Microperoxisomes were found to be abundant in the retinal pigment epithelium of the human, rhesus monkey, mice, rats, domestic fowl, and frog by ultrastructural histochemistry. They were rare in other cells of the retina and choroid. These organelles had a granular matrix, ranged in diameter from 0.15 μm to 0.30 μm, and were bound by a single tripartite membrane which often maintained slender connections with the smooth endoplasmic reticulum and other microperoxisomes. They exhibited a positive reaction (electron opaque product) following incubation in diaminobenzidine and H₂O₂, for the demonstration of the peroxidatic activity of catalase (Novikoff et al., J. Histochem. Cytochem. 20: 1006, 1972). The reaction was inhibited by: (1) aminotriazole; (2) dichlorophenol-indophenol; (3) preheating at 95° C.; or (4) elimination of H₂O₂. Microperoxisomes, like the well-known peroxisomes (microbodies) of liver cells have been implicated in various aspects of lipid metabolism and the detoxification of H₂O₂. We demonstrated for the first time that microperoxisomes respond to drug-induced changes in lipid metabolism, as previously shown for peroxisomes. Nafenopin is a recently utilized drug which greatly decreases serum lipids, increases hepatic catalase activity, and induces an increased size and number of hepatic peroxisomes. Black, beige, albino, and obese mutant mice of the C57BL/6J strain treated with nafenopin for several weeks showed a two- to threefold increase in the number of microperoxisomes in the retinal pigment epithelium. Microperoxisomes of the retinal pigment epithelium may be involved in the transport, storage, and rapid turnover of lipids associated with the maintenance of photoreceptor outer segment disc membranes.

Peroxisomes (microbodies), which range in diameter from 0.2 μm to 0.8 μm and contain para-crystalline nucleoid cores are well-known components of kidney and liver cells. Recently, microperoxisomes, which differ from peroxisomes mainly in size (usually 0.15 μm to 0.25 μm) and their lack of nucleoids, have been established as essentially ubiquitous cell organelles. They have spherical to ovoid shapes, a granular matrix and size according to cell catalase content and the delimiting membrane which is continuous with the endoplasmic reticulum. Typically they contain the hydrogen peroxide-destroying enzyme, catalase, and various oxidases which generate hydrogen peroxide, as demonstrated by advanced techniques in fine structure histochemistry and cell fractionation. The role of microperoxisomes is not clearly defined, but these organelles are more numerous in cells with high lipid content or turnover, and often they associate with lipid droplets and lipofuscin granules.

Hepatic peroxisomes vary in number and size according to cell catalase content and the level of serum lipids. We confirmed reports that microperoxisomes are numerous in retinal pigment epithelium of rodents, and initiated comparative and functional studies in humans, rhesus monkeys, mice, rats, domestic fowl, and frogs. A general abundance of microperoxisomes in retinal pigment epithelium is of
interest considering their implication in lipid metabolism, \(^1\), \(^2\), \(^4\) and the active role of pigment epithelium in the turnover of rod outer segment membranes.\(^6\)

Experiments were designed to test the relation of microperoxisomes to lipid metabolism. Since catalase activity can be increased and serum lipids decreased with nafenopin,\(^8\) this drug was fed to cogenic mice. We compared the responses of retinal microperoxisomes to the responses of hepatic peroxisomes in four mutants of the C57BL/6J strain. Histochemistry for the intracellular localization of the peroxidatic activity of catalase\(^2\) was utilized in conjunction with electron microscopy and light microscopy.

**Materials and methods.**

**Tissue source.** A human eye enucleated for ocular melanoma from a 25-year-old white female was obtained within 30 minutes of surgery. Fetal, newborn, and adult rhesus monkeys (Macaca mulatta) as well as Sprague-Dawley rats of various ages were utilized. Cogenic mice of the C57BL/6J strain with defined mutations and mice of the C3H/HeJ strain were obtained from the Jackson Laboratory, Bar Harbor, Me. These mice were maintained in the laboratory at room temperature and 12-hour cyclic illumination which never exceeded four foot candles. All the albinos and many of the others were raised from birth under these conditions. They were sacrificed at 30 to 90 days of age, except as noted. A white leghorn rooster one year of age and chicks two days after hatching were obtained locally. Male frogs of the subspecies *Rana pipiens pipiens* were maintained in the laboratory at 5° C. for a few weeks before use. All animals were sacrificed by injection of a lethal dose of sodium pentobarbital. Cell cultures of chick retinal pigment epithelium were prepared and maintained in Ham’s F-12 medium as described previously.\(^7\)

**Drug treatment.** Nafenopin (2-methyl-2-[\(p\)-(1,2,3,4-tetrahydro-1-naphthyl) phenoxyl]-propionic acid; Su 13437) was obtained gratis from the CIBA-Geigy Corporation, Summit, N. J. It was mixed thoroughly with pulverized Wayne Labbrox rat and mouse ration from Allied Mills, Inc., Chicago, Ill., in concentrations of 0.15 per cent to 0.3 per cent. The ingestion of drug was approximately 0.2 mg. to 0.4 mg. per gram of body weight per day. Control mice were fed pulverized ration alone. All were given water ad libitum.

**Electron microscopy.** Portions of retina and liver were excised and fixed for approximately four hours at 4° C. in either 2.5 per cent glutaraldehyde and 6 per cent sucrose buffered to pH 7.2 with 50 mM sodium cacodylate or in 4 per cent glutaraldehyde buffered with 150 mM Na-K phosphate. The tissues were washed in buffer for several hours, cut with a sharp razor blade into pieces 200 \(\mu\)m to 400 \(\mu\)m, and incubated as indicated below. Following incubation they were washed in buffer (pH 7.2), postfixed in 1 per cent osmium tetroxide with 150 mM Na-K phosphate buffer (pH 7.2), and embedded in epon for electron microscopy. Only the first 100 \(\mu\)m of each piece was sectioned in order to avoid artifacts related to limited penetration by the histochemical media. Sections were examined and photographed with a JEM 100B electron microscope, unstained or after staining with uranyl acetate and lead citrate.

**Histochemistry.** Microperoxisomes were demonstrated by electron opaque reaction product following incubation for 90 to 120 minutes at 37° C. in 3,3-diaminobenzidine tetrahydrochloride (Sigma No. D-8128) and H\(_2\)O\(_2\) (Baker No. 2186) buffered at pH 9.7 with 2-amino-2-methyl-1,3-propanediol (Sigma No. A-9754).\(^7\) Potassium cyanide (5 mM) was included to prevent reaction of the cytochrome oxidase of mitochondria. The peroxidase reaction was inhibited in control tissues by 20 mM 3-amino-1,2,4-triazole (Sigma No. A-4383), 5 mM 2,6-dichlorophenol-indophenol (Sigma No. D-1878), elimination of H\(_2\)O\(_2\) or heating at 95° C. for 10 minutes prior to incubation. Acid phosphatase histochemistry was carried out as described previously.\(^5\)

**Results.** Membrane-bound organelles resembling classical microbodies (peroxisomes) but ranging in size from 0.15 \(\mu\)m to 0.3 \(\mu\)m were common in retinal pigment epithelium. They were distributed throughout the cytoplasm but were more abundant in the basal regions and along the lateral cell boundaries. They were spherical to oval and had granular matrices. Occurring in clusters, they often exhibited slender continuities with the smooth endoplasmic reticulum and with each other. These bodies were identified as microperoxisomes since they stained intensely following incubation at pH 9.7 in a medium containing diaminobenzidine, hydrogen peroxide, and potassium cyanide. Their staining was reduced following control incubations. Thus, these organelles exhibited the peroxidatic activity of catalase typical of peroxisomes and microperoxisomes. Unlike peroxisomes, which contain a characteristic paracrystalline nucleoid structure, these microperoxisomes had a matrix free of inclusions. Although hepatocytes contained both peroxisomes and microperoxisomes, no peroxisomes were found in the retinal pigment epithelium.

Microperoxisomes were found only rarely in cells of the neural retina and choroid.

Erythrocytes and certain parts of secondary lysosomes and lipofuscin granules as well as the microperoxisomes showed reaction product following incubations in diaminobenzidine. Since only the reaction of microperoxisomes was eliminated in control incubations, the other staining must have been nonspecific and not indicative of peroxidase activity.
activity. No other nonenzymatic reaction was observed.

*Human and monkey.* The human eye utilized had a juxtafoveal malignant melanoma measuring 6 mm. by 6 mm., by 3 mm. Areas of the retina and choroid more than 1.0 mm. from the lesion were processed for electron microscopy and ultrastructural histochemistry. The fine structure of the retinal pigment epithelium appeared normal. Annulate lamellae were present consistently in the lower regions of the cells near their lateral boundaries. Lipofuscin granules as well melanin granules were abundant in the middle and apical regions, respectively.

Histochemically reacted microperoxisomes in the basal portion of the cell are seen in Figs. 1 and 2. The mitochondria and other organelles remained unreacted owing to the high pH of the incubation medium and the presence of cyanide. The coated vesicles, polyribosomes, and smooth endoplasmic reticulum of these micrographs were made visible by routine staining of the sections. Unstained sections of histochemically reacted tissue showed only the reacted microperoxisomes and dense melanin granules against a very low-contrast background.

Adult monkeys had somewhat fewer microperoxisomes in their retinal pigment epithelium than did the human. Fetal and neonatal monkeys had fewer still. Monkey microperoxisomes reacted significantly less with diaminobenzidine in the presence of hydrogen peroxide and cyanide at pH 9.7 at comparable incubation times. Often the reaction was so weak that treated tissues could barely be distinguished from controls.

*Other vertebrates.* The retinal pigment epithelium from mice of the C57BL/6J and C3H/HeJ strains and from Sprague-Dawley rats was generally similar to human retinal pigment epithelium. The main differences were a reduction in number of lipofuscin granules, and an absence of annulate lamellae. Numerous microperoxisomes which reacted strongly to catalase histochemistry were distributed mainly in the basal regions and along intercellular junctions (Fig. 3). Some were spherical, but most were rod-shaped with average dimensions of 0.10 μm by 0.20 μm.

Albino mice (C57BL/6J-c<sup>+/-</sup>/c<sup>+</sup>−) and mice lacking rod outer segments (C3H/HeJ-rd/rd and C57BL/6J-rd/rd) had normal numbers of microperoxisomes compared to the standard black mouse. Obese mice (C57BL/6J-ob/ob) had somewhat
Fig. 2. Microperoxisomes in human retinal pigment epithelium showing direct connections (arrows) with tubules of the smooth endoplasmic reticulum. The small microperoxisome in micrograph B connects with the endoplasmic reticulum on both sides. (×76,000.)

Fig. 3. Retinal pigment epithelium of the beige mouse showing histochemically reacted microperoxisomes (arrows) distributed mainly near the basal cell infoldings and along a lateral cell boundary (CB). No significant associations of microperoxisomes with the phagosomes (Ph) or the giant “Chediak-Higashi” granules (G) typical of this mutant were noted. (×17,400.)
faster. Generally, older animals (about 15 months) had more microperoxisomes than did younger ones. Beige mice (C57BL/6J-hg/bg) had more than any other mutant. Notably the microperoxisomes of the beige mutant did not undergo multiple fusions or fuse with the giant "Chediak-Higashi" granules, as did the lysosomes, and they showed no acid phosphatase activity by histochemical procedures.

In the domestic fowl, microperoxisomes of the retinal pigment epithelium were similar in structure to those of the human, but they were less numerous, showed less histochemical reaction for catalase, and did not accumulate in the basal regions. Fewer were present in the hatching chick than in the rooster. These organelles were spherical or oval with diameters of 0.10 μm to 0.30 μm, or oval to pearshaped with dimensions of about 0.19 μm in width by 0.35 μm in length. In addition to typical association with the smooth endoplasmic reticulum, these microperoxisomes often maintained membranous connections with myeloid bodies which consisted of membrane lamellae about 1.9 μm in length stacked to approximately 0.70 μm high in the rooster, and measuring 0.72 μm by 0.17 μm in the hatching chick. A few microperoxisomes were found near Golgi bodies, but no significant association was noted.

Tissue explants of retinal pigment epithelium from six-day-old chick embryos were incubated histochemically for catalase after eight weeks of growth in culture. Cultured retinal pigment epithelium lacked basal infoldings, had few microvilli, and contained a greatly reduced amount of smooth endoplasmic reticulum. Microperoxisomes were found to be very rare and only moderately reacted. They had typical connections with the smooth endoplasmic reticulum.

The pigment epithelium of the frog retina under conditions of hibernation contained a few lightly reacted microperoxisomes of typical structure. Most of them were adjacent to the myeloid bodies, but no direct connections were observed.

**Drug experiments.** Nafenopin, a drug which simultaneously induces increased liver catalase activity and lowered serum lipid levels, was fed to black, beige, albino, and obese mice of the C57BL/6 strain with similar results. The treated mice gained weight more slowly than did the control mice, and their livers became significantly enlarged compared with controls. After two weeks the treated mice began to lose weight while their littersmates on the standard diet continued to gain.

After four to ten weeks of continuous nafenopin administration the treated mice and their untreated littersmates were sacrificed. The eyes and livers were processed for catalase histochemistry and electron microscopy. The hepatocytes of treated animals were replete with densely reacted peroxisomes. The number of peroxisomes per cell was increased manifold over controls in all the treated animals; as readily seen by either light or electron microscopy.

The number of microperoxisomes increased, but their distribution remained unaltered in the retinal pigment epithelium of the treated mice (Fig. 4). The number of microperoxisomes in the retinal pigment epithelium averaged two to three times greater in all the mice that were fed nafenopin than in their untreated littersmates. Lipid droplets often accumulated in junctional regions along with the microperoxisomes. No significant differences were observed among animals treated with 0.15 per cent and 0.3 per cent nafenopin, or among those treated for four weeks compared to ten weeks. Drug treatment did not alter the low number of microperoxisomes in cells of the neural retina or choroid.

**Discussion.** We found microperoxisomes to be abundant in the retinal pigment epithelium and to be rare in other cells of the retina and choroid. This study confirmed work on rodent retinal pigment epithelium; added descriptions of retinal microperoxisomes in the human, other mammals, and lower vertebrates; and provided experimental evidence relevant to determining possible functions of microperoxisomes.

Although microperoxisomes are ubiquitous, apparently an abundance equivalent to that observed in the retinal pigment epithelium is attained only in certain kidney cells, in hepatocytes, and in several cells of the gastrointestinal tract. A well-developed smooth endoplasmic reticulum (SER) is typical of most of the cells in which these bodies are numerous. Direct connections between microperoxisomes and the SER were found consistently, and we observed continuities with myeloid bodies in rooster retinal pigment epithelium. Cultured pigment epithelial cells from the chick had greatly reduced amounts of SER and very few microperoxisomes. Such associations suggest that microperoxisomes and the SER may be related functionally. We experimentally induced an increase in the number of microperoxisomes in retinal pigment epithelium. The inducing agent (nafenopin) simultaneously caused hepatic peroxisome (microbody) proliferation, increased liver catalase activity, and decreased serum lipid levels.

Similarly, an increased microperoxisome number in correlation with increased cell catalase activity was reported for normal smooth muscle cells which became foam cells in atheromatous aortas of cholesterol-fed rabbits. Topographical association of microperoxisomes with lipid droplets and lipofuscin granules has been reported repeatedly. Our findings provide experimental evidence for the suggestions that microperoxisomes are probably involved in some aspects of lipid metabolism, lipid transport, and lipid storage, including the detoxification of H₂O₂.
Fig. 4. Retinal pigment epithelium of a beige mouse following five weeks of 0.15 per cent nafenopin treatment. Compared to its littermate control, this animal showed a threefold increase in the number of microperoxisomes (arrows), and some accumulation of lipid droplets (asterisks). Microperoxisome size remained essentially the same, but more clustering occurred. (x38,000.)

However, the involvement of microperoxisomes with lipid metabolism may not be direct. It is notable that we found only slightly decreased numbers of microperoxisomes in obese mice which had congenital hyperlipidemia. Among other possibilities, perhaps the induced increases in hepatic peroxisomes and in retinal microperoxisomes resulted from processes of drug detoxification or from other drug-induced changes that are not directly related to serum lipid or cholesterol levels. Currently we are determining the number of hepatic peroxisomes and retinal microperoxisomes...
in mouse strains which have been specially selected over many generations for low and high serum cholesterol levels, respectively.

The abundance of microperoxisomes in the retinal pigment epithelium, and the paucity in neural retina and choroid suggest that these organelles may have an important role in the eye. Considering their response to induced hyperlipidemia and evidence implicating them with metabolic disorders of lipid metabolism,\(^9\) \(^10\) it is tempting to speculate that microperoxisomes are associated in some way with the digestion or renewal of rod outer segment membranes. The fewer microperoxisomes observed in prenatal and neonatal animals compared to adults may reflect differences in renewal kinetics and not simply developmental changes. The persistence of microperoxisomes in the retinal pigment epithelium of hibernating frogs and also in mice with congenital retinal degeneration indicates that active turnover of rod outer segments is not required to maintain the capabilities provided by microperoxisomes. In any case, probably these organelles have various functions, some of which are unrelated to rod membrane turnover. Microperoxisomes are sensitive intracellular indicators of metabolic state, and they can be useful in distinguishing normal from pathologic conditions,\(^9\) \(^10\) but their precise relation to lipid metabolism remains unknown.

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REFERENCES


Use of a platelet-fibrinogen-thrombin mixture as a corneal adhesive: experiments with sutureless lamellar keratoplasty in the rabbit.\(^*\) A. RALPH ROSENTHAL, CHRISTINA HARBUGY, PETER R. EGBERT, AND EDWARD RUBENSTEIN.

A platelet-fibrinogen-thrombin mixture utilizing autologous platelets was studied for its potential as a corneal adhesive. In the rabbit it demonstrated sufficient adhesive properties to allow 50 per cent of lamellar keratoplasties (autotransplants) to remain in place without the use of sutures. The mixture retains significant adhesive properties for four to six days. It is simple to prepare and apply. It also appears nonantigenic and nontoxic to the cornea; it does not incite inflammation, nor interfere with corneal clarity or the regrowth of corneal epithelium.

Thrombin\(^1\) and a mixture of plasma and thrombin\(^2\) have been used in the past as tissue adhesives in ophthalmic surgery. These agents did not gain acceptance, presumably because of unsatisfactory adhesive properties. Work at our institution has