MICROPEROXISOMES

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It is fitting that a symposium on peroxisomes be organized by the Histochemical Society and that the symposium papers be published in the JOURNAL OF HISTOCHEMISTRY AND CYTOCHEMISTRY. The JOURNAL published the original 3,3'-diaminobenzidine (DAB) procedure of Graham and Karnovsky (13), then the modification of the DAB medium which is most widely used to visualize peroxisomes (19) and recently a further improvement of the medium (20).

Cytochemistry has served to bridge morphology (electron microscopic study of microbodies (15)) and biochemistry (density gradient separation of particles containing catalase, a hydrogen peroxide-destroying enzyme and oxidases producing hydrogen peroxide (7, 8) and therefore named peroxisomes (6)). Incubation of aldehyde-fixed tissues in alkaline DAB media reveals peroxisomes and microperoxisomes because of the peroxidatic activity of their catalase. Cytochemistry has also established that these organelles are ubiquitous in mammalian cells (16, 20, 21, 23, 24). Aided by cytochemistry, we were able to describe the detailed structure of the microperoxisomes and particularly their relations to the endoplasmic reticulum (ER).

It is now possible to identify microperoxisomes by electron microscopy, without cytochemistry. A microperoxisome is a roughly circular or elongate cytoplasmic body delimited by a tripartite membrane. This membrane is continuous, by numerous slender continuities, with the smooth ER membrane. The organelle has a moderately electron-opaque matrix which lacks the nucleoid used by electron microscopists to identify microbodies in hepatocytes of the rat and other mammals. Usually, but not invariably, microperoxisomes are small, about 0.15-0.25 μ. The largest microperoxisomes thus far encountered are those of human hepatocytes, where they may approach 1 μ in diameter (24). Almost without exception (20), they give a positive reaction when incubated in alkaline DAB. Those microperoxisomes that are very small or few in number are easier to locate after DAB incubation. The continuities of the delimiting microperoxisome membrane and smooth ER are more readily seen when the specimen is viewed at different tilt angles and varying degrees of rotation. Thus, the continuities shown in Figures 1 and 2 (arrowheads) were not evident in either the untilted micrographs or in the micrographs taken at other tilt angles.

By serial sectioning we have demonstrated unequivocally that rat hepatocytes have nucleoid microperoxisomes as well as the well known nucleoid-containing peroxisomes which served as the basis for the biochemical definition of the peroxisome (24). We have suggested that microperoxisomes are progenitors of peroxisomes in this tissue, and a possible course of development of one into the other has been proposed. Similar studies in proximal convolution cells of the renal tubule are in progress.

We have indicated elsewhere (20, 23, 24) the appeal to us of the term, microperoxisome. These organelles are present in all mammalian cells studied and in all they are structurally similar. The new term reflects a new situation, namely that these organelles can now be recognized solely by their fine structure. The term, microperoxisome, encompasses both morphology (the first part of microbody) and biochemistry (the peroxisomal enzyme, catalase and possibly others). Connock and Kirk (5) have shown that in homogenates of epithelial cells of the guinea pig intestine the microperoxisomes, because they are considerably smaller than mitochondria and lysosomes, sediment more slowly than both lysosomes and mitochondria. Dr. T. J. Peters has shown that in homogenates of epithelial cells of the guinea pig intestine the microperoxisomes, because they are considerably smaller than mitochondria and lysosomes, sediment more slowly than both lysosomes and mitochondria. Dr. T. J. Peters has shown that a catalase-enriched fraction of this tissue is similarly rich in D-amino acid oxidase activity.

Naturally, the term by which these ubiqui-

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2 Peters TJ: Private communication.
tous organelles are called is less important than the understanding of the functions which they play in cell metabolism. In contrast to plant cells, little has been learned about peroxisomes of liver since de Duve and Baudhuin's review in 1966 (7). Beyond the ubiquity ofmicroperoxisomes we know that their number varies greatly among different cell types (21). The metabolism, transport and storage of lipids are major functions of many cell types possessing large numbers of microperoxisomes. These include intestinal mucosa (23), hepatocytes (24), adrenal gland (2-4, 17), interstitial cells of the testis (26, 28) and interstitial cell tumors (27). It is known that the microsome fractions separated from several of these tissues, and derived largely from the ER, contain numerous enzymes of lipid metabolism. This is probably the underlying biochemical function related to the spatial relation of microperoxisomes and ER. Other, more specific, spatial relations probably reflect more specific functional roles. This includes the presence of microperoxisomes on the surfaces of stored fat droplets (Fig. 4; also see References 20 and 24 and Fig. 13 in Reference 29) and their presence close to lipofuscin granules of human hepatocytes, known to accumulate characteristic lipids (Fig. 3; also Reference 22), and on the surface of zymogen granules in guinea pig pancreas (21). Conceivably, the close contact of smooth ER, microperoxisomes and zymogen granules (Fig. 2) may be involved in withdrawal of lipid. It is known that mature zymogen lacks lipid (14).

Thus, cytochemistry has again made our laboratory focus upon the ER and its incredibly varied functions. Phosphatase cytochemistry helped us delineate GERL, a specialized region of ER with acid phosphatase activity, from the Golgi apparatus which shows nucleoside diphosphatase and thiamine pyrophosphatase activities in neurons and other cell types (25). DAB cytochemistry permitted us to describe the microperoxisomes. Although structurally more separated from the ER than GERL is, the microperoxisomes may also be considered specialized portions of smooth ER.

We wish to conclude with speculations to which we were brought by cytochemistry—phosphatase cytochemistry and DAB cytochemistry. The ER appears to be involved in three types of autophagy. The sequestration of organelles such as mitochondria and peroxisomes, or of portions of organelles such as ER, forms the well known autophagic vacuoles—to which we refer as type 1 (18). Another type of autophagy involves internalization of smooth ER membrane, bringing into the vacuole (referred to as type 2) components of the cytosol (18, 25) (cf. Reference 11). Finally, microautophagy, suggested conceptually by de Duve and Wattiaux (9), may be involved in the uptake of glycogen into lysosomes in rat hepatocytes.

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**Fig. 1.** A portion of a normal human hepatocyte following incubation in alkaline DAB (from Reference 24). This is one of 14 micrographs of the same section taken at 10° tilts intervals with and without rotating the stage by 90°, utilizing the goniometer stage. It was taken at +30°, without rotation. The continuity of smooth ER (ER) and the delimiting membrane of the microperoxisome (arrowhead) was evident in this one micrograph. Other such continuities were seen in six other micrographs, each evident in only one micrograph. The delimiting membrane of the microperoxisome is evident at the right half; the rest of the membrane was seen in other micrographs. A portion of a mitochondrion is seen at M. x85,000.

**Fig. 2.** Guinea pig pancreas following DAB incubation. A portion of a zymogenic cell is seen. This is one of six micrographs taken, at 10° intervals, from the same section. Note the manner in which the microperoxisomes are flattened upon the surface of the mature zymogen granules (Z). Note also the proximity of smooth ER (ER) to the zymogen granules. Sometimes the ER appears to be in the form of tubules (short arrows) which show circular profiles when sectioned transversely. The longer white arrow indicates the proximity of a microperoxisome to smooth ER. An arrowhead shows a continuity of smooth ER and a microperoxisome. The continuity is not evident in five other micrographs. The interdigitating processes of adjacent cells are labeled I. ×43,000.

**Fig. 3.** A portion of a normal human hepatocyte, unincubated. Note the close spatial relation of the smooth ER to a microperoxisome (MP) and to dense areas that are either grazing portions of the C component (22) of one or two lipofuscin granules or an early stage in the development of lipofuscin granules from swollen areas of ER. Note the presence of free ferritin-like grains in the cytosol. Arrows indicate some grains that may be in the process of incorporation into the lipofuscin granule by microautophagy (18, 22). In regions of the dense areas some of the individual ferritin-like grains may be discerned. That the grains are indeed ferritin are indicated by their staining with the alkaline bismuth staining method of Ainsworth and Karnovsky (1). This is seen in the inset, which shows a portion of a lipofuscin granule. x77,000.

**Fig. 4.** A portion of a normal human hepatocyte, unincubated. Note portions of two large lipid droplets (L), each with two microperoxisomes on its surface (numbered 1 to 4). A mitochondrion (M) is seen on the surface of one of the droplets. x30,000.
(Shin and Novikoff; see Reference 18) and ferritin into lipofuscin granules of human hepatocytes (22). The ferritin-containing areas of the lipofuscin granules are DAB-positive (22), probably nonenzymatically (12). These areas also show acid phosphatase activity (10). As indicated above, microperoxisomes are found close to the surface of lipofuscin granules and microperoxisomes. It is tempting to think that the microperoxisomes may be involved in lipid storage in the lipofuscin granules and that smooth ER, via microautophagy, may bring ferritin and other cytosol molecules into these "wear and tear" granules.

REFERENCES


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