Microbodies: Peroxisomes and Glyoxysomes

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Microbodies were first reported at the ultrastructural level in the proximal convoluted tubule of mouse kidney by Rhodin in 1954 (1) and in hepatic parenchymal cells by Rouiller and Bernhard in 1956 (2) at about the time The Journal of Cell Biology was established. They were reported in plants by Porter and Caulfield in 1958 (3) and by Mollenhauer et al. in 1966 (4). Microbodies are now recognized as ubiquitous subcellular respiratory organelles in eukaryotic cells. Microbodies from all tissues appear morphologically similar and have similar enzymatic properties, but the metabolic pathways within this subcellular compartment vary, depending upon the tissue. Microbodies (peroxisomes and glyoxysomes) were one of the last major subcellular compartments to be recognized, and it was not until the end of the 1960s that their significance was established by several reviews. Most important were the following two summaries: “Peroxisomes (Microbodies and Related Particles)” by de Duve and Baudhuin in 1966 (5), and “The Peroxisome: a New Cytoplasmic Organelle” by de Duve in 1969 (6). The Nobel Prize that de Duve received was based on his pioneering work in the discovery and isolation of subcellular organelles, such as microbodies. Material in these two papers is essential reading for new students in the field. Also in 1969, the morphological literature was assembled into a book, Microbodies and Related Particles by Hruban and Rechcigl (7), which summarized the evidence for the widespread distribution of the particle. Another landmark in 1966, also from de Duve’s group (8), was the development of procedures for isolating microbodies. The first research symposium, “The Nature and Function of Peroxisomes (Microbodies, Glyoxysomes),” was held in 1969 (9).

Recently there has been such a proliferation of papers about the many aspects of microbodies that in this article we cite only reviews or use only an initial reference to a specific subject. Some of the general reviews are on development and enzymatic content (10), microbodies in leaves (11–13), germinating seeds (14, 15), algae (16), fungi (17), and protozoa (18); other reviews or use only an initial reference to a specific subject. Nevertheless, we have little knowledge today of the physiological role of microbodies in cellular metabolism. Properties and characteristics of microbodies are still incompletely described, and much of the recent literature has not been confirmed or well established by the few biologists working in this field.

Nomenclature

From the titles cited above, it is apparent that there has been long been uncertainty about naming this organelle. Morphologists sometimes used the nonspecific term microbody until a more specific name came to be established through functional and biochemical studies. At present, microscopists are continuing to use the term microbody for the organelle that is variously called, by others studying its biochemical properties, mammalian peroxisomes (5), leaf peroxisomes (12), glyoxysomes (12, 15), or glycerophosphate oxidase bodies (18). The term peroxisomes was proposed by de Duve for the organelle because it produced and consumed hydrogen peroxide. The in vivo potential for its catalase to metabolize peroxidatively other organic substrates with H₂O₂ generated internally by flavin oxidases, was used as an assay, based on the conversion of [³⁴C]HCOOH to [³⁴C]CO₂. Novikoff and Novikoff (19) have used the term microperoxisome to describe similar smaller particles. The use of the term leaf peroxisome was adopted by Tolbert (12) because the morphological and enzymatic properties of microbodies from leaves were consistent with de Duve’s description of the organelle. In addition to meeting de Duve’s criteria for peroxisomes, microbodies in germinating fatty seeds have as one of their metabolic pathways the glyoxylate cycle, and were termed glyoxysomes by Breidenbach and Bevers (20). Initially, Müller et al. (21) referred to those in Tetrahymena as peroxisomes (9), but the term glyoxysome is now generally used for microbodies containing at least malate synthetase or isocitrate lyase, the two unique enzymes of the glyoxylate cycle.

Microbodies from all sources have a somewhat similar appearance, but they will have different metabolic pathways, depending on the tissue and its function. In current usage, the term microbody is assigned to the particle that has not been biochemically characterized from a given tissue, or is used as a general term to include both peroxisomes and glyoxysomes. Peroxisomes are microbodies that are known to contain catalase and at least one flavin oxidase; glyoxysomes are microbodies that contain, in addition, isocitrate lyase and/or malate synthetase, two enzymes of the glyoxylate cycle. Abandoned terms for microbodies are phragmosome, c₉₃some, peroxidosome, and crystal-containing body.

 Whereas microbody respiration must be significant and different from mitochondrial respiration or other O₂ uptake processes in the cell, there is no convenient method for measuring it specifically, nor is there a general physiological nomenclature for it. Because of big changes in the total number of microbod-
ies and amounts of their substrates, this respiration must vary in different tissues, at different stages of development, and in plants at different periods of the day. Through spectrophotometric measurements of catalase turnover in perfused liver, a member of Chance’s group (22) has estimated that hepatic peroxisomal respiration may account for up to 10% of the total \( O_2 \) uptake. In leaves, the term photorespiration, referring to glycolate biosynthesis in the chloroplasts and its oxidation in the peroxisomes and mitochondria, may be fivefold greater than dark mitochondrial respiration, but all of photorespiration cannot be designated as peroxisomal respiration because of the participation of other organelles in this process. Although precise figures are not available, glyoxysomal respiration in germinating seeds, which would be the \( O_2 \) uptake associated with the conversion of long-chain fatty acids to \( C_4 \) acids, probably is also much greater than mitochondrial respiration during this period.

**Morphology**

Microbodies are morphologically characterized as particles ranging in diameter from 0.1 to 1.5 \( \mu m \) (average about 0.5 \( \mu m \)), which are delimited by a single tripartite membrane and contain a finely granular matrix. In liver, an estimation of 1,000 microbodies per hepatocyte has been made (23). They show a close spatial relationship to the endoplasmic reticulum. Their catalase can be demonstrated cytochemically. Because microbodies without inner membranes must be pliable, they usually appear spherical or ovoid, but in the cell they may also be irregular in shape or show unusual projections. Hepatic (Fig. 1) and plant (Fig. 2) microbodies contain, in addition to the granular matrix, an electron-dense core (nucleoid) in which a series of parallel membranes or lattice structures are sometimes observed. De Duve and Baudhuin (5, 24) considered that urate oxidase in rat-liver peroxisomes is associated with the nucleoid. Newcomb’s group (25) showed cytochemically that the core in plant microbodies is rich in catalase. Although usually structureless, fine branching filaments (4-5 nm) or short fibrils have also been described in the microbody matrix (7).

The hepatic microbody is delimited by a tripartite membrane approximately 6–8 nm in thickness. In contrast to lysosomes, the delimiting membrane appears to be thinner, and no electron-lucid zone is found between the membrane and the matrix of the microbodies (Fig. 1). Freeze-fracture replicas of hepatic microbodies revealed numerous particles (7–8 nm), often in clusters, on the protoplasmic face of the delimiting membrane, whereas the extracellular face is almost devoid of such particles (26). These features, which resemble those seen in other types of membranes, are also found on the corresponding fracture faces of these portions of endoplasmic reticulum which are located adjacent to the microbody membrane.

The microbodies of rat kidney frequently show tubular protrusions and circular profiles at the margin of the matrix (7). Barrett and Heidger (27) showed that the tubular protrusion rods were absent from rat renal microbodies when fixed by perfusion, but the circular and tubular profiles were consistently demonstrable. According to Tisher et al. (28), the morphology of the renal microbody can vary markedly, depending on the method of fixation. Recent freeze-fracture studies, which provide a three-dimensional view of these inclusions (100–125 nm), appear to confirm the impression that the tubular and

**Figure 1** Peroxisomes in mouse liver. Note characteristic configuration of nucleoids. X 43,000.

**Figure 2** Peroxisome in parenchyma cell of a tobacco leaf. Electron micrograph courtesy of Dr. S.E. Frederick. X 40,000.
circular profiles represent sections through different planes of the same elements (29). As in the case of hepatic microbodies, freeze-fracture replicas of renal proximal tubule cells reveal more particles on the protoplasmic face than on the extracellular face of the delimiting membrane.

Much interest has been focused on the central core, or nucleoid, of plant and hepatic microbodies that consists of homogeneous, membranous, or lamellar forms. In hepatic microbodies, the lamellar type may appear as a crystalloid consisting of a polytubular substructure. Hruban and Rechcigl (7) examined the form and complexity of crystalloids in microbodies in various species, and attempted to classify microbodies according to the presence or absence of a nucleoid and to determine the pattern formed by the elements of the crystalloid. In many species these can be grouped into the following two categories “coarsely polytubular” or “finely polytubular.” The nucleoids of hepatic microbodies were isolated by Tsukada et al. (30) and were shown to consist of parallel bundles of highly dense, hollow tubules, which, in cross section, have a honeycomb appearance. In the microbodies of guinea-pig liver, which correspond to the second type, the nucleoid consists of microtubules, approximately 4.5 nm in diameter, arranged in a regular hexagonal lattice with spacings of about 11 nm between the axes of contiguous tubules (5, 24).

In 1964, Hruban and Swift (31) reported that the microbody nucleoids of rat hepatocytes and of certain transplantable rat hepatomas were similar in structural organization and dimensions to commercial preparations of urate oxidase from hog liver, and suggested that the core was composed of the crystalline enzyme. However, Baudhuin et al. (24) felt that structures seen in the urate oxidase preparations probably represented preformed microbody nucleoids that had been concentrated by the purification procedure, rather than crystals of the enzyme. The question was reexamined by Lata et al. (32) with sections through highly purified preparations of rat-liver urate oxidase that revealed a polytubular structure similar to that present in the microbody nucleoids of the same species and to that described by Hruban and Swift (31). Comparative ultrastructural studies also suggest a correlation between the presence or absence of a nucleoid and the level of urate oxidase activity. The occurrence and substructure of nucleoids and the presence or absence of urate oxidase activity were correlated by Afselius (33) and Shnitka (34) in hepatic microbodies of various species. Among rodents, carnivores, and ungulates, there was a positive correlation between the presence of a nucleoid and the presence of urate oxidase activity. Humans and birds, whose hepatocytes contain anucleoid microbodies, are without hepatic urate oxidase activity. Although urate oxidase is now considered a reasonably constant component of the nucleoid, the functional significance of the structural variations encountered among different species remains unclear.

Another characteristic structure sometimes observed in microbodies is the “marginal plate.” It is usually located at the microbody periphery and consists of a relatively straight, thickened region that sometimes shows periodic substructure. Marginal plates have been described in microbodies of liver and kidney from several species (7). The functional significance of the marginal plate remains obscure.

In 1958, Porter and Caulfield (3) described membrane-bound bodies (“phragmosomes”) in dividing onion root cells that measured 0.25–0.5 μm in diameter and contained a granular matrix. Organelles of similar appearance were subsequently identified in other higher plants, algae, and fungi by Mollenhauer et al. (4), who also drew attention to the similarity of these bodies to the microbodies of animal cells. A delimiting tripartite lipid bilayer, 6–7 nm thick, and a granular matrix are virtually constant features of microbodies in plant cells (for reviews see references 25 and 35). As in animal cells, plant microbodies show a close spatial relationship to the endoplasmic reticulum. The presence of a marginal plate has also been described in fungal microbodies. As in liver and kidney, plant microbodies may be grouped into three categories: anucleoid microbodies, those containing noncrystalline cores, and those with crystalloid cores characterized by an organized substructure. Examples of anucleoid microbodies have been found especially in meristematic and differentiating plant cells, such as root cells (4, 35), and those with crystalloid cores were first reported in oat coleoptiles (36) and, subsequently, in various other plant cells (25). The crystalloid core of plant microbodies gives a strong cytochemical reaction for catalase and has been variously interpreted as layers of parallel sheets, or as tubules which are organized into hexagonal, tetragonal, or rectangular patterns (7, 25, 35).

**Cytochemistry**

A number of attempts have been made, with varying degrees of success, to localize enzyme activities within microbodies by cytochemical means. In 1965, Graham and Karnovsky (37) localized uricase (urate oxidase) activity in microbodies by light microscopy. Horseradish peroxidase was used to catalyze the oxidation of 3-amino-9-ethy carbazole by H$_2$O$_2$ generated at the sites of urate oxidase activity. Allen and Beard (38) reported on the light-microscope localization of α-hydroxyacid oxidase in renal peroxisomes by use of a method based on reduction of nitro blue tetrazolium. Although subsequently modified, this method was not useful for electron-microscope localization (39). Shnitka and Talibi (40) introduced a method for the light- and electron-microscope localization of α-hydroxybutyrate oxidase. In this reaction, ferrocyanide, produced by enzymatic reduction of ferricyanide, is captured by copper to yield insoluble, electron-dense, cupric ferrocyanide. Although subsequently modified to yield more reproducible results (41), the method was unsatisfactory, in part because of the sensitivity of the enzyme to glutaraldehyde and the prevalence of nonspecific precipitate. An electron-microscope method for demonstrating NADH oxidase, based on the use of cerium ions, has now been modified for the localization of d-amino acid oxidase (42). The cytochemical localization of peroxisomal oxidases has been reviewed recently by Hand (43).

Although catalase had been demonstrated in hepatocytes by direct assay and immunocytochemical techniques, the limitations of the methods provided only hints of possible localization in microbodies. In 1968 Novikoff and Goldfischer (44, 45) described a procedure for the cytochemical visualization of microbodies at the light and electron microscope levels using a modification of the diaminobenzidine (DAB) procedure originally described by Graham and Karnovsky (46) for peroxidase (Figs. 3 and 4). This modification made it possible for Novikoff and Goldfischer (44, 45) to confirm the identity of catalase in particles considered to correspond with microbodies in various animal cells, and has facilitated the analysis of their number, distribution, and relationship to other structures, particularly the endoplasmic reticulum. The cytochemical localization of microbody catalase was also reported independently by Hirai (47) and, subsequently, by Fahimi (48). In tissues such as liver,
the identification of microbodies after incubation for catalase activity was unequivocal, because the tubular substructure of the nucleoid was still recognizable. Microbody staining is almost completely inhibited by aminotriazole, a potent and relatively specific inhibitor of catalase. Distinguishing the peroxidative activity of catalase from that of peroxidase by cytochemical means has been investigated by a number of workers (for a review see Fahimi [49]). Frederick et al. (25) and Vigil (35) used the alkaline DAB medium and also demonstrated an aminotriazole-sensitive staining of the granular matrix of peroxisomes in leaf cells, as well as a localization of catalase in the crystalloid of microbodies in oat coleoptile cells and in endosperm cells of germinating castor beans.

Incubation of tissue in the alkaline DAB medium results in deposition of reaction product in the matrix of the microbody and over the central nucleoid, if present. Although the procedure is now widely used, the mechanism of the reaction remains unclear. It is assumed that catalase, acting peroxidatively, oxidizes the DAB to a conjugated double-bond structure that binds large amounts of OsO₄ and appears opaque by electron microscopy. Fahimi (48) has suggested that the alkalinity of the medium serves to enhance the oxidation of certain substrates. It has been suggested by Goldfischer and Essner (50) that the alkaline medium causes dissociation of microbody catalase into subunits, which, despite loss of catalatic activity, have enhanced peroxidase activity, as occurs with preparations of hepatic catalase. Additional evidence that microbody catalase is dissociated into peroxidatic subunits came from studies of mutant acatalasemic mice. The catalase in these mutants is temperature-sensitive, and its catalatic activity is rapidly destroyed at 37°C. Goldfischer and Essner (51) found that hepatic and renal microbodies of these mice exhibited stronger staining in DAB medium, even at neutral pH, than did those of the wild-type tissues, and suggested that the microbody catalase in the mutant existed in vivo in a partially degraded form that showed enhanced peroxidase activity.

Microperoxisomes

Small microbody-like organelles, usually lacking a nucleoid, had been observed in various tissues by a number of earlier electron microscopists. In 1968, Kuhn (52) described small particles in the dog perianal gland, which were similar to microbodies and which sometimes showed a marginal plate or a dense nucleoid and had continuities with the endoplasmic reticulum. Hruban et al. (53) used the alkaline DAB method to demonstrate small, anucleoid microbodies in a variety of vertebrate cell types and noted their prominence in cells that were engaged in the metabolism of cholesterol, steroids, and lipids. By using a modification of the DAB procedure for the demonstration of catalase activity, Novikoff et al. (54) were able to identify similar particles in a variety of cell types (55) (Fig. 5). They drew attention to the fact that these particles were generally smaller than the nucleoid-containing peroxisomes, and showed frequent continuities of their delimiting membrane.
with that of the endoplasmic reticulum. Based on these hallmarks, they suggested the term "microperoxisome" (19). Microperoxisomes are found in virtually all cells with the exception of erythrocytes and, possibly, other specialized end-stage cells (55). Although their functions are not known, microperoxisomes appear to be involved in the metabolism, transport, and storage of lipid. Relatively large numbers of these particles are found in cells engaged in lipid metabolism and, in some instances, may show a close spatial relationship to lipid droplets (56) or to lipofuscin granules (57). Organelles with the appearance of microperoxisomes also have been described in root meristem cells and in differentiated plant parenchyma cells, where they are found together with nucleoid-containing microbodies (25). For a brief review of microperoxisomes see Novikoff and Novikoff (58).

One of the most significant and characteristic features of microperoxisomes is their frequent, often multiple, continuities with the endoplasmic reticulum (ER). These connections are wider and therefore easier to demonstrate than are the slender connections seen between microbodies and ER in liver and kidney. As is the case for microbodies, the continuities are regarded as local dilatations of the smooth ER that lack ribosomes and in which peroxisomal constituents accumulate. Whether microperoxisomes exist separately from the ER, are always in continuity with it, or are in a state of change between the two phases is not known. According to Novikoff et al. (57), microperoxisomes are also found in liver and kidney, where they may represent the progenitors of the larger microbodies. The process would involve enlargement of the microperoxisome together with formation of an electron-dense area (nucleoid) in which a tubular substructure later develops. The sequence may be similar to that described for the transformation of anucleoid to nucleoid microbodies in the liver during late fetal development (59, 60) and to the process described by Frederick et al. (25) in root parenchyma cells. Further possible evidence for the role of microperoxisomes as progenitors of microbodies comes from studies of liver from rats treated with clofibrate (see Development section). Many of the large numbers of microbodies formed in response to administration of this agent have the morphological characteristics of microperoxisomes, such as multiple continuities to and occurrence in clusters.

Isolation and Assay

The development of buoyant density or isopycnic centrifugation was essential for the isolation of microbodies sufficiently separated from other particles for examination of enzymatic composition. Because the subject is reviewed in this volume by de Duve, only some salient points relative to microbody isolation are mentioned here. Between 1964 and 1968, de Duve's group (8), using a zonal rotor designed by Beaufay, developed a complex procedure for partial purification of microbodies that has been the basis of all subsequent procedures. The development of commercial zonal rotors (B-29 and B-30) and centrifuges during the same period was based on Anderson's (61) adaptations of buoyant density centrifugation for the isolation of biological particles. Swinging-bucket rotors, such as Beckman's SW 25.2, for 10- or 55-ml tubes have been in use for small-scale preparations of microbodies. Several advantages of the zonal rotor were discussed by Anderson, such as a larger

**FIGURE 5** Absorptive cell from guinea pig duodenum showing cluster of microperoxisomes (arrows) in proximity to smooth endoplasmic reticulum. Electron micrograph courtesy of Dr. P. M. Novikoff. X 43,000.
volume and size of preparation, and good separation of the bands of organelles. The earlier work by de Duve's group showed that the "light mitochondrial" particles obtained by rate sedimentation were a mixture of mitochondria, lysosomes, and microbodies, as well as a good deal of contaminating ER; however, the term light mitochondrial fraction is no longer used extensively. Preparations of the light mitochondrial fraction from differential centrifugation may be used for partial separation of microbodies before isopycnic centrifugation, but such manipulations decrease the yield of the fragile microbodies. More recently, Methods in Enzymology (particularly volume 31) have devoted many chapters to isolation of subcellular particles, and several discussions of the isolation of microbodies are available (62-66).

The final equilibrium density of a particle is dependent on its composition (protein, lipid, and bound water). In addition, microbodies and lysosomes, which have only a single bounding membrane, may more rapidly lose bound water to the sucrose solution of the gradient than do the mitochondria. Thus microbodies sediment to the highest density in the gradient because of their low lipid composition and dehydration. Sucrose gradients have generally been used, because in gradients of large molecular weight-compounds, microbody isolation is based more on rate sedimentation. The equilibrium density of microbodies from all sources is generally about 1.24 g·cm⁻³ (about 51% sucrose) but it must be remembered that these isolated particles have lost bound water and probably many soluble components.

The separation of the hepatic microbodies and lysosomes of nearly similar equilibrium density was accomplished by de Duve's group by injecting a detergent, Triton WR-1339, into the rat two days before sacrifice. Lysosomes containing the engulfed detergent then had a lower equilibrium density than the microbodies. However, for other biological tissues, and now for most mammalian peroxisomal preparations, investigators use no detergents and rely only on marker enzyme profiles to evaluate their microbody purity. Plant lysosomes (vacuoles) do not generally survive grinding. Liver lysosomes distribute over two major areas: a small (10%) part of the lysosomes bands at the edge of the peroxisome peak and the rest among the ER. Although the specific activity of peroxisomal enzymes is increased 10- to 50-fold by isopycnic centrifugation, the reported specific activity values would be still higher if all the contaminating organelles could be removed. Besides lysosomes, the other major contaminant in the microbody peak is a small part of the total ER. This ER almost represents the total activity in the tissues because of losses from incomplete grinding and previous differential centrifugation. For total activity, a separate analysis should be run on a totally homogenized fraction.

Enzymatic Composition and Metabolic Pathways

CATALASE AND FLAVIN OXIDASES: The terminal oxidase for O₂ uptake by microbodies from all tissues examined has been a H₂O₂-producing flavin oxidase associated with catalase. In addition, some microbodies have copper-containing urate oxidase. The flavin oxidase varies with the tissue and with the available substrates. An exception is that glyoxysomes from some fungi and molds may contain only part of the glyoxylate cycle and no terminal oxidase system. In 1966, de Duve (5) illustrated this concept by the following scheme (slightly modified), which indicates one oxidase reaction and two possible catalase reactions with the H₂O₂:

\[
\begin{align*}
\text{O}_2 & \xrightarrow{\text{oxidase}} \text{H}_2\text{O}_2 \\
\text{RH}_2 & \xrightarrow{\text{catalase}} \text{R}' + \text{R}'\text{H}_2
\end{align*}
\]

The identity of the various substrates, RH₂, for the oxidase will be cited later for each metabolic cycle. Whether a second substrate, R'RH₂, is peroxidically oxidized in vivo is not established. However, the original in vitro assay (8) was the quantitative peroxidative oxidation of [¹⁴C]HCOOH to [¹⁴C]CO₂. More recently, isolated peroxisomal fractions have been quantitated by peroxidation of volatile [¹⁴C]CH₂OH to a nonvolatile product (67). Catalase has been estimated to be about 33% of the hepatic peroxisomal protein (5), so it is present in the particle in great excess. This important enzyme for peroxisomal respiration was exhaustively characterized and studied by Chance (68), Theorell (69), and others before the realization that it resided in a subcellular organelle. In the reaction mechanism for catalase, a stable complex, compound 11, of catalase H₂O₂ is first formed; it then reacts peroxidatively with either another H₂O₂ or R'RH₂. The amount of the catalase H₂O₂ complex formed is increased by high catalase concentration, as in the peroxisomes. The complex has a characteristic absorption spectrum (22), which has been monitored in vivo by Chance's group, who used perfused liver provided with substrates (RH₂) for H₂O₂ generation by peroxisomal oxidases (glycolate or urate). These measurements indicated that hepatic peroxisomal respiration could be as much as 10% of the total respiration.

The extent of peroxidative metabolism of a second substrate, R'RH₂, remains to be established by physiological experiments. In support of a peroxidative mechanism, methanol detoxification in rats (but not in monkeys) was reduced when catalase was poisoned by aminotriazole (70). However, ethanol detoxification was not inhibited, probably because of the alternative alcohol dehydrogenase reaction. During photosynthesis in plant peroxisomes or during the glyoxylate cycle in seed glyox-
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the hepatic peroxisomal system is about one-fourth to one-third as active as the mitochondrial system in the rat (85), and the two pathways are about equal in activity in the mouse (10, 86). Speculatively, the mitochondrial β-oxidation may proceed via acetyl CoA to CO₂ and ATP, whereas the peroxisomal system, which only degrades the fatty acids to C₆ or C₄ acids, may provide acetyl CoA or acetylcarnitine for other synthetic processes in the cell. Such exciting developments certainly indicate that microbodies are essential organelles.

**Alcohol and Alkane Oxidases:** When yeast is grown on methanol, the cells contain mainly gigantic microbodies containing a flavoprotein, methanol oxidase, and catalase (87, 88). As expected, growth on a sugar represses these microbodies. Growth on long-chain alkanes (oils) also induces the development of microbodies containing an oxidase to initiate alkane oxidase (89).

**Ureide Metabolism:** Enzymes for ureide metabolism, including xanthine oxidase, urate oxidase, allantoicase, and allantoicase have been found in microbodies of some tissues. The H₂O₂-producing oxidase, indicative of microbodies, is urate oxidase. Indeed urate oxidase (see Morphology) is a major peroxisomal enzyme from liver of rat and presumably all ureotelic animals. Scott et al. (90) originally reported that the whole ureide pathway was in avian hepatic peroxisomes, but the results have not been confirmed, There is now one report of the ureide pathway of metabolism in peroxisomes from fish liver (91). The end products of this pathway are CO₂, NH₃, and glyoxylate, and the formation of glyoxylate is also indicative of a microbody system (see next section on Glyoxylate). Xanthine oxidase in liver generally appears to be in the cytoplasm, and there are no publications, except for the one on avian hepatic peroxisomes, that locate it in the microbodies.

**Glyoxylate Metabolism and Aminotransferases:** Many, if not all, reactions involving glyoxylate biosynthesis and metabolism are compartmentalized in microbodies. This is true for the glycolate pathway of photorespiration in plants, for the glycolate cycle, for glycolate oxidase in the liver, and for ureide metabolism. Likewise, the aminotransferases of microbodies are relatively specific for glyoxylate as the amino acceptor resulting in the formation of glycine. The equilibrium of the aminotransferases lies almost totally in the direction of glycine formation. In leaf peroxisomes, there are two different, active aminotransferases: a glutamate:glyoxylate, and a serine:glyoxylate enzyme (92). In hepatic peroxisomes, a leucine:glyoxylate aminotransferase was reported from rats (93), and an alanine:glyoxylate aminotransferase from humans (94). All aminotransferases in microbodies seem to be able to use alanine to some extent as an amino donor for glyoxylate.

Glyoxylate oxidation by glycolate oxidase in the peroxisomes to oxalate is considered a side reaction of C₂ metabolism. Possibly the glyoxylate may be peroxidatively oxidized to CO₂ and formate. Much earlier work in the metabolism of added glycolate by animals ought to be reevaluated with the knowledge that it is an excellent substrate for the abundant lactate dehydrogenase and for other aminotransferases of the cytoplasm, but it is doubtful whether glycolate is ever formed in vivo outside of the metabolic pathways of the microbodies.

**Metabolic Generalizations:** From the current biochemical knowledge about microbodies, a few tentative generalizations can be made. (a) The metabolic pathways within the microbody are catabolic; however, the end products of microbody pathways, e.g., acetylcarnitine or a C₄ acid, may be used for synthetic processes elsewhere in the cell. (b) Some of the enzymes of microbodies from different tissues vary greatly, depending on physiological parameters and tissue function. That is, there is no constancy for the same metabolic pathway in microbodies from different tissues as there is in mitochondrial composition. (c) The metabolic pathways so far described in microbodies represent one of dual or alternative pathways for metabolism of a substrate. Examples are the two β-oxidation systems, the citric acid cycle versus the glyoxylate cycle, and postulated peroxidation of alcohol versus alcohol dehydrogenase. Some of these differences may be essential because of different use of the end product of the metabolic pathway in the microbody. (d) The unique microbody enzymes are those associated with O₂ uptake, namely the flavin oxidases and catalase. Other enzymes are either slightly different or isoenzymic with their counterparts elsewhere in the cell; however, they are never identical. Examples are isoenzymes of NAD:malate dehydrogenase in leaf peroxisomes versus leaf mitochondria and different fatty acyl CoA enoylhydrolases in hepatic peroxisomes and mitochondria.

**Transport, Shuttles, Latency, and Outer Membrane**

Active membrane transport by translocases, as in the mitochondria and chloroplasts, has not been discovered so far in microbodies. One concept is that microbody substrates and products may diffuse passively across the single bounding membrane. Microbodies may be simply compartments for clustering enzymes for specialized metabolic pathways associated only with catabolism. De Duve (5, 6) was of the opinion that most enzymatic assays showed no latency or initial lag during assay with isolated microbodies, as if the rates were not limited by membrane diffusion. This is a difficult problem to assess because fully intact particles may never be isolated or they may be broken at the beginning of the subsequent enzyme assays. Assays in dense sucrose are diffusion-limited, whereas dilution and handling before assay damages the organelle. In the author’s (N. E. Tolbert) laboratory, assays are performed after dilution into a buffered detergent (Triton X-100) to dissolve the particle for maximum enzyme activity.

It seems likely that shuttles of organic and amino acids may exist between the inside of the microbody and the cytoplasm, but still the actual membrane transport could be by passive diffusion. Several such microbody shuttles have been investigated. A malate, oxaloacetate, aspartate shuttle has been proposed for plant microbodies similar to the one for mitochondria (12). Both leaf peroxisomes and seed glyoxysomes contain large amounts of an isoenzyme of NAD:malate dehydrogenase that is unique to microbodies. Except for catalase, this is the most active enzyme yet measured in leaf peroxisomes (about 50 μmol min⁻¹ mg⁻¹ peroxisomal protein). Such a shuttle seems to be the only way to oxidize NADH produced in the microbody, because no NADH oxidase has been detected in them. Hepatic peroxisomes do not contain malate dehydrogenase, but they do contain a small part of the total NAD:glycerol-P dehydrogenase (95). The existence of a hepatic peroxisomal glycerol-P shuttle has not yet been fully elaborated.

Fatty acids are transported across the mitochondrial membranes as acylcarnitine derivatives, for which there are three enzymes—a carnitine acyltransferase, a carnitine octanoyltransferase, and a carnitine palmitoyltransferase. Rat liver peroxisomes, but not renal peroxisomes, contain considerable amounts of the short-chain and medium-chain transferases (96, 97). The function of these two peroxisomal transferases is not
clear, particularly since they are located in the peroxisomal matrix and not in the membrane. Palmitoyl CoA oxidation by isolated hepatic peroxisomes is not stimulated by carnitine. A working hypothesis at the present is that during hepatic peroxisomal β-oxidation, acetyl CoA and octanoyl CoA are formed and converted to the carnitine derivatives to conserve intragranellar CoA, and that the carnitine derivatives then diffuse out of the peroxisomes to the cytoplasm and the mitochondria.

The composition of the single tripartite peroxisomal membrane is similar to that of the ER, from which it is presumed to arise by budding. The peroxisomal membrane contains phosphatidylcholine, phosphatidylethanolamine, plus phosphatidylinositol (98, 99) and some antiyvin A-insensitive cytochrome b₅ reductase. At first, it appeared that an isolated peroxisomal fraction would be an easy way to obtain a pure membrane fraction. This has proven not to be the case, because even the best peroxisomal preparations when examined by electron microscopy, contain a significant amount of ER relative to the small amount of peroxisomal outer membrane. Several groups of investigators (15, 100) have broken microbodies gently by osmotic shock and observed occluded membrane protein in the ghosts, which may be related to preferential retention of certain enzymatic activities with the ruptured particles. Such enzymes are readily solubilized from this “membrane fraction” by MgCl₂ solutions. Perhaps differential rates of loss of matrix enzymes from microbodies may account for some puzzling results, including the very rapid loss of catalase. Muto and Beevers (101) have clearly shown, however, that a monoglyceride lipase remains with isolated glyoxysomal membrane from germinating castor bean seeds.

**Biogenesis and Development**

**ASSOCIATION WITH ER:** Almost from the time of the initial descriptions of the microbody, the question of their mode of origin has occupied the attention of numerous investigators. In earlier studies, microbodies were thought to originate from the Golgi apparatus, multivesicular bodies, mitochondria, or dense bodies (lysosomes) (see reference 7), but none of these modes of origin has been substantiated. Evidence for nucleic acid in microbodies has so far been negative. For over a decade, however, evidence for the origin of animal and plant microbodies from the ER has been accumulating (Fig. 4). Early electron microscopists noted “projections” of smooth ER associated with the microbody membrane (reviewed by Hruban and Rechcigl in reference 7). In 1964, Novikoff and Shin (102) studied rat hepatocytes after partial hepatectomy and demonstrated numerous continuities, which often appeared in ringlike or hooklike configurations, between the delimiting membranes of microbodies and those of the ER. They suggested that the moderately opaque material characteristic of microbodies is deposited within dilated portions of smooth ER that further enlarge to form microbodies. These then separate from the ER or remain attached via narrow, tortuous connections. So frequently were these continuities observed that the authors raised the possibility that microbodies are always attached to smooth ER in vivo. Such connections might, however, be broken during homogenization. In fetal mouse liver, Essner (59) described continuities between the ER and anucleoid microbodies, which form late in gestation, and suggested that the microbody constituents accumulated in regions of the rough ER. After dilating and losing ribosomes, a nucleoid formed within the bulge. A similar sequence was described by Tsukada et al. (60) in fetal rat liver. These authors also found that the specific activity of urate oxidase in isolated nucleoid fractions from liver was significantly lower at earlier stages of postnatal growth (when anucleoid forms are found) than at later stages. Following a report by Hess et al. (103) that the number of microbodies increased after administration of the hypolipidemic drug clofibrate, Svoboda and Azarnoff (104) described irregular dilatations of the ER that contained material similar in appearance to the microbody matrix. The relationship between microbodies and ER was documented in a series of papers by Svoboda, Reddy, and co-workers (see Reddy [105] for a brief review).

In 1970, Rigatuto et al. (106), who studied hepatocytes of clofibrate-fed male rats, described small, catalase-positive, smooth-walled “vesicles” that contained a microbody-like matrix and were adjacent to or in continuity with microbodies. They suggested that microbodies proliferated by a process of fragmentation or budding from preexisting microbodies. In addition, cytochemically demonstrable catalase activity was detected in association with both the membrane surface and portions of attached ribosomes of the ER that were adjacent to the microbody matrix. In subsequent studies (107) these observations were expanded, and the findings were interpreted to indicate that catalase was synthesized on regions of the rough ER adjacent to the microbody membrane and, after accumulating in the surrounding cytosol, was transferred directly into the microbody without having entered the cisternae of the ER.

In 1972, Novikoff et al. (108) demonstrated that DAB reaction product (oxidized DAB) could diffuse from sites where it had been deposited originally, especially if such sites contained heavy accumulations, and that DAB was adsorbed to other sites, such as ribosomes, which normally lack oxidative activity. They considered it likely that staining of ribosomes was caused by diffusion and subsequent adsorption of oxidized DAB, rather than of the enzyme itself. Ribosomal staining after intravenous injection of horseradish peroxidase was also described by Bock (109). He and also Seligman et al. (110) argued in favor of diffusion of the hemoprotein, rather than of oxidized DAB. In 1974, Fahimi (111) demonstrated that when glutaraldehyde-fixed tissue was stored in buffer for prolonged periods of time, catalase diffused from microbodies and adsorbed to adjacent ribosomes, as well as to mitochondria and ER. It is now evident that ribosomal staining, whether caused by diffusion of oxidized DAB or of catalase, represents an artifact and cannot be offered as evidence for the synthesis of the enzyme on ribosomes.

A general concept for microbody biogenesis is that the enzymes, after synthesis on the ribosomes, move to the budding or developing microbody. Whether protein synthesis is by bound or free ribosomes and whether the transport is through the ER channel or through the cytoplasm to the microbodies by some selective mechanism, continue to be investigated. Recent data from Goldman and Blobel (112) indicated that catalase and uricase were immunoprecipitated from translation products directed by the free polysomes, but not from products of membrane-bound polysomes. Their data are taken to mean that those two peroxisomal enzymes could neither be synthesized by ribosomes bound to the ER nor selected during cotranslational segregation by the microsomal membranes. Rather, a mechanism of "post translational" transfer from the cytoplasm during passage through the peroxisomal membrane would have to be involved. Further insight into this process.
will greatly contribute to the understanding of the physiological phenomena described in the next section.

Development: A large body of physiological literature during the past decade has focused on the development of microbodies. One generality is that microbodies form during tissue development and differentiation. The near absence of microbody enzyme activities in young tissue or in poorly differentiated hepatomas, such as the Morris 3683, needs to be further explored. Another generality has already been discussed in the section on Metabolism, namely that microbody development and enzyme content may be substrate-dependent or induced.

During seed germination, after RNA and ER proliferation, development of glyoxysomes begins on day 2, and glyoxysomal activity reaches maximum at days 4 and 5 during lipid degradation. When the seedling continues to develop in the dark, the glyoxysomal enzymes and particles disappear on days 6 to 8. During glyoxysomal development there is de novo synthesis of its proteins. The most recent review concerning the rise and decline of the glyoxysomal population is by Beevers (15).

Another example from plants is leaf peroxisomes, which greatly increase in activity in the light during greening of a new etiolated leaf. This development is largely independent of chloroplast development, but both processes seem to be controlled in part by phytochrome (15). Fatty seeds have cotyledons that develop in the light into cotyledonary leaves. Consequently, in the light, there will be an increase in leaf peroxisomal activity whereas glyoxysomal enzymes decrease. These two biochemical classes of microbodies are morphologically similar and cannot be separated by centrifugation. Thus, these changes during development can only be observed by enzyme assays of the microbody fraction. There has been much speculation as to whether the existence of two different biochemical populations of microbodies were a result of de novo formation of leaf peroxisomes or whether the glyoxysomes were being changed into peroxisomes by an alteration of their enzymatic composition. Because no evidence could be found for two populations of microbodies during de novo labeling of the newly formed enzymes, the possibility had to be considered that the microbody enzymes all change from glyoxysomal to peroxisomal types in the whole population of microbodies.

Postnatal development of hepatic peroxisomes has been described in terms of enzymatic composition (60, 113), but not in a molecular or physiological context. Peroxisomes and all peroxisomal enzymes in the rat liver are very low or not detectable at birth. The peroxisomal enzymes for β-oxidation and catalase increase rapidly during the first two postnatal weeks, whereas urate oxidase increases more slowly over a four-week period.

The development and turnover of hepatic peroxisomal catalase has been extensively investigated by several laboratories. De Duve's group (114, 115) has observed that, during catalase biogenesis, an apomonomer is formed in the extraperoxisomal pool with a half-life of about 14 minutes. The addition of heme and tetramerization of catalase takes place in the peroxisomes. The intracellular site for the apomonomer pool is unknown, but it is in the soluble fraction after cell breakage. It has been proposed that liver peroxisomes are all interconnected through the ER channels so that any alteration of the enzymes would be distributed to all the peroxisomes. Thus one would not detect differences between young or old, and large or small, hepatic peroxisomes. Such a scheme would be comparable also to the conversion of glyoxysomes into peroxisomes in greening cotyledons. Masters and Holmes (10) and Rechcigl and Heston (116) have examined the isoenzymic forms of catalase and phenotypic changes induced by structural gene mutations. Masters and Holmes (10) in genetic studies have also utilized polymorphisms of α-hydroxyacid oxidase in different strains of mice. They conclude that the peroxisomal enzyme loci in mice are not closely localized on a linkage group and are not associated in the form of one operon regulating peroxisomal enzyme synthesis. This conclusion may be consistent with the multiple metabolic pathways and rates of development of microbodies in different tissues.

Because metabolic activity of microbodies seems to be very readily modified, like some ER oxidase systems, investigators of microbodies have utilized chemical treatments as a way to elucidate microbody function. In the earlier work of Reddy, Svoboda, and Azarnoff (104, 105), it was discovered that feeding certain hypolipidemic agents, particularly clofibrate (CPIB or ethyl-p-chlorophenoxyisobutyrate), increased the number of peroxisomes or microperoxisomes in liver of male (but not female) rats by two- or threefold within two weeks. Upon withdrawal of clofibrate, the number of peroxisomes returns to normal. The mechanism of action is totally unknown, but this compound has been repeatedly used when measuring peroxisomal activity. More recently, several other analogues of clofibrate have been reported to be equal or more potent stimulators of peroxisomal number, although they may be toxic to man (Fig. 6). In general, the effect of all hypolipidemic agents on peroxisomes ought to be examined. Hashimoto's group (82) has used the plasticizer di(2-ethylhexyl)phthalate, which is mildly hypolipidemic, to induce hepatic peroxisomes in their studies of peroxisomal β-oxidation. Clofibrate, or the plasticizer in relatively large dosage, increases the total activity of the enzymes associated with β-oxidation in the hepatic peroxisomes of both male and female rats about tenfold. This includes the enzymes for the β-oxidation reactions, the two carnitine acyltransferases, and glycerol-P dehydrogenase. It also increases the mitochondrial β-oxidation activity two- or threefold. Clofibrate does not greatly alter the total catalase and urate oxidase activities. Thus, clofibrate may be increasing hepatic peroxisomal fatty acid oxidation capability without affecting other peroxisomal activities and, in the case of the female rat, without causing peroxisomal proliferation in number. The effects of other drugs and hormones on peroxisomal activity have not yet been reported.

Function and Metabolic Diseases

It is presumed that microbodies must have important functions because of their ubiquitous distribution in eukaryotic cells. The exact relationship of microbody respiration to the rest of the cell is beginning to appear to be a very complex interrelationship with the whole cell. Although several metabolic diseases could be cited as related to peroxisomal metabolism, no significant disease-oriented research has yet developed based on peroxisomes. Studies by Goldfischer's group (86, 117) reported the absence of peroxisomes in hepatocytes and renal proximal tubule cells associated with the fatal cerebrohepatorenal syndrome of infants.

In his first reviews, de Duve (5, 6) speculated that microbodies might be a primitive respiratory organelle. We now know they are present in most aerobic eukaryotic cells and are absent in prokaryotes. Leaf peroxisomes are in all photosynthetic cells of higher plants, whereas most unicellular algae do not have
peroxisomes or nearly as much catalase, but oxidize glycolate by a dehydrogenase not linked to O₂ uptake and H₂O₂ production (16). Such data do not support de Duve's original hypothesis. Based on the fact that peroxisomes seemed to be present in gluconeogenic tissue (liver, kidney, and leaves), de Duve also called for consideration of the role of microbodies in gluconeogenesis. This hypothesis has been supported in plants, where seed glyoxysomes are active in the conversion of the stored lipid reserves into sucrose. During photorespiration in leaves, the carbon flow in the peroxisomes eventually leads to resynthesis of the sugars. There has been little support for this hypothesis in animals.

In leaf peroxisomes, photorespiration accompanying photosynthesis in high O₂ has suggested that microbodies are part of the protective processes against excess oxygen (11, 12). In this protection they may also participate with superoxide dismutase, which converts O₂⁻ to H₂O₂ that must, in turn, be removed in the microbody. In photosynthesis, the primary benefit seems to be for the chloroplast electron transport system, which is kept from becoming oxidized by continuous photorespiration involving carbon metabolism in the peroxisomes. None of these data indicate that microbody respiration removes a significant amount of the large excess of O₂, but rather that it functions in some manner to balance the cellular redox potential by respiration.

All microbody metabolic pathways to date are degradative and include one irreversible, flavin-oxidase, H₂O₂-producing step. These pathways often duplicate or complement another reversible metabolic sequence linked by pyridine nucleotide dehydrogenases for energy transfer. In the microbody pathway, the metabolic function seems to direct or to push carbon flow into a given sequence at the expense of the energy lost at the initial flavin oxidase step. Other reactions of a given metabolic pathway in the microbody are generally not energy-wasting. Thus, it can be calculated that the oxidation of a fatty acid initiated by the β-oxidation system in hepatic peroxisomes will cost only about 6% to 7% of the energy that would have been conserved as ATP if all the fatty acid β-oxidation had occurred in the mitochondria. Certainly the microbody flavin oxidase/catalase system has no energy-conserving mechanisms comparable to the coupling in the mitochondria to electron flow and ATP generation. But the initial concept that microbody respiration was simply wasteful or a way to lose energy is too simplistic to be substantiated by recent data. It is true that if leaf peroxisomal photorespiration is blocked, the microbodies photosynthesize and grow twice as fast. The increase in hepatic peroxisomal β-oxidation by clofibrate is also consistent with this hypothesis, but treated animals seldom lose much weight (97). In a series of studies of genetically obese mice (118), the total hepatic peroxisomal β-oxidation activity was actually twice that in the liver of the lean litter mates. In this case, the obese syndrome could not be blamed on lowered peroxisomal activity, but is probably a result of excess fatty acid synthesis.

Because the microbody enzymes vary immensely, depending on the tissue, it is not yet possible to discuss microbody function in tissues of the body other than liver. Renal peroxisomes do not contain a β-oxidation system, and the α-hydroxyacid oxidase is different from that in the liver. Microbodies (micro-peroxisomes) from the intestinal mucosa, myocardium, skeletal muscle, retinal pigment epithelium, and other tissues where
they abound will all have to be isolated and each group characterized enzymatically.

Little is known about metabolic imbalance and diseases that can be attributed to microbodies. Alterations in hepatic peroxisomal β-oxidation may be related to the many disease aspects of fat and lipid metabolism. But to date no direct peroxisomal alteration has been related to a metabolic disorder of this nature, except that the obese mouse is not deficient in hepatic peroxisomes (118). Some changes in hepatic peroxisomal activity have been recorded during starvation or diet change. Feeding long-chain fatty acids induces more long-chain substrate specificity for β-oxidation by the hepatic peroxisomal system. Similarly, hepatic β-oxidation may be related to the many disease aspects of fat and lipid metabolism. But to date no direct peroxisomal metabolisminvolves glycolate oxidation to oxalate (84). From numerous reviews (119) on ethanol metabolism, there are at least two pathways: a cytosolic alcohol dehydrogenase with a low Km and a peroxidative pathway with H2O2 and catalase; however, the latter pathway has not been investigated as a distinct peroxisomal system.

REFERENCES

1. Rhodin, J. 1954. Akriebolaget Godvill Stockholm. Karolinska Institute. Dissertation.

2. Rouiller, C., and W. Bernhard. 1956. J. Biophys. Biochem. Cytol. 2(Suppl.): 355-359.

3. Porter, R., and J. B. Caulfield. 1958. Proceedings of the fourth International Congress Electron Microscopy. Springer-Verlag, Berlin 2:503.

4. Mollenhauer, H. H., D. J. Morre, and A. G. Kelley. 1966. Protoplasma. 62: 44-52.

5. de Duve, C., and P. Baudhuin. 1966. Physiol. Rev. 26:323-357.

6. de Duve, C. 1969. Proc. R. Soc. Biol. Sci. B 173:71-83.

7. Hruban, Z., and M. Rechi, Jr. 1969. Int. Rev. Cytol. Suppl. 1: 296.

8. Leighton, B. P., Boole. H. Beaufay, P. Baudhuin, J. W. Coffey, S. Fowler, and C. de Duve. 1968. J. Cell Biol. 28:482-513.

9. Hoff, J. F., editor. 1969. Ann. N. Y. Acad. Sci. 168:299-381.

10. Masters, C., and R. Holmes. 1970. J. Cell Biol. 57:816-877.

11. Tolbert, N. E. 1969. Ann. N. Y. Acad. Sci. 163:325-341.

12. Tolbert, N. E. 1971. Annu. Rev. Plant Physiol. 22:45-74.

13. Gerhardt, B. 1978. Microorganisms/Peroxomes Pflanzliche Zellen. Springer-Verlag, Berlin.

14. Bteers, H. 1969. Ann. N. Y. Acad. Sci. 168:313-324.

15. Bteers, H. 1970. Annu. Rev. Plant Physiol. 20:159-193.

16. Tolbert, N. E. 1972. In Algal Physiol. Biochem. W. D. P. Steward, editor. Blackwell Scientific Publications Ltd., Oxford. 474-504.

17. Maxwell, D. P., V. N. Armentrout, and L. B. Graves, Jr. 1977. Annu. Rev. Phytopathol. 15:119-134.

18. Muller, M. 1975. Annu. Rev. Microbiol. 29:267-483.

19. Novikoff, P. M., and A. B. Novikoff. 1972. J. Cell Biol. 53:532-560.

20. Breidenbach, R. W., and H. Beevers. 1967. Biochem. Biophys. Res. Commun. 7:462-69.

21. Muller, M., J. F. Hogg, and C. de Duve. 1968. J. Biochim. 5:365-3595.

22. Sies, H. 1974. Angew. Chem. Int. Ed. Engl. 13:706-718.

23. Sternlieb, I. 1979. Prog. Liver Dis. 11:81-104.

24. Christiansen, R. Z., E. Christiansen, and J. Bremer. 1973. J. Biol. Chem. 248:3425-3432.

25. Fredericke, S. E., P. J. Gruber, and E. H. Newcomb. 1975. Protoplasma. 31:754-759.

26. Reddy, J. K., J. P. Tewari, D. Svoboda, and S. K. Malhotra. 1974. Lab. Invest. 31:285-291.

27. Barrett, J., and W. H. Martin. 1974. Cell Tissue Res. 157:283-305.

28. Fish, C. H., R. M. Finkel, S. Rosen, and E. M. King. 1968. Lab. Invest. 20:91-96.

29. Kalmbach, P., and H. D. Fahimi. 1978. Cell Biol. Int. Rep. 2:399-396.

30. Tsukada, H., Y. Mochizuki, and T. Konishi. 1968. J. Cell Biol. 37:231-243.

31. Anderson, N. G., editor. 1966. Nail. Cancer Inst. Monogr. 21.

32. Novikoff, P. M., A. B. Novikoff, N. Quintana, and C. Davis. 1973. J. Histochem. Cytochem. 21:1010-1020.

33. Afzelius, B. A. 1965. J. Cell Biol. 26:835-843.

34. Shnitka, T. K. 1966. J. Ultrastruct. Res. 15:598-625.

35. Vigil, E. L. 1973. Sub-Cell. Biochem. 2:237-285.

36. Thornden, R. D., and K. W. Thirman. 1964. J. Cell Biol. 20:345-350.

37. Graham, R. C., Jr., and M. J. Karnovsky. 1965. J. Histochem. 13:448-453.

38. Allen, J. M., and E. M. Beard. 1965. Science (Wash. D.C.). 149:1507-1509.

39. Novikoff, A. B., and A. B. Novikoff. 1969. J. Cell Biol. 52:1-518.

40. Shnitka, T. K., and G. G. Talibi. 1971. Histochemistry. 27:137-158.

41. Hand, A. R. 1975. Histochemistry. 41:195-206.

42. Venhuis, M. and S. D. Wendelaar-Bonga. 1977. Histochem. J. 9:171-181.

43. Hand, A. R. 1979. J. Histochem. Cytochem. 27:1367-1370.

44. Novikoff, A. B., and S. Goldfischer. 1968. J. Histochem. Cytochem. 16:597(abstr.).

45. Novikoff, A. B., and S. Goldfischer. 1969. J. Histochem. Cytochem. 17:675-680.

46. Graham, R. C., Jr., and M. J. Karnovsky. 1966. J. Histochem. Cytochem. 14:291-302.

47. Hirai, K. I. 1969. J. Histochem. Cytochem. 17:585-590.

48. Fahimi, H. D. 1969. J. Cell Biol. 42:275-288.

49. Fahimi, H. D. 1975. Tech. Biochem. Biophys. Morphol. 2:197-245.

50. Goldfischer, S., and E. Esterer. 1969. J. Histochem. Cytochem. 17:681-685.

51. Goldfischer, S., and E. Esterer. 1970. J. Histochem. Cytochem. 18:482-499.

52. Kuhn, C. 1968. Z. Zellforsch. Mikrosk. Anat. 90:534-562.

53. Hruban, Z., E. L. Vigil, A. Slesers, and E. Hopkins. 1972. Lab. Invest. 28:184-191.

54. Novikoff, A. B., P. M. Novikoff, C. Davis, and N. Quintana. 1972. J. Histochem. Cytochem. 20:1006-1023.

55. Novikoff, A. B., P. M. Novikoff, C. Davis, and N. Quintana. 1973. J. Histochem. Cytochem. 21:727-755.

56. Novikoff, P. M., A. B. Novikoff, N. Quintana, and C. Davis. 1973. J. Histochem. Cytochem. 21:540-558.

57. Novikoff, A. B., P. M. Novikoff, C. Davis, and N. Quintana. 1973. J. Histochem. Cytochem. 21:1010-1020.

58. Novikoff, A. B., P. M. Novikoff, C. Davis, and N. Quintana. 1973. J. Histochem. Cytochem. 21:963-966.

59. Baker, E. 1967. Lab. Invest. 17:1-81.

60. Shnitka, T. K., and G. G. Talibi. 1971. Histochemistry. 27:137-158.
98. Donaldson, R. P., N. E. Tolbert, and C. Schnarrenberger. 1972. Arch.
Biochem. Biophys. 152:199–215.
99. Donaldson, R. P., and H. Beevers. 1977. Plant Physiol. (Bethesda). 59:259–
263.
100. Bieglmayer, C., G. Nahler, and H. Ruis. 1974. Hoppe-Seyler's Z. Physiol.
Chem. 355:1121–1128.
101. Muto, S., and H. Beevers. 1974. Plant Physiol. (Bethesda). 54:23–28.
102. Novikoff, A. B., and W. Y. Shin. 1964. J. Micro. (Paris). 3:187–206.
103. Hess, R., W. Staubli, and W. Riess. 1965. Nature (Lond.). 208:856–858.
104. Svoboda, D., and D. Azarnoff. 1966. J. Cell Biol. 30:442–450.
105. Reddy, J. K. 1973. J. Histochem. Cytocem. 21:967–971.
106. Rigattuso, J. L., P. G. Legg, and R. L. Wood. 1970. J. Histochem. Cytocem.
18:993–990.
107. Legg, P. G., and R. L. Wood. 1970. J. Cell Biol. 45:118–129.
108. Novikoff, A. B., P. M. Novikoff, N. Quintana, and C. Davis. 1972. J.
Histochem. Cytocem. 20:745–749.
109. Bock, P. 1972. Acta Histochem. 43:92–97.
110. Seligman, A., A. W. Shannon, Y. Hoshino, and R. Plapinger. 1973. J.
Histochem. Cytocem. 21:756–759.
111. Fahimi, H. D. 1974. J. Cell Biol. 63:675–683.
112. Goldman, B. M., and G. Blobel. 1978. Proc. Natl. Acad. Sci. U.S.A. 75:
5066–5070.
113. Krahlng, J. B., R. Gee, J. A. Gauger, and N. E. Tolbert. 1979. J. Cell.
Physiol. 101:375–390.
114. de Duve, C. 1973. J. Histochem. Cytocem. 21:941–948.
115. de Duve, C., P. B. Lazarow, and B. Poole. 1974. Adv. Cytopharmacol. 2:
219–223.
116. Rechcigl, M., and W. E. Heston. 1963. J. Natl. Cancer Inst. 30:855–864.
117. Goldfischer, S., A. B. Johnson, E. Esnser, C. Moore, and R. H. Ritch. 1973.
J. Histochem. Cytocem. 21:972–977.
118. Murphy, P. A., J. B. Krahlng, R. Gee, J. R. Kirk, and N. E. Tolbert. 1979.
Arch. Biochem. Biophys. 193:179–185.
119. Thurman, R. G. 1977. Fed. Proc. 36:1640–1646.