CYTOLOGICAL STUDIES ON TWO FUNCTIONAL HEPATOMAS

Interrelations of Endoplasmic Reticulum, Golgi Apparatus, and Lysosomes

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ABSTRACT

The Reuber hepatoma H-35 and Morris hepatoma 5123 have been studied by electron microscopy and by cytochemical staining methods for a number of phosphatases. These studies emphasize the resemblances of the two tumors to rat liver, but they also indicate distinctive features in each of the three tissues. Secretory product accumulates within the cisternae of the Golgi apparatus that dilate to form the Golgi vacuoles. The vacuoles apparently separate, and secretory material undergoes further condensation within them. These "secretory vacuoles" possess acid phosphatase activity and may thus be considered lysosomes. The membranes of the Golgi apparatus are without acid phosphatase activity but show high levels of thiaminepyrophosphatase activity. The endoplasmic reticulum also hydrolyzes thiaminepyrophosphate but at a lower rate; it hydrolyzes the diphosphates of uridine, guanosine, and inosine rapidly. These observations and the electron microscopic images are consistent with the view that the cytomembranes are in a dynamic state of flux, movement, and transformation in the living cell, and that smooth surfaced derivatives of the endoplasmic reticulum become refashioned into the Golgi membranes as the Golgi membranes are being refashioned into those that delimit secretory vacuoles. The variations encountered in the two hepatomas are described. The electron microscope literature dealing with the relations of the Golgi apparatus to secretory granules, on the one hand, and the endoplasmic reticulum, on the other, is reviewed briefly.

INTRODUCTION

The Morris 5123 hepatoma (20) and Reuber H-35 hepatoma (38) are attracting much attention from oncologists because of their resemblance to liver, biochemically and morphologically (37, 47, 34). This communication extends the morphological studies to cover fine structure and cytochemical staining reactions for enzyme activities.

In the course of these studies it became apparent that the cells of these tumors are unusually favorable for analysis of the cytological aspects of secretion.

In concluding his masterful review of 1929, R. H. Bowen (4) wrote, "Secretion is in essence a phenomenon of 'granule' or droplet formation. Starting with a single such secretory droplet about to be expelled from the cell, we find it possible to trace its origin step by step to a minute

vacuole, which has thus from the beginning served as a segregation center for a specific secretion-material. The primordial vacuole is found to arise in that zone of the cell characterized by the presence of the Golgi apparatus, and the evidence indicates, if it does not demonstrate, that the primary vacuole arises through the activity of the Golgi substance and undergoes a part at least of its development in contact with, or embedded in, the Golgi apparatus."

Electron microscopy, beginning with the studies of Sjöstrand and Hanzon (41) and Haguenau and Bernhard (13) and extending through numerous investigations (see Palay (32), Dalton (7), and Kurosumi (18)), has clarified the morphological origin of this "primary vacuole." It is a separated Golgi vacuole that arises by dilatation of a Golgi cisterna. It is delimited by a membrane apparently derived from the membrane that had previously surrounded the cisterna. Sometimes the secretory material it contains has a characteristic appearance in the electron microscope (17, 40, 43, 49). By coupling electron microscopy and radioautography, Caro (5) has shown the

FIGURE 1

Reuber H-35 hepatoma. Hematoxylin-eosin preparation of frozen section. Arrows indicate green bodies within parenchymal cells. × 460.

FIGURE 2

Reuber H-35 hepatoma. Frozen section incubated 40 minutes at 37°C for TPNH-nitro-BT reductase activity (24). The spheres containing bile pigment appear black (arrows) in such preparations. At lower right (arrow), the spheres are within lining (Kupffer?) cells. The others are within parenchymal cells and are concentrated along the bile canaliculi; some of the unstained canaliculi are visible. × 460.

FIGURE 3

Reuber H-35 hepatoma. Frozen section incubated 10 minutes at 37°C for nucleosidediphosphatase activity, with inosinediphosphate as substrate (28, 29). Except for bile canaliculi (*B*) and sinusoids (*S*), staining is restricted to irregular strands in the cytoplasm (endoplasmic reticulum) and nuclear membrane (unmarked arrows) (see Fig. 18). × 1570.

FIGURE 4

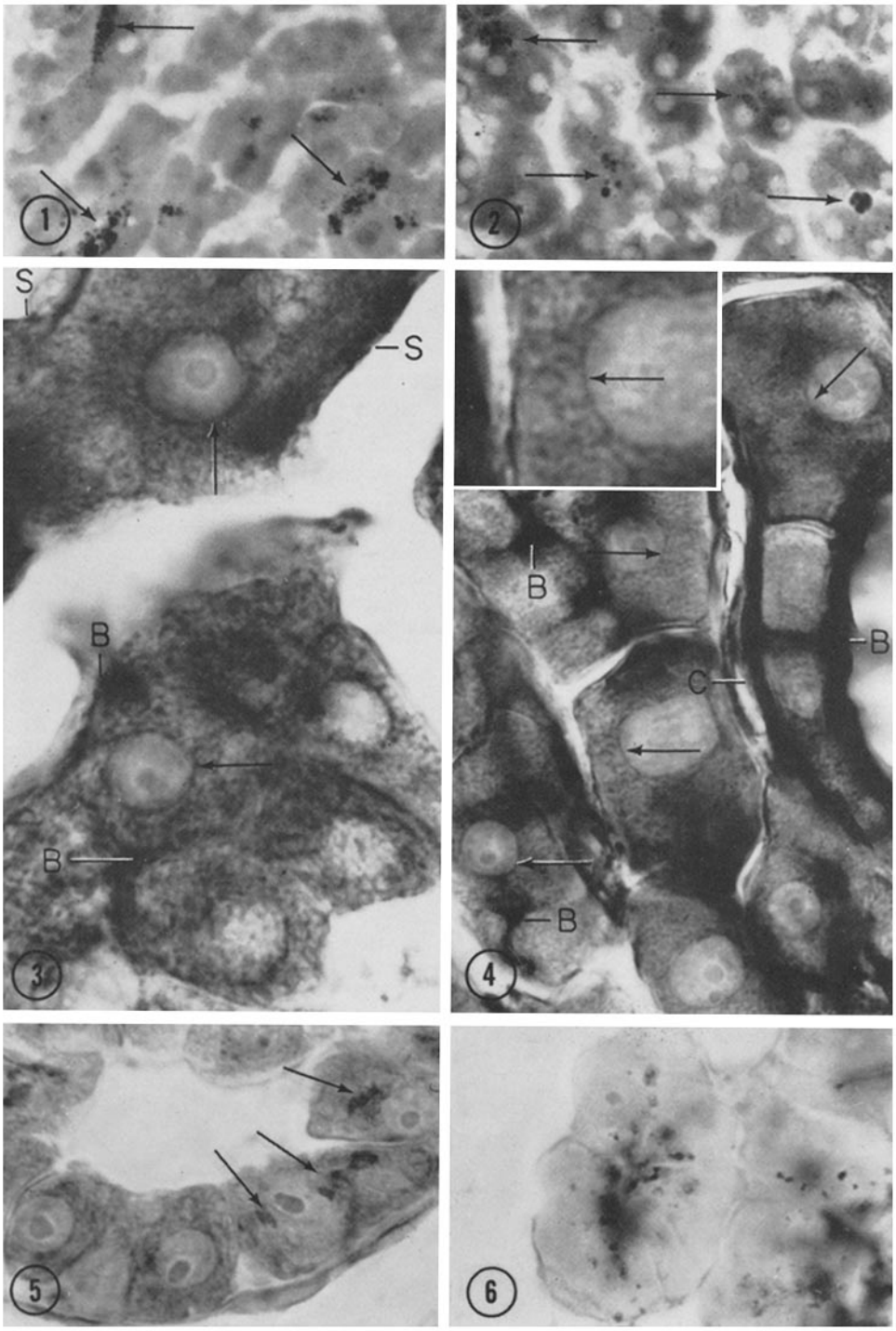
Morris 5123 hepatoma. Frozen section incubated 20 minutes at 37°C for nucleosidediphosphatase activity, with inosinediphosphate as substrate (28, 29). Intense staining is shown by altered bile canaliculi (*B*) and capillaries (*C*). Within the parenchymal cells, staining is restricted to irregular strands in the cytoplasm (endoplasmic reticulum) and nuclear membrane (unmarked arrows) (see Fig. 17). × 1300. *Insert.* Enlargement of part of cell in center of figure to show stained nuclear membrane and endoplasmic reticulum. × 2600.

FIGURE 5

Morris 5123 hepatoma. Frozen section incubated 30 minutes at 37°C for thiamine-pyrophosphatase activity (28). Acinar arrangement of cells around dilated bile canaliculus (21) is evident. The Golgi apparatus, generally between nucleus and acinus lumen, is darkly stained (arrows). Staining is also seen in endoplasmic reticulum, nuclear membrane (*cf.* Fig. 4), and nucleolus (see (27)). × 830.

FIGURE 6

Morris 5123 hepatoma. Frozen section incubated 15 minutes at 37°C for acid phosphatase activity (12). The concentration of granules (lysosomes) along the bile canaliculi is high, but most individual ones are difficult to make out in the photograph. Those farther removed from the canaliculi are more evident. × 1360.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secretory material to contain injected labeled amino acid. The secretory material, when condensed into a granule, will be referred to as a "secretory granule," and the vacuole containing it as a "secretory vacuole."

The present paper presents evidence[1] suggesting that, in cells of the Reuber H-35 hepatoma (38) and Morris 5123 hepatoma (20), the enzymatic activity of the Golgi membrane is altered when it becomes the membrane delimiting the secretory vacuole. The Golgi membrane has high levels of thiaminepyrophosphatase activity. The membrane of the secretory vacuole shows no such activity. Instead it has acid phosphatase characteristic of lysosomes (9, 22). The evidence is consistent with the view that the Golgi membranes are in a state of dynamic equilibrium. It is suggested that as these membranes yield up membrane to the secretory vacuoles, or lysosomes, they gain membrane from smooth surfaced derivatives of the endoplasmic reticulum.

MATERIALS AND METHODS

Electron Microscopy

Areas of the Reuber hepatoma H-35 (38) and of the Morris hepatoma 5123 (20) were dissected from rats under pentobarbital anesthesia. Small pieces were rapidly removed for electron microscopy. These were immersed in cold, 1 per cent buffered osmium tetroxide (pH 7.2) containing 4.5 per cent sucrose (6), cut into smaller pieces, and fixed for approximately 1 hour. After rinsing in veronal-acetate buffer, the tissue blocks were dehydrated in graded concentrations of alcohol and infiltrated and embedded in methyl-butyl methacrylate (1:7) containing 75 mg per 100 ml of uranyl nitrate (44). Polymerization took place at 60°C overnight. Thin sections were prepared on a Porter-Blum microtome, equipped with a diamond knife, and were mounted on collodion-coated copper grids. The sections were stained by floating the grids face down on a saturated solution of potassium permanganate, freshly prepared and filtered before use. When dry, the sections were "sandwiched" by evaporation of a light layer of carbon (46). Specimens were examined in an RCA EMU-3B electron microscope. Micrographs were taken at magnifications of from 3800 to 14,000 and enlarged photographically.

Cytochemical Procedures

Portions of tumor were fixed (*a*) in Bouin's fluid for hematoxylin-eosin staining and routine histological study; (*b*) in Aoyama's fluid for classical Golgi preparations; and (*c*) in cold formol-calcium (2) or formol-phosphate (19), with or without the addition of 5 per cent sucrose (16), for cytochemical preparations. The latter were made of tissue fixed overnight at 4°C. Frozen sections were prepared on a Bausch and Lomb or Sartorius freezing microtome and incubated at 37°C. The incubation media contained lead ions (0.11 or 0.12 per cent) to trap the phosphate ions released by enzymes in the section. The phosphate esters used were α, β, or β-glycerophosphate in the medium of Gomori (12), for lysosomes; inosinediphosphate for endoplasmic reticulum (27) in the medium of Novikoff and Goldfischer (28); and thiaminepyrophosphate for Golgi apparatus in the medium of Novikoff and Goldfischer (28, 27). For demonstration of other

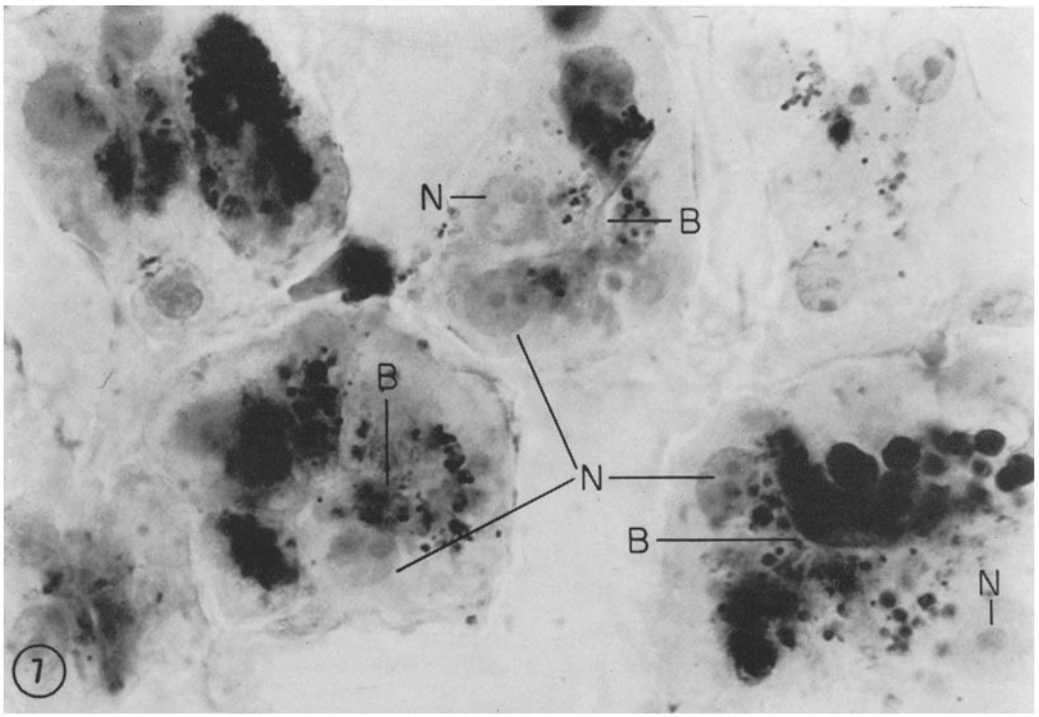
[1] This work has been presented at the Gordon Research Conference on Cancer, September 1, 1961, the International Society for Cell Biology (27), and the American Society for Cell Biology (11). Supporting evidence for the proposed relation between endoplasmic reticulum and Golgi apparatus has been reported recently by Zeigel, R. F., and Dalton, A. J. (*J. Cell Biol.*, 1962, **15**, 45).

FIGURE 7

Reuber H-35 hepatoma. Frozen section incubated 20 minutes at 37°C for acid phosphatase activity (12). Note concentration of lysosomes, particularly smaller ones, between nuclei (*N*) and bile canaliculi (*B*). Large granules at lower right measure roughly 4 μ in diameter. × 1270.

FIGURE 8

Reuber H-35 hepatoma. Electron micrograph of formol-calcium-fixed frozen section incubated 7 minutes at room temperature for acid phosphatase activity (12). Dense accumulations of reaction product are not seen in nucleus (*N*), mitochondria (*M*), or endoplasmic reticulum (*ER*). Such accumulations are evident (unmarked arrows) in the membranes of vacuoles containing an electron-opaque material presumed to be secretory material. Note proximity of endoplasmic reticulum to mitochondria. × 24,000.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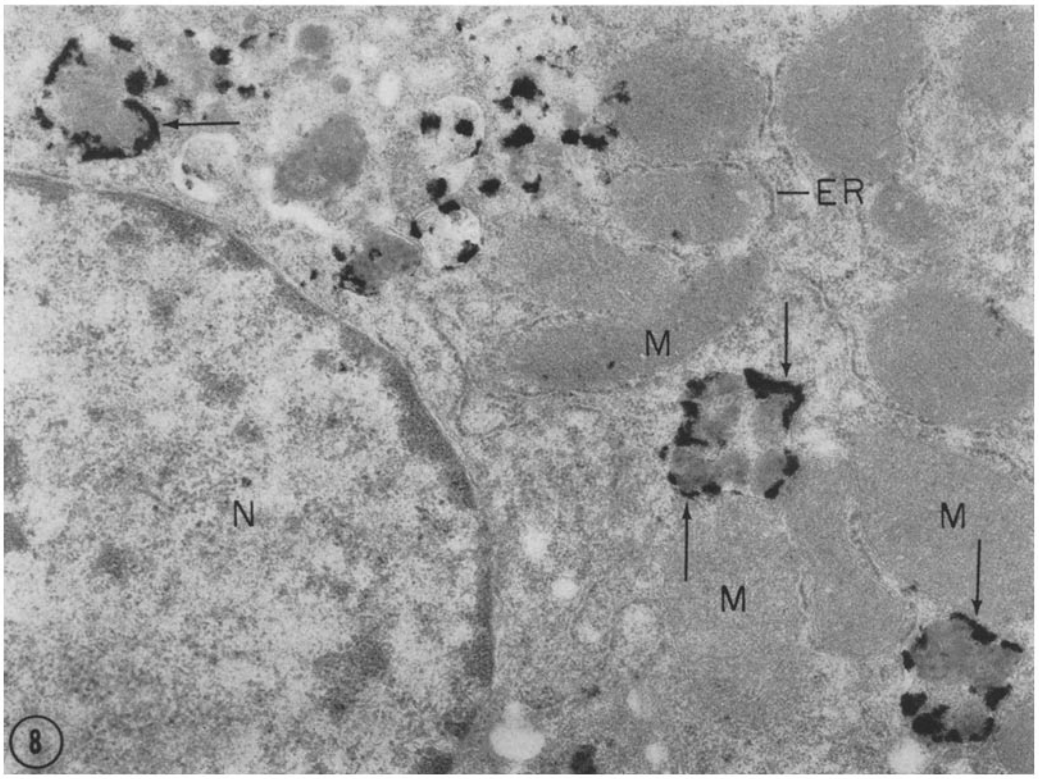
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structures such as bile canaliculi, adenosinemonophosphate and adenosinetriphosphate were also used as substrates, in the medium of Wachstein and Meisel (45) or Novikoff and Goldfischer (28).

For light microscopy, the frozen sections, 10 μ thick, were cut into water. Following incubation, they were rinsed in water, immersed in dilute ammonium sulfide for a few minutes, rinsed, and mounted in Kaiser's glycerogel (19).

For electron microscopy 40 μ frozen sections were used. They were cut into water or, if the fixative contained sucrose, into 5 per cent sucrose. In the latter case, the media also contained 5 per cent sucrose. Following incubation, the sections were rinsed in sucrose and immersed in buffered osmium tetroxide with 4.5 per cent sucrose for 30 minutes, generally without prior treatment with ammonium sulfide. Further processing for electron microscopy was the same as that described above for unincubated tissue. When the sections were in ethanol they were cut into small pieces for methacrylate embedding.

OBSERVATIONS

Reuber Hepatoma H-35

General Cytology

The Reuber hepatoma H-35 shows considerable morphologic and functional resemblance to rat liver, as first described by Reuber (38) and emphasized by the staining procedures used in the present studies. Bile canaliculi are present between adjacent cells. As in liver, they hydrolyze nucleoside mono-, di-, and triphosphates (Fig. 3). The relative rates of hydrolysis are, however, different from those of liver canaliculi, with the tumor canaliculi cleaving the monophosphates more rapidly than the di- and triphosphates. Apparently, the canaliculi do not join in the organized fashion typical of liver; there is, of course, no drainage system into a bile duct; and there is no visible evidence of secretion into the canaliculi.

Reuber (38) described accumulations of bile pigment in macrophages of adjacent connective tissue and in the tumor cells. Those of sufficient size are readily distinguished by their yellowish-green color, both in unstained frozen sections and in hematoxylin-eosin sections. In some areas, generally near the connective tissue surrounding the tumor (38), many tumor cells show such spheres (Fig. 1). They may range in size from the diameter of usual hepatic lysosomes to a diameter of 3 μ or more. We shall refer to these as secretory granules or vacuoles, because of their green color and because they stain for bilirubin by the method of Hall (14). They are stained black in frozen sections incubated for TPNH-nitro-BT reductase (Fig. 2); the basis of this reaction was not studied.

When sections are incubated for acid phosphatase activity, the green color of the secretory vacuoles is no longer detectable. The large vacuoles, easily identified because of their size, show high levels of acid phosphatase activity (Fig. 7). It is not possible, in the light microscope, to determine how many of the smaller acid phosphatase-rich granules are secretory vacuoles.

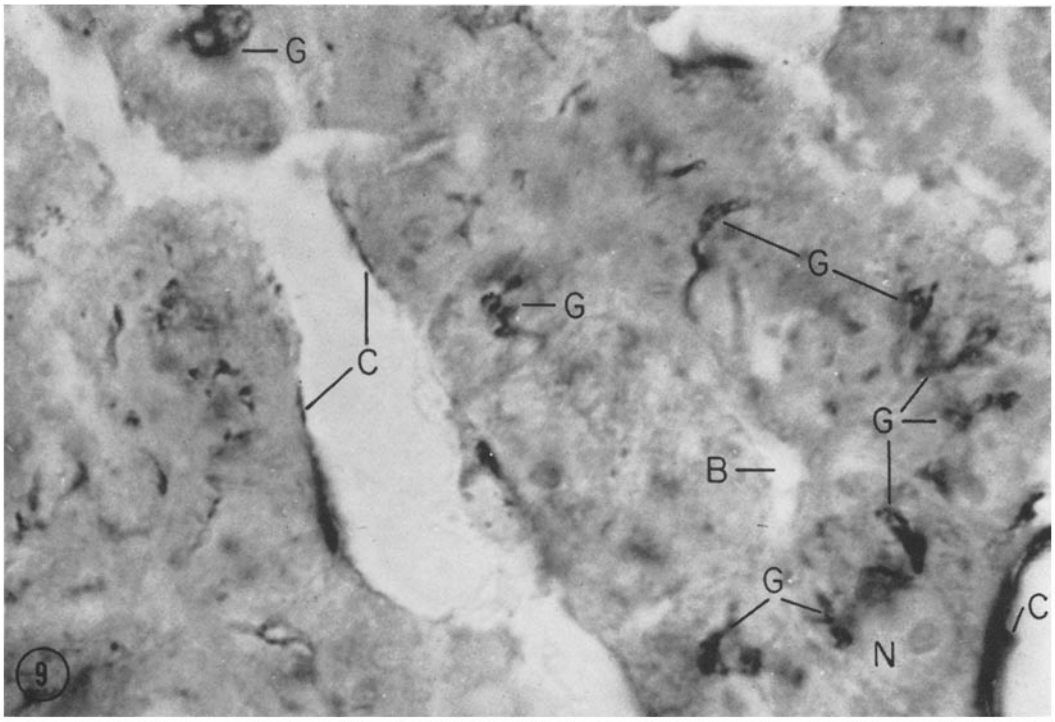
The secretory vacuoles, and whatever other lysosomes there may be in these cells, lie along the bile canaliculus and in the cytoplasm between canaliculus and nucleus, with the larger granules often farthest from the canaliculus (Fig. 7). The Golgi apparatus, large and "reticular" (Figs. 9 and 10), lies in this area of lysosome concentration. The Golgi lamellae (35) frequently lie parallel to the nuclear membrane and extend

FIGURE 9

Reuber H-35 hepatoma. Frozen section incubated 10 minutes at 37°C for thiaminepyrophosphatase activity (28). The polarized arrangement of the Golgi lamellae (*G*) is evident in the lower right cell, where the nucleus (*N*) is evident, and the unstained bile canaliculus (*B*) and darkly staining capillary (*C*) at the base may be seen. × 1760.

FIGURE 10

Reuber H-35 hepatoma. Electron micrograph from formol-calcium-fixed frozen section incubated 20 minutes at 37°C for thiaminepyrophosphatase activity (28). The field shows several areas in which membranes of the Golgi apparatus (*G*) contain reaction product. The nuclei (*N*) and mitochondria (*M*) contain no reaction product, but small amounts are deposited in the endoplasmic reticulum (*ER*) (see Figs. 17 and 18). Methacrylate embedded; potassium permanganate staining. × 15,000.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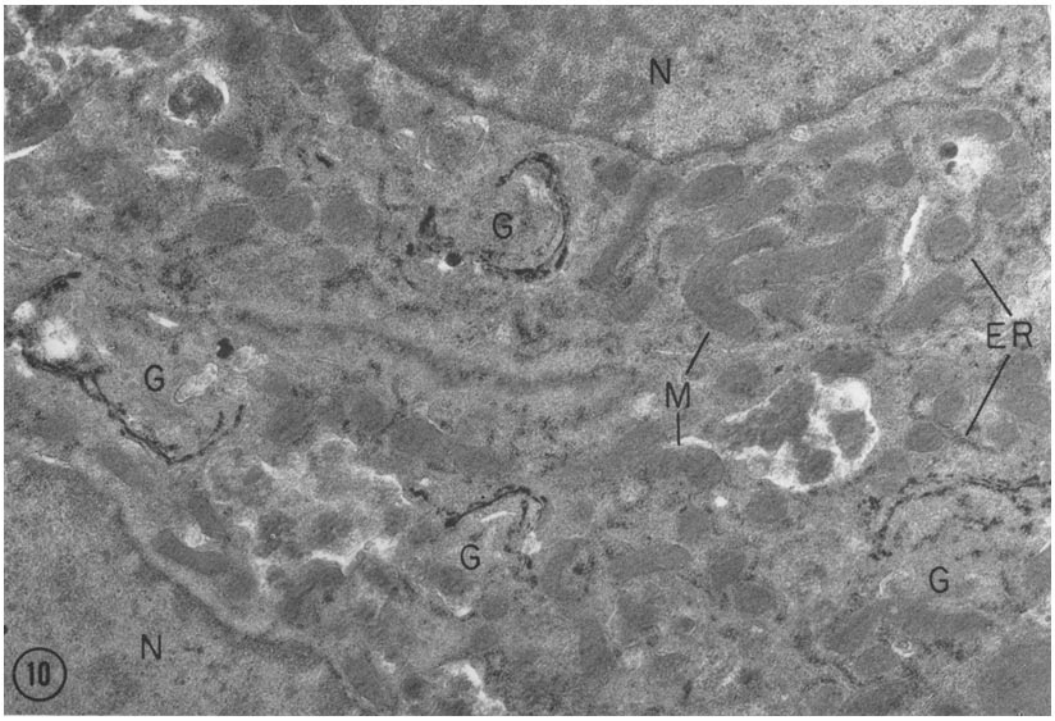
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toward the bile canaliculus (Fig. 9). They hydrolyze thiaminepyrophosphate but not the diphosphates of uridine, guanosine, and inosine. By first incubating sections for acid phosphatase activity and then for thiaminepyrophosphatase activity it is seen that many lysosomes lie extremely close to, possibly in contact with, the Golgi lamellae, as in many other tissues (27).

The Reuber H-35 hepatoma resembles liver in possessing a specific nucleosidediphosphatase in the endoplasmic reticulum that splits the diphosphates of uridine, guanosine, and inosine rapidly, and thiaminepyrophosphate slowly (27, 29). The endoplasmic reticulum is not highly concentrated in localized areas as it is in many liver cells, where it constitutes the basophilic "clumps" of classical cytology. Rather, it appears as strands coursing irregularly through the cytoplasm (Fig. 3). The nuclear membrane also shows nucleosidediphosphatase activity (Fig. 3).

Fine Structure

The *microvilli* of the bile canaliculi show considerable variation in structure. Some are much like those of normal liver; others are quite abnormal; and most are swollen and irregular. Sometimes, small deposits of electron-opaque material are encountered within the bile canaliculi, but it is not known whether these are related to the bile pigment–containing bodies of the tumor cells. Nothing suggestive of transfer of secretory materials from intact cells into bile canaliculi was encountered.

The *mitochondria* are relatively numerous and their structure appears similar to that of mitochondria of liver cells. They possess the usual cristae and intramitochondrial bodies (Figs. 11 to 15).

Rough surfaced endoplasmic reticulum, bearing particles presumed to contain ribonucleoprotein (RNP), is present in most of the areas examined. It generally appears as loosely formed parallel arrays of membranes representing flattened cisternae (30) or as individual elements which course through the cytoplasmic matrix among and around the mitochondria (Figs. 11 to 16, and 18). Apparently unattached particles are present in small numbers in the cytoplasm; these may be free RNP particles (Figs. 12 and 14).

Cytoplasmic *bodies limited by "single" membranes* are quite numerous (Figs. 11 to 14). The matrix of these bodies is of low electron opacity but contains irregular masses of electron-opaque material. The larger of these bodies correspond in size, shape, and location to the secretory granules seen in light microscope preparations. The electron-opaque areas may represent some biliary pigments. Multivesicular bodies (42; see 22) and microbodies (39) are also encountered. The microbodies are generally smaller than the mitochondria, are limited by "single" membranes, and have opaque central cores, or nucleoids, often with parallel arrays of material. They appear identical with the particles described by Rouiller and Bernhard (39) in rat liver.

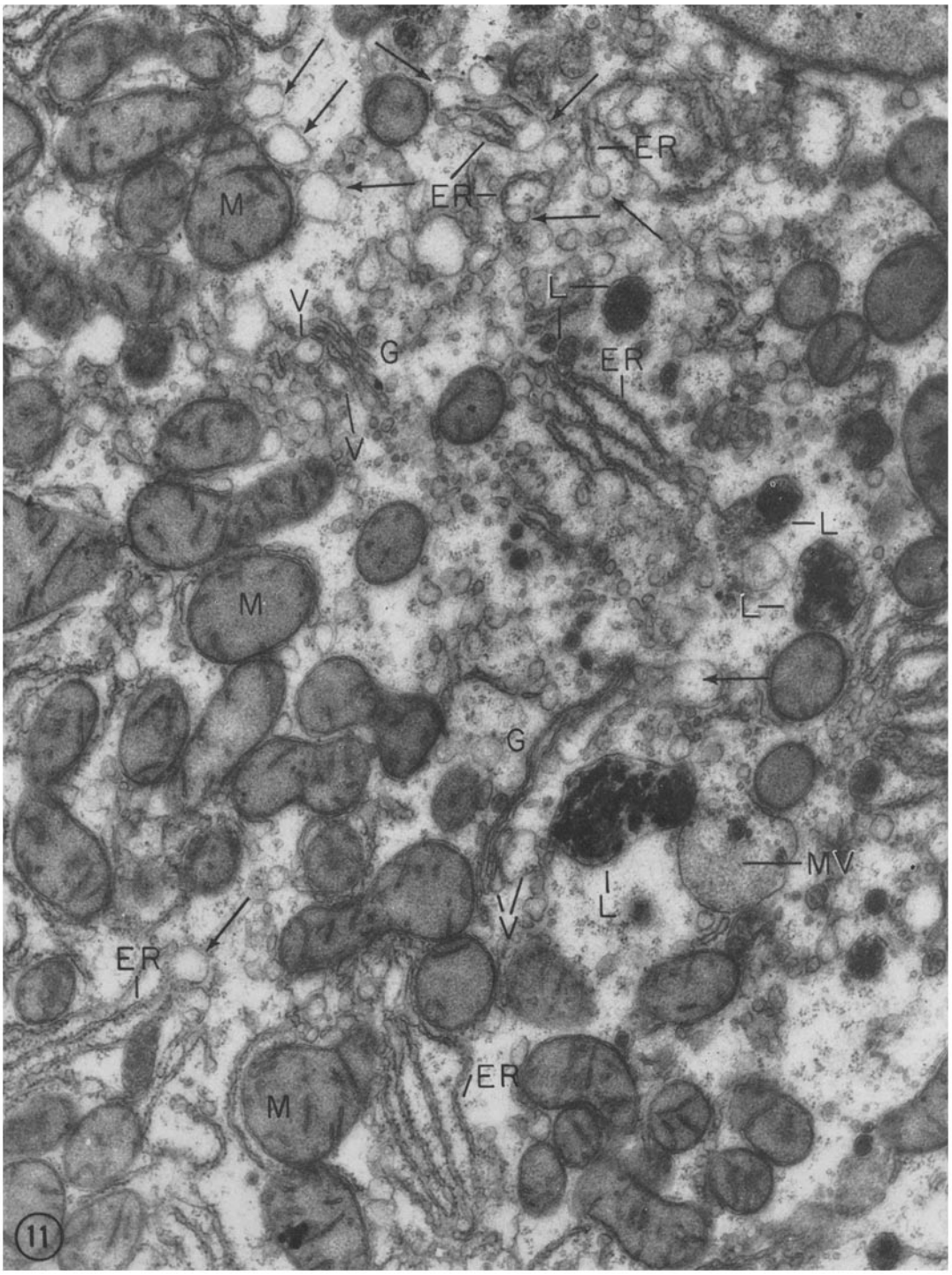
The cytoplasm also contains large numbers of *smooth surfaced vesicles and sacs* (Figs. 11 to 16). These will be discussed more fully below.

The Golgi apparatus consists of the usual arrays of closely packed smooth membranes interpreted as profiles through flattened cisternae or saccules (Figs. 12 to 14). As observed in many cells by other investigators, there is less variability in the distance between adjacent cisternae than in the depth of the cisternae. The number of cisternae is roughly constant, three or four, and there is no evidence of communications or connections between adjacent ones.

In their usual form, the cisternae contain a homogeneous matrix of low or moderate electron opacity (Figs. 13 and 14). Frequently, however, small, discrete particles of moderate opacity are

Figure 11

Reuber H-35 hepatoma. Low power electron micrograph. The field contains numerous vesicular elements. Those vesicles marked *V* lie close to existing membranes of the Golgi apparatus (*G*) and are interpreted as forming new Golgi membranes. Unmarked arrows show free vesicles and similar vesicles that appear to form from the cisternae of the rough surfaced endoplasmic reticulum (*ER*). Numerous small and large bodies containing electron-opaque material and limited by single membranes are seen (*L*). Numerous mitochondria (*M*) and a multivesicular body (*MV*) are indicated. Note proximity of endoplasmic reticulum to mitochondria. Methacrylate embedded; potassium permanganate staining. × 13,000.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observed within the cisternae (Fig. 12). These are more numerous in the dilated ends of the cisternae, or Golgi vacuoles. Similar small particles are present in isolated vacuoles that lie both adjacent to the Golgi membranes and a bit removed from them (Figs. 12 to 14). Each of these vacuoles is limited by a smooth membrane that appears single at the usual resolution of this study. Within them the small granules, interpreted as initial condensation products of material presumably manufactured in the endoplasmic reticulum, are surrounded by another material. This material varies from one that is essentially electron-transparent to one that is sufficiently opaque to mask the small granules almost completely (Figs. 11, 13, and 14). It may be assumed that other granules are masked completely. The opaque material is separated from the outer membrane by a narrow, electron-transparent area. These *secretory vacuoles* appear to be separated individually from the Golgi membranes. Occasionally, however, secretory vacuoles are arranged in rows adjacent to undilated Golgi cisternae, suggesting that a cisterna may pinch into several vacuoles simultaneously (Fig. 12; cf. reference 17).

There is very little rough surfaced endoplasmic reticulum in the immediate area of the Golgi apparatus, but the region contains numerous *smooth surfaced sacs* (Figs. 11 to 14). Although such sacs may be observed elsewhere in the cytoplasm, their presence is a constant and characteristic feature of the Golgi region. They are readily distinguished from secretory vacuoles by their number, distribution, irregular shape, apparently folded limiting membranes, and inner matrix of low electron opacity. Frequently such sacs appear to have preferential orientations, lying in close apposition to each other and to the outermost Golgi membranes. This is well illustrated in Fig. 14. The roughly parallel arrangement of apposed membranes suggests to us that the sacs may be flattening progressively, to become Golgi membranes. It is recognized that deducing such details of process from static electron micrographs is hazardous. Yet we believe it possible also to suggest the origin of these smooth membrane sacs. They appear to arise by dilatation, or vesiculation, of the rough surfaced reticulum (Figs. 11, 12, 15, and 16). The major portion of the rough reticulum appears unaltered in the process.

In a few instances smooth surfaced sacs are seen as if separating from the outer nuclear membrane (Figs. 15 and 16).

Electron Microscopic Examination of Enzyme Reaction Product

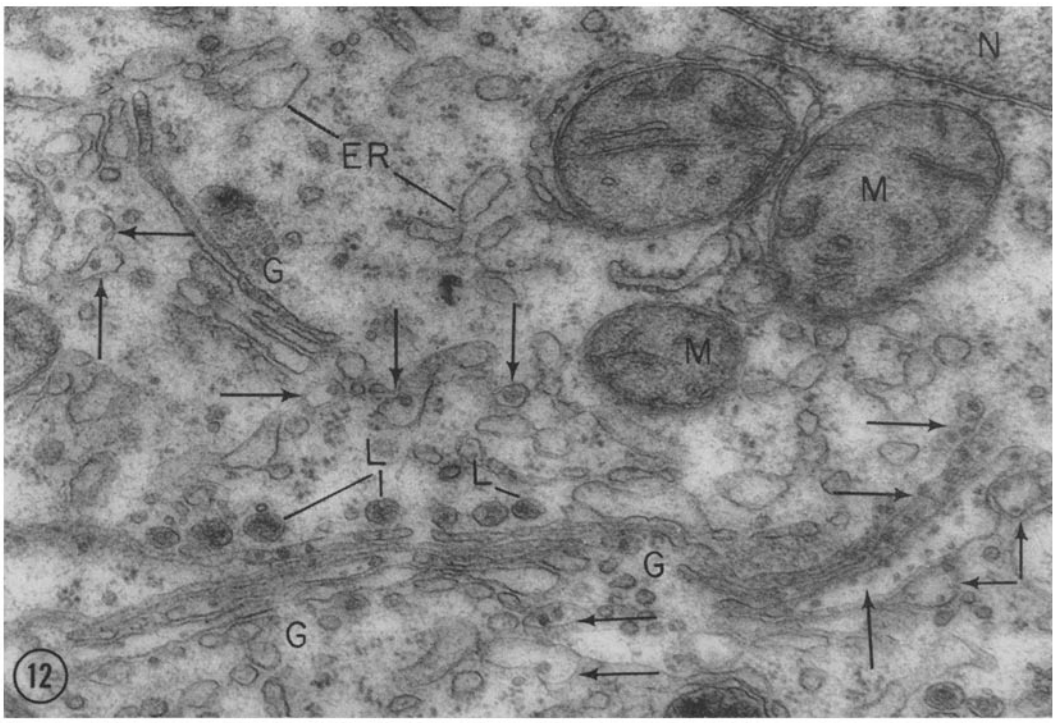
When the incubation time is reduced so that the deposits of reaction product are patchy rather than continuous, and when areas are examined in which the membranes are cut transversely, reaction product may be found in the cytomem-

FIGURE 12

Reuber H-35 hepatoma. Relatively long lengths of Golgi membranes (*G*) are shown in the lower part of the micrograph. Unmarked arrows point to dilated Golgi cisternae containing small particles interpreted as initial products of secretion; note the frequency with which these granules lie against the membrane. Other unmarked arrows point to isolated vesicles containing similar particles which have probably pinched off from the dilated ends of the Golgi cisternae. *L* indicates small bodies interpreted as early secretory vesicles in which the contents are undergoing further condensation. Part of the nucleus (*N*), some mitochondria (*M*), elements of the smooth and rough surfaced endoplasmic reticulum (*ER*); and particles (probably RNP particles) apparently unattached to membranes are also seen. Note proximity of endoplasmic reticulum to mitochondria. Methacrylate embedded; potassium permanganate staining. × 55,000.

FIGURE 13

Reuber H-35 hepatoma. Several areas containing Golgi membranes (*G*) are shown. Small secretory vesicles (*L*) with electron-opaque content are seen in proximity to and in the vicinity of the Golgi membranes. Mitochondria (*M*), elements of the smooth surfaced endoplasmic reticulum (ER), and a multivesicular body (MV), apparently with some secretory material within it, are seen. Note proximity of endoplasmic reticulum to mitochondria. Methacrylate embedded; potassium permanganate staining. × 35,000.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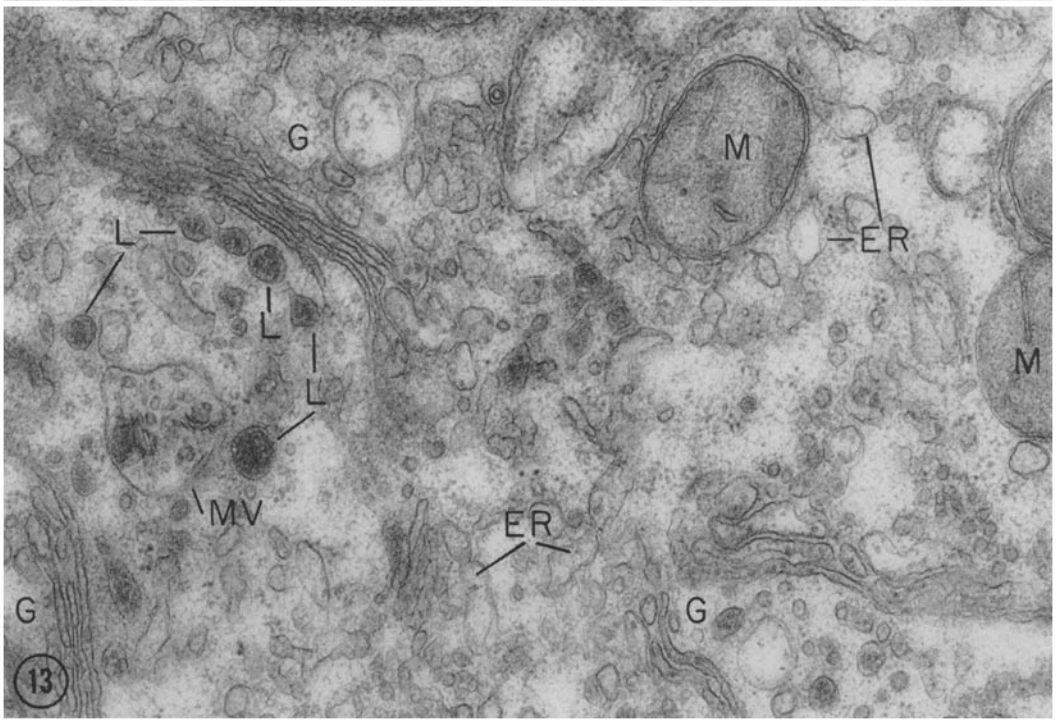
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branes of Golgi cisternae (thiaminepyrophosphatase) and endoplasmic reticulum (inosinediphosphatase) and not in the cavities that they enclose (Fig. 18). With longer incubation periods, reaction product covers both the membranes and the cavities (Figs. 10 and 17). Even with prolonged incubation time, it is the membrane of the secretory vacuole (lysosome) that shows most of the acid phosphatase reaction product (Fig. 8).

Only within the lysosomes is an inner content visible (Fig. 8). Presumably this is the secretory material. Whether lysosomes without secretory material were present in these cells could not be determined unequivocally. In such incubated sections, for unexplained reasons the ribosomes are often not visible in the areas where the reaction product forms on the membranes.

Morris Hepatoma 5123

General Cytology

The similarity of the cytology of the Morris hepatoma 5123 to that of liver is emphasized by Morris *et al.* (20) and Novikoff (21). Novikoff's studies included cytochemical and electron microscopic analyses. However, they were performed before the present methods were available for staining Golgi apparatus and endoplasmic reticulum in frozen sections.

The cytology of the tumor is remarkably similar to, but not identical with, that of the Reuber hepatoma H-35. Bile canaliculi are present and they show nucleosidephosphatase activities like those of the Reuber tumor. Unlike those of the Reuber tumor, the canaliculi show numerous large dilatations or acini (21). Occasionally these are filled with material giving positive reactions for lipid (oil red O), mucopolysaccharide (periodic acid–Schiff–positive, saliva-resistant), and adenosinemonophosphatase activity. Small to medium-sized acid phosphatase–rich lysosomes are abundant over a wide area of pericanalicular cytoplasm (Fig. 6) but none reaches the size of the large spheres containing bile pigment in the Reuber tumor. The Hall method (14) reveals positive granules only very rarely. The Golgi apparatus is large and reticular, and it generally lies close to the nucleus; extensions toward the

FIGURE 14

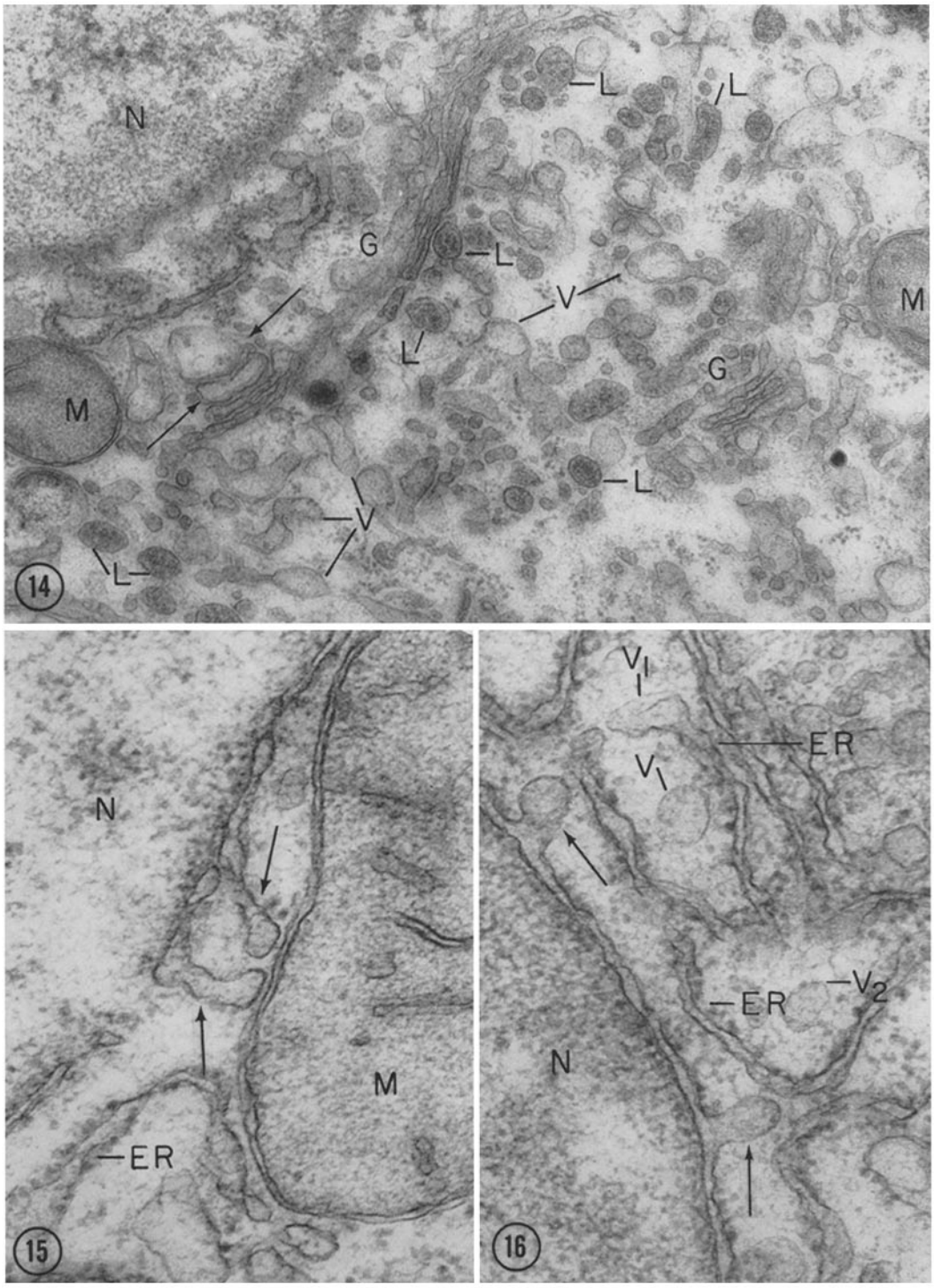
Reuber H-35 hepatoma. The membranes of the Golgi apparatus (*G*) are surrounded by numerous vesicular elements. The unmarked arrows indicate vesicles which may be interpreted as undergoing transformation into new Golgi membranes. Smooth surfaced vesicles (*V*) of the endoplasmic reticulum are present in large numbers. Some of these vesicles lie close to the Golgi membranes. *L* indicates isolated secretory vesicles that are presumably derived, by dilatation, from the Golgi cisternae. In some of these vesicles, particularly those which lie near Golgi membranes, the small particles representing initial products of secretion are still evident (cf. Figs. 12 and 13). A portion of the nucleus (*N*), mitochondria (*M*), and apparently unattached particles (probably RNP particles) are seen. Methacrylate embedded; potassium permanganate staining. × 35,000.

FIGURE 15

Reuber H-35 hepatoma. The unmarked arrows indicate areas of the outer nuclear membrane which may be forming isolated, smooth surfaced vesicles. Part of the nucleus (*N*), a mitochondrion (*M*), and part of the rough surfaced endoplasmic reticulum (*ER*) are seen. Note proximity of endoplasmic reticulum to mitochondria. Methacrylate embedded; potassium permanganate staining. × 89,000.

FIGURE 16

Reuber H-35 hepatoma. The unmarked arrows show two vesicles which may be separating from the outer nuclear membrane. Similar vesicles (*V*, V_1, and V_2) are also evident in the cytoplasm. The vesicle marked V_1 appears to be forming from the rough surfaced endoplasmic reticulum (*ER*) (see Fig. 11). The vesicle marked V_2 may still bear attached ribosomes. *N*, nucleus. Methacrylate embedded; potassium permanganate staining. × 89,000.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bile canaliculus are less common than in cells of the Reuber tumor (Fig. 5). It hydrolyzes thiaminepyrophosphate rapidly. The close association of lysosomes with the lamellae of the Golgi apparatus is evident in sections stained for both acid phosphatase and thiaminepyrophosphatase activities. As in the Reuber tumor, the endoplasmic reticulum shows nucleosidediphosphatase activity. In frozen sections incubated with uridine-, guanosine-, or inosinediphosphate as substrate, or for a longer time with thiaminepyrophosphate, the reticulum is seen as irregular strands in the cytoplasm (Fig. 4). The nuclear membrane also shows nucleosidediphosphatase activity (Fig. 4).

Fine Structure

In their ultrastructure, the *mitochondria, endoplasmic reticulum*, and canalicular *microvilli* are essentially the same as they are in the Reuber tumor (Figs. 17 and 19 to 21). This is true also of the close proximity of the endoplasmic reticulum to the mitochondria (compare Figs. 17 and 19 to 21 with Figs. 10 to 15 and 18), and the presence of *apparently unattached particles* that are probably RNP particles (Figs. 19 to 21).

The *Golgi apparatus* consists of relatively long lengths of closely packed, parallel, smooth surfaced membranes that are probably profiles of flattened cisternae. Numerous fenestrations are apparent in these membranes (Figs. 19 to 21). In the cavities of the cisternae, particularly where they are dilated to form Golgi vacuoles, there is a homogeneous, presumably secretory, material of moderate electron opacity. A narrow space separates this material from the limiting "single" membrane of the separated *secretory vacuoles*.

As in the Reuber hepatoma, cells of the Morris hepatoma contain an abundance of *smooth surfaced endoplasmic reticulum*, especially in the Golgi areas. Isolated sacs and vesicles, particularly frequent in Reuber tumor cells, are relatively uncommon in these cells. Most of the smooth reticulum takes the form of an anastomosing network, probably of both tubules and cisternae (Figs. 19 to 21). In many areas the reticulum forms a loose, irregular arrangement readily distinguishable from the ordered arrays of the Golgi membranes (Fig. 19). However, in other areas, where the membranes are more closely packed and particularly where fenestrations are present (Figs. 20 and 21), it is often difficult to know whether the membranes are part of the reticulum or belong to the Golgi apparatus. This distinction is not very important if our view is correct, namely, that the loosely arranged membranes of the reticulum are reoriented and become part of the parallel arrays of Golgi membranes. We were unable to verify this suggested transformation by cytochemical staining, using high thiaminepyrophosphatase activity as a marker for

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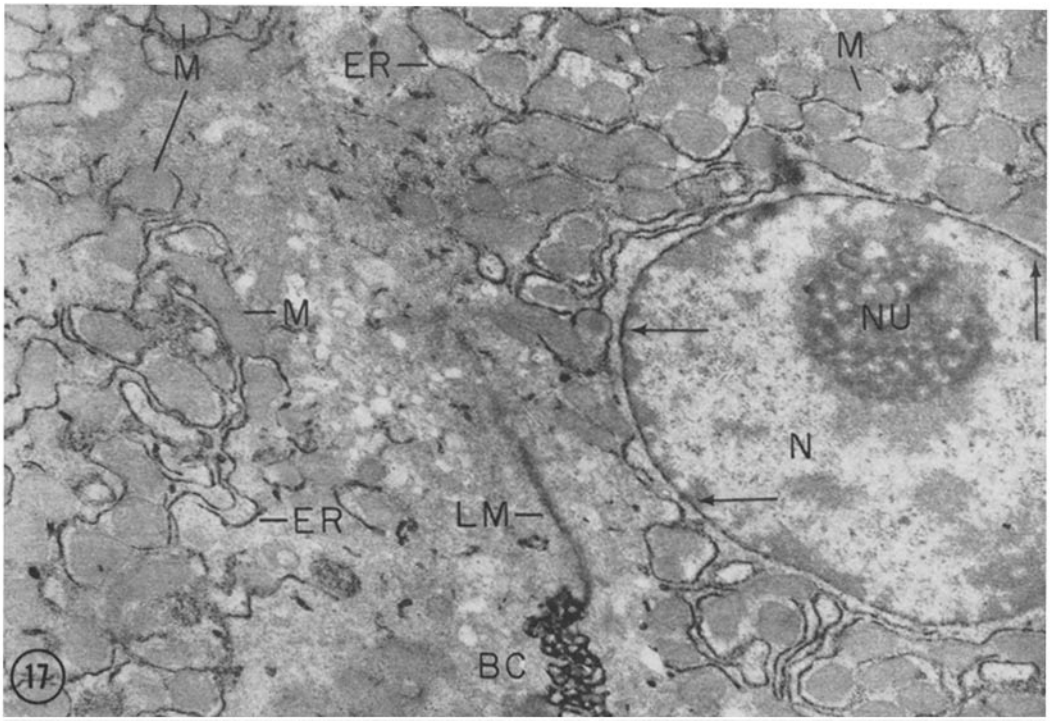
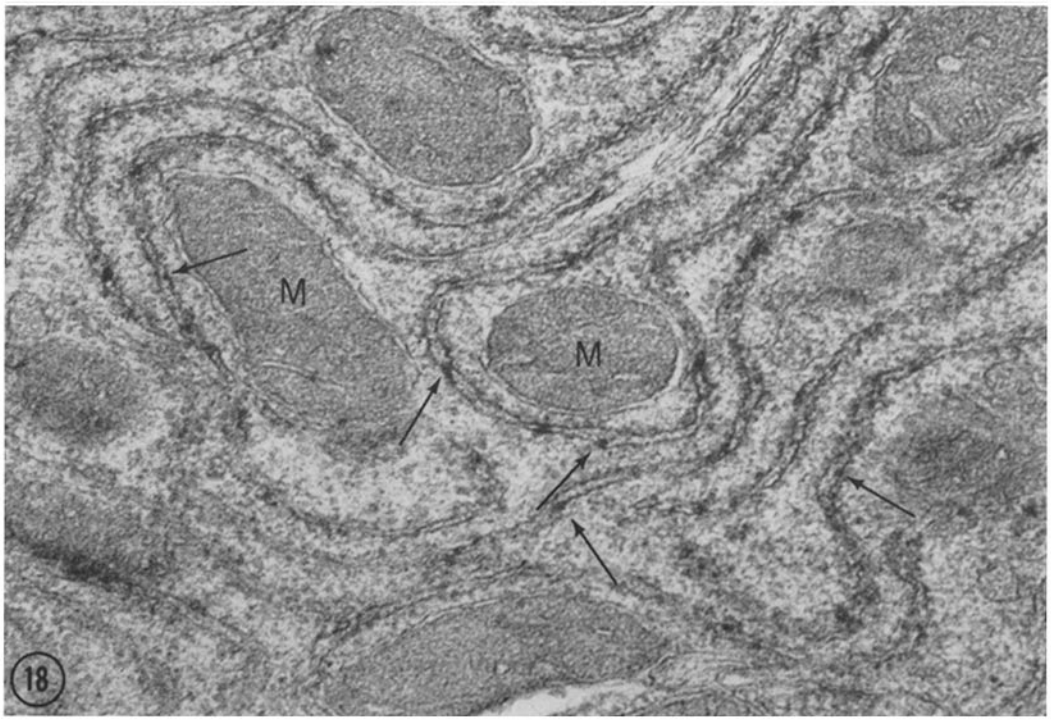
FIGURE 17

Morris 5123 hepatoma. Electron micrograph from formol-calcium-fixed frozen section incubated 15 minutes at room temperature for nucleosidediphosphatase activity, with inosinediphosphate as substrate (28, 29). Reaction product is restricted to the endoplasmic reticulum (*ER*) (see Fig. 18) and nuclear membrane (unmarked arrows). No reaction product is present within the nucleus (*N*), nucleolus (*NU*), or mitochondria (*M*). Note proximity of endoplasmic reticulum to mitochondria. Reaction product is present in the bile canaliculus (*BC*), presumably in the plasma membranes which delimit the microvilli. Lateral cell membranes are seen at *LM*, but they have no enzyme reaction product. Methacrylate embedded; potassium permanganate staining. × 17,000.

FIGURE 18

Reuber H-35 hepatoma. Electron micrograph from frozen section of formol-calcium-fixed tissue incubated 8 minutes at room temperature for nucleosidediphosphatase activity with inosinediphosphate as substrate (28, 29). Initial deposits of reaction product associated with the endoplasmic reticulum are shown. Arrows indicate sites where reaction product is present in the membranes but not in the cavities of the cisternae. No reaction product is seen in or on the mitochondria (*M*). Note proximity of endoplasmic reticulum to mitochondria. Methacrylate embedded; potassium permanganate staining. × 57,000.


M
ER
M
M
NU
N
ER
LM
BC
17


M
M
18

Golgi membranes and nucleosidediphosphatase activity as a marker for endoplasmic reticulum. In the Morris hepatoma, as in the Reuber hepatoma, when tissues were incubated for enzyme activities the ribosomes were generally not visible in the areas where reaction product formed on the membranes. Thus smooth surfaced vesicles could not be identified with certainty.

Owing to the static nature of electron micrographs and to the absence of serial sections, it is not possible to decide whether the areas of smooth reticulum that apparently transform into Golgi membranes retain their connections to the remainder of the endoplasmic reticulum, *i.e.*, the rough reticulum. In this tumor, unlike the Reuber tumor, there is no suggestion of extensive movement of smooth surfaced elements away from the rough reticulum, but this cannot be excluded by electron micrographs.

Electron Microscopic Examination of Enzyme Reaction Products

Only the reaction product resulting from nucleosidediphosphatase activity (with inosinediphosphate) was studied with the electron microscope (Fig. 17). As in the case of the Reuber H-35 tumor (Fig. 18), it was found in endoplasmic reticulum, nuclear membrane, and bile canaliculi. With short incubation periods, the product was present in patches. Then one could find areas in which the membranes, but not the cavities of the cisternae, had reaction product.

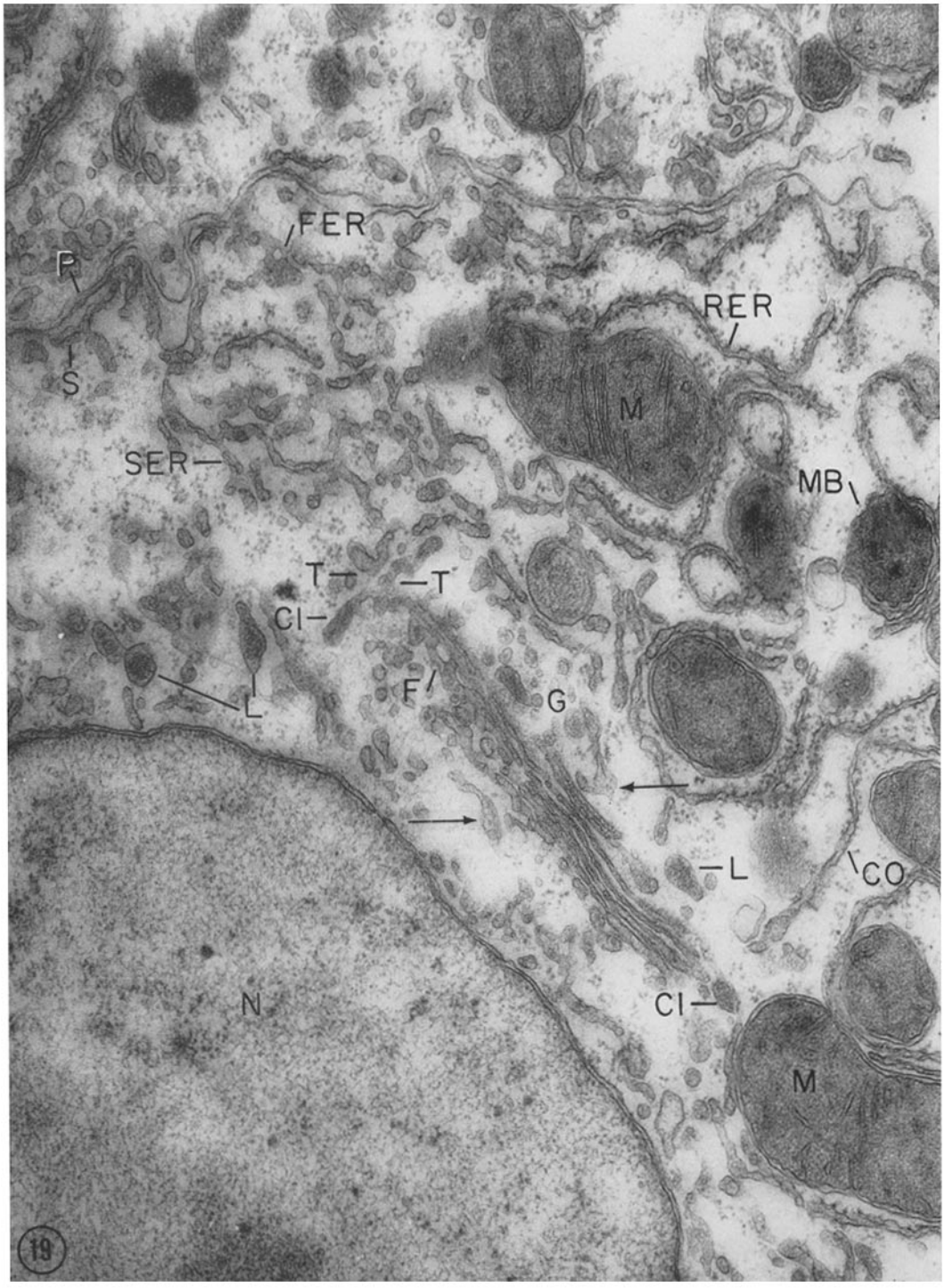
DISCUSSION

The Reuber H-35 and Morris 5123 hepatomas constitute favorable material for morphological and enzymatic staining studies of the cytomembranes involved in the secretory process. In these tumors, unlike liver, secretory products accumulate within small or large granules in the cytoplasm, either because the products have a composition different from that of the secretory material of liver or because the cell's mechanisms for releasing secretory products are defective. The Golgi apparatus is a large organelle lying close to the nucleus and often extending to the altered bile canaliculus, particularly in the Reuber tumor. The diphosphatase activities of Golgi apparatus and endoplasmic reticulum, as well as the distribution of these organelles in the cell, permit their ready study by both light microscopy and electron microscopy. Identification of some of the secretory vacuoles in electron micrographs of tissue previously incubated for acid phosphatase activity is facilitated in the Reuber tumor. They have been shown to possess acid phosphatase activity and to be delimited by "single" membranes, the two features used for identifying lysosomes (9) in sections (10, 16, 22). It is not now known whether these vacuoles possess the other acid hydrolases characteristic of lysosomes (9), but some, at least, possess an esterase activity characteristic of other lysosomes (unpublished observations).

These findings initiated the study of a variety

FIGURE 19

Morris 5123 hepatoma. The area adjacent to the nucleus (*N*) contains relatively long lengths of Golgi membranes (*G*) with typical parallel orientation. A fenestra within the membrane is seen at *F*. Dilated cisternae (*CI*), containing homogeneous material of moderate electron opacity, are seen at both ends. Material of similar appearance is present within small, isolated vesicles (*L*) in the vinicity of the Golgi membranes. Above and to the right of the Golgi apparatus rough surfaced endoplasmic reticulum (*RER*) may be seen. To the left and above, smooth surfaced endoplasmic reticulum (*SER*) may be seen. A fenestration (*FER*) in the smooth surfaced endoplasmic reticulum similar to that in the Golgi membrane is visible. Apparent continuity between rough and smooth surfaced endoplasmic reticulum is indicated at *CO*. The zone indicated by lines at *T* marks an area of possible transformation of elements of the smooth surfaced endoplasmic reticulum into Golgi membranes. Unmarked arrows indicate additional smooth surfaced vesicles that may be transforming into Golgi membranes. Mitochondria (*M*) are also shown; note proximity of endoplasmic reticulum to mitochondria. Apparently unattached particles (probably RNP particles) may be seen. A portion of the smooth surfaced endoplasmic reticulum (*S*) lies close to the plasma membrane (*P*). A microbody (*MB*) with internal nucleoid is also seen. Methacrylate embedded; potassium permanganate staining. × 33,000.


FER
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S
RER
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Cl
L
F
G
L
CO
N
Cl
M
19

of secretory cells and neurons by light and electron microscopy. These studies led us to the suggestion that lysosomal hydrolases are involved in the "condensation" or other aspects of secretory material accumulation in the early secretory vacuoles, and we have also suggested that the vacuole membrane may play a metabolic role because the reaction product is generally concentrated in the membrane (23, 27, 26, 25). Apparently in other secretory cells acid phosphatase activity of the membrane is lost before the secretory vacuole matures. It may be that persistence of enzyme in the hepatoma is linked with the apparent inability of the cells to discharge the granules.

Two features facilitate study of the membranous structures in the region of the Golgi apparatus: their distinctive fine structure and the presence of visible material in the cavities enclosed by some of them. The Golgi vacuoles can readily be identified by the particulate secretory product in the Reuber hepatoma (Figs. 12 to 14) and the more diffuse material in the Morris hepatoma (Figs. 19 to 21). As in cells generally, the Golgi cisternae and rough surfaced endoplasmic reticulum are readily identified by characteristic ultrastructural features (36). In addition, the smooth surfaced structures can be identified by a characteristic appearance in this material of the delimiting membrane in the Reuber tumor (Figs. 11 to 14) and by numerous anastomoses in the Morris tumor (Figs. 19 to 21).

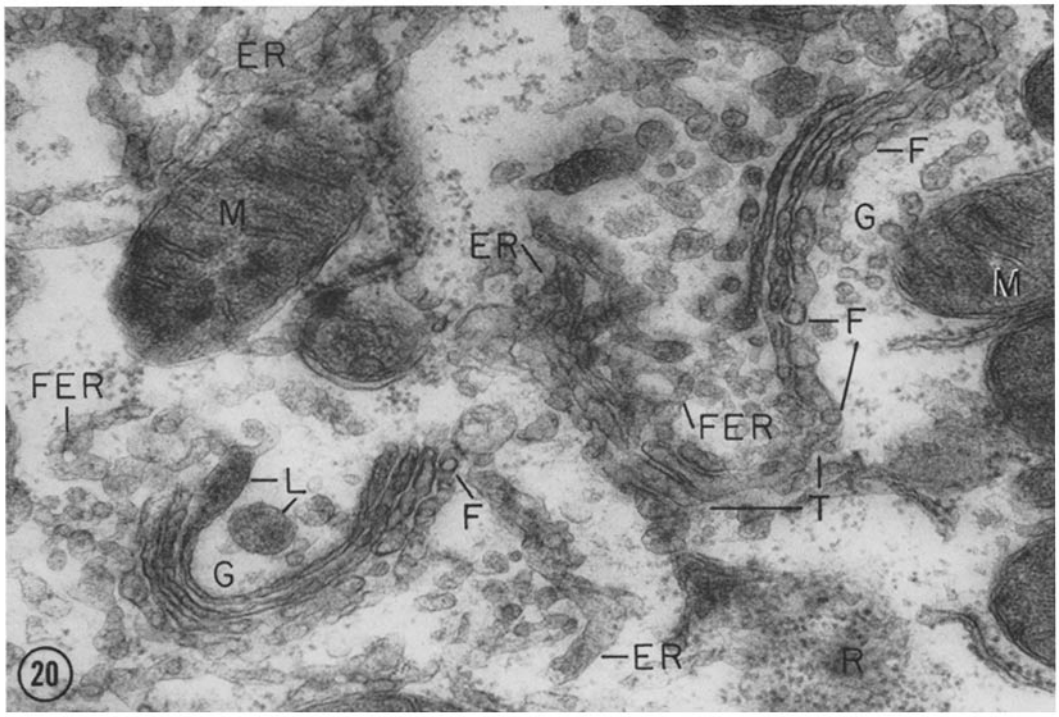
These favorable features do not outweigh completely the uncertainties inherent in deductions of *process* from static photographs, but they have permitted a concrete formulation that can guide further study of the functional relations between endoplasmic reticulum and Golgi apparatus. The idea that the two organelles are related is an old one. Palade, who considered the Golgi membranes to be a specialization of the endoplasmic reticulum with which it maintains structural continuity (30), early suggested that the Golgi apparatus is a membrane "depot" into which the membranes of pinocytotic and phagocytotic vacuoles flow (30). More recently, Palade (31) has noted that among the cytological events involved in the secretory process one of the least understood is the manner in which secretory products move "from the rough surfaced cisternae

FIGURE 20

Morris 5123 hepatoma. Smooth surfaced membranes of the Golgi apparatus (*G*) occupy much of the field. Typical fenestrations (*F*) in the Golgi membranes are indicated. Similar fenestrations (*FER*) in elements of the smooth surfaced endoplasmic reticulum (*ER*) are also seen. The two lines at *T* delimit a region which may represent conversion of smooth endoplasmic reticulum (left of line) into Golgi membranes (right of line). The lines at *L* mark one dilated Golgi cisterna with electron-opaque content (secretory material) and an isolated secretory vesicle, with similar contents, which may have separated from the Golgi cisterna. Mitochondria (*M*) and a cluster of ribosomes (*R*) are also seen; note proximity of endoplasmic reticulum to mitochondria. Apparently unattached particles (probably RNP particles) may be seen. Methacrylate embedded; potassium permanganate staining. × 41,000.

FIGURE 21

Morris 5123 hepatoma. In this micrograph the Golgi apparatus (*G*) occupies a position above the nucleus (*N*) and consists of relatively long lengths of smooth membranes. Fenestrations marked *F* are in membranes (also see Fig. 20), which may be transforming from smooth endoplasmic reticulum into Golgi membranes. Similar fenestrations (*FER*) occur in membranes which appear to be part of the smooth surfaced endoplasmic reticulum (*SER*). Other areas of apparent continuity (transition) between smooth surfaced endoplasmic reticulum and Golgi membranes are evident. The unmarked arrows indicate isolated secretory vesicles similar to those shown in Figs. 19 and 20 and in Figs. 12 to 14. Rough surfaced endoplasmic reticulum (*RER*) and mitochondria (*M*) are also shown; note proximity of endoplasmic reticulum to mitochondria. Apparently unattached particles (probably RNP particles) may be seen. Methacrylate embedded; potassium permanganate staining. × 20,000.


ER
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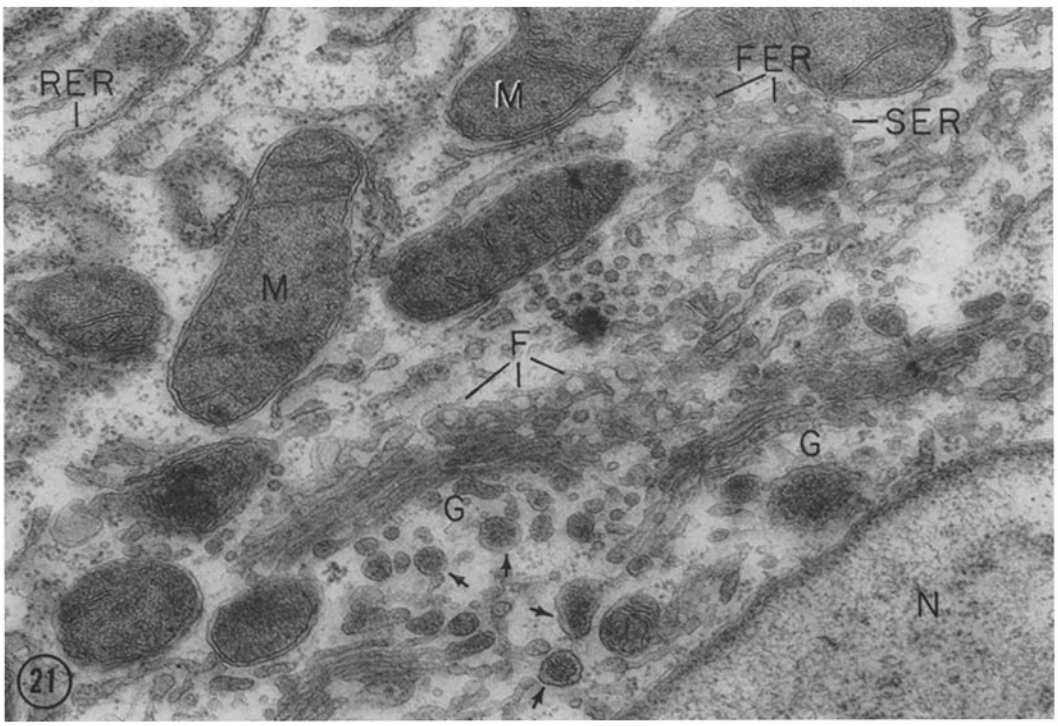
20


RER
M
FER
SER
M
F
G
G
N
21

through the centrosphere region." He considers it likely that smooth endoplasmic reticulum, viewed as "transitional elements," "join the two main parts" of the endoplasmic reticulum, rough surfaced reticulum and the Golgi apparatus. "The filling material may come from the rough surfaced endoplasmic reticulum, possibly in the form of intracisternal granules, as suggested by the presence of such granules in partly rough and partly smooth surfaced channels at the periphery of the centrosphere region. Images of granules 'in transit' are, however, rarely encountered and as such the point requires additional evidence."

Hirsch (15) has recently reviewed the possible modes of transit in the centrosphere region, which he calls "das Lamellen-Vakuolen-Feld."

In common with these views, our suggestion stresses that the relative stability of the Golgi apparatus, as seen by light microscopy, taken with the electron microscopic evidence suggesting separation from the Golgi apparatus of membranes delimiting the vacuoles, implies a simultaneous reconstitution of the Golgi membranes. Since Bennett's original description (3), "membrane flow" has generally been conceived as a process in which membrane circulates as on a conveyer belt. The essential modification in the hypothesis we are proposing is that, at least in secretory cells, the conveyer system is not a belt but a series of smooth surfaced derivatives, derived mostly or entirely from the granular endoplasmic reticulum and reoriented and flattened into Golgi membranes. It is possible to interpret other electron micrographs in the literature in this fashion, including those in which structural continuity has been claimed for the membranes of endoplasmic reticulum and Golgi apparatus (these micrographs are enumerated elsewhere (27)).

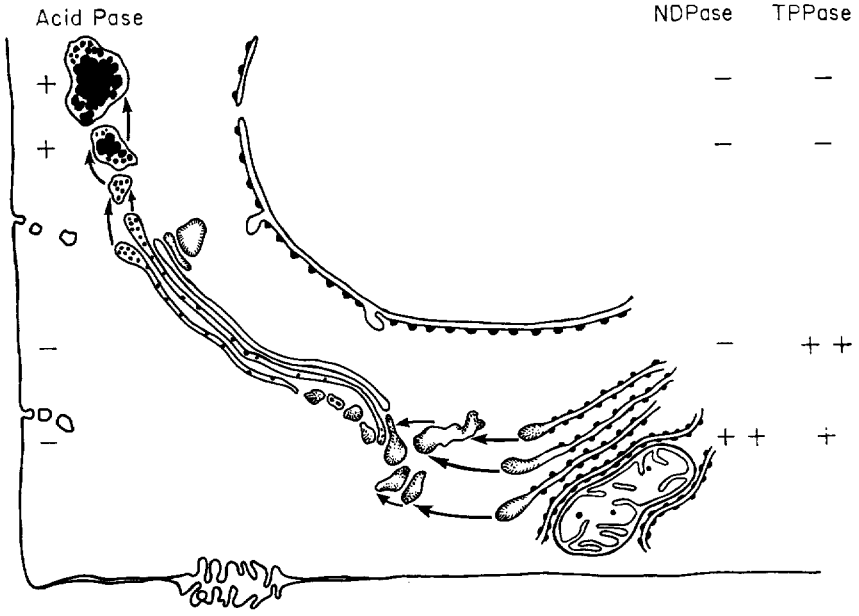


FIGURE 22

Schema of a Reuber H-35 hepatoma cell to suggest relations (*a*) between smooth surfaced vesicles, derived from the rough surfaced endoplasmic reticulum (ergastoplasm), and the Golgi cisternae, and (*b*) between Golgi cisternae and secretory granules (lysosomes). The presence (+) or absence (−) of acid phosphatase (acid Pase), nucleosidediphosphatase (NDPase), and thiaminepyrophosphatase (TPPase) activities is indicated for the following structures, reading from top to bottom: large secretory granules, small secretory granules, Golgi membranes, and ergastoplasm. Also shown is a portion of the nucleus (upper right), one mitochondrion (lower right), and a bile canaliculus (left bottom). Note two smooth surfaced extensions of the nuclear membrane (see Figs. 15 and 16) and pinocytotic vesicles forming from the plasma membrane on the left. The intimate relation between mitochondria and endoplasmic reticulum is indicated.

Figs. 22 and 23 illustrate the proposed cytomembrane relations as they seem to occur in cells of the Reuber hepatoma and the Morris hepatoma, respectively. In the Reuber tumor the flow of smooth membrane from the granular reticulum is discontinuous in the sense that the smooth surfaced membranes surround isolated vesicles. In the Morris tumor the membrane transformation and reorientation apparently occurs in a continuous fenestrated membranous system. It is, however, not known that this transforming portion of the endoplasmic reticulum retains continuity with the rough endoplasmic reticulum.

Thus, the flow of smooth membrane derivatives from the endoplasmic reticulum maintains the structural integrity of the Golgi apparatus in the face of intense secretory activity. Beneath the apparent constancy there lies a constant turnover of membrane.

If this view proves correct, separation of smooth surfaced derivatives and their transformation into Golgi elements would constitute the mechanism by which secretory materials synthesized in the endoplasmic reticulum gain access to the Golgi apparatus. This would permit even large granules or droplets to reach the Golgi cisternae without breakdown, as apparently is true of the lipid droplets in the intestinal mucosa cells (48, 33).

The forces controlling the balance between membrane loss and membrane reconstitution are the subject matter of future research. High resolution methods for studying the molecular biology of cytomembranes should make it possible to answer questions such as: Do existent Golgi membranes act as templates for the new ones? How much of newly forming lysosome membrane consists of molecules derived from sources other than the Golgi membrane? Does the cytoplasm contain a pool of membranes capable of flowing along different pathways, dependent upon the metabolic activities of the cell? Is the flow to the Golgi apparatus continuous or intermittent?

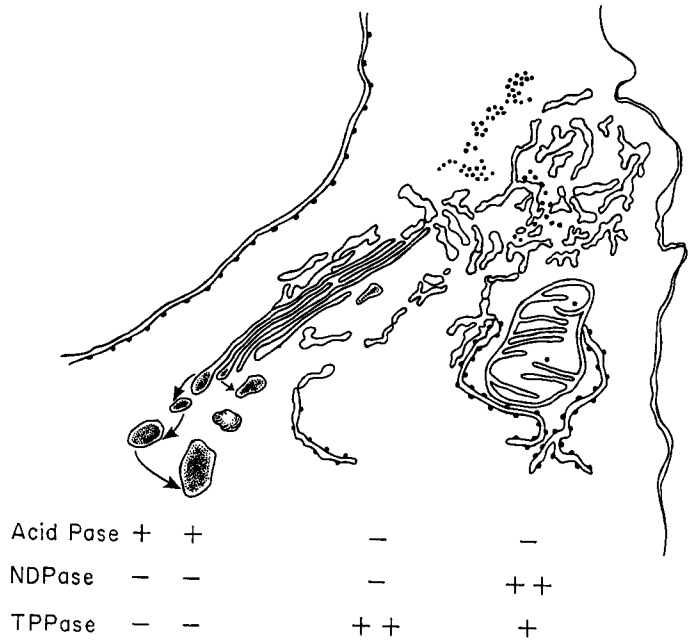


FIGURE 23

Schema of a Morris 5123 hepatoma cell to suggest relations (*a*) between smooth surfaced anastomosing tubules, derived from the rough surfaced endoplasmic reticulum, and the Golgi cisternae, and (*b*) between Golgi cisternae and secretory granules (lysosomes). The presence (+) or absence (−) of enzyme activities (see Fig. 22 for abbreviation) is shown, from left to right, for large secretory granules, small secretory granules, Golgi membranes, and ergastoplasm. Nuclear membrane is shown at upper left and plasma membrane at right. The intimate relation between mitochondria and endoplasmic reticulum is indicated.

If the view is correct that endoplasmic reticulum is derived from the nuclear membrane, the latter might, in a sense, be considered the source of Golgi membranes. Occasionally an appearance is encountered in the Reuber tumor that is consistent with a more direct flow of membrane from nucleus to Golgi apparatus (Figs. 15 and 16). It is conceivable that such direct flow is of greater quantitative significance in non-secretory cells, where there is little endoplasmic reticulum. In such cells, Golgi membranes may also be derived from pinocytotic vacuoles. Electron microscope images in epithelial cells of rat ileum (Novikoff and Biempica, unpublished) are consistent with such flow from plasma membrane to Golgi apparatus. In these non-secretory cells the Golgi apparatus is relatively small and its cisternae are not dilated into Golgi vacuoles.

It may be noted that the term "Golgi vacuole," suggested by Dalton and Felix (8), has been used throughout this communication, but not the term "Golgi vesicle." Diverse structures may look like small vesicles: transverse sections of the Golgi cisternae; derivatives of endoplasmic reticulum; or, as suggested elsewhere (27), on the basis of occasional images encountered in the Reuber tumor and in other secretory cells (23), micropinocytotic vacuoles.

In the cells of the Reuber and Morris hepatomas, as in rat liver and some absorptive cells in rat ileum, the Golgi lamellae do not appear to hydrolyze the diphosphates of uridine, guanosine, or inosine, but rapidly hydrolyze thiaminepyrophosphate (27). In contrast, the endoplasmic reticulum of the hepatomas (and liver) rapidly hydrolyzes these nucleosidediphosphates while hydrolyzing thiaminepyrophosphate very slowly. It would seem that, in these cells, as the reticulum membranes transform into Golgi membranes they lose the ability to hydrolyze nucleosidediphosphates while their capacity to hydrolyze thiaminepyrophosphate increases. This is consistent with the view that the thiaminepyrophosphatase and nucleosidediphosphatase activities of the Golgi membranes are due to different enzymes (28, 27, 1).

It would also appear that as the Golgi cisternae dilate to enclose the secretory material the vacuole membranes acquire acid phosphatase activity. We have considered elsewhere (23, 25) an alternative possibility, namely, that the Golgi apparatus makes secretory vacuoles *and* lysosomes. If so, the lysosomes must empty into the secretory vacuoles almost as the latter are being formed, since the vacuoles containing secretory product and acid phosphatase activity lie immediately adjacent to the Golgi membranes.

The observations recorded in this manuscript illustrate the extent to which, in some instances, enzyme histochemistry has been transformed into enzyme cytochemistry, and the manner in which they may supplement electron microscopy in suggesting dynamic interrelationships among cell organelles.

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BIBLIOGRAPHY

1. ALLEN, J., A cytochemical and electrophoretic analysis of Golgi associated thiaminepyrophosphatase and nucleosidediphosphatase in cells of the mouse, *J. Histochem. and Cytochem.*, in press.
2. BAKER, J. R., The histochemical recognition of lipine, *Quart. J. Micr. Sc.*, 1946, **87**, 441.
3. BENNETT, H. S., The concepts of membrane flow and membrane vesiculation as mechanisms for active transport and ion pumping, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4, suppl., 99.
4. BOWEN, R. H., The cytology of glandular secretion, *Quart. Rev. Biol.*, 1929, **4**, 299.
5. CARO, L. G., Electron microscopic radioautography of thin sections: The Golgi zone as a

site of protein concentration in pancreatic acinar cells, *J. Biophysic. and Biochem. Cytol.*, 1961, **10**, 37.

6. CAULFIELD, J. B., Effects of varying the vehicle for OsO_4 in tissue fixation, *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 827.
7. DALTON, A. J., Golgi apparatus and secretion granules, *in* The Cell, (J. Brachet and A. E. Mirsky, editors), New York, Academic Press, Inc., 1961, **2**, 603.
8. DALTON, A. J., and FELIX, M. D., Cytologic and cytochemical characteristics of the Golgi substance of epithelial cells of the epididymis —in situ, in homogenates and after isolation, *Am. J. Anat.*, 1954, **94**, 171.
9. DE DUVE, C., Lysosomes, a new group of cytoplasmic particles, *in* Subcellular Particles, (T. Hayashi, editor), New York, Ronald Press Co., 1959, 128.
10. ESSNER, E., and NOVIKOFF, A. B., Localization of acid phosphatase activity in hepatic lysosomes by means of electron microscopy, *J. Biophysic. and Biochem. Cytol.*, 1961, **9**, 773.
11. ESSNER, E., and NOVIKOFF, A. B., "Membrane flow": endoplasmic reticulum, Golgi apparatus and secretory granules, (abstract) First Annual Meeting of the American Society for Cell Biology, 1961, 55.
12. GOMORI, G., Microscopic Histochemistry, Principles and Practice, Chicago, University of Chicago Press, 1952.
13. HAGUENAU, F., and BERNHARD, W., L'appareil de Golgi dans les cellules normales et cancéreuses de vertebrés, *Arch. anat. micr. et morphol. exp.*, 1955, **44**, 27.
14. HALL, M. J., A staining reaction for bilirubin in sections of tissue, *Am. J. Clin. Path.*, 1960, **34**, 313.
15. HIRSCH, G. C., Das Lamellen-Vakuolen-Feld, *in* Dynamik der tierischen Zellen, chap. 7, Handbuch der Biologie, 1962, **1**, 409.
16. HOLT, S. J., and HICKS, R. M., Studies on formalin fixation for electron microscopy and cytochemical staining purposes, *J. Biophysic. and Biochem. Cytol.*, 1961, **11**, 47.
17. KARRER, H. E., Electron microscopic observations on developing chick embryo liver: The Golgi complex and its possible role in the formation of glycogen, *J. Ultrastruct. Research*, 1960, **4**, 149.
18. KUROSUMI, K., Electron microscopic analysis of the secretion mechanism, *Internat. Rev. Cytol.*, 1961, **11**, 1.
19. LILLIE, R. D., Histopathologic Technic and Practical Histochemistry, New York, McGraw-Hill Book Co., 1954.
20. MORRIS, H. P., SIDRANSKY, H., WAGNER, B. P., and DYER, H. M., Some characteristics of transplantable rat hepatoma no. 5123 induced by ingestion of *N*(2-fluorenyl)phthalamic acid, *Cancer Research*, 1960, **20**, 1252.
21. NOVIKOFF, A. B., Enzyme localization in tumor cells, *in* Cell Physiology of Neoplasia, Austin, Texas, University of Texas, 1960, 219.
22. NOVIKOFF, A. B., Lysosomes and related particles, *in* The Cell, (J. Brachet and A. E. Mirsky, editors), New York, Academic Press, Inc., 1961, **2**, 423.
23. NOVIKOFF, A. B., Cytochemical staining methods for enzyme activities: their application to the rat parotid gland, *Bull. Jewish Memorial Hosp.*, 1962, **6**, 70.
24. NOVIKOFF, A. B., Electron transport enzymes: Biochemical assays and tetrazolium staining studies, *Ist Internat. Cong. Histochem. and Cytochem.*, 1960, in press.
25. NOVIKOFF, A. B., and ESSNER, E., Pathological changes in cytoplasmic organelles, *Fed. Proc.*, 1962, in press.
26. NOVIKOFF, A. B., ESSNER, E., BIEMPICA, L., and IACIOFANO, P., Lysosomes and secretory granules: Cytochemical and electron microscopic studies, *J. Histochem. and Cytochem.*, in press.
27. NOVIKOFF, A. B., ESSNER, E., GOLDFISCHER, S., and HEUS, M., Nucleosidephosphatase activities of cytomembranes, *Symp. Internat. Soc. Cell Biol.*, 1962, **1**, 149.
28. NOVIKOFF, A. B., and GOLDFISCHER, S., Nucleosidediphosphatase activity in the Golgi apparatus and its usefulness for cytological studies, *Proc. Nat. Acad. Sc.*, 1961, **47**, 802.
29. NOVIKOFF, A. B., HEUS, M., ESSNER, E., and IACIOFANO, P., Nucleosidediphosphatase activity in the endoplasmic reticulum and nuclear membrane of liver and other cells, (abstract) First Annual Meeting of the American Society for Cell Biology, 1961, 155.
30. PALADE, G. E., The endoplasmic reticulum, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4, suppl., 85.
31. PALADE, G. E., The secretory process of the pancreatic exocrine cell, *in Symposium on Electron Microscopy*, British Anatomical Association, London, Arnold Press, 1960, 176.
32. PALAY, S. L., The Morphology of Secretion, *in* Frontiers in Cytology, (S. L. Palay, editor), New Haven, Yale University Press, 1958, 305.
33. PALAY, S. L., and KARLIN, L. J., An electron microscopic study of the intestinal villus. I. The fasting animal, *J. Biophysic. and Biochem. Cytol.*, 1959, **5**, 363.
34. PITOT, H., Molecular pathogenesis of experimental carcinoma, *Fed. Proc.*, 1962, in press.
35. POLLISTER, A. W., and POLLISTER, P. F., The

structure of the Golgi apparatus, *Internat. Rev. Cytol.*, 1957, **6**, 85.

36. PORTER, K. R., The ground substance: Observations from electron microscopy, *in* The Cell, (J. Brachet and A. E. Mirsky, editors), New York, Academic Press, Inc., 1961, **2**, 621.
37. POTTER, V. R., Enzyme studies on the deletion hypothesis of carcinogenesis, Bertner Lecture, *Texas Rep. Biol. and Med.*, in press.
38. REUBER, M. D., A transplantable bile-secreting hepatocellular carcinoma in the rat, *J. Nat. Cancer Inst.*, 1961, **26**, 891.
39. ROUILLER, C., and BERNHARD, W., "Microbodies" and the problem of mitochondrial regeneration in liver cells, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4, suppl., 355.
40. SCHARRER, E., and BROWN, S., Neurosecretion. XII. The formation of neurosecretory granules in the earthworm, *Lumbricus terrestris* L., *Z. Zellforsch.*, 1961, **54**, 530.
41. SJÖSTRAND, F. S., and HANZON, V., Ultrastructure of Golgi apparatus of exocrine cells of mouse pancreas, *Exp. Cell Research*, 1954, **7**, 415.
42. SOTELO, J. R., and PORTER, K. R., An electron microscope study of the rat ovum, *J. Biophysic. and Biochem. Cytol.*, 1959, **5**, 327.
43. STRUNCK, S. W., The formation of intracellular crystals in midgut glands of *Limnoria lignorum*, *J. Biophysic. and Biochem. Cytol.*, 1959, **5**, 385.
44. WARD, R. T., Prevention of polymerization damage in methacrylate embedding media, *J. Histochem. and Cytochem.*, 1958, **6**, 398.
45. WACHSTEIN, M., and MEISEL, E., Histochemistry of hepatic phosphatases at a physiological pH with special reference to the demonstration of bile canaliculi, *Am. J. Clin. Path.*, 1957, **17**, 13.
46. WATSON, M. L., Reduction of heating artifacts in thin sections examined in the electron microscope, *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 1017.
47. WEBER, G., Behavior of liver enzymes in hepato carcinogenesis, *Adv. Cancer Research*, 1961, **6**, 403.
48. WEISS, J. M., The role of the Golgi complex in fat absorption as studied with the electron microscope with observations on the cytology of duodenal absorptive cells, *J. Exp. Med.*, 1955, **102**, 775.
49. WELLINGS, S. R., and DEOME, K. B., Milk protein droplet formation in the Golgi apparatus of the C3H/Crgl mouse mammary epithelial cells, *J. Biophysic. and Biochem. Cytol.*, 1961, **9**, 479.