The endoplasmic reticulum: A cytochemist's view (A Review)*

(electron microscopy/enzyme localizations/GERL/Golgi apparatus)

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ABSTRACT Enzyme cytochemistry has been used, at the light and electron microscope levels, to "mark" cytoplasmic organelles of mammalian cells. Catalase cytochemistry permitted identification of microperoxisomes, apparently ubiquitous organelles that are attached by numerous slender connections to the endoplasmic reticulum. Thiamine pyrophosphatase and acid phosphatase cytochemistry can be used to distinguish between the Golgi apparatus and a specialized acid-phosphatase-rich region of smooth endoplasmic reticulum (ER) that appears to be involved in: (a) the formation of lysosomes and melanin granules; (b) the processing and packaging of secretory materials in endocrine and exocrine cells; and (c) the metabolism of lipid. The acronym GERL has been given to this region of smooth ER because it is located at the inner or "trans" aspect of the Golgi apparatus and because it appears to produce various types of Lysosomes.

Cytochemistry, "marker enzymes," and specialized endoplasmic reticulum regions

By making possible the visualization of organelles in situ, cytochemistry has aided interpretation of biochemical analyses of subcellular fractions isolated from homogenates. It has added a new dimension to understanding electron microscope observations. Cytoplasmic organelles not usually identifiable by light microscopy can be observed in normal and altered cell states.

In our laboratory we have chosen enzyme activities that survive the sufficiently long aldehyde fixation required for adequate structural preservation of the organelles and that stain ("mark") one or more cytoplasmic organelle (Table 1). The manner of preparing the tissue sections and incubation media is described in the references listed in the table.

During 1966-1970 a series of publications drew attention to an effect of lead ions employed to trap the phosphate released in the course of the phosphatase procedures: that the lead ions could themselves induce hydrolysis of ATP or other phosphate esters. These papers (12-15) cast doubt upon the validity of the cytochemical procedures. In reply I pointed to: (a) specificities of the localizations obtained with specific substrates; (b) the similar localizations obtained under conditions where lead-induced hydrolysis does not occur; and (c) other observations which made me confident that the observed localizations did indeed reveal the intracellular sites of the different phosphatases (16-18). Thus, in 1963 we described the NDPase localization in the endoplasmic reticulum (ER) of rat liver (Fig. 1) and we confirmed it by assay of subcellular fractions (1). In addition, when purified from isolated microsome fractions the enzyme demonstrates the same substrate specificity and optimal conditions for activity as when studied by the cytochemical procedure (2).

Abbreviations: AcPase, acid phosphatase; ER, endoplasmic reticulum; NDPase, nucleoside diphosphatase; TPPase, thiamine pyrophosphatase.

* By invitation. From time to time, reviews on scientific and technological matters of broad interest are published in the PROCEEDINGS.

The contributions of AcPase cytochemistry to elucidating the forms and functions of lysosomes have been described by de Duve (19). Light microscopy of lysosomes with the AcPase procedure and of the Golgi apparatus with the TPPase procedure, in the small neurons of rat dorsal root ganglia, initiated a series of publications which led to appreciation of specialized regions of ER.

GERL and lysosomes

Light microscopic studies of a wide variety of cell types led us to identify a close relationship between the location of lysosomes and the form and distribution of the Golgi apparatus (20, 21). In AcPase preparations of rat dorsal root ganglia not only the stained spherical lysosomes but also some larger stained areas were encountered. From their distribution, size, and shape I considered that these areas could be fitted within the crescent-shaped regions formed by portions of the Golgi apparatus.

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<th>Table 1. Cytochemical marker enzymes</th>
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FIGS. 1–8. (Legend appears at bottom of the following page.)
(22). This was confirmed by incubating sections for AcPase activity and then for TTPase activity. With electron microscopy it was determined that the AcPase-rich region was smooth ER. I named it GERL (23) because this region of smooth ER is located at the inner or "trans" (24) aspect of the Golgi apparatus and because it appears to produce various types of lysosomes (25).

The structural relation of GERL and Golgi apparatus has been studied best in the neurons of dorsal root ganglia of the rat (5) and mouse (26). In these cells the relationship appears to be more complex than in other cell types studied in our laboratory (6, 4, 27). Figs. 5-7 illustrate some aspects of this relationship; also see Fig. 40 in ref. 5. Smooth-surfaced tubules, probably part of GERL, extend into each polygonal compartment of the trans element of the Golgi apparatus. The polygonal arrangement provides a large surface area where molecular interchanges between GERL and Golgi apparatus could occur. However, the functional significance of the spatial relations of GERL and Golgi apparatus remains to be elucidated. This is particularly important for understanding events in the secretory cells considered in the next section.

Four types of lysosomes seem to arise from GERL: (a) residual bodies (Fig. 8) in which presumably indigestible residues of intracellular digestion are visible; (b) coated vesicles which may carry lysosomal hydrolases to other cell structures (if so they would be primary lysosomes); (c) autophagic vacuoles, type I, in which portions of GERL envelop regions of cytoplasm containing organelles like mitochondria, ER, or peroxisomes. (When sealed off, lysosomal hydrolases are apparently released into the vacuole and the sequestered structures undergo degradation to become a residual body),; and (d) autophagic vacuoles, type 2, in which a region of GERL enlarges and portions of its membrane are internalized, bringing in bits of cytoplasm like cytosol or glycogen. Degradation by lysosomal enzymes leads to residual body formation, as in autophagic vacuoles, type 1. For a fuller description see another review (25).

An assumption made by cytochemists, apparently valid thus far, is that when AcPase activity is found in a cell organelle other lysosomal hydrolases are probably there as well. Unfortunately, only two cytochemical procedures validated at the ultrastructural level are currently available for other lysosomal enzymes, in most cells, and even these are not as convenient as the AcPase procedure. These enzymes are arylsulfatase and an esterase which hydrolyzes thiolacetate. Decker (28) has demonstrated both enzyme activities in GERL of neurons. Bentfield and Bainott (29) have shown arylsulfatase activity in GERL of megakaryocytes. In addition to the demonstrable acid hydrolase activities common to GERL and lysosomes, GERL often shows other hallmarkst of the residual body type of lysosome: a relatively thick delimiting membrane, an electron-lucent "halo" between membrane and residual body contents, and the presence in the contents of small ferritin-like grains, myelin-like figures, and other structures (25).

GERL and secretion processing and packaging

The cytochemical visualization of TTPase and AcPase activities has shed new light on the packaging, and perhaps the final processing, steps of secretory materials. With E. Essner, in the early sixties, we had demonstrated the presence of AcPase activity in nascent secretory granules of both endocrine and exocrine glands (20). Recently we have returned to studies engendered by these observations. In cells where TTPase activity is demonstrable in only one element of the Golgi apparatus it is the trans element, adjacent to GERL, that shows the activity. Thus, by incubating sections for TTPase activity and parallel ones for AcPase activity it can readily be determined whether secretory granules arise from GERL or from the trans element of the Golgi apparatus. Thus far the findings are different from what is generally assumed (30, 31).

In an insulin-producing transplantable hamster tumor we conclude (32) that most secretory granules arise from GERL and none from the Golgi apparatus, because the granules show AcPase activity and not TTPase activity (Fig. 9).

In the β cells of the rat pancreas the granules also arise from GERL and not the Golgi apparatus as widely thought. Again the nascent granules show AcPase activity (Fig. 11) and no TTPase activity. Unlike the insulinoma, however, the more mature secretory granules do not have demonstrable AcPase activity.

We (32) have indicated possible advantages of the transplantable insulinoma for biochemical studies required to test the assumption that when acid phosphatase activity is present in granules it is likely that other lysosomal enzymes are there as well, and to resolve the issues considered by Steiner et al. (31) in their discussion of the possible involvement of lysosomes in converting proinsulin to insulin. Unfortunately there is currently no valid cytochemical procedure generally accepted for visualizing, at the electron microscope level, the sites of the proteolytic enzymes involved in converting proinsulin to insulin.

The presence of AcPase only in the nascent secretory granules is also seen in the exocrine cells of the pancreas. Fig. 10 shows the absence of TTPase activity and Fig. 11, the presence of AcPase in the nascent secretory granules in the pancreas of the untreated guinea pig. The same is true in the fasted and refeed guinea pig pancreas and in untreated hamster, rabbit, and rat (33). The nascent granules are the "condensing vacuoles" considered by Palade and coworkers to be sites of processing and packaging of secretory granules; for a review see Palade (30). We view the condensing vacuole portions as expanded cisternal portions of GERL, tubular ER elements attached to them often take the form of "rigid lamellae" described by Claude (34) (arrows in Figs. 10 and 12).

Figs. 1-8 (on preceding page). Fig. 1. Section of rat liver incubated for NADPase activity, with inosine diphasosphate as substrate, from Novikoff and Heus (1). Reaction product is seen in the endoplasmic reticulum (ER) and nuclear envelope (NM). The mitochondria (M) are unreactive; the nucleolus is also labeled (Nu). X23,000. Fig. 2. Section of retina of C57 black mouse incubated for AcPase activity. The melanolysosomes of the pigment epithelium cell are ringed with reaction product, evidently a paper in the rat, X22,000. Fig. 3. Section of mouse Harding-Passey melanomas incubated for tyrosinase activity, from Novikoff et al. (6). The Golgi apparatus (G) and rough endoplasmic reticulum (RER) are unreactive. Reaction product is present in GERL (GE) and in premelanosomes and melanosomes (ME), X22,000. Fig. 4. Section of rat thyroid incubated for peroxidase activity, from Novikoff et al. (4). Reaction product is present in the endoplasmic reticulum (ER) and "A granules" (A), but is absent from the "B granules" and residual bodies (L). A portion of the nucleus is seen at N, X16,000. Figs. 5-7. Serial sections of a rat dorsal root ganglion incubated for TTPase activity, from P. M. Novikoff et al. (5). Reaction product is restricted to the trans element of the Golgi apparatus. GERL (GE) is unreactive. Long arrows indicate tubular structures, probably part of GERL. The short arrows indicate a small gap in the Golgi apparatus in Figs. 6 and 7 but this is closed over in Fig. 5, demonstrating that even at the electron microscope level the Golgi apparatus is a continuous organelle throughout the cytoplasm. X32,000. Fig. 8. A section, 0.5 μm thick, of a rat dorsal root ganglion incubated for acid phosphatase activity, from P. M. Novikoff et al. (5). Reaction product is seen in GERL which shows a cisternal portion (C), tubular elements (T), and budding residual bodies (arrows). X40,000.
Fig. 9. Section of a transplanted hamster insulinoma, incubated for TPPase activity, from Novikoff et al. (32). Only the innermost element of the Golgi apparatus (G) shows reaction product. Most of the crescent-shaped area enclosed by the Golgi apparatus is occupied by GERL. It consists of tubular elements (arrowheads), with nascent secretory granules (S) attached to some, and coated vesicles (C), some of which are attached to tubular elements and others apparently free in the cytosol. Also labeled are two separated secretory granules (GR); note that they are unreactive. X26,000. Fig. 10. Section of guinea pig pancreas, incubated for TPPase activity, showing part of an exocrine cell. Reaction product is present only in the inner element of the Golgi apparatus (G). Condensing vacuoles are indicated by V and "rigid lamellae" by arrows; both are unreactive. X26,000. Fig. 11. Section of rat pancreas, incubated for AcPase activity, showing part of a β cell, from Novikoff et al. (32). The Golgi apparatus (G) is unreactive. Reaction product is seen in GERL (arrows) and nascent secretory granules (GR). Mature unreactive secretory granules are not included in this field. X57,000. Fig. 12. Section of guinea pig pancreas, incubated for AcPase activity, showing part of an exocrine cell. The Golgi apparatus (G) is unreactive. Reaction product is seen in three condensing vacuoles (V), in "rigid lamellae" (arrows), and in a residual body (RB). X30,000. Fig. 13. Section of liver from a male hamster fed a high-cholesterol diet for 2 days, incubated for AcPase activity, from Nehemiah and Novikoff (27). The Golgi apparatus (G) is unreactive. Much reaction product is seen in GERL (GE) and lysosomes (LL), within which the lipid site is electron-lucent. Portions of GERL containing very low density lipoprotein-like particles show slight deposits of reaction product (arrows). X29,000.

Strikingly similar results have been reported and conclusions drawn in a preliminary report by Hand and Oliver (35) for the exorbital lacrimal gland. Earlier AcPase results on epinephrine-secreting cells of adrenal medulla were reported by Holtzman and Dominitz (36).

Interesting questions raised by these findings are: (a) Do the elements of the Golgi apparatus contribute to the secretory material in GERL? (b) If so, what is the nature of this contribution and by what mechanisms does transfer from Golgi to GERL occur? (c) What roles do the coated vesicles derived from GERL play?

Despite such questions, it seems most likely to us that some secretion processing and the secretion packaging in a variety of secretory cells will prove to be a function of GERL, implying direct access from ER to secretory granules for at least some secretory components. The functions of the Golgi apparatus in these particular instances remain to be elucidated. Again we are limited by the exceedingly few cytochemical procedures available. Thus, it would be highly desirable to demonstrate cytochemically the glycosyl transferases known to be concentrated in isolated subcellular fractions enriched in portions of the Golgi apparatus, but this is presently not possible.

GERL, lysosomes, and lipid transformations in hepatocytes

We have studied two model situations which suggest an involvement of GERL and lysosomes [identified cytochemically (AcPase) as well as by ultrastructural hallmarks] in lipid
transformations within hepatocytes. However, we will begin by referring to the studies of Essner and Oliver (37) on hepatocytes of the beige mouse, regarded as an analogue to the human Chediak-Higashi disease. They wrote, "In addition to marked enlargement and increased complexity, GERL (and lysosomes) of beige hepatocytes was characterized by accumulations of lipid-like inclusions, dense grains, and membranous material . . . ." Essner and Oliver suggest that the pathogenesis of Chediak-Higashi disease may involve a defect in lipid metabolism resulting in the accumulation of lipid within the greatly enlarged GERL and the huge lysosomes (residual bodies) that arise from it.

J. Nehemiah and I (37) have studied the hepatocytes of the Syrian golden hamster, untreated and fed a high cholesterol diet. Unlike any other mammalian hepatocytes we know of, in the hamster hepatocytes lipid accumulates within lysosomes. The lipid is stainable by Oil Red O and is birefringent; in electron micrographs its site is electron-lucent. Such lipid is present in the perportal hepatocytes of untreated male hamsters. On the high cholesterol diet all hepatocytes, in either male or female hamsters, show enlargement of GERL and accumulation of cholesterol-rich lipid in the residual bodies arising from GERL (also in adjacent areas of ER); cholesterol levels in the liver rise dramatically. Fig. 13 shows part of the Golgi zone in a hepatocyte of a male hamster fed the cholesterol-rich diet for 2 days. AcPase reaction product can be seen in GERL, portions of which contain lipid-like (very low density lipoprotein?) particles, and in lysosomes (residual bodies) filled with large electron-lucent lipid. We have termed such lipid-filled lysosomes liposomes. Recently, they have been described in human hepatocytes of patients with Wilson's disease (38).

Judging from ultrastructure and their development as described by Wake (39), the vitamin-A-containing "lipid droplets" in the "lipocytes" of rat liver are also liposomes.

The studies of P. M. Novikoff et al. (40) also suggest a role of GERL in lipid alteration within hepatocytes. A fatty liver is induced in rats by feeding a semisynthetic diet rich in 6-ornithic acid. The hypolipidemic drug clofibrate (ethyl chlorophe- noxyisobutyrate) produces a milky fatty liver on this semisynthetic diet. If the two agents are fed together a fatty liver fails to develop. In hepatocytes of the untreated rat GERL is not particularly conspicuous, but in rats fed clofibrate with the orotate GERL is very much enlarged and swollen with material interpreted as lipid undergoing transformation. Stiffing of GERL with lipid-like particles is even more prominent when a fatty liver is first induced in the rat by orotate and then clofibrate is added to the diet, causing the fatty liver to disappear and hepatocyte ultrastructure to revert to normal (P. M. Novikoff and D. Edelstein, unpublished work). In the reversal studies GERL was identified not only by characteristic ultrastructural features but also by AcPase cytochemistry (P. M. Novikoff, personal communication).

Biochemical, autoradiographic, and morphological evidence (summarized in ref. 40) has established the ER as the organelle through which lipid is transported by the hepatocyte. In GERL, and in lysosomes derived from it, the lipid acquires a different appearance. The biochemical events associated with the transformation cannot be revealed by cytochemistry. Isolation procedures are required.

GERL in melanocytes

In 1968 we reported that in the B-16 and Harding-Passey mouse melanomas GERL possesses tyrosinase ("dopa oxidase") activity as well as AcPase activity (6). Both enzyme activities are also demonstrable in the premelanosomes and melanosomes (Fig. 3). These findings have been confirmed, in general, in other laboratories for other melanomas and for normal melanocytes (41–47). Since premelanosomes and melanosomes show AcPase (Fig. 2) as well as the typical tripartite delimiting membrane of residual bodies, P. Leuenberger and I (48) proposed the term melanolysosome. Maul (41) and Maul and Romsdahl (42) suggest that AcPase may be involved in degrading enzymes after melanization is completed or in degrading old melanin granules but they present no evidence for the proposals.

Microperoxisomes in mammalian cells

The cytochemical 3,3'-diaminobenzidine procedure of Graham and Karnovsky (49) has contributed to many areas of cell biology. In our laboratory we modified the procedure to stain peroxisomes and other organelles (5, 7, 10). The alkaline 3,3'-diaminobenzidine procedure visualizing sites of peroxisome catalase (7, 8) served to bridge morphology and biochemistry in studying peroxisomes (50). Unlike the nucleoid-containing peroxisomes found among mammalian cells only in liver and kidney, anucleoid peroxisomes, or microperoxisomes, were first revealed by the dianimobenzidine procedure (Figs. 14–16). Microperoxisomes are present in all cells save for mature erythrocytes and perhaps other specialized end-stage cells. They differ from the nucleoid-containing peroxisomes in two other respects. They are generally smaller and they possess numerous slender connections to the ER. So frequent are the continuities of their delimiting membranes with the ER membrane that microperoxisomes may well be considered as specialized regions of ER in which catalase and other peroxisomal enzymes are compartmentalized. The roles of these enzymes in animal cells, unlike those in plant cells (51), are still to be clarified. They appear in some manner to be involved in the metabolism, transport, and storage of lipid.

ER in thyroid epithelium cells

If our interpretation (4) is correct there are two secretory pathways that by-pass the Golgi apparatus in the thyroid epithelial cells. One is like that in pancreas exocrine or endocrine cells considered above. So-called "B" granules (Fig. 4) are considered to arise from GERL and to carry noniodinated thyroglobulin to the follicular lumen where it is released by exocytosis. The second pathway involves the ER generally which in this cell type has endogenous peroxidase activity. Peroxidase-positive "A" granules (Fig. 4), smaller than the peroxidase-negative "B" granules, appear to bud directly from the apical ER and to bring the peroxidase to the lumen where it is secreted via exocytosis.

Conclusion

We have briefly reviewed how cytochemistry, by its demonstration of specific enzyme localizations in situ, can help solve problems in cell biology. We have emphasized a current focus of our laboratory, namely, the complexity of the ER. In addition to its well-known roles in protein synthesis, the ER appears to have regions with specialized functions. For example, in virtually all mammalian cells, microperoxisomes attached to the ER are sites where catalase and probably other peroxisomal enzymes are localized. In diverse cell types—including neurons, exocrine and endocrine cells, hepatocytes, and melanocytes—the region of ER near the Golgi apparatus known as GERL possesses AcPase and morphological features in common with lysosomes (residual bodies) that appear to arise from it. We interpret our observations as revealing different transformations
within GERL—producing autophagic vacuoles and coated vesicles as well as residual bodies, packaging secretory materials, changing the nature of lipid in transit within the ER, and forming premelanosomes. Yet another type of secretory mechanism is seen in thyroid epithelial cells, where some secretory granules appear to bud from the apical ER. As in the mechanisms involving GERL, the Golgi apparatus would appear to be bypassed. The functional relations of the Golgi apparatus to GERL remain to be elucidated in all cell types we have studied.

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