UNUSUAL RESPONSES OF RAT

HEPATIC AND RENAL PEROXISOMES TO RMI 14, 514,

A NEW HYPOLIPIDEMIC AGENT

DONALD J. SVOBODA

From the Department of Pathology, University of Kansas, College of Health Sciences and Hospital, Kansas City, Kansas 66103

ABSTRACT

RMI 14, 514 ([5-tetradecycloxy]-2-furancarboxylic acid) represents a new class of hypolipidemic agents which cause unusual ultrastructural changes in liver of male rats and in selected peroxisomal enzymes in liver and kidney of both sexes. Among the principal ultrastructural changes in peroxisomes of male rat liver were (a) cavitation and compartmentalization of the matrix, often giving the appearance of a peroxisome-within-a-peroxisome, and (b) narrow, dense extensions of canaliculi or cisterns from the periphery of the peroxisome, forming partial circlets or surrounding irregular areas of cytoplasm. The unusual enzyme responses were (a) elevation of catalase activity in liver and kidney in female rats, (b) increased activity of three hydrogen peroxide-producing oxidases (urate oxidase, L-α-hydroxy acid oxidase, and D-amino acid oxidase) in the liver of both sexes, and (c) elevation of activity of the last two oxidases in male kidney. The peculiar ultrastructural changes in liver peroxisomes combined with the responses of selected peroxisomal enzymes represent unusual modulations or adaptations of these organelles to a hypolipidemic agent, the effects of which have not been reported extensively.

KEY WORDS   peroxisomes · liver · kidney · hypolipidemia · RMI 14, 514 · oxidases

The proliferation of peroxisomes, especially in male rat or mouse liver, after administration of Clofibrate (CPIB, ethyl-α-p-chlorophenoxyisobutyrate; Ayerst Laboratories, New York) (23, 24, 42) or other hypolipidemic drugs with chemical structure similar to that of Clofibrate (12, 47) or to that of hypolipidemic drugs unrelated to Clofibrate (37), has been amply documented. Characteristically, in the absence of other drugs that bind catalase or inhibit its synthesis, the proliferation of hepatic peroxisomes is accompanied by a simultaneous increase in activity of catalase, one of the principal enzymes of these organelles, and by a variety of subtle morphological alterations such as absence of a nucleoid, changes in matrix density, appearance of matrix tubules or striations, marginal plates, and elongated profiles.

One purpose of this paper is to report unusual ultrastructural abnormalities in hepatic peroxisomes of male rats given RMI 14, 514 ([5-tetradecycloxy]-2-furancarboxylic acid) (Fig. 1). The principal ultrastructural abnormalities of hepatic peroxisomes illustrated in this paper are peculiar and have not been reported previously, to our knowledge. RMI 14, 514 represents a new class of drugs which produce hypolipidemic effects in
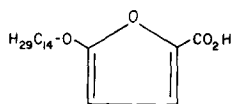
$H_{29}C_{14}$-O $\diagup$ $\diagdown$ CO_2H

O

FIGURE I

rats and monkeys. In rats, the drug produces marked reduction in plasma cholesterol and triglyceride levels and reduces hepatic fatty acid synthesis to one-third of control levels. Hepatomegaly and effects on liver fat are less than after Clofibrate treatment. RMI 14, 514 is reported to be more effective than Clofibrate in reducing plasma cholesterol levels, whereas plasma triglycerides are reduced to approximately the same degree by CPIB and RMI 14, 514. Preliminary studies indicate that RMI 14, 514 not only differs from Clofibrate and its aryloxyisobutyrate analogues in chemical structure but also in its probable mechanism of action (18, 32, 33). RMI 14, 514 also differs in chemical configuration from the novel hypolipidemic, non-Clofibrate-like drugs (tibric acid, Wy 14643) reported by Reddy and Krishnakantha (37) to cause peroxisomal proliferation and increased catalase activity in liver cells of rats and mice.

The second purpose of this paper is to report certain changes in activity of selected peroxisomal enzymes that occur in hepatic and renal cells in rats given RMI 14, 514, changes that differ from behavior of these enzymes after administration of other hypolipidemic agents of various chemical configurations and modes of action. Early biochemical studies indicated that peroxisomes contained not only catalase but also urate oxidase, D-amino acid oxidase, L-α-hydroxy acid oxidase, and isocitrate dehydrogenase (4, 25). With few exceptions (14), despite the characteristic increase in hepatic peroxisomes and catalase activity in male rats given Clofibrate, some of the oxidases of hepatic peroxisomes, notably D-amino acid oxidase, urate oxidase and L-α-hydroxy acid oxidase (in those instances in which these enzymes were studied), remained unchanged or decreased significantly (2, 8, 9, 10, 11, 24, 28, 44). In contrast, after animals are given RMI 14, 514, there is significant elevation in urate oxidase, L-α-hydroxy acid oxidase, and D-amino acid oxidase in the liver of both males and females (Table I) and, moreover, activity of the latter two oxidases increases in kidneys of males (Table III), an observation not previously reported. Although the elevation of catalase activity and peroxisome response in male rat liver after RMI 14, 514 treat-

ment is not unexpected in view of similar findings with several other hypolipidemic drugs, the spectrum of other changes produced by RMI 14, 514, namely (a) the ultrastructural abnormalities in hepatic peroxisomes, (b) the elevation of catalase activity in female rat liver and kidney, and (c) the increased activity of three hydrogen peroxide-producing oxidases in liver of both sexes and of two peroxisomal oxidases in male rat kidney, constitutes unusual adaptations of peroxisomes and adds to the currently available knowledge regarding various modulations of these organelles to experimental manipulations.

MATERIALS AND METHODS

F-344 rats of both sexes weighing between 126 and 140 g were housed in individual cages, given water and Purina Rat Chow (Ralston Purina Co., St. Louis., Mo.) ad lib., and weighed daily. RMI 14, 514, obtained from Merrell-National Laboratories, Cincinnati, Ohio, was suspended in ethyl alcohol and given by gavage each morning at 8 a.m. at a dose of 150 mg/kg body weight once a day for 7 successive days. Control rats of both sexes were given equal amounts of alcohol without RMI 14, 514 by daily gavage. At 10 a.m. on the 7th day, the animals were sacrificed, the livers were weighed, and samples of liver, kidney, and intestinal mucosa were taken from each animal for biochemical and ultrastructural study. 2 ml of blood were collected from the aorta of 13 control and five experimental animals of each sex for determination of triglycerides, cholesterol, and proteins. The number of control and experimental animals of each sex used for study of liver and kidney is indicated in Tables I and III, respectively. Because a previous study from this laboratory had shown that Clofibrate caused significant increase in microperoxisomes and in catalase activity in the mucosa of jejunum and ileum of male rats and, to a lesser extent, in the jejunal mucosa of female rats (41), samples of mucosa from jejunum and ileum were taken from nine control and five experimental rats of each sex to compare the effect of RMI 14, 514 to that of Clofibrate on intestinal catalase activity. Pure samples of mucosa were prepared and confirmed by light microscopy as previously described (41).

Biochemical and Electron Microscope Methods

For measurement of catalase activity, samples of liver, intestinal mucosa, and kidney were homogenized in M/150 phosphate buffer at 1°-4°C in a Potter-Elvehjem homogenizer (Potter Instrument Co., Inc., Plainview, N.Y.) in a 1:10 or 1:20 dilution. Catalase activity was measured by the spectrophotometric method of Lück (27). Total protein in liver, kidney, intestinal mucosa, and serum were determined by the Lowry method (26). Serum triglycerides were measured by the method of

TABLE I

Effects of RMI 14,514* on F-344 Rat Liver

	Male						Female					
	Control		Experimental		Change		Control		Experimental		Change	
	No. rats	Liver	No. rats	Liver	P value	%	No. rats	Liver	No. rats	Liver	P value	%
% tissue wt/body wt	15	3.4 ± 0.1	9	3.9 ± 0.1	>0.005	+15	14	3.4 ± 0.14	10	3.9 ± 0.07	>0.01	+15
Catalase (U/mg protein)	15	36.9 ± 1.0	9	77.1 ± 3.3	>0.001	+109	14	29.6 ± 1.3	10	56.7 ± 3.1	>0.001	+92
Uricase (U/mg protein)	10	1.9 ± 0.1	9	3.5 ± 0.2	>0.001	+84	9	2.1 ± 0.1	10	3.5 ± 0.2	>0.001	+67
α-Hydroxy acid oxidase (U/h/mg protein)	10	0.12 ± 0.006	9	0.21 ± 0.02	>0.001	+75	9	0.06 ± 0.005	10	0.09 ± 0.006	>0.005	+50
D-Amino acid oxidase (U/h/mg protein)	10	0.23 ± 0.01	9	0.50 ± 0.04	>0.001	+117	9	0.23 ± 0.02	10	0.43 ± 0.04	>0.001	+87
lipids (%/g wet tissue)	6	4.3 ± 0.6	5	2.5 ± 0.2	>0.025	-42	6	4.7 ± 0.4	5	3.0 ± 0.3	>0.01	-36

* 150 mg/kg/day for 7 days, by gavage.

Fletcher (7). Urate oxidase activity was assayed by the method of Arima and Nose (1), and D-amino acid oxidase and L-α-hydroxy acid oxidase activities were assayed by the method of Robinson et al. (39). For total liver lipids, liver was dried to constant weight, extracted with Bloor's ethanol-ether solvent, then weighed again, and the lipid content was determined by difference. Serum cholesterol was determined by the method of Huang et al. (17).

For routine electron microscopy, small samples of tissue were fixed in 2% osmium tetroxide buffered with S-collidine, and then dehydrated through a graded series of alcohols and propylene oxide. They were embedded in Epon, and ultrathin sections were stained with lead. Semithin (0.5 μm) sections of Epon-embedded tissues were stained with basic fuchsin-crystal violet after removal of the Epon with sodium methoxide. For cytochemical demonstration of the peroxidatic activity of catalase, samples of liver, kidney, and intestinal mucosa were processed according to the methods of Novikoff and Goldfischer (29). Controls consisted of: (a) preincubation for 15 min in propanediol buffer containing 0.02 M 3-amino-1,2,4-triazole followed by incubation in standard Diaminobenzidine (DAB) medium also containing 0.02 M aminotriazole, (b) preincubation for 10 min in propanediol buffer containing 0.1 M KCN followed by incubation in the standard DAB mixture also containing 0.1 M KCN, and (c) incubation in the standard DAB incubation mixture lacking hydrogen peroxide.

RESULTS

Biochemical

The results of enzyme assays on liver are given in Table I. It is apparent that RMI 14, 514 caused significant increase in catalase activity in livers not only in male rats but in intact females as well. The percentage increase in catalase activity in experimental animals compared to controls was 109 and 92% in males and females, respectively. Similarly, there was significant increase in activity of uricase, L-α-hydroxy acid oxidase and D-amino acid oxidase in both sexes. There was a slight increase in liver weight (15%) in both sexes given RMI 14, 514. Total liver lipids decreased slightly more in males than in females.

The results for serum cholesterol, triglycerides, and proteins are given in Table II, where it is apparent that there was significant lowering of serum triglycerides, due to RMI 14, 514, in females. There was a slight decrease in serum cholesterol in both sexes but it was not of the same magnitude as the decrease in triglycerides. The changes in serum triglycerides and cholesterol are consistent with previous reports on the use of this agent (18, 32).

The results of assay of activities of selected renal peroxisomal enzymes are given in Table III, where it is evident that, in males, not only catalase but L-α-hydroxy acid oxidase activity and D-amino acid oxidase activity increased significantly. RMI 14, 514 also caused significant elevation in renal catalase and D-amino acid oxidase activity in females. There was no significant change in L-α-hydroxy acid oxidase in females.

The catalase activity of jejunal and ileal mucosa of male and female rats did not change significantly after 7 days of RMI 14, 514. In control males, the catalase activity (in units per milligram of protein) in jejunum and ileum was 0.65 ± 0.02 and 0.78 ± 0.04, respectively. The values in experimental males were 0.77 ± 0.06 in the jejunum and 1.21 ± 0.2 in the ileum. In control females, the catalase activity in jejunum and ileum was 0.90 ± 0.08 and 0.93 ± 0.08, whereas in experimental females the values for jejunum and ileum, respectively, were 0.91 ± 0.09 and 1.16 ± 0.16.

Morphological

Ultrastructural abnormalities in peroxisomes were observed only in the liver. Peroxisomes in kidney and in intestinal mucosa appeared normal in size and structure, though they were increased in number in renal tubular epithelium.

Although the mean diameter of hepatic peroxisomes in experimental animals was 0.3–0.5 μm, several reached a diameter of 0.9 μm. Abnormalities in peroxisomes were present in all zones of the hepatic lobule and were not confined to any specific region of individual cells. At low magnifications, the most characteristic change in peroxisomes was the presence of irregularly shaped cavities or defects in their matrix (Fig. 2). In some instances, peroxisomes contained a dense core in their matrix (Fig. 3), but many lacked a typical crystalline nucleoid. At higher magnifications, many peroxisomes contained, in addition to cavities, one or more circular compartments partially but distinctly separated from the remaining matrix by condensations of matrix material surrounded by a limiting membrane, in some instances (Figs. 3 and 4). Occasionally, the material in the compartments was surrounded by a relatively electron-transparent halo (Figs. 4 and 5). In most peroxisomes, in addition to matrix compartments or cavities, there extended, from the periphery of the organelle, narrow linear densities coursing through adjacent cytoplasm and enclosing partial

TABLE II

Effects of RMI 14,514* on Serum Cholesterol, Triglycerides and Protein of F-344 Rats

	Male						Female					
	Control		Experimental		Change		Control		Experimental		Change	
	No. rats	Blood	No. rats	Blood	P value	%	No. rats	Blood	No. rats	Blood	P value	%
Cholesterol‡	13	38.2 ± 3.5	5	31.6 ± 4.2	>0.40§	−17	13	68.6 ± 5.0	5	53.8 ± 4.2	>0.20§	−22
Triglycerides‖	13	43.8 ± 9.8	5	18.6 ± 5.9	>0.20§	−58	13	55.2 ± 7.0	5	12.0 ± 3.2	>0.005	−78
Protein g/100 ml	13	6.18 ± 0.2	5	6.10 ± 0.1	§	−1	13	6.7 ± 0.2	5	6.0 ± 0.1	>0.05	−10

* 150 mg/kg/day for 7 days, by gavage.
‡ mg/dl.
§ Not significant.
‖ mg/100 ml.

TABLE III

Effects of RMI 14,514* on F-344 Rat Kidney

	Male						Female					
	Control		Experimental		Change		Control		Experimental		Change	
	No. rats	Kidney	No. rats	Kidney	P value	%	No. rats	Kidney	No. rats	Kidney	P value	%
Catalase (U/mg protein)	5	14.58 ± 1.13	5	22.4 ± 0.3	>0.001	+54	5	11.16 ± 0.40	5	16.8 ± 0.6	>0.001	+51
α-Hydroxy acid oxidase (U/h/mg protein)	5	0.408 ± 0.008	5	0.61 ± 0.02	>0.001	+49	5	0.374 ± 0.006	5	0.42 ± 0.002	>0.20‡	+12
D-Amino acid oxidase (U/h/mg protein)	5	2.51 ± 0.16	5	3.6 ± 0.2	>0.01	+43	5	2.41 ± 0.06	5	2.9 ± 0.1	>0.01	+20

* 150 mg/kg/day for 7 days, by gavage.
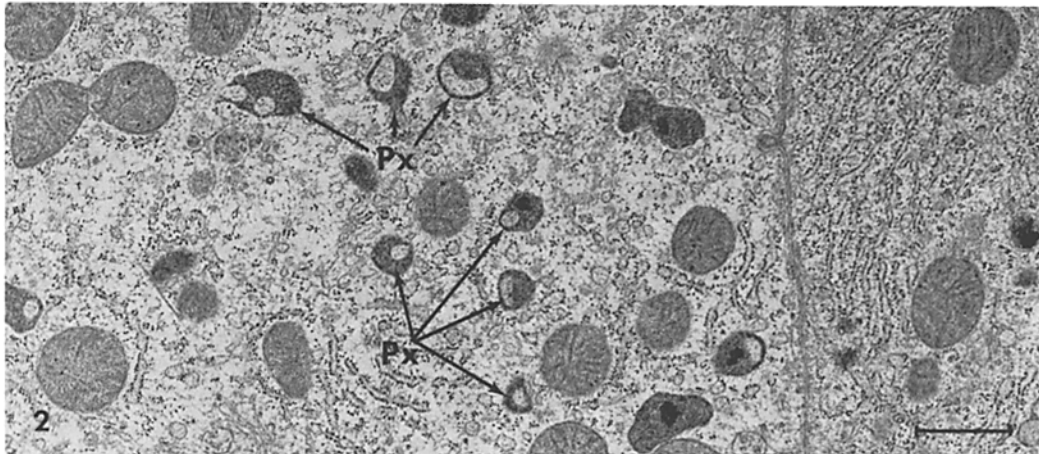‡ Not significant.

FIGURE 2 Several peroxisomes (Px) contain circular or irregularly shaped cavities in their matrix. × 22,800. All illustrations are from sections of liver stained with lead. All bars, 0.5 μm.
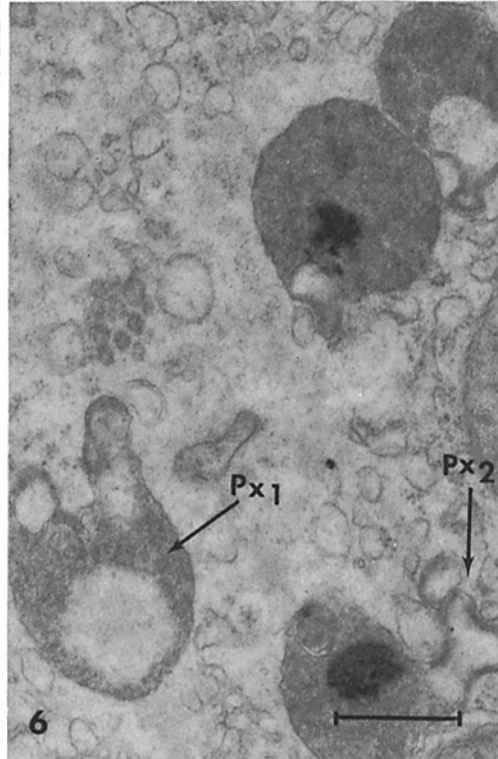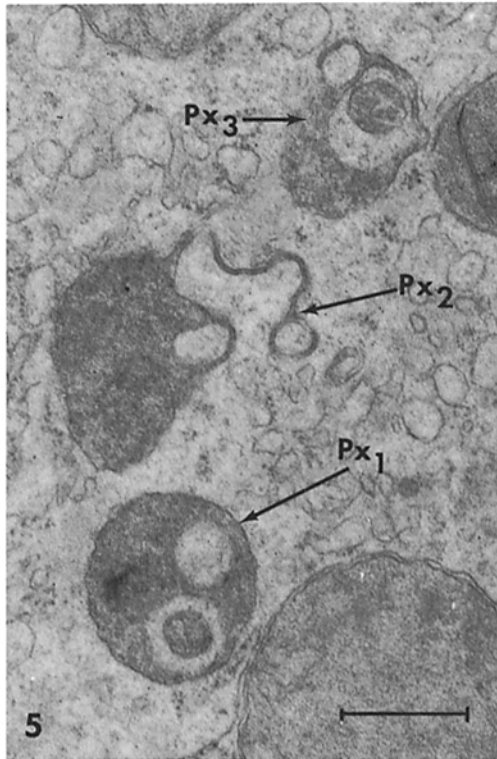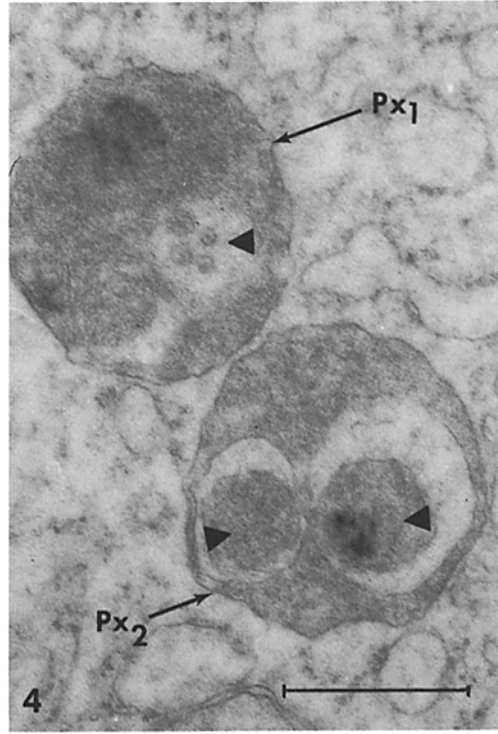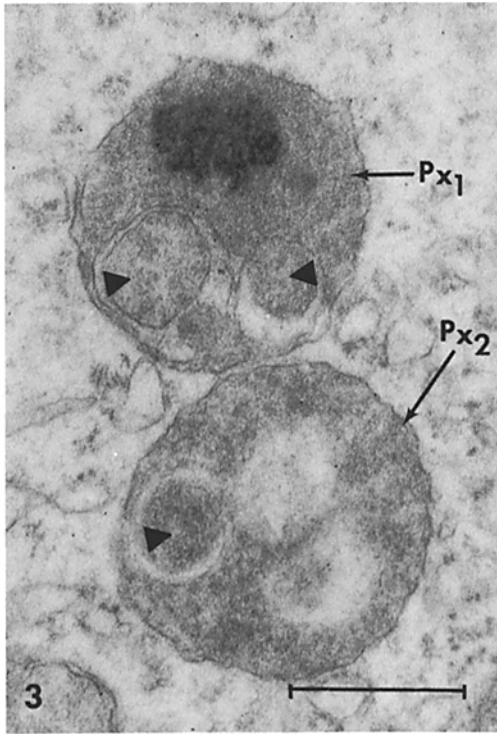
or complete circlets (Figs. 5, 6, 9, and 10). In many peroxisomes, the cavities were large and eccentric, leaving most of the circumference of the organelle consisting of a narrow attenuated band of material of a density similar to that of the matrix (Figs. 7 and 8). The cavities contained filamentous (Fig. 7) or amorphous (Fig. 8) material similar to surrounding hyaloplasm. In some instances, the peroxisomes, save for a small amount of matrix, consisted of several linear or narrow, membrane-bounded canaliculi extending into and enclosing portions of cytoplasm (Fig. 8, open arrowhead, and Fig. 9, Px 3). In many cells, the cavities surrounded a second circular matrix body, giving the appearance of peroxisome-within-a-peroxisome (Fig. 10). In such instances, the inner matrical mass appeared membrane bounded. Similarly, the interior aspect or space within which the smaller matrical mass was located was confined by a membrane. Such changes may represent a more advanced stage of the alterations illustrated in Figs. 3 and 4. No marginal plates, matrix striations, or circumferential "budding" of peroxisomes was seen. Cytochemical preparations demonstrating the peroxidatic activity of catalase confirmed the identity of peroxisomes in their varied configurations, but ultrastructural details illustrated were clearer with primary osmium tetroxide fixation. A slight increase in smooth endoplasmic reticulum was apparent. No mitochondrial alterations were observed.

DISCUSSION

Several studies have reported that peroxisomes are associated with or involved in lipid metabolism in rodents (13, 16, 23, 24, 31, 37, 42). More recently, Sternlieb and Quintana (40) observed that peroxisomes may enlarge or undergo changes in electron opacity of the matrix in association with the steatosis characteristic of early stages of Wilson's disease, suggesting that, in humans also, peroxisomes may be associated with lipid metabolism. Though RMI 14,514 differs in chemical structure and mode of action from hypolipidemic agents reported thus far, its profound effects, especially on hepatic peroxisomes, provide further evidence for a relationship between peroxisomes and lipid metabolism, regardless of the nature of the hypolipidemic chemical.

Studies with RMI 14,514 add to the increasing evidence for the heterogeneity of peroxisomes not only in their morphological alterations but also in their enzymatic responses to different drugs, in different tissues and in different sexes. For example, there are obvious differences between the effects of RMI 14,514 and Clofibrate on peroxisomal enzymes. Catalase activity does not increase in the liver of female rats given 0.25% Clofibrate in their diet unless they are castrated and given testosterone (43), whereas with RMI 14,514 intact females showed a significant increase in hepatic catalase activity comparable to that occurring in males given the same dose. Reddy et al. reported an increase in hepatic catalase in intact females given Nafenopin, an analog of Clofibrate (36). In that study, however, the activities of other peroxisomal oxidases were not investigated. The significant elevation of hepatic uricase, L-α-

hydroxy acid oxidase and D-amino acid oxidase in both sexes given RMI 14,514 also differs from the usual response of these enzymes to Clofibrate or to other hypolipidemic drugs in that, instead of decreasing or remaining unchanged, their activities increased significantly in both sexes. Whether in female rats the metabolism of RMI 14,514 is different from the metabolism of other hypolipidemic drugs has yet to be determined.

Little has been reported on the effects of Clofibrate or other hypolipidemic drugs on renal peroxisomes and their enzymes. In an early report, Svoboda et al. (44) noted only slight increase in male rat renal peroxisomes after 8-10 wk of Clofibrate treatment. Reddy et al. (38), using methyl clofenapate, found marked proliferation in renal peroxisomes in male (wild type) mice, but catalase activity was elevated <30%. In view of the paucity of information regarding the response of L-α-hydroxy acid oxidase and D-amino acid oxidase in rat kidneys after administration of more familiar hypolipidemic agents, it is impossible to compare the renal peroxisomal response to RMI 14,514 in kidneys of male rats with other data. In animals, the catalase activity of kidney is usually lower than that of liver, and the same is true after RMI 14,514 treatment.

Another difference between Clofibrate and RMI 14,514 is their contrasting effect on intestinal mucosal catalase activity. After Clofibrate treatment, the jejunal and ileal mucosal catalase activity in males increased 82 and 92%, respectively (41), whereas after RMI 14,514 treatment, neither sex responded in terms of intestinal catalase activity. It is apparent that the same mechanisms may not influence peroxisomal functions or response in different tissues or in different sexes.

Although elevations in hepatic peroxisomal urate oxidase and D-amino acid oxidase after RMI 14,514 administration are unusual, they are not unique to this chemical. Hruban et al. reported elevation of hepatic urate oxidase and D-amino acid oxidase in livers of male rats given W-1372 (N-[benzyloxy]-N-3[phenylpropyl]acetamide) and 1-benzylimidazole, two compounds affecting blood cholesterol, as well as in rats given cyclizine derivatives (14). Urate oxidase is localized in the core or the nucleoid of peroxisomes (3, 45), and the nucleoid has been reported to increase in size in the peroxisomes of liver in rats given azaserine (15) or terephthalanalides (16). Despite the elevation of hepatic urate oxidase activity after RMI 14,514, no increase in the size of nucleoids was seen.

A number of ultrastructural abnormalities in hepatic peroxisomes in rats given a wide variety of compounds intended to alter the metabolic systems of peroxisomal enzymes was reported by Hruban et al. (16), and the spectrum of morphologic changes in hepatic peroxisomes in humans was compiled and illustrated by Sternlieb and Quintana (40) and by Novikoff et al. (30). Despite some similarities, none of the changes included in these comprehensive reports were identical to those occurring in hepatic peroxisomes after RMI 14,514 treatment. Close structural relationships and continuities between peroxisomes and the smooth endoplasmic reticulum have been observed by several investigators (6, 19, 25, 30, 46), and it has been hypothesized that peroxisomes may form condensations within localized outpocketings of cisterns of the smooth endoplasmic reticulum (5, 35). Moreover, Poole et al. suggested that peroxisomes might not exist as

FIGURE 3 Two peroxisomes contain matrix cavities or compartments. In Px 1, there is a dense core and two matrix compartments (solid arrowheads); the one to the left appears limited by a membrane. In Px 2, there are two cavities containing amorphous material, and one compartment (solid arrowhead) containing material of density similar to that of the matrix. × 44,600.

FIGURE 4 Px 1 contains a core of a density slightly greater than that of the surrounding matrix and a cavity (solid arrowhead) containing circular structures. Px 2 contains two compartments of matrixlike density (solid arrowheads) surrounded by a halo of relative electron transparency. × 44,600.

FIGURE 5 Px 1 contains both a cavity and a compartment containing matrix-like material. Px 2 has a small, eccentric cavity. A narrow, dense band or canaliculus extends from it into the cytoplasm and encloses a circlet at its terminus. Px 3 also has a compartment and a protuberant circlet at its upper periphery. × 31,800.

FIGURE 6 Px 1 has a large, electron-transparent cavity and two irregular extensions at the upper part of its profile. Px 2 has a bandlike linear extension that extends from the periphery to enclose a small portion of cytoplasm. × 31,800.
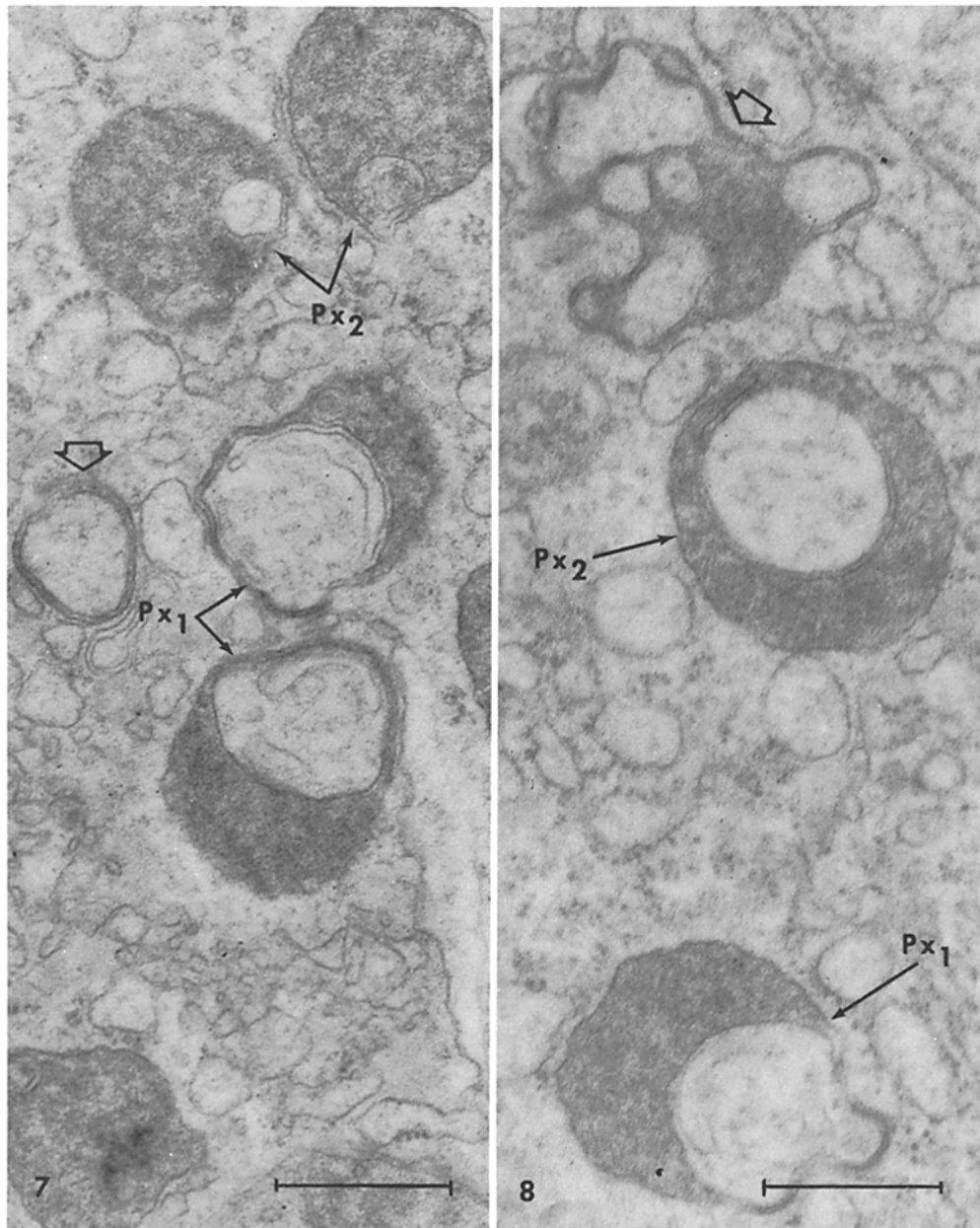
FIGURE 7 *Px 1* illustrates two peroxisomes with large eccentric cavities containing filamentous material. Most of the circumference consists of an attenuated band of material having a density similar to that of the matrix. *Px 2* illustrates two peroxisomes with matrix cavities. The circular, linear density (open arrowhead) may represent a cistern of endoplasmic reticulum containing peroxisome matrix or the periphery of a peroxisome whose major matrical portion is out of the plane of section. × 45,600.

FIGURE 8 *Px 1* and *Px 2* contain large cavities containing amorphous material. The peroxisome indicated by the open arrowhead has an irregularly shaped matrical portion from which extend several membrane-bounded, dense canaliculi enclosing several portions of cytoplasm. × 45,600.
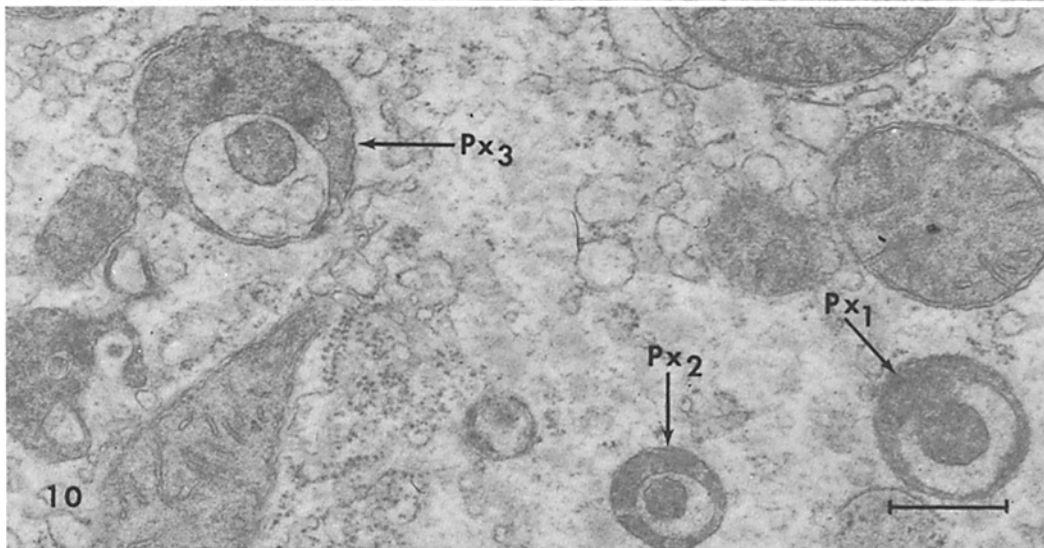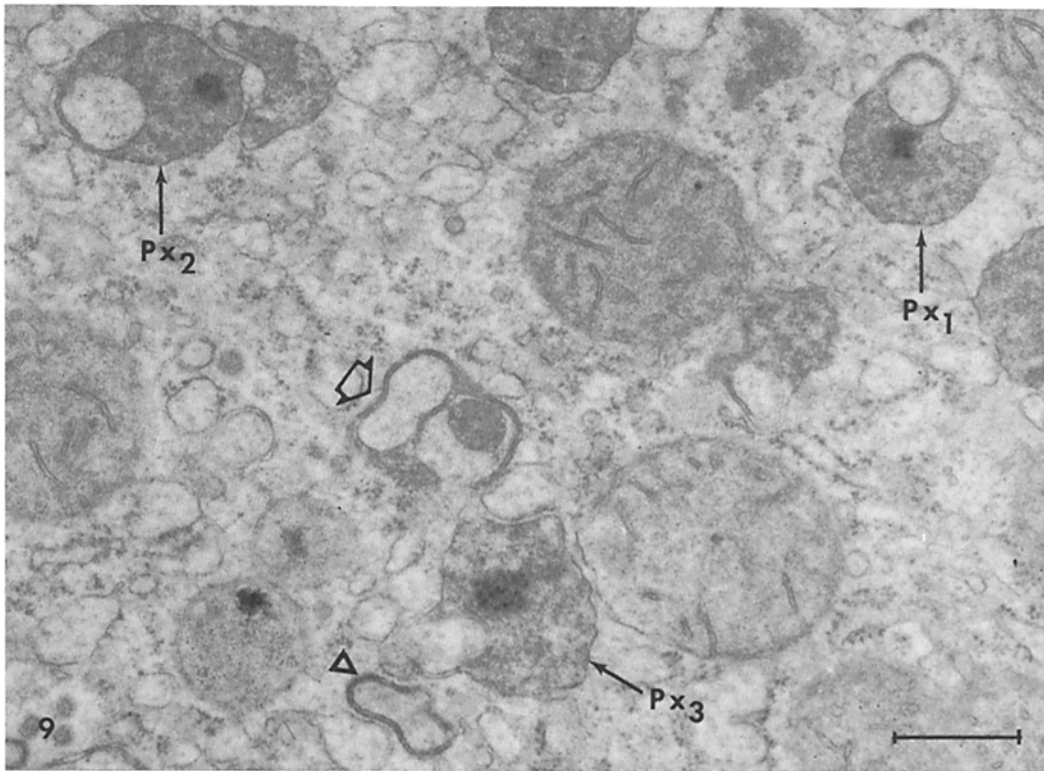
FIGURE 9 *Px 1* and *Px 2* contain small, dense cores and matrix cavities. *Px 3* has linear, dense canaliculi extending from it (large open arrowhead) and partially enclosing a small circular peroxisome-like structure to the left. The small open arrowhead indicates a similar canaliculus or cistern in close proximity to *Px 3*. × 31,800.

FIGURE 10 *Px 1, 2*, and *3*, though of differing diameter in this plane, each contain a small circular, membrane-bounded compartment containing material of a density similar to that of the matrix and imparting a peroxisome-within-a peroxisome appearance. × 31,800.

independent entities but as interconnected clusters of specialized portions of endoplasmic reticulum, with their protein components constituting a single common pool within which there is rapid continuous or intermittent exchange (34). Furthermore, to quote de Duve, "to mention an extreme possibility, it is not inconceivable that peroxisomes form in each cell a single membrane-bound space, of highly contorted and possibly continually varying shape, associated with a special ER region engaged in the manufacturing of the protein constituents" (5). With these observations in mind, the narrow, dense canaliculi that extend from the periphery of some peroxisomes into, and partially or totally surround portions of, cytoplasm and form circlets (Figs. 5, 6, 8, and 9) may represent "flow" or "exchange" of peroxisomal proteins within narrow cisterns of the smooth endoplasmic reticulum, inasmuch as the density of such extensions resembles that of peroxisome matrix and these extensions appear to be continuous with the parent matrix via the narrow channels or canaliculi. If such were the case, it would appear that one of the possible effects of RMI 14,514 is to induce profound perturbations in the cisterns of the smooth endoplasmic reticulum associated with peroxisomes or in the process of protein exchange occurring between peroxisomes and smooth ER.

Aside from the narrow, dense channels extending from peroxisome matrix, the other abnormalities consisted principally of (a) cavitation or defects of the matrix, (b) formation of one or more compartments in the matrix, or (c) the appearance of a targetlike peroxisome-within-a-peroxisome. It is not certain whether these abnormalities represent primary modulations in the smooth endoplasmic reticulum and consequent interference in normal processes of synthesis and assembly in the morphogenesis of peroxisomes or whether they represent injury to or degenerative changes in previously normal peroxisomes. The relatively short half-life of hepatic peroxisomes (~1.5 days) would permit interference with normal morphogenesis during the 7 days in which the animals were given RMI 14,514. The relative scarcity of normal peroxisomes in any hepatic cells after 7 days further suggests continuous interference with their normal formation or assembly. Serial studies with daily or more frequent sacrifice of animals after short intervals on the drug might clarify this problem. The studies of Poole et al. indicating that peroxisomes are destroyed in a random fashion, with an older organelle no more likely to be destroyed at any given time than a younger one (35), indicate that structural abnormalities in peroxisomes need not reflect an age-dependent degeneration or injury.

Hruban et al. described "gastruloid" cisterns as special forms of smooth endoplasmic reticulum (reference 14, Fig. 31, diagrams D and E) consisting of a double-walled loop with an inner pair of concentric rings which bears some resemblance to Fig. 10. Without careful and extensive study of consecutive serial sections, it is impossible to formulate conclusive evidence for the three-dimensional dynamics of such an appearance of "gastruloid" cisterns or the peroxisome-within-a-peroxisome effect as seen after RMI 14,514 administration. Their curious appearance may represent in situ synthesis of peroxisomal matrix proteins within concentric cisterns of the smooth endoplasmic reticulum. The fact that both the inner matrical mass and the interior aspect of the space in which it was located were both membrane-bounded suggests assembly of matrical protein simultaneously in concentric or connected cisterns of the smooth endoplasmic reticulum. Alternatively, the appearance of compartments or targetlike peroxisomes may represent sections through invaginations or indentations into spherical peroxisomes.

Among the most significant and original observations on peroxisomes and their relationship to lipid metabolism are the recent reports by Lazarow and de Duve (21) and by Lazarow (20). Lazarow and de Duve indicated that fatty acyl-CoA oxidizing activity is present in rat liver peroxisomes and increases significantly with treatment of the rats with Clofibrate. There was simultaneous increase in the number of hepatic peroxisomes (21). Also, Lazarow demonstrated that, like Clofibrate, tibric acid and Wy-14,643, given to male rats, caused an 11- to 18-fold increase in the capacity of their livers to oxidize palmitoyl-coenzyme A, and simultaneously caused an increase in hepatic peroxisomes (20). Recently, Lazarow reported that rat liver peroxisomes may be specialized for β-oxidation of long-chain fatty acids (22). These reports shed new light not only on a mechanism of action of Clofibrate, tibric acid, and Wy-14,643 but also on the significance of peroxisome proliferation and the role of peroxisomes in lipid metabolism. In view of the chemical structure of RMI 14,514 and its effects on the ultrastructure and enzyme activity of hepatic peroxisomes, it would be of interest to determine

whether RMI 14,514, like Clofibrate and other hypolipidemic drugs unrelated to the aryloxyisobutyrate group, has a similar effect on hepatic palmitoyl-coenzyme A oxidizing activity. Also, investigation of the effects of long-term administration of RMI 14,514 and serial studies after its withdrawal might prove informative.

REFERENCES

1. ARIMA, K., and K. NOSE. 1968. Studies on bacterial urate: oxygen oxidoreductase. I. Purification and properties of the enzyme. *Biochim. Biophys. Acta.* **151**:54–62.

2. AZARNOFF, D., and D. SVOBODA. 1969. Microbodies in experimentally altered cells. VI. Thyroxine displacement from plasma proteins and clofibrate effect. *Arch. Int. Pharmacodyn. Ther.* **181**:386–393.

3. BAUDHUIN, P., H. BEAUFAY, and C. DE DUVE. 1965. Combined biochemical and morphological study of particulate fractions from rat liver. *J. Cell Biol.* **26**:219–243.

4. BAUM, H. 1974. Mitochondria and peroxisomes. *In* The Cell in Medical Science. F. Beck and J. B. Lloyd, editors. Academic Press Inc., New York. **1**:183–272.

5. DE DUVE, C. 1973. Biochemical studies on the occurrence, biogenesis and life history of mammalian peroxisomes. *J. Histochem. Cytochem.* **21**:941–948.

6. ESSNER, E. 1967. Endoplasmic reticulum and the origin of microbodies in fetal mouse liver. *Lab. Invest.* **17**:71–87.

7. FLETCHER, M. J. 1968. A colorimetric method for estimating serum triglycerides. *Clin. Chim. Acta.* **22**:393–397.

8. GOLDENBERG, H., M. HÜTTINGER, P. KAMPFER, R. KRAMAR, and M. PAVELKA. 1976. Effect of clofibrate application on morphology and enzyme content of liver peroxisomes. *Histochemistry.* **46**:189–196.

9. GOTOH, M., C. GRIFFIN, and Z. HRUBAN. 1975. Effect of citrate and aminotriazole on matrical plates induced in hepatic microbodies. *Virchows Arch. B Cell Pathol.* **17**:279–294.

10. HAYASHI, H., T. SUGA, and S. NIINOBE. 1975. Studies on peroxisomes. V. Effect of ethyl p-chlo-

11. HESS, R., W. STAUBLI, and W. RIESS. 1965. Nature of the hepatomegalic effect produced by ethylchlorophenoxy-isobutyrate in the rat. *Nature (Lond.).* **208**:856–858.

12. HIRAI, K. I., and K. OGAWA. 1975. Ultrastructural studies on the morphogenesis of peroxisomes in mouse hepatocytes treated with simfibrate. *Acta Histochem. Cytochem.* **8**:18–29.

13. HRUBAN, Z., M. GOTOH, A. SLESERS, and S. CHOU. 1974. Structure of hepatic microbodies in rats treated with acetylsalicylic acid, clofibrate, and dimethrin. *Lab. Invest.* **30**:64–75.

14. HRUBAN, Z., Y. MOCHIZUKI, M. GOTOH, A. SLESERS, and S. CHOU. 1974. Effects of some hypocholesterolemic agents on hepatic ultrastructure and microbody enzymes. *Lab. Invest.* **30**:474–485.

15. HRUBAN, Z., H. SWIFT, and A. SLESERS. 1965. Effect of azaserine on the fine structure of the liver and pancreatic acinar cells. *Cancer Res.* **25**:708–723.

16. HRUBAN, Z., H. SWIFT, and A. SLESERS. 1966. Ultrastructural alterations of hepatic microbodies. *Lab. Invest.* **15**:1884–1901.

17. HUANG, T., C. CHEN, V. WEFLER, and A. RAFTERY. 1961. A stable reagent for the Liebermann-Burchard reaction. *Anal. Chem.* **33**:1405–1407.

18. KARIYA, T., R. PARKER, J. GRISAR, J. MARTIN, and L. WILLE. 1975. Laboratory studies with RMI 14,514, a new hypolipidemic agent. *Fed. Proc.* **34**:789. (Abstr.)

19. KARTENBACK, J., and W. W. FRANKE. 1974. Membrane relationships between endoplasmic reticulum and peroxisomes in rat hepatocytes and Morris hepatoma cells. *Cytobiologie.* **10**:152–156.

20. LAZAROW, P. B. 1977. Three hypolipidemic drugs increase hepatic palmitoyl-coenzyme A oxidation in the rat. *Science (Wash. D.C.).* **197**:580–581.

21. LAZAROW, P. B., and C. DE DUVE. 1976. A fatty acyl-CoA oxidizing system in rat liver peroxisomes; enhancement by clofibrate, a hypolipidemic drug. *Proc. Natl. Acad. Sci. U.S.A.* **73**:2043–2046.

22. LAZAROW, P. B. 1978. Rat liver peroxisomes catalyze the β oxidation of fatty acids. *J. Biol. Chem.* **253**:1522–1528.

23. LEGG, P., and R. WOOD. 1969. New observations on microbodies. A cytochemical study on CPIB-treated rat liver. *J. Cell Biol.* **45**:118–129.

24. LEIGHTON, F., L. COLOMA, and C. KOENIG. 1975. Composition, physical properties, and turnover of proliferated peroxisomes. A study of the trophic effects of SU-13437 on rat liver. *J. Cell Biol.* **67**:281–309.

25. LEIGHTON, F., B. POOLE, H. BEAUFAY, P. BAUDHUIN, J. COFFEY, S. FOWLER, and C. DE DUVE. 1968. The large-scale separation of peroxisomes,

mitochondria, and lysosomes from the livers of rats injected with Triton WR-1339. *J. Cell Biol.* **37**:482-513.

26. LOWRY, O., N. ROSEBROUGH, A. FARR, and R. RANDALL. 1957. Protein estimation with the Folin-Ciocalteu reagent. *In* Methods in Enzymology. S. Colowick and N. Kaplan, editors. Academic Press, Inc., New York. **3**:448-450.

27. LÜCK, H. 1965. *In* Catalase Methods of Enzymatic Analysis. H. U. Bergmeyer, editor. Academic Press, Inc., New York. 885-894.

28. MOODY, D. E. and J. K. REDDY. 1976. Comparative effects of hypolipidemic drugs on hepatic peroxisomal enzymes. *Fed. Proc.* **35**:381. (Abstr.)

29. NOVIKOFF, A., and S. GOLDFISCHER. 1969. Visualization of peroxisomes (microbodies) and mitochondria with diaminobenzidine. *J. Histochem. Cytochem.* **17**:675-680.

30. NOVIKOFF, P., A. NOVIKOFF, N. QUINTANA, and C. DAVIS. 1973. Studies on microperoxisomes. III. Observations on human and rat hepatocytes. *J. Histochem. Cytochem.* **21**:540-558.

31. NOVIKOFF, A., and W. SHIN. 1964. The endoplasmic reticulum in the Golgi zone and its relations to microbodies, Golgi apparatus, and autophagic vacuoles in rat liver cells. *J. Microsc. (Paris).* **3**:198-206.

32. PARKER, R., T. KARIYA, J. GRISAR, and V. PETROW. 1975. 5-(tetradecyloxy)-2-furancarboxylic acid (RMI 14,514) and related hypolipidemic fatty acid-like alkyloxyarylcarboxylic acids. *Abstr. Pap. Amer. Chem. Soc.* **170**:25.

33. PARKER, R., T. KARIYA, J. GRISAR, and V. PETROW. 1977. 5-(tetradecyloxy)-2-furancarboxylic acid and related hypolipidemic fatty acid-like alkyloxyarylcarboxylic acid. *J. Med. Chem.* **20**:781-791.

34. POOLE, B., T. HIGASHI, and C. DE DUVE. 1970. The synthesis and turnover of rat peroxisomes. III. The size distribution of peroxisomes and the incorporation of new catalase. *J. Cell Biol.* **45**:408-415.

35. POOLE, B., F. LEIGHTON, and C. DE DUVE. 1969. The synthesis and turnover of rat liver peroxisomes. II. Turnover of peroxisome proteins. *J. Cell Biol.* **41**:536-546.

36. REDDY, J., D. AZARNOFF, D. SVOBODA, and J. PRASAD. 1974. Nafenopin-induced hepatic microbody (peroxisome) proliferation and catalase synthesis in rats and mice. *J. Cell Biol.* **61**:344-348.

37. REDDY, J., and T. KRISHNAKANTHA. 1975. Hepatic peroxisome proliferation: induction by two novel compounds structurally unrelated to clofibrate. *Science (Wash. D.C.).* **190**:787-789.

38. REDDY, J., T. KRISHNAKANTHA, and M. RAO. 1975. Microbody (peroxisome) proliferation in mouse kidney induced by methyl clofenapate. *Virchows Arch. B Cell Pathol.* **17**:295-306.

39. ROBINSON, J., L. KEAY, R. MOLINARI, and I. SIZER. 1962. L-a-hydroxy acid oxidase of hog renal cortex. *J. Biol. Chem.* **237**:2001-2010.

40. STERNLIEB, I., and N. QUINTANA. 1977. The peroxisomes of human hepatocytes. *Lab. Invest.* **36**:140-149.

41. SVOBODA, D. 1976. The response of microperoxisomes in rat small intestinal mucosa to CPIB, a hypolipidemic drug. *Biochem. Pharmacol.* **25**:2750-2752.

42. SVOBODA, D., and D. AZARNOFF. 1966. Response of hepatic microbodies to a hypolipidemic agent, ethyl chlorophenoxyisobutyrate (CPIB). *J. Cell Biol.* **30**:442-450.

43. SVOBODA, D., D. AZARNOFF, and J. REDDY. 1969. Microbodies in experimentally altered cells. II. The relationship of microbody proliferation to endocrine glands. *J. Cell Biol.* **40**:734-746.

44. SVOBODA, D., H. GRADY, and D. AZARNOFF. 1967. Microbodies in experimentally altered cells. *J. Cell Biol.* **35**:127-152.

45. TSUKADA, H., Y. MOCHIZUKI, and S. FUJIWARA. 1966. The nucleoids of rat liver cell microbodies. Fine structure and enzymes. *J. Cell Biol.* **28**:449-460.

46. TSUKADA, H., Y. MOCHIZUKI, and T. KONISHI. 1968. Morphogenesis and development of microbodies of hepatocytes of rats during pre- and postnatal growth. *J. Cell Biol.* **37**:231-243.

47. TUCHWEBER, B., P. KOUROUNAKIS, and J. LATOUR. 1976. Drug metabolism and morphologic changes in the liver of nafenopin-treated rats. *Arch Int. Pharmacodyn. Ther.* **222**:309-321.