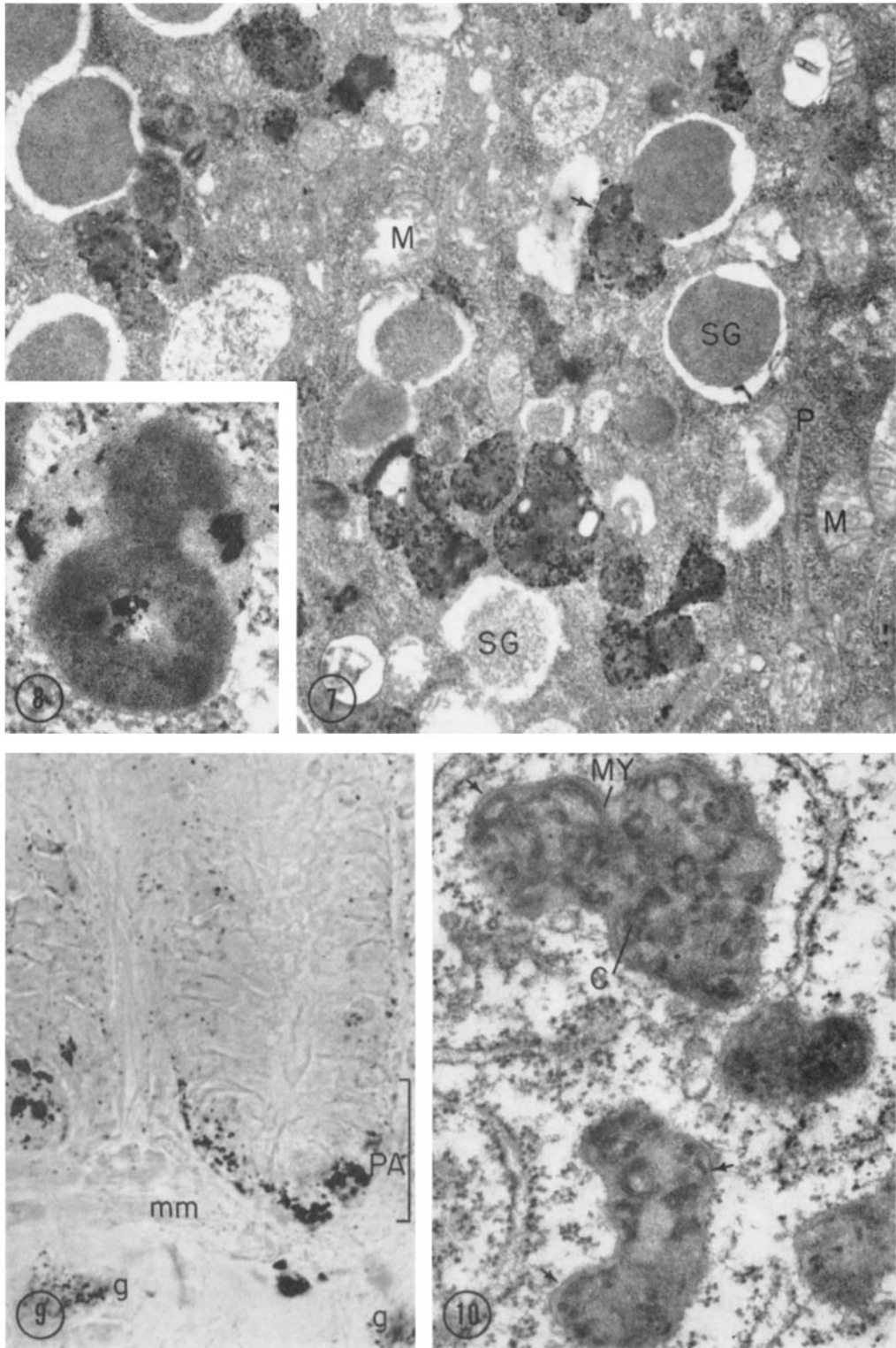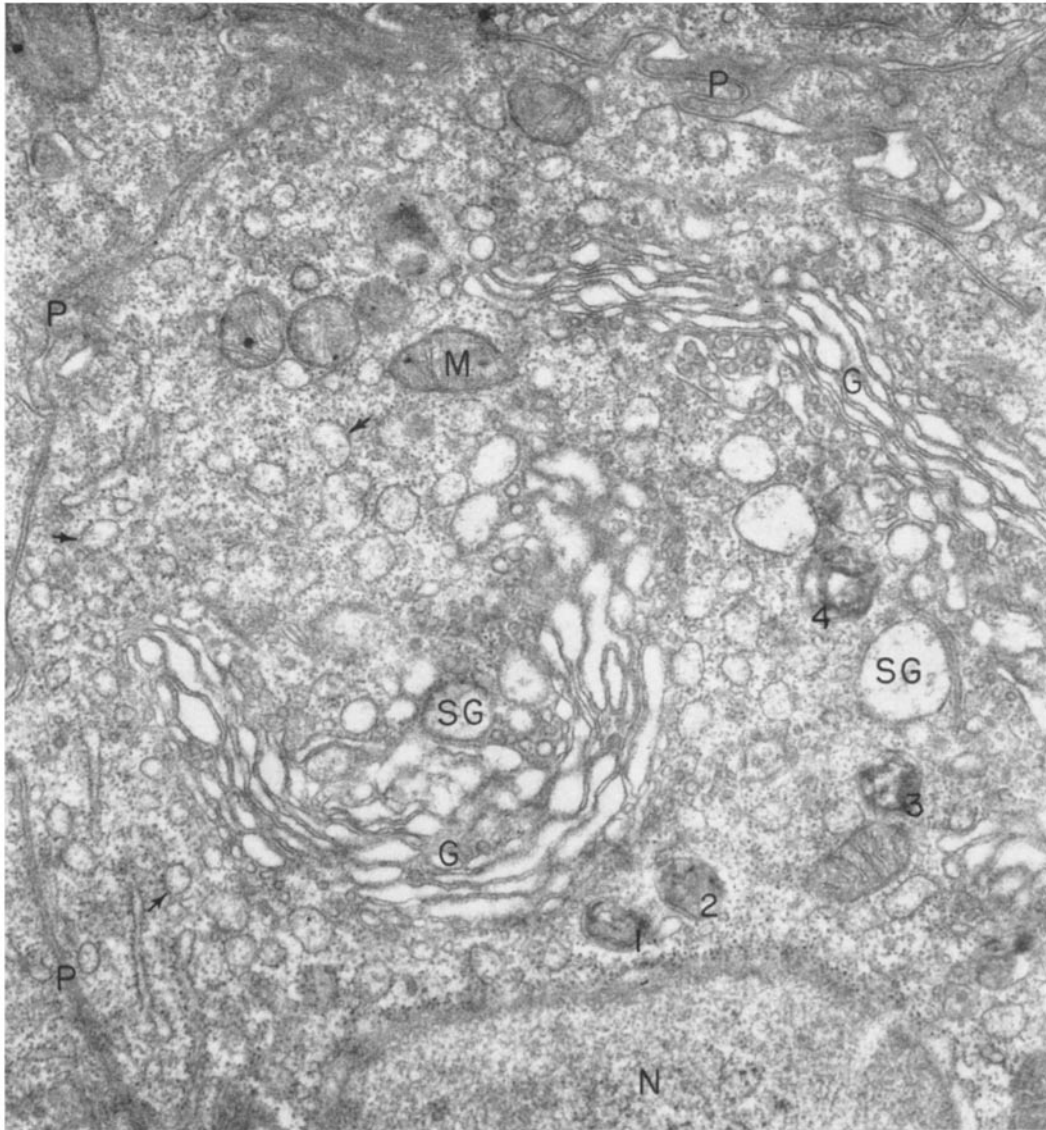
FIGURE 11 This micrograph shows a part of a young Paneth cell in a relatively early stage of differentiation. The rough-surfaced endoplasmic reticulum is mainly represented by round or oval profiles (arrows). The two divisions of the Golgi complex (G) seen in the section are well developed. Some early secretory granules (SG) and four lysosomes (1 to 4) are seen. N: nucleus; M: mitochondrion; P: plasma membrane. O, E, K. × 27,000.

regularity (Figs. 16, 18). In oblique sections, the contents of the oriented cisternae were seen as broader belts, in many instances presenting oblique or transverse striations. In sections cut roughly tangentially to the cisternae, the contents likewise present a delicate pattern of parallel lines or stripes (Fig. 17), and sometimes two sets of stripes crossing each other were seen within the same area.

The material described was most frequently observed in oriented cisternae with profiles as straight as arrows, but sometimes it was also pres-
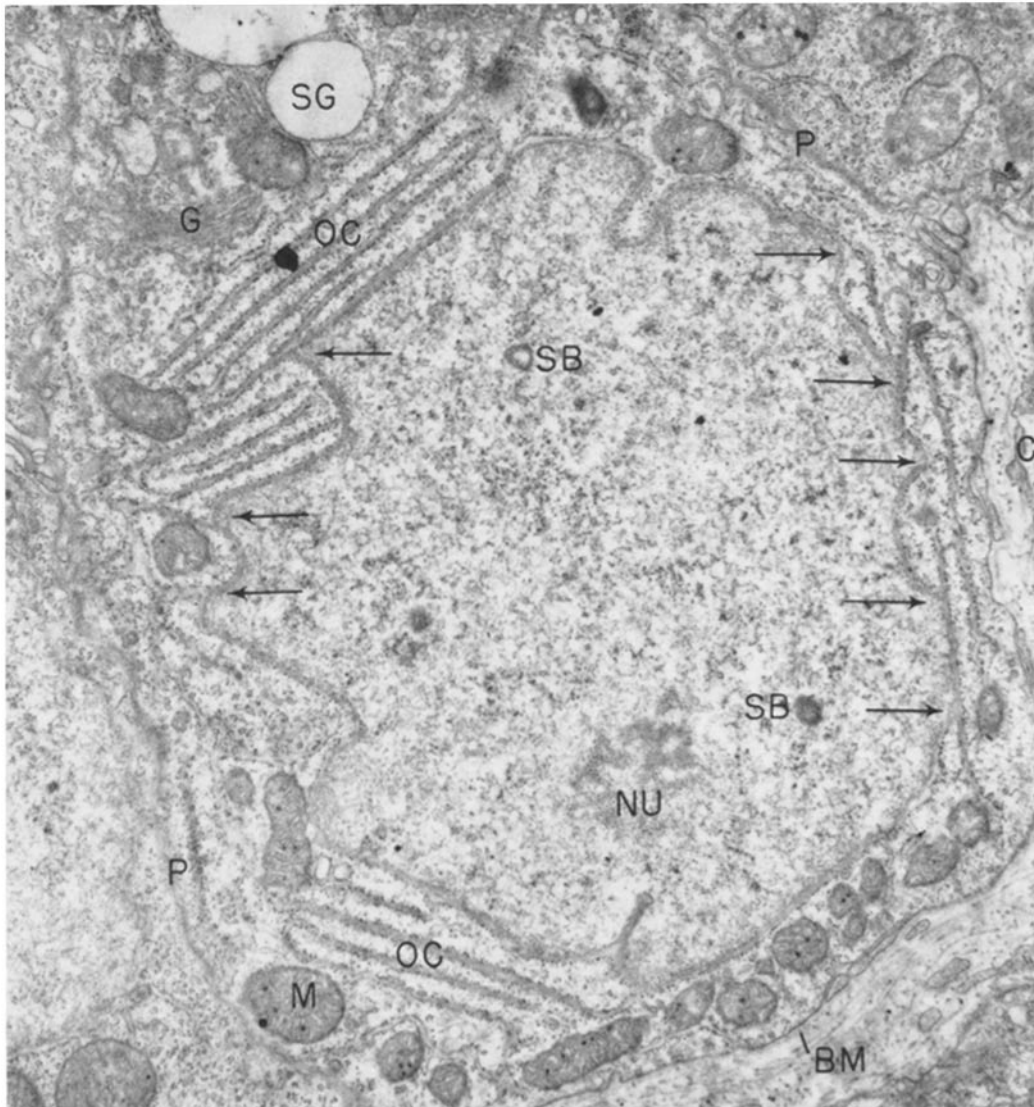
FIGURE 12 Survey micrograph of the basal part of a young Paneth cell. The arrows indicate communications between the nuclear envelope and flat, oriented cisternae (OC). All cisternae, which show communication with the nuclear envelope, and the nuclear envelope itself contain material (see text). Two spherical nuclear bodies are seen (SB). At C, a part of a capillary. A subdivision of the Golgi complex (G) and secretory granules (SG) are seen at the upper left. NU: nucleolus; BM: basement membrane of crypt; M: mitochondrion; P: plasma membrane. O, E, K. × 16,500.

ent for short distances in unoriented cisternae. As mentioned earlier, the majority of the latter are empty, but some contain a sparse, membrane-like material (Fig. 15). Finally, striated material was found in the perinuclear space, from which it continued directly into the oriented cisternae

(Figs. 15, 18). Several segments of the perinuclear space contain material, and corresponding to these segments the surface of the nucleus is straight or nearly so, while between the segments it may be rather uneven (Fig. 12). A narrow zone of fine granular chromatin was often observed close to the

nuclear aspect of the inner nuclear membrane (Figs. 15, 18).

Occasionally, small, dense, homogeneous particles were found in the cisternae in addition to the striated material (Figs. 16, 17). They were seen in all parts of the endoplasmic reticulum and in the perinuclear space, and they usually appeared together with the striated material. The diameter of the particles is 400 to 600 A.

The Golgi complex in the young Paneth cells consists of groups of smooth, flattened cisternae and spherical vesicles. The cisternae within a group are arranged parallel to each other in stacks and are usually curved, forming more or less shallow concavities in which one or more premature secretory granules were observed (Fig. 11). The vesicles were seen on all sides of the stacks, but are most frequently located at the margins of the cisternae and in the concavities. The individual groups of cisternae and vesicles seem to be morphological and functional units. They increase in number during the maturation of the cells, the margins of the groups often adjoining, thereby forming large curved or sinuous systems. In cells building up lamellar endoplasmic reticulum, groups of rough-surfaced cisternae were often seen in intimate relationship with the Golgi cisternae. Numerous vesicles were generally observed in these areas, and pinching-off of smooth-surfaced vesicles from agranular areas of the ergastoplasmic cisternae seems to take place. In this connection, it should be mentioned that striated material with the same 100 A intrinsic periodicity as the material in the cavities of the ergostoplasm was observed within the Golgi cisternae and secretory granules (Fig. 19).

Lysosomes were regularly observed in the differentiating Paneth cells, although in these functionally young cells they are usually smaller and less numerous. They are bordered by a single membrane and contain a homogeneous, rather dense substance, in which wrinkled membranes were often seen (Fig. 11). The older the cells became, the more the interior of the bodies became dominated by myelin figures. Not infrequently, ring- and C-shaped figures, which in some cells were found near the Golgi complex, show connection with a lysosome (Fig. 15). Serial sections indicated that these figures represent sections at different levels through a cup-like structure, with an inner and an outer membrane, enclosing a space filled with a homogeneous substance. Although no acid-phosphatase activity was found in these cup-like structures, they are presumed to represent early stages in the development of some of the lysosomes. No evidence was found that the lysosomes of the Paneth cells might be remnants of the cytoplasmic bodies present in epithelial cells of the small intestine of the rat during certain earlier stages of differentiation (23).

Microvilli, desmosomes, and asymmetry of the plasma membrane were found in all stages of development. Tonofilaments are rather sparse in the youngest Paneth cells, and spherical nuclear bodies were encountered less frequently in Paneth cells with endoplasmic reticulum of the vesicular type.

DISCUSSION

Of all the organelles of the Paneth cell, the endoplasmic reticulum is subjected to the most extensive reorganization during the process of differentiation. The mature Paneth cells are equipped with a lamellar reticulum similar to the highly specialized reticulum found in cells that synthesize and secrete proteins (24, 25). On the other hand, the very immature Paneth cells contain a reticulum

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FIGURE 13   The figure shows a young Paneth cell in a slightly later stage of differentiation than the cell seen in Fig. 12. The cell contained only one cisterna with material (OC). The lamellated ergastoplasmic membranes seen at the base of the cell (bottom of micrograph) are similar to those seen in the mature Paneth cell (Fig. 3). RB: ring-shaped body in Golgi region (G); SG: secretory granules; P: plasma membrane; N: nucleus, BM: basement membrane of the crypt; Ne: nucleus of endothelial cell of a capillary in relation to the Paneth cell. O, E, K. × 18,000.

FIGURE 14   Part of Paneth cell nucleus with spherical nuclear body (SB). NE: nuclear envelope. O, E, K. × 37,000.
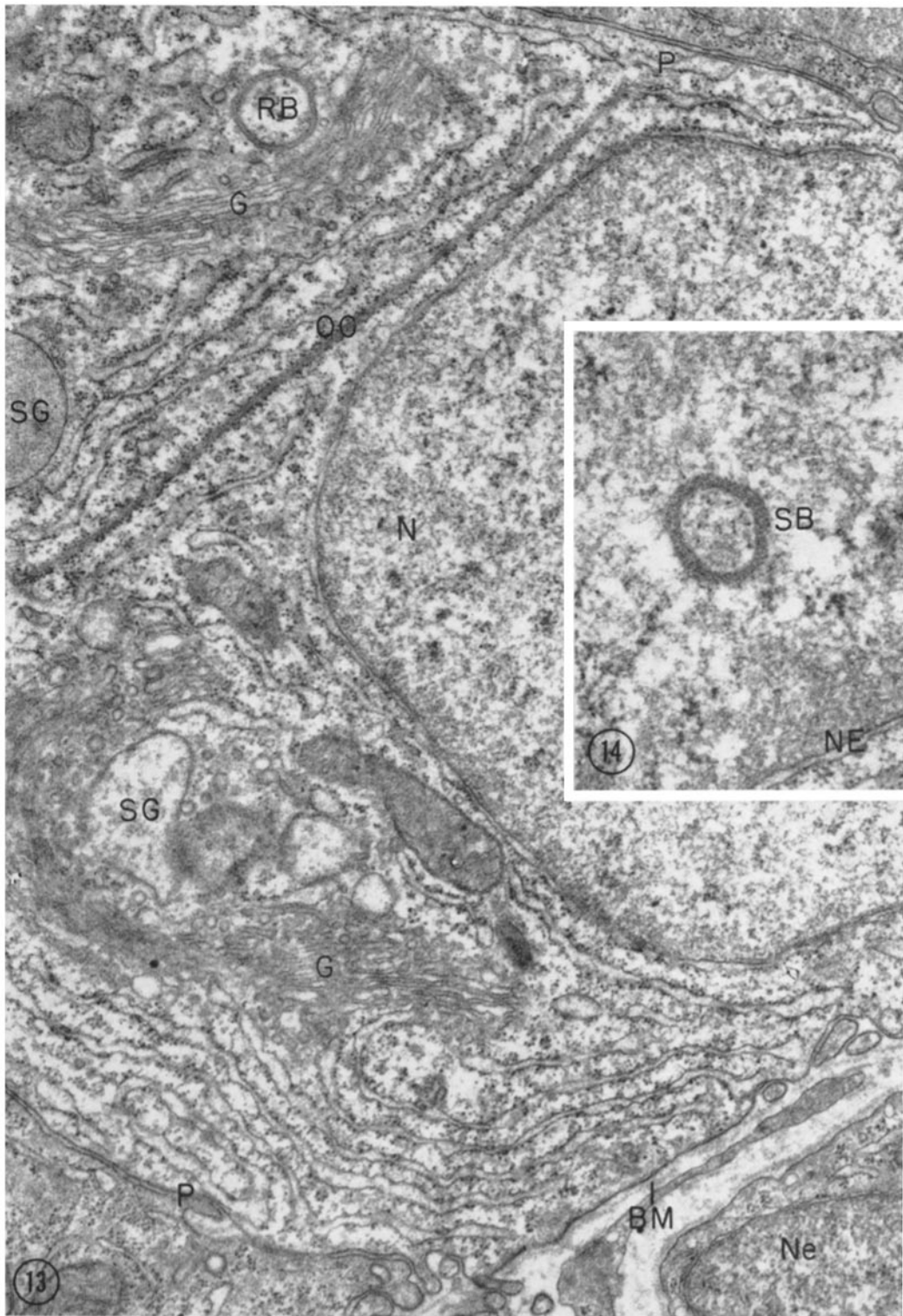
consisting of round and oblong, ribosome-covered vesicles dispersed in most of the cytoplasm. Endoplasmic reticulum of this very unorganized type occurs in several cell types in early stages of differentiation, as shown by Hay (26), Slautterback and Fawcett (27), Godman and Porter (28), and Eakin (29). It has been suggested that vesicular endoplasmic reticulum may originate by coalescence of smooth-surfaced, minute vesicles and tubules arising in the cytoplasm (30) or originating from pre-existing membranous elements in the Golgi region of the cell (26). Ribosomes are assumed subsequently to become associated with the surface of the vesicles (28–30). It has been suggested, furthermore, that this "primitive" reticulum may develop into highly organized, lamellar reticulum by continued fusion of vesicles and tubules and spatial rearrangements of the lamellae (27, 30, 31).

Parts of the lamellar ergastoplasm in Paneth cells may possibly originate in a similar way, as the most immature cells have abundant vesicular elements which eventually disappear. The nuclear envelope seems, however, to play a major role in the development of the ergastoplasm of the Paneth cells. Supporting this assumption is the fact that, during the formation of the lamellar reticulum, cisternae of all sizes—from single, short lamellae to large complexes of oriented cisternae—were very frequently seen in communication with the nuclear envelope, suggesting an outgrowth of cisternae from the outer membrane of the envelope. At the same time, the perinuclear space and the lumen of the cisternae contain a crystal-like material which, in the osmium-fixed condition, looks like plates made of closely packed

laminae, resembling a Venetian blind. Such plates seem to possess considerable rigidity, as the areas of the nuclear envelope and the cisternae which contain structured material are always straight and flat. The hypothesis of the formation of endoplasmic reticulum from the nuclear envelope is not a new one (see Porter, 35), but very few observations supporting this hypothesis have been reported. Probably the best support is to be found in a paper published by Parks (36). He examined the parotid gland in mice re-fed after 4 days of starvation and found, in some of the acinous cells, large, complicated, ergastoplasmic formations which had many connections with the nuclear envelope. Parks mentions that the cisternae may take their origin from the nuclear envelope and adds that the ergastoplasmic formations later on appear to fragmentize and to detach from the nuclear envelope. Fragmentation of organized, lamellar reticulum has also been described by Hay (26) and by Slautterback and Fawcett (27). Our observations indicate that a similar fragmentation and rearrangement of the ergastoplasmic complex of the Paneth cells takes place during the final stages of differentiation, resulting in the disposition typical of the mature cell.

It is generally assumed that the ergastoplasmic reticulum produces and segregates secretory substances and also functions as a pathway for intracellular transport. Usually, the contents of its cavities manifest themselves as a structureless material of low or moderate electron opacity. As shown by Sabatini, Bensch, and Barrnett (39) and also in the present work, the intracisternal content is more electron-opaque if the tissue is prefixed in glutaraldehyde before osmium fixation;

Figure 15   Part of a young Paneth cell, showing three communications (large arrows) between the nuclear envelope and oriented cisternae (OC) containing material. The cisterna to the left shows a fine line (arrow 3) in its cavity. The cavities in the remaining cisternae are filled with a rather dense material, which in places shows transverse striations. In the perinuclear space, two bands of material are seen (arrows 1 and 2), which continue into a cisterna of the reticulum. DBR: combined ring-shaped and dense body; G: Golgi region; SG: secretory granule. O, E, K. × 42,000.

Figure 16   Higher magnification of a part of three oriented cisternae, showing the periodicity of the material contained in the cisterna to the left. This cisterna contains two bands of material, between which two osmiophilic granules are seen (arrows). O, E, K. × 75,000.
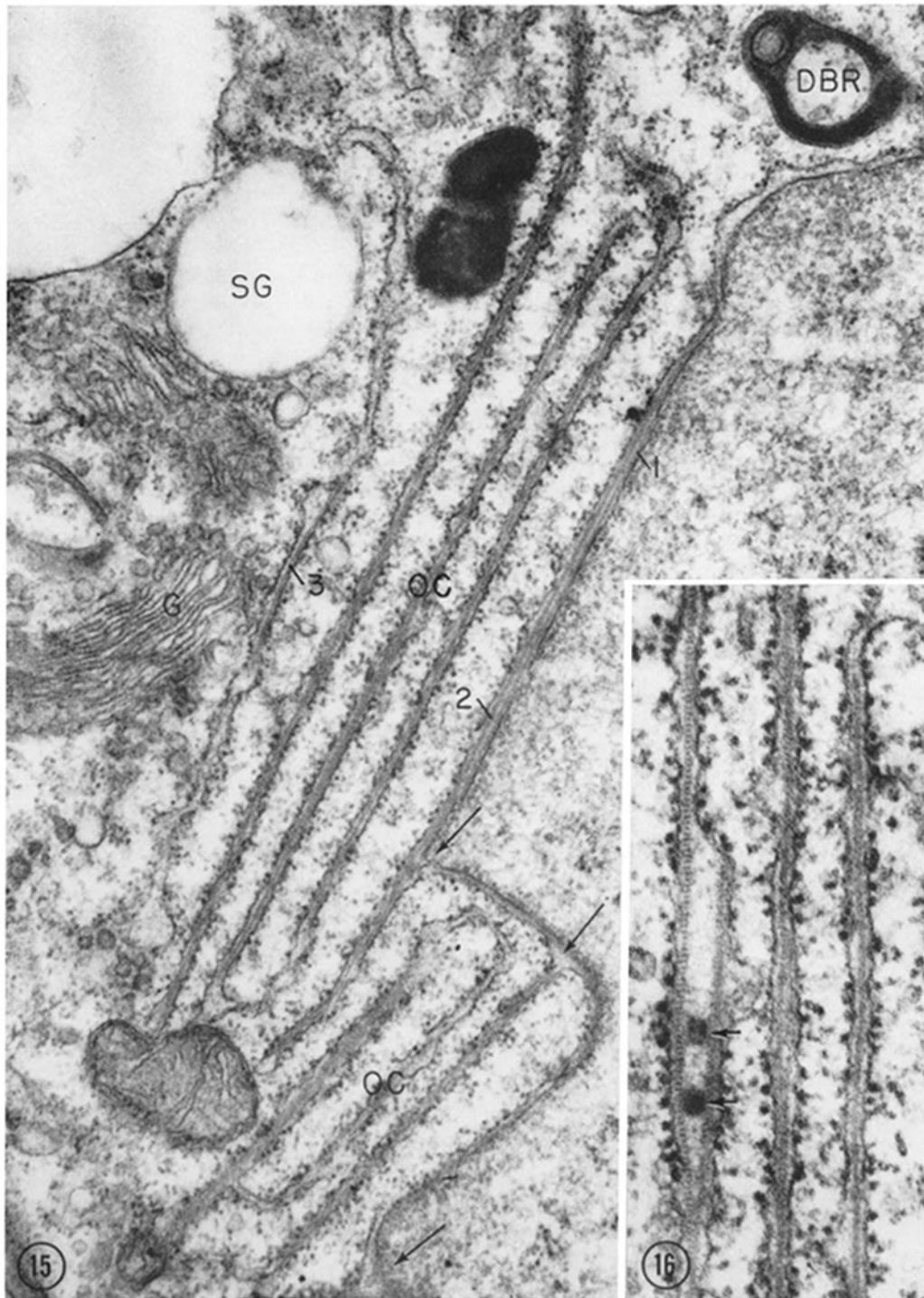
but it still seems to have no distinct structure. The finding of the above-described delicately structured, intracisternal material and spherical particles in tissue fixed only in osmium tetroxide was therefore, surprising. This material was found at a stage of differentiation at which the formation of lamellated endoplasmic reticulum seemed to be lively. Its occurrence in the cisternal as well as the perinuclear space, and its uniform structure independent of localization may indicate that the material is elaborated both by the nucleus or the nuclear envelope and by the cisternae. Another possibility is that it is formed exclusively in the envelope and moves more or less concomitantly with the cisternae during their development.

An intimate relationship between the newly formed, lamellar, endoplasmic reticulum and the Golgi system was conspicuous in many cells. The two membrane systems lie close to each other in cytoplasmic regions, which show an abundance of small vesicles. The impression was gained that a lively "budding" or "blebbing" process (37, 38) takes place at this stage of differentiation, as agranular areas of the ergastoplasmic membranes and "blebs" in these areas are common in the vicinity of the Golgi system.

The structured material in the Paneth cells probably consists of secretory substances which, for some reason, are precipitated in a crystalline pattern; its appearance both in the cavities of the ergastoplasm and in the Golgi cisternae and secretory granules seems to indicate a transfer of the material from the ergastoplasm to the secretory granules *via* the Golgi cisternae. In guinea pig pancreas, Palade (40) found numerous large granules in the cavities of the endoplasmic reticulum during the period of recovery after secretion. When these granules were isolated, it was found that they have the properties of pancreas zymogen (41, 42). Intracisternal granules believed to repre-

sent the precursors of proteinaceous yolk have been reported in the endoplasmic reticulum in crayfish oocytes by Beams and Kessel (43), who suggested that the granules "flowed" from oriented into unoriented cisternae, where they collected and underwent transformation into yolk bodies.

Cytoplasmic bodies containing myelin figures have been reported in a wide variety of cells, under both physiological and pathological conditions (23, 44–50). Furthermore, some investigators have reported the presence of acid phosphatase in such bodies (15, 51, 52). The presence of acid phosphatase activity in the dense, cytoplasmic bodies of the Paneth cells characterizes these bodies as lysosomes.

Novikoff *et al.* (4) and Novikoff and Essner (5) have demonstrated acid phosphatase activity localized in early secretory granules, and their results have been confirmed by other investigators (55). In our material, however, we have not observed acid phosphatase activity in either early or mature secretory granules (Fig. 7).

Morphological heterogeneity of lysosomes has often been reported in the literature (for review, see 53). The lysosomes of the fully differentiated Paneth cells show a striking morphological homogeneity, indicating that they have distinct chemical constituents. Their PAS-positivity and their strong affinity for basic dyes, seen in the light microscope, along with the fine structure of the bodies, make it seem probable that the contents of the bodies are largely phospholipids. It seems unlikely that the lysosomes of the Paneth cells are storage granules derived from segregated foreign material. It appears more probable that the contents are of endogenous origin. One might speculate as to whether the bodies are residual bodies derived from lysosomes active in the turnover of cell organelles, the myelin figures thus being remnants of lipoprotein systems liberated from

FIGURE 17 Oriented cisternae sectioned obliquely. The lines indicate the direction, in the individual cisternae, of the striation of the material contained. The arrows point to osmiophilic granules in the cisternae. O, E, K. × 71,000.

FIGURE 18 Profiles of oriented cisternae. The periodicity of the material contained is clearly seen in the cisterna in the middle. Along the nuclear side of the inner membrane of the nuclear envelope, condensed nuclear material is seen. The arrow points to a communication between the perinuclear space and a cisterna. *N:* nucleus. O, E, K. × 61,000.
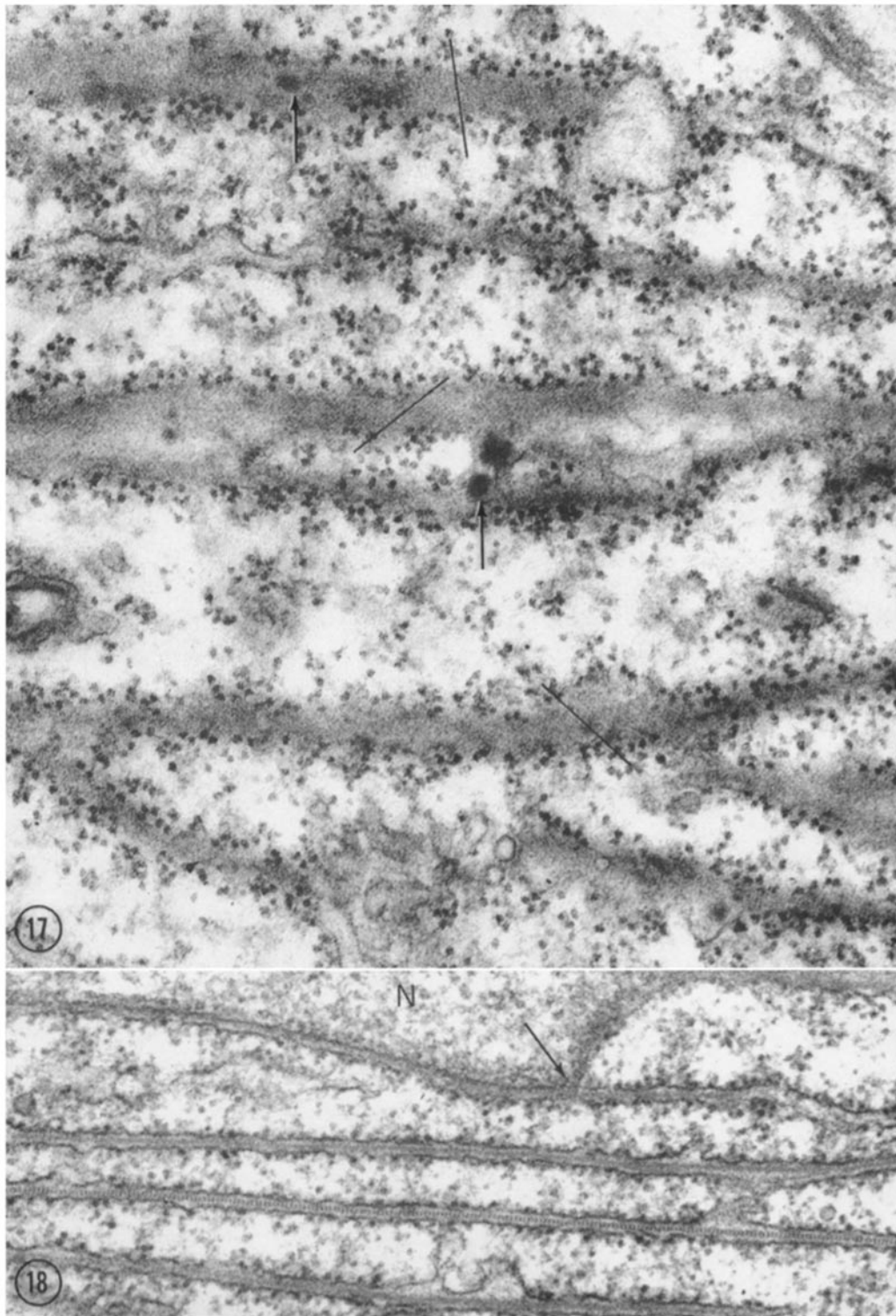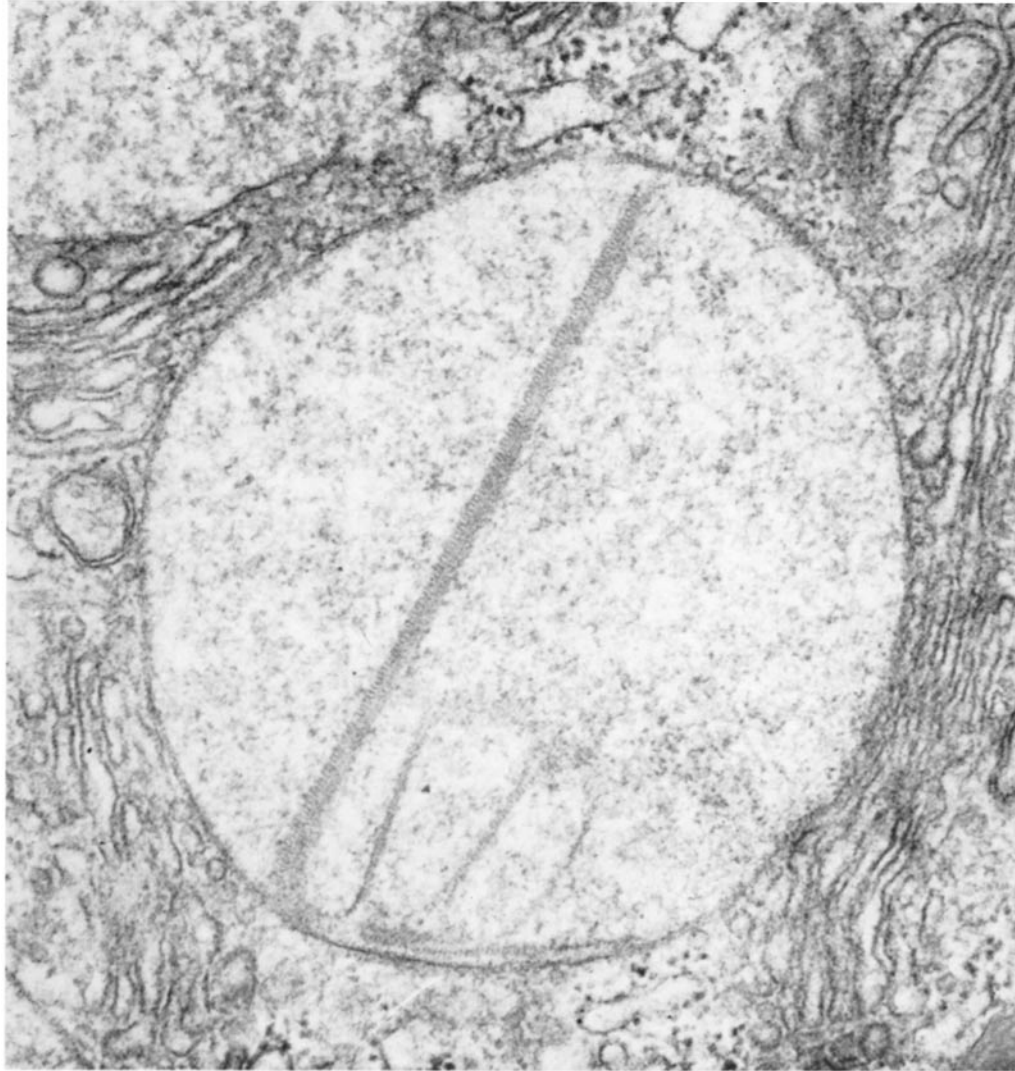
FIGURE 19 This micrograph shows a secretory granule in a Paneth cell from a non-fasting rat, 20 days old. The granule contains a band of material which exhibits the same 100 A periodicity as that of the oriented cisternae. O, E, K. × 69,000.

autolyzed organelles. The large number of lysosomes might then be a consequence of the Paneth cells becoming "old" cells, not participating in the rapid turnover of the epithelium of the small intestine in the rat (54). Against this assumption, however, remains the fact that we have never observed the incorporation of mitochondria, endoplasmic reticulum, or other organelles into the bodies; moreover, newly differentiated Paneth cells, at most 7 to 8 days old, often contain great numbers of lysosomes. The fate of the lysosomes is obscure. They do not seem to be expelled from the cells, and they are very seldom observed in the apical cytoplasm among the secretory granules.

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