Induction of Hepatic Peroxisome Proliferation in Nonrodent Species, Including Primates

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It is well established that the administration to rodents of a variety of structurally diverse chemicals possessing hypotriglyceridemic properties results in hepatomegaly, the induction of hepatic peroxisome (microbody) proliferation, and the development of hepatocellular carcinomas. Studies have led to the hypothesis that persistent proliferation of peroxisomes serves as an endogenous initiator of neoplastic transformation in liver by increasing the intracellular production of H_2O_2 by the peroxisomal oxidase(s). The objective of the present study was to determine whether hepatic peroxisome proliferation can be induced in cats, chickens, pigeons, and two species of monkeys (rhesus and cynomolgus). The hypolipidemic drug ciprofibrate (2-[4-(2,2-dichlorocylopropyl)phenoxyl]2-methylpropionic acid) induced peroxisome proliferation in the livers of cats (dose, >40 mg/kg body weight for 4 weeks); chickens (dose >25 mg/kg body weight for 4 weeks); pigeons (300 mg/kg body weight for 3 weeks), rhesus monkeys (50 to 200 mg/kg body weight for 7 weeks) and cynomolgus monkeys (400 mg/kg body weight for 4 weeks). In all five species examined in this study, a marked but variable increase in the activities of peroxisomal catalase, carnitine acetyltransferase, heat-labile enoyl-CoA hydratase, and the fatty acid β-oxidation system was observed. These results suggest that peroxisome proliferation can be induced in the livers of several species and that it is a dose-dependent but not a species-specific phenomenon. (Am J Pathol 1984, 114:171–183)

PEROXISOME (microbody)[1] was once considered a "fossil organelle" by DeDuve and Baudhuin,[2] but this view has changed in recent years, in part due to the identification of the highly remarkable hepatic peroxisome proliferative property of several structurally dissimilar hypolipidemic drugs[3–7] and certain phthalate ester plasticizers.[8] These chemicals, which serve as simple and reproducible means of increasing the number of peroxisomes and synthesis of peroxisomal enzymes in the livers of rodents, have provided considerable insight into the structure and function of peroxisomes.[6,9–12] Studies from our laboratory demonstrated the hepatocarcinogenicity in rats and/or mice of six hypolipidemic drugs with hepatic peroxisome proliferative properties.[13–15] These studies led to the suggestion that potent hepatic peroxisome proliferators, as a class, are carcinogenic.[14,15] The inability of these compounds to induce mutations[16] in the *Salmonella*/microsome assay[17] or interact with DNA,[16] unlike a majority of chemical carcinogens,[18] resulted in the hypothesis that persistent proliferation of peroxisomes serves as an endogenous initiator of neoplastic transformation of hepatocytes by increasing the intracellular production of H_2O_2 by the peroxisomal oxidases.[15] Because hepatic peroxisome proliferation was not observed in several nonrodent species given the hypolipidemic drug clofibrate in a preliminary screening study by Svoboda et al,[9] it has been stated recently by several workers that peroxisome proliferation and the resulting liver carcinogenicity is a unique, atypical toxic phenomenon, restricted to rodents, without any predictive value for man.[19,20] Since cellular reactions to various xenobiotics may depend on species, strain, sex, and a variety of other parameters, information on the interspecies responses to peroxisome proliferators should prove to be of con-

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siderable value in the evaluation of the implications of peroxisome proliferation and peroxisomal enzyme induction.

The objective of the work described here was to determine whether hepatic peroxisome proliferation could be induced in cats, chickens, pigeons, and two species of monkeys. The results demonstrate that peroxisome proliferation and peroxisome-associated enzymes are inducible in all species examined in this study.

Materials and Methods

Chemicals

The hypolipidemic compound ciprofibrate (2-[4-(2,2-dichlorocyclopropyl)phenoxy]-2-methylpropionic acid)[21] was a generous gift from Sterling-Winthrop Research Institute (Rensselaer, NY). Crotonyl CoA, NAD, NADP, CoA, palmitoyl CoA, and carnitine were obtained from Sigma (St. Louis, Mo). [1-^{14}C] palmitoyl CoA (sp. act. 59 mCi/mmol) was obtained from Radiochemical Centre, Amersham, Arlington Heights, Illinois. All other chemicals were purchased from sources listed elsewhere.[22]

Animals and Treatment

Male cats weighing about 500–700 g were obtained from Sleepy Hollow Cattery, Mundelein, Illinois. Pigeons were obtained from Al-Mon's Feeding and Racing Pigeon Supply, Chicago, Illinois. Male chickens were obtained from Roth Hatchery, Watseka, Illinois. Adult, male rhesus monkeys (*Macaca mulatta*) were a generous gift from Searle Research and Development Division of G. D. Searle and Co., Skokie, Illinois; and male cynomolgus monkeys (*Macaca fascicularis*) were purchased from Charles River Research Primate Corporation, Long Island, New York. The animals and birds were housed in individual cages with free access to water and food and maintained on 12-hour light/dark period in the Center for Experimental Animal Research of Northwestern University Medical School.

Cats were given ciprofibrate orally in gelatin capsules in doses of 10–200 mg/kg body weight daily for up to 4 weeks. Pigeons were administered ciprofibrate by gavage (300 mg/kg body weight) for 3 weeks. Chickens received ciprofibrate (25 to 150 mg/kg body weight) orally in capsule for 4 weeks.

Rhesus monkeys were allowed to eat ciprofibrate mixed in fruit jelly and bread, at graded dose levels of 50–200 mg/kg body weight per day over a 7-week period (50 mg/kg body weight, 1 week; 100 mg/kg, 3 weeks; 200 mg/kg, 3 weeks). Cynomolgus monkeys were given ciprofibrate in jelly and bread at a dose level of 400 mg/kg body weight per day for about 4 weeks.

All animals were sacrificed under ketamine-chloride-induced anesthesia.

Morphology and Morphometry

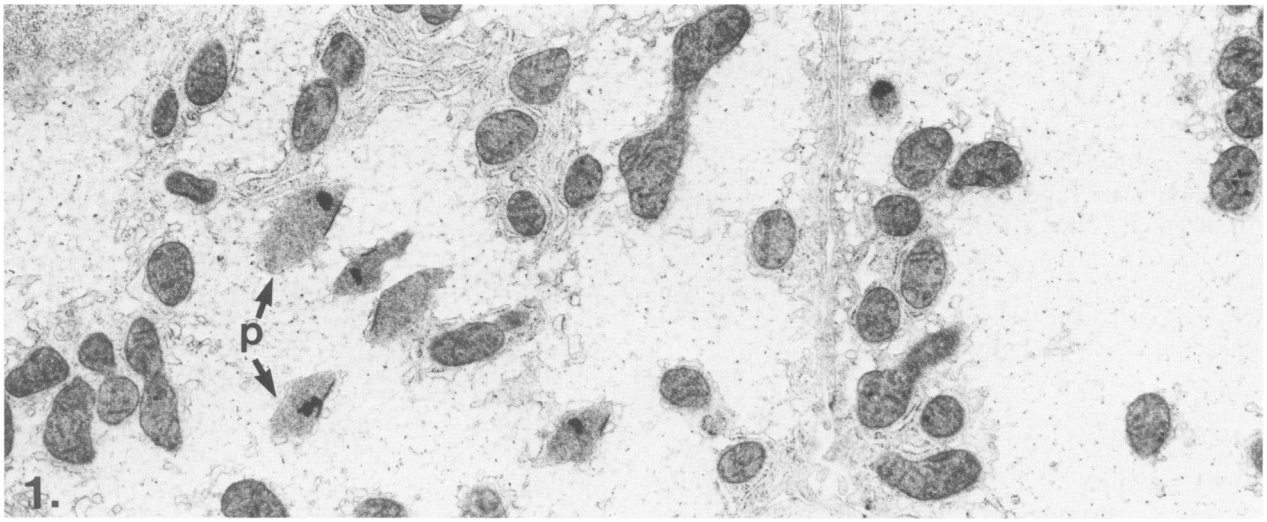
Small pieces of liver were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, for 1 hour and postfixed in 1% OsO_4 in 0.1 M *s*-collidine buffer, pH 7.4, for 1 hour at 4 C. Thin sections of Epon-embedded liver tissue, stained with uranyl acetate and lead citrate, were observed under the electron microscope. For the cytochemical localization of peroxisomal catalase, liver tissue was fixed in 2.5% glutaraldehyde for 4 hours and processed according to Novikoff and Goldfischer,[23] as previously described.[24] Incubations in alkaline, 3,3′-diaminobenzidine HCl medium were performed at 37 C to 42 C.[25]

For morphometric analysis of changes in peroxisome volume density, 30 randomly photographed electron micrographs of cytoplasm of liver cells from normal and ciprofibrate-treated cats, chickens, and monkeys (10 electron micrographs form 1 to 2 blocks per animal; 3 animals per group) were obtained. Micrographs were taken at ×5000 and enlarged 2.5 times at printing to a final magnification of ×12,500. Points of intersection overlying cytoplasm, mitochondria, and peroxisomes were counted with the use of a 5-mm spaced lattice grid.[24,26] The volume density of mitochondria and peroxisomes was determined in relation to cytoplasmic volume according to the method described by Weibel.[27]

Subcellular Fractionation of Liver

The livers were homogenized in ice-cold 0.25 M sucrose (10% homogenates wt/vol) with a Potter-Elvehjm homogenizer. These homogenates were fractionated into nuclear (700*g* for 10 minutes); heavy

Figure 1 – Electron micrograph of liver cell cytoplasm of a normal male cat. Peroxisomes (*p*) display a marginal plate and a crystalloid core. (Uranyl acetate and lead citrate, ×10,400) **Figure 2** – Liver cell cytoplasm of an adult male cat treated with ciprofibrate (200 mg/kg body weight) for 2 weeks. Notice the presence of numerous peroxisomes (*p*); several of these do not contain any inclusions. (Uranyl acetate and lead citrate, ×10,000) **Figure 3** – Ciprofibrate treated cat liver (as in Figure 2), incubated at 37 C for the cytochemical localization of peroxisomal catalase in alkaline 3,3′-diamino-benzidine (DAB) medium. The reaction product is present in all peroxisomes (*p*) counterstained with lead citrate. (×12,800)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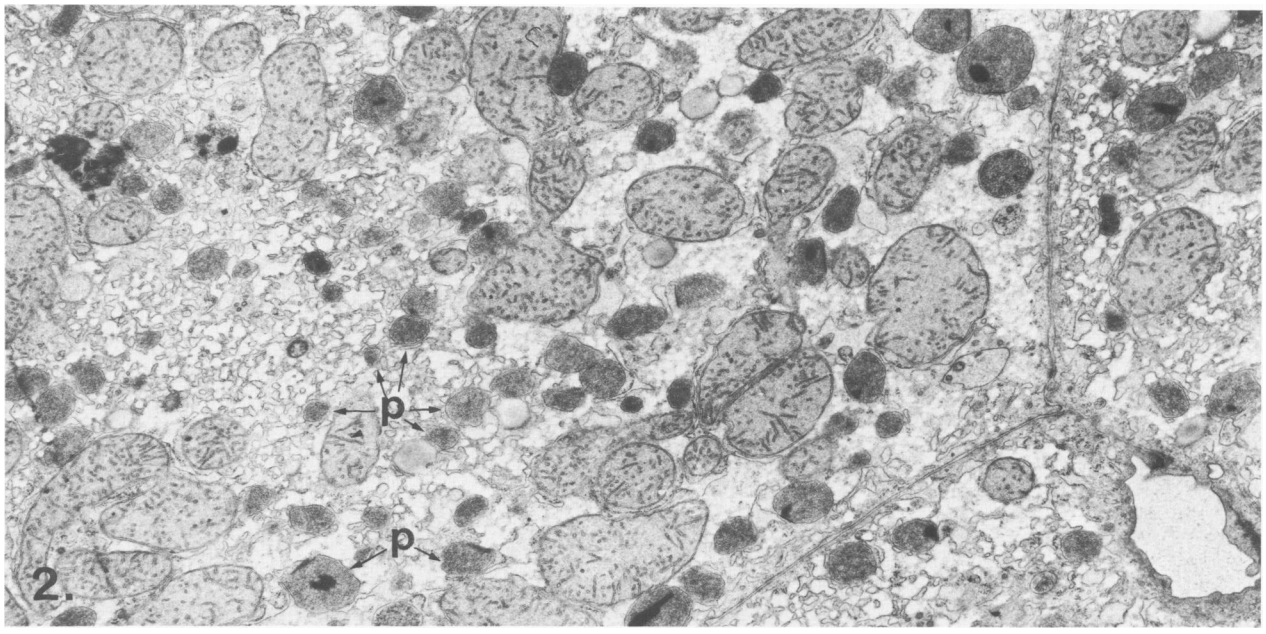
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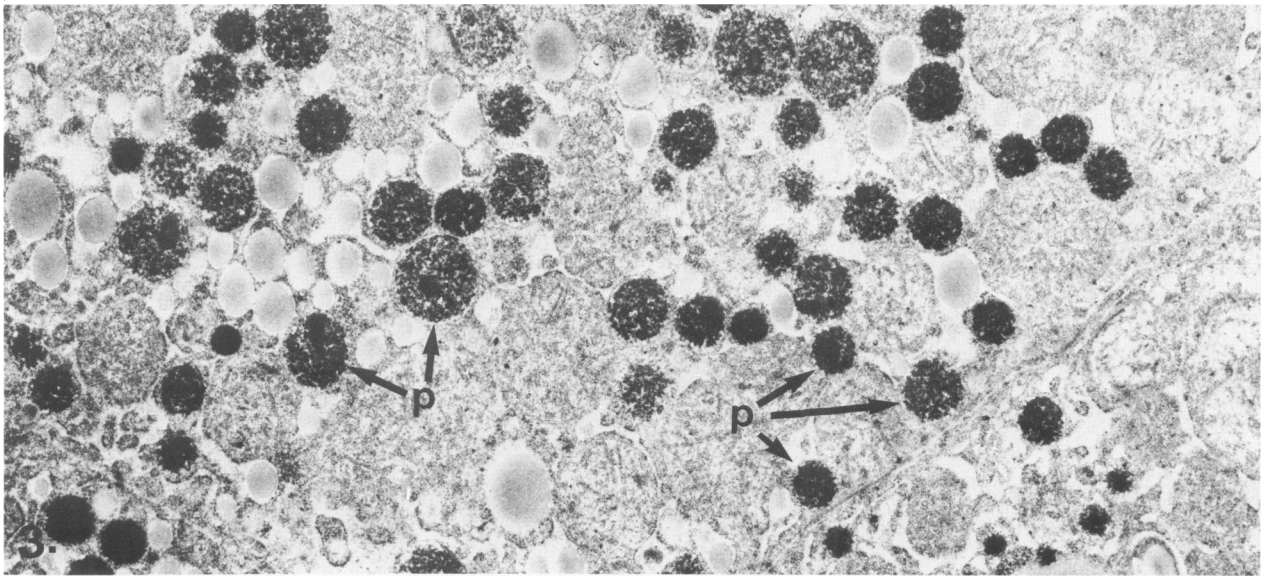
p
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mitochondrial (11,000*g* for 3 minutes) and light mitochondrial (15,000*g* for 15 minutes) fractions in Beckman J-21C centrifuge at 4 C according to the procedure outlined by Baudhuin et al.[28] The post-light mitochondrial supernatants were centrifuged at 105,000*g* for 1 hour with a Type 50 Ti rotor in a Beckman L5-65 ultracentrifuge for the purpose of obtaining the microsomal fraction and postmicrosomal cytosol. All pellets were resuspended in 0.25 M sucrose and recentrifuged at the respective *g* forces.

Enzyme Assays

The activities of peroxisome-associated enzymes catalase,[29] carnitine acetyltransferase,[30] urate oxidase,[31] heat-labile enoyl-CoA hydratase,[32] and palmitoyl-CoA oxidation[33] were measured in homogenates and subcellular fractions as previously described.[22,26] Protein concentrations were determined by the method of Lowry et al[34] with the use of crystalline bovine serum albumin as the standard.

SDS-Polyacrylamide Gel Electrophoresis

We analyzed the large particle fractions[35] by SDS-polyacrylamide gel electrophoresis according to the method of Laemmli[36] to identify the peroxisome-proliferation-associated M_r 80,000 polypeptide.[35]

Results

Electron-Microscopic Observations

Hepatic peroxisomes of control and ciprofibrate-treated cats stained positively for the peroxidatic activity of catalase by the alkaline DAB method when incubated at 37 C, whereas optimal DAB staining of chicken, pigeon, and primate hepatic peroxisomes was obtained when incubation temperatures ranged between 39 and 42 C.

Cats

Peroxisomes in normal cat liver cells are few and display a characteristic appearance with a marginal plate and a crystalloid nucleoid (Figure 1). In cats treated with ciprofibrate (at dose levels >40 mg/kg body weight), the number of peroxisomes in hepatic parenchymal cells increased considerably. Several of these peroxisomes appeared as small accumulations of electron-dense material delimited by a single membrane, but lacking a nucleoid (Figure 2). In larger peroxisomes, the marginal plate as well as the crystalloid core were present (Figure 2). All these organelles stained positively for the peroxisomal marker enzyme, catalase (Figure 3). The volume density of peroxisomes in the liver cells of male cats treated with ciprofibrate at 50 mg/kg body weight for 1 week and 100 mg/kg body weight for an additional 2-week period showed a fourfold increase as compared with cells from untreated controls (Table 1). In addition to increases in peroxisome volume and numerical density, there was a substantial increase in the smooth endoplasmic reticulum and in the volume density of mitochondria (approximately a twofold increase) in ciprofibrate-treated cats.

Chickens

Peroxisomes in liver cells of normal chickens are usually spherical and measure ~0.1–0.3 μ in diameter. The matrix is slightly electron-dense, and in an occasional peroxisome a core or nucleoid is discernible. In

Table 1—Morphometric Analysis of Ciprofibrate Induced Peroxisome Proliferation in Livers of Male Cats, Chickens, and Rhesus Monkeys*

Species	Group	Volume density	
		Mitochondria	Peroxisomes
Cat	Control	14.87 ± 0.09	1.33 ± 0.21
	Ciprofibrate-treated (50 mg/kg body weight 1 week, 100 mg/kg body weight 2 weeks)	30.7 ± 7.0†	5.83 ± 0.64†
Chicken	Control	22.17 ± 4.25	0.39 ± 0.09
	Ciprofibrate-treated (100 mg/kg body weight 6 weeks)	47.66 ± 6.59†	7.26 ± 1.21†
Rhesus monkey	Control	19.4 ± 1.7	1.9 ± 0.44
	Ciprofibrate-treated (50 mg/kg body weight 1 week, 100 mg/kg body weight 3 weeks, 200 mg/kg body weight 3 weeks)	21.0 ± 2.1‡	5.6 ± 0.58†

* Male cats, chickens, and rhesus monkeys were fed ciprofibrate as described in Materials and Methods. Thirty electron micrographs of randomly selected areas of liver cell cytoplasm from each group (3 animals per group, 10 micrographs per animal) were subjected to morphometric measurement as described by Weibel.[27] Points overlying cytoplasm, mitochondria, and peroxisomes were determined to obtain the volume density of mitochondria and peroxisomes. The values are expressed as percent of cytoplasmic volume.

† The values are mean ± SE and $P < 0.05$, as determined by a two-tailed *t* test for small samples.

‡ The difference is not significant, compared with control.

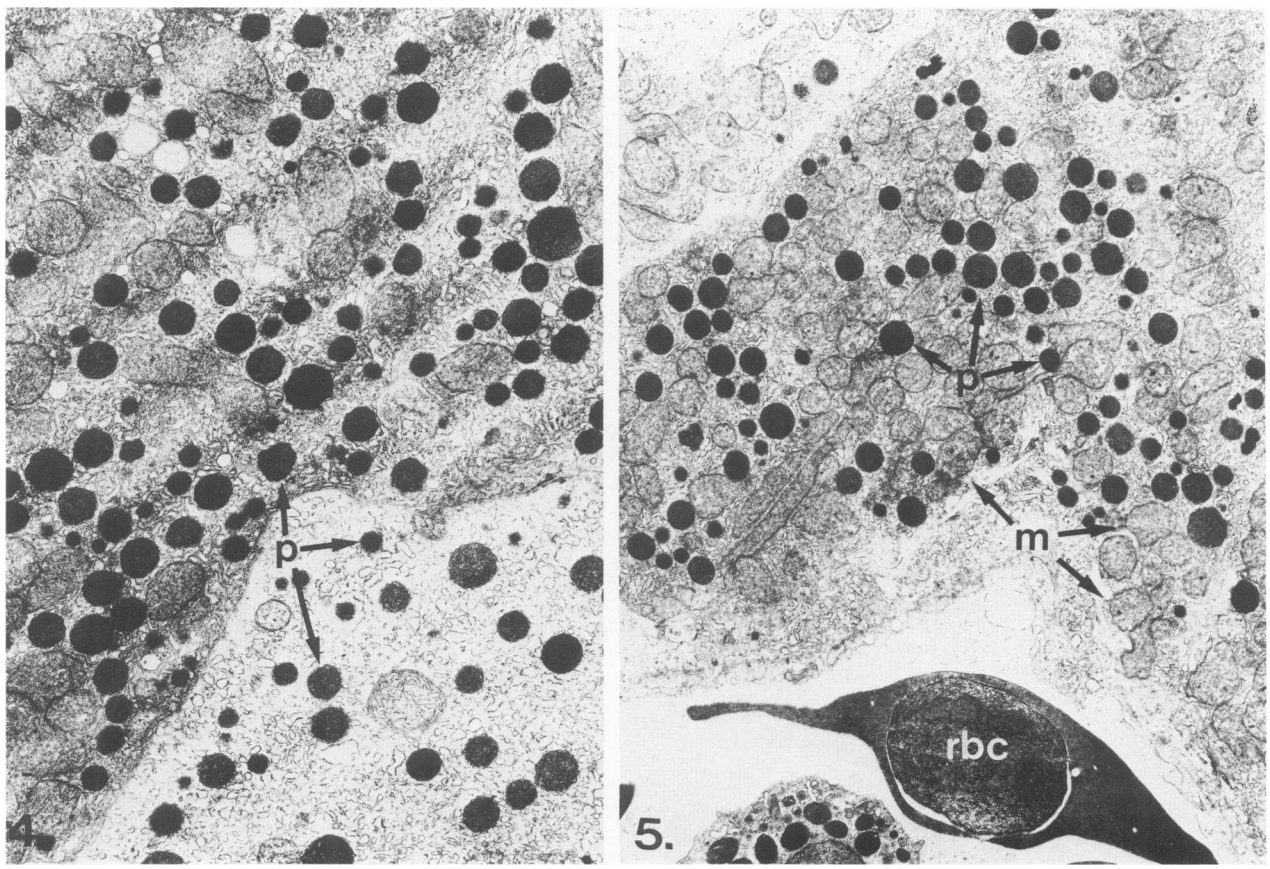
chickens treated with ciprofibrate at 25 mg/kg body weight for 2 weeks, peroxisome proliferation was minimal. A marked increase in peroxisome population occurred in chickens given ciprofibrate at 50, 75, or 100 mg/kg body weight dose levels (Figures 4 and 5). Peroxisomes in normal and treated chickens stained positively for catalase at 37 C, but the reaction was weak. When incubated at 40 C, the reaction product was intense. The morphometric data on peroxisome and mitochondrial volume changes in chickens treated with ciprofibrate are presented in Table 2. In this species, ciprofibrate at 100 mg/kg body weight for 6 weeks induced ~18-fold increase in hepatic peroxisome volume density, whereas the mitochondrial volume density was elevated ~2-fold.

Pigeons

Pigeon liver peroxisomes are very small in diameter (0.1–0.2 μ). Peroxisome proliferation was noted in these birds when ciprofibrate was administered at 300 mg/kg body weight for 3 weeks.

Rhesus and Cynomolgus Monkeys

In the liver cells of normal rhesus and cynomolgus monkeys, peroxisomes appear as single-membrane-limited structures measuring 0.2–0.8 μ in diameter. These organelles lack the typical urate-oxidase–containing nucleoid or core (Figure 6). Matrix densities[37] are present in an occasional peroxisome. In both rhesus (Figures 7–10) and cynomolgus (Figures 11 and 12) monkeys a substantial increase in peroxisome number occurred in liver cells following ciprofibrate administration. Although peroxisome proliferation was evident in all hepatocytes, some areas of hepatocyte cytoplasm displayed abundant numbers of these organelles with numerical densities matching those seen in rat and mouse hepatocytes following exposure to peroxisome proliferators.[6] The DAB cytochemical staining of monkey liver peroxisomes was intense when liver tissue was incubated in the reaction mixture at 40 C (Figure 10), when compared with an incubation at 37 C (Figures 9 and 12). Morphometric data on rhesus monkey liver peroxisomes are pre-



Figures 4 and 5 — Portions of liver cells of male chicken treated with ciprofibrate at 100 mg/kg body weight for 6 weeks. Numerous peroxisomes (*p*) are present. *rbc*, nucleated red blood cell. *m*, mitochondria. (Uranyl acetate and lead citrate, **Figure 4**, ×8900; **Figure 5**, ×6400)

Table 2—Alterations in the Liver Weight and Liver Peroxisomal Enzymes in Cats, Chickens, Pigeons, Rhesus Monkeys, and Cynomolgus Monkeys Treated With the Hypolipidemic Compound Ciprofibrate

Species*	Liver weight (g/100 g body weight)	Catalase (U/mg protein)	Carnitine acetyl transferase (U/mg protein)	Enoyl-CoA hydratase (U/mg protein)	Palmitoyl-CoA oxidation (μmol/min/g liver)	Urate oxidase (U/g liver)
Cats†						
Control (3)	5.4 ± 0.02**	27.8 ± 5.6	4.1 ± 1.9	0.12 ± 0.02	0.45 ± 0.01	1.47 ± 0.15
Treated (3)	6.9 ± 1.0††	41 ± 7.8	54.8 ± 13.3	0.54 ± 0.07	2.24 ± 0.24	1.9 ± 0.26
Chickens‡						
Control (4)	1.78 ± 0.24	12.0 ± 1.7	6.3 ± 1.9	0.6 ± 0.2	0.3 ± 0.02	–
Treated (6)	2.18 ± 0.29	18.3 ± 4.3	41.3 ± 4.8	8.4 ± 3.4	4.7 ± 1.4	–
Pigeons§						
Control (3)	1.56 ± 0.15	15.82 ± 1.0	22 ± 6	0.4 ± 0.2	0.38 ± 0.13	–
Treated (4)	3.66 ± 0.18	82.35 ± 10.0	108 ± 27	13.7 ± 4.6	3.93 ± 13.7	–
Rhesus Monkey‖						
Control (3)	1.66 ± 0.17‖	86 ± 7	Not detectable	0.05 ± 0.02	0.14 ± 0.03	0.49 ± 0.1
Treated (3)	2.16 ± 0.006	161 ± 34	29.1 ± 6.1	0.31 ± 0.06	0.69 ± 0.07	1.02 ± 0.03
Cynomolgus¶ monkey						
Control (2)	2.39 ± 0.55	105 ± 25	Not detectable	0.07 ± 0.01	0.57 ± 0.12	–
Treated (4)	4.18 ± 0.72	149 ± 32	13.89 ± 3.8	0.49 ± 0.07	7.2 ± 1.72	–

* Number of animals is given in parenthesis. The enzyme activities were determined on liver homogenates as described in Materials and Methods.

† Male cats were given ciprofibrate (10 mg/kg body weight per day for 1 week followed by 20 mg/kg body weight for 2 weeks, 40 mg/kg body weight for 1 wk) in gelatin capsules.

‡ White leghorn chicken given ciprofibrate 50 mg per kg body weight per day in a gelatin capsule for 4 weeks.

§ Pigeons were given ciprofibrate 300 mg/kg body weight in DMSO by gavage daily for 2 weeks.

‖ Rhesus monkeys were fed ciprofibrate 50 mg/kg body weight per day for 1 week, 100 mg/kg body weight per day for 3 weeks, and 200 mg/kg body weight for 3 weeks.

¶ Cynomolgus monkeys were fed ciprofibrate 400 mg/kg body weight per day for 2 weeks.

** The values represent the mean ± SD.

†† The difference between control and experimental was not significant. All other values are significantly different from controls ($P < 0.05$).

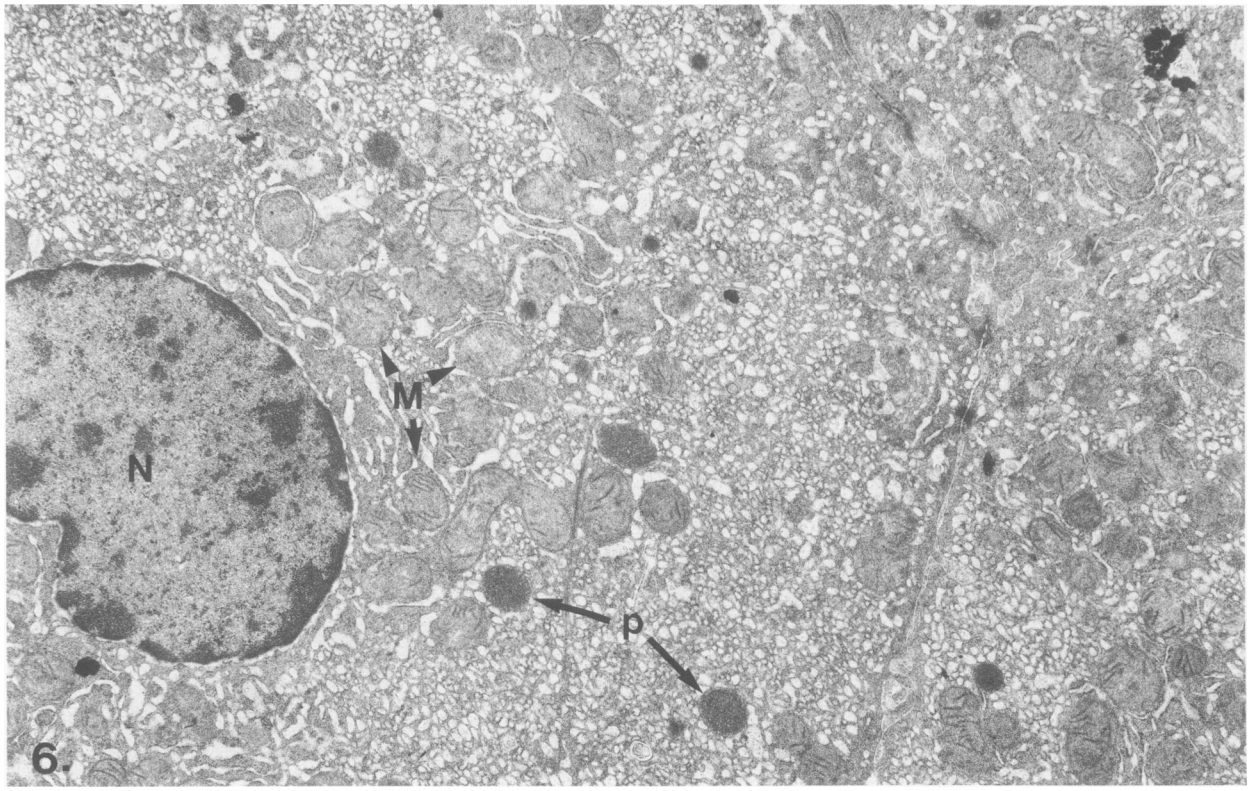
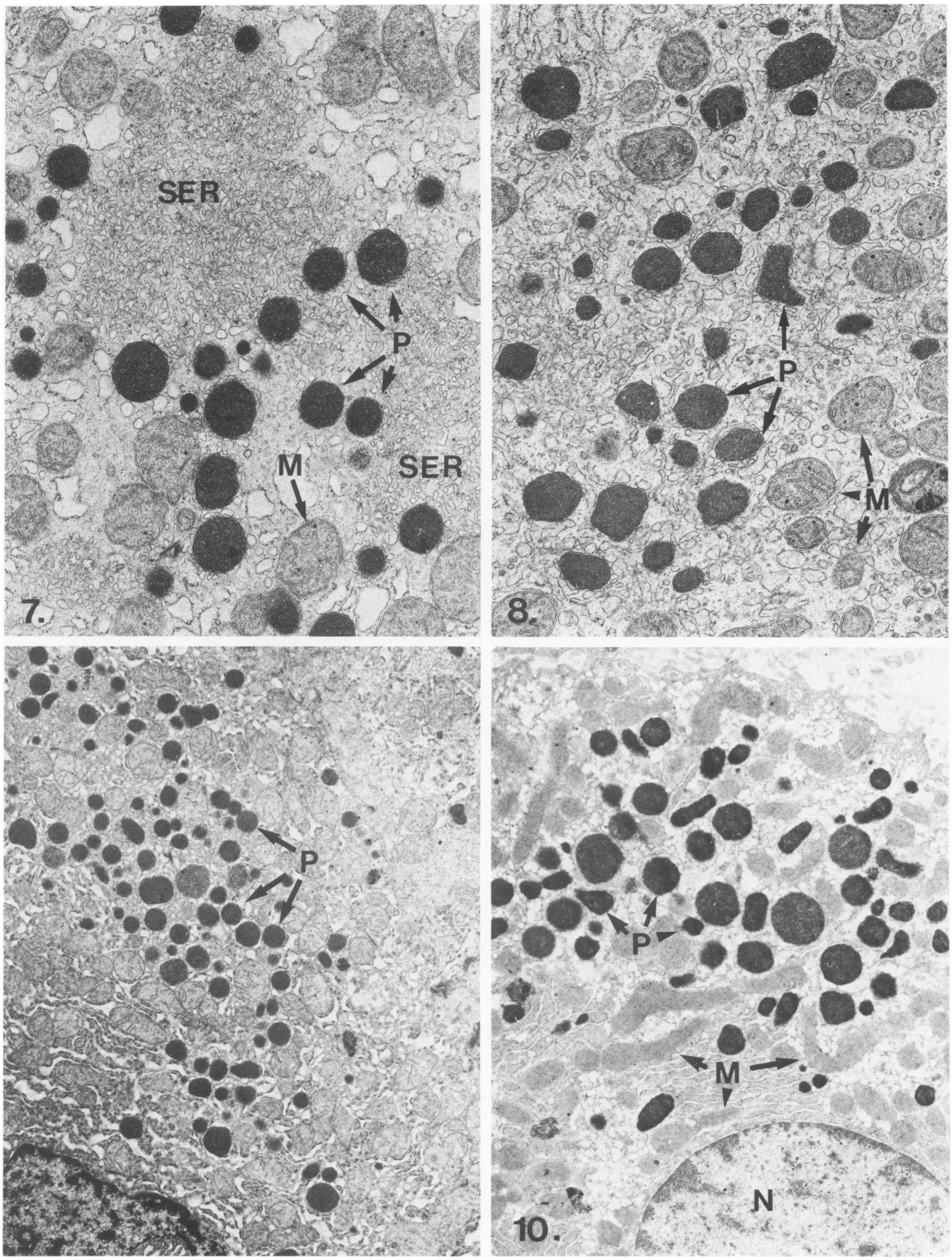
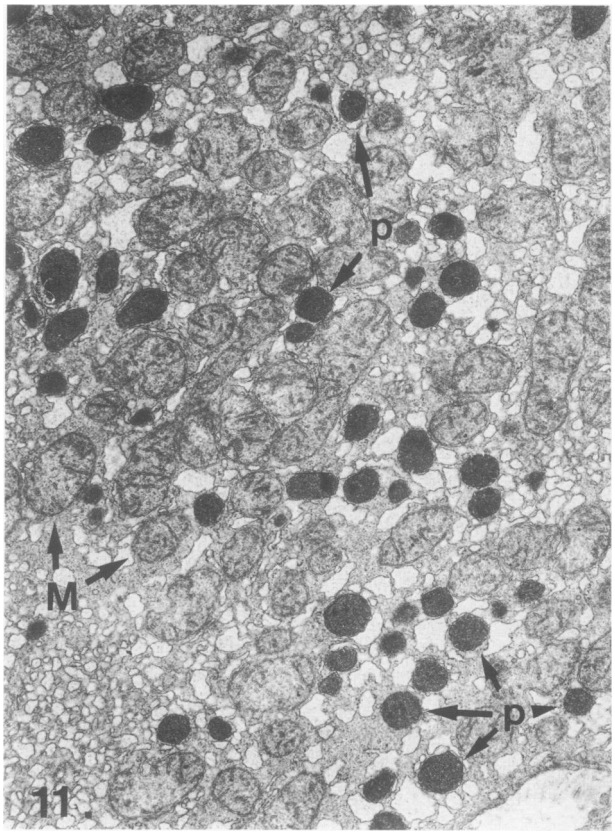


Figure 6—Rhesus monkey liver (control). Peroxisomes (*p*) are single-membrane-limited structures with granular matrix and lack a crystalloid core. *M*, mitochondria. *N*, nucleus. (Uranyl acetate and lead citrate, × 10,300) (With a photographic reduction of 7%)



Figures 7–10 – Rhesus monkey treated with ciprofibrate for 7 weeks. Focal proliferations of smooth endoplasmic reticulum (*SER*) appear as organoid structures (**Figure 7**). Peroxisome (*P*) proliferation is present in all these electron micrographs. When sections of rhesus monkey liver are incubated in alkaline DAB medium at 37 C (**Figure 9**) for the cytochemical localization of peroxisomal catalase, the reaction product is not well formed; intense cytochemical reaction product is seen in peroxisomes, when sections are incubated at 40 C (**Figure 10**). *M*, mitochondria. *N*, nucleus. **Figures 7** and **8**, uranyl acaetate and lead citrate, ×13,500; **Figures 9** and **10**, DAB-incubated sections, counterstained with lead citrate, **Figure 9**, ×7000, **Figure 10**, ×8800)



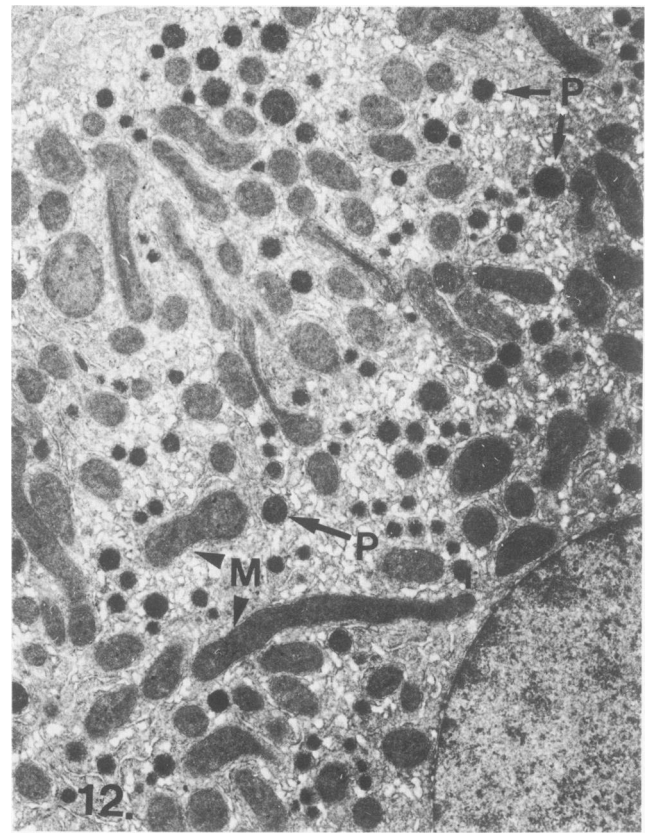


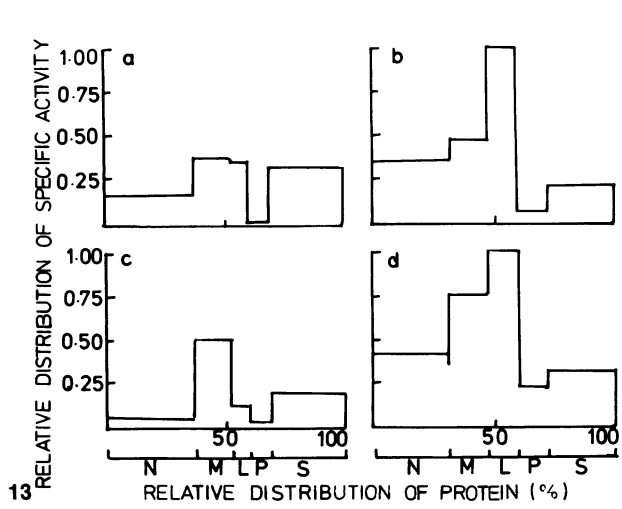
Figure 11 – Cynomolgus monkey treated with ciprofibrate for 3 weeks. Increase in peroxisome (*P*) population is evident. *M*, mitochondria. (Uranyl acetate and lead citrate, × 10,000) **Figure 12** – Cynomolgus monkey treated with ciprofibrate for 3 weeks. Liver sections incubated in alkaline DAB medium at 37 C for cytochemical localization of catalase. Reaction product in peroxisomes (*P*) is not very intense. *M*, mitochondria (M) (Counterstained with lead citrate, × 12,000) (Both with a photographic reduction of 4%)

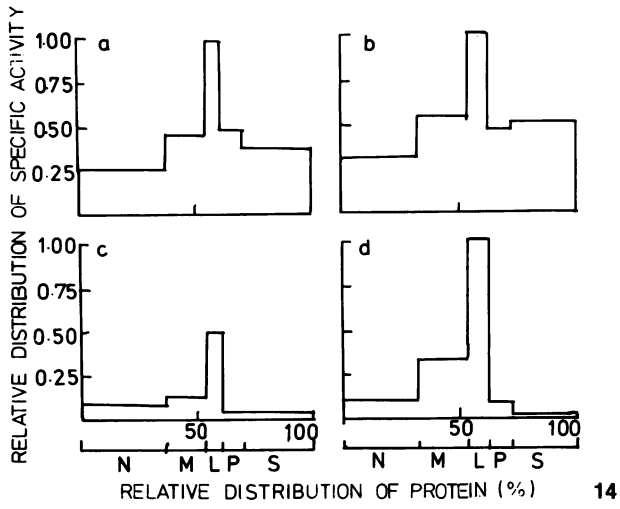
sented in Table 1. Morphometric analysis of cynomolgus monkey liver peroxisomes was not undertaken. In addition to alteration in peroxisome number, there was a marked increase in the smooth endoplasmic reticulum in the liver cells of ciprofibrate-treated rhesus and cynomolgus monkeys. In rhesus monkeys given ciprofibrate, peculiar organoid patterns of SER were present in several hepatocytes (Figure 7).

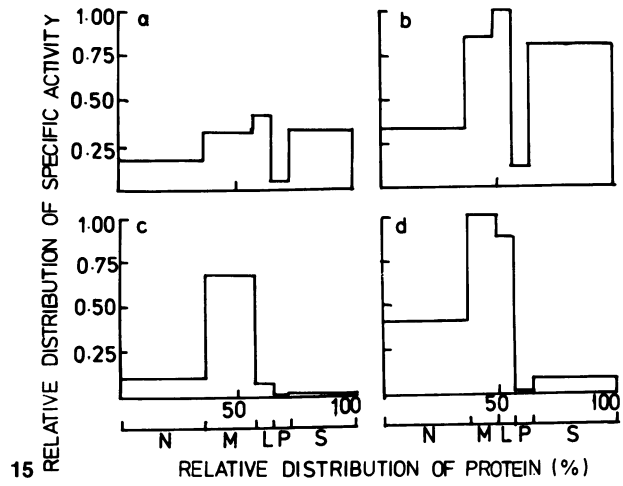
Changes in Liver Peroxisomal Enzymes

The alterations in liver weight and in the specific activities of peroxisome-associated enzymes in the liver homogenates of male cats, chickens, pigeons, and in both species of primates treated with the hypolipidemic drug ciprofibrate are shown in Table 2. The liver homogenates of all animals treated with ciprofibrate demonstrated significantly higher activities of catalase, carnitine acetyltransferase, heat-labile peroxisomal enoyl-CoA hydratase, and the peroxisomal palmitoyl-CoA oxidizing system when compared with liver homogenates obtained from respective controls. In cats and rhesus monkeys given ciprofibrate the uricase activity showed a significant elevation (Table 2).

In this study we also investigated the subcellular distribution of catalase and palmitoyl-CoA oxidation system in the livers of normal and ciprofibrate-treated cats, chickens, and rhesus and cynomolgus monkeys (Figures 13–16). In control livers, the highest activities of catalase and the peroxisomal palmitoyl-CoA oxidation system were found in the heavy and light mitochondrial fractions. Figure 17 illustrates a representative light mitochondrial fraction of liver obtained from a ciprofibrate-treated chicken. Numerous peroxisomes are present in this fraction. In all animals, a substantial activity of these enzymes were also recovered in soluble fraction, which is attributed to breakage of peroxisomes during homogenization. The distribution of these enzymes in the livers of ani-







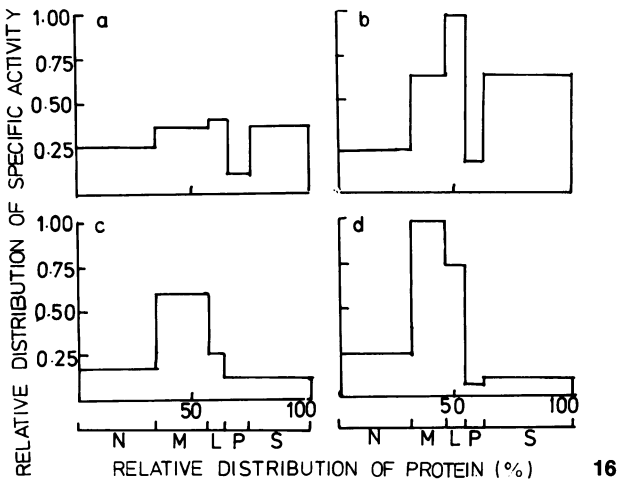


Figure 13 — Subcellular distribution in the control (**a** and **c**) and ciprofibrate-treated (**b** and **d**) cat liver of the relative specific activities of catalase (**a** and **b**) and the peroxisomal palmitoyl-CoA oxidation system (**c** and **d**). Homogenates were fractionated according to the method of Baudhuin et al.[28] The *ordinates* represent the distribution of specific activities in subcellular fractions in normal liver, relative to the highest activity in the ciprofibrate-treated liver. The *abscissas* indicate the relative distribution of protein content of nuclear (*N*), mitochondrial (*M*), light mitochondrial (*L*), microsomal (*P*), and supernatant (*S*) fractions. **Figure 14** — Subcellular distribution in the control (**a** and **c**) and ciprofibrate-treated (**b** and **d**) chicken liver of the relative specific activities of catalase (**a** and **b**) and the peroxisomal palmitoyl-CoA oxidation system (**c** and **d**). For other details see the legend to Figure 13. **Figure 15** — Subcellular distribution in the control (**a** and **c**) and ciprofibrate-treated (**b** and **d**) rhesus monkey liver of the relative specific activities of catalase (**a** and **b**) and the peroxisomal palmitoyl-CoA oxidation system (**c** and **d**). For other details see the legend to Figure 13. **Figure 16** — Subcellular distribution in the control (**a** and **c**) and ciprofibrate-treated (**b** and **d**) cynomolgus monkey liver of the relative specific activities of catalase (**a** and **b**) and the peroxisomal palmitoyl-CoA oxidation system (**c** and **d**). For other details see the legend to Figure 13.

mals following ciprofibrate treatment was similar to that seen in controls. This compound caused a marked elevation in the activities of these enzymes.

Peroxisome-Proliferation-Associated 80,000 mol wt Polypeptide

In rat, mouse, and hamster liver, proliferation of peroxisomes induced by hypolipidemic drugs and phthalate ester plasticizers[6,35] is accompanied by a significant increase in the quantity of a M_r 80,000 protein. In this study, we analyzed the large-particle fractions of liver obtained from control and ciprofibrate-treated cats, chickens, pigeons, and monkeys by SDS-polyacrylamide gel electrophoresis to determine whether this polypeptide is also induced in conjunction with peroxisome proliferation in species other than rodents. As illustrated in Figure 18, this polypeptide was induced in the livers of all species examined in this study.

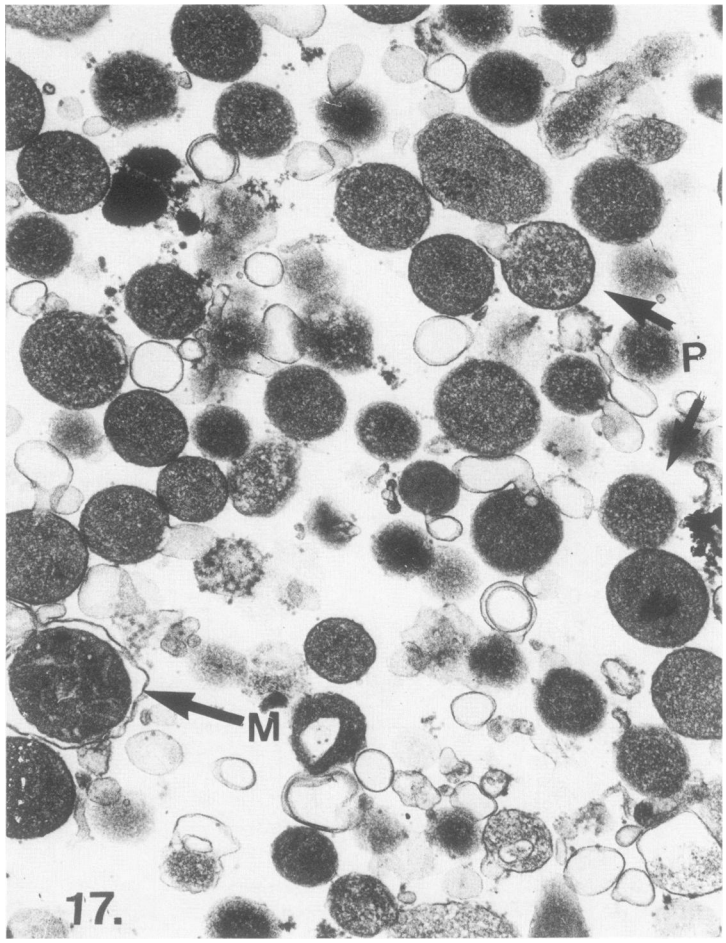


Figure 17 – Electron micrograph of peroxisome (*P*)-enriched fraction obtained from a ciprofibrate-treated chicken. *M*, mitochondria. (Uranyl acetate and lead citrate, × 30,000)

Discussion

Svoboda et al,[9] in a preliminary screening study conducted several years ago with clofibrate, did not observe peroxisome proliferation in the livers of subhuman primates and certain other species. Although the above study on species range of peroxisome induction was limited in scope, and exploratory in nature, several recent studies have cited the study to emphasize that hypolipidemic drug-induced peroxisome proliferation and hepatocarcinogenesis are peculiar to rodents.[19,20] The results presented in the present communication demonstrate clearly that hepatic peroxisome proliferation is inducible in cats, chickens, pigeons, and two species of nonhuman primates, and that it is not limited to rodents. We also demonstrate the existence of peroxisomal fatty acid β-oxidation system in the livers of all five species, and that the hypolipidemic drug ciprofibrate is capable of increasing the activity of this enzyme system in their livers. The cyanide-insensitive fatty acyl-CoA oxidizing system,[11] which differs from that of mitochondria,[11,38] has been shown to be enhanced several times in the livers of rats, mice, and hamsters[7,12,22,39,40] and in the kidney cortex of mice treated with various peroxisome proliferators.[22] The increases in the activities of catalase, carnitine acetyltransferase, heat-labile enoyl-CoA hydratase, and in fatty acyl-CoA oxidizing system in cats, chickens, pigeons, and monkeys in the present investigation are reminiscent of the increases noted in the livers of rodents with peroxisome proliferation.

The present studies also demonstrate that ciprofibrate causes a detectable increase in the amount of peroxisome-proliferation–associated 80,000 mol wt polypeptide in the livers of all species examined. This polypeptide appears immunochemically identical to the bifunctional protein that displays the activity of the second and third enzymes (heat-labile enoyl-CoA hydratase and β-hydroxyacyl CoA-dehydrogenase) of the peroxisomal β-oxidation system.[41] This enzyme system is localized exclusively in peroxisome matrix, as revealed recently by the immunocytochemical procedures.[42]

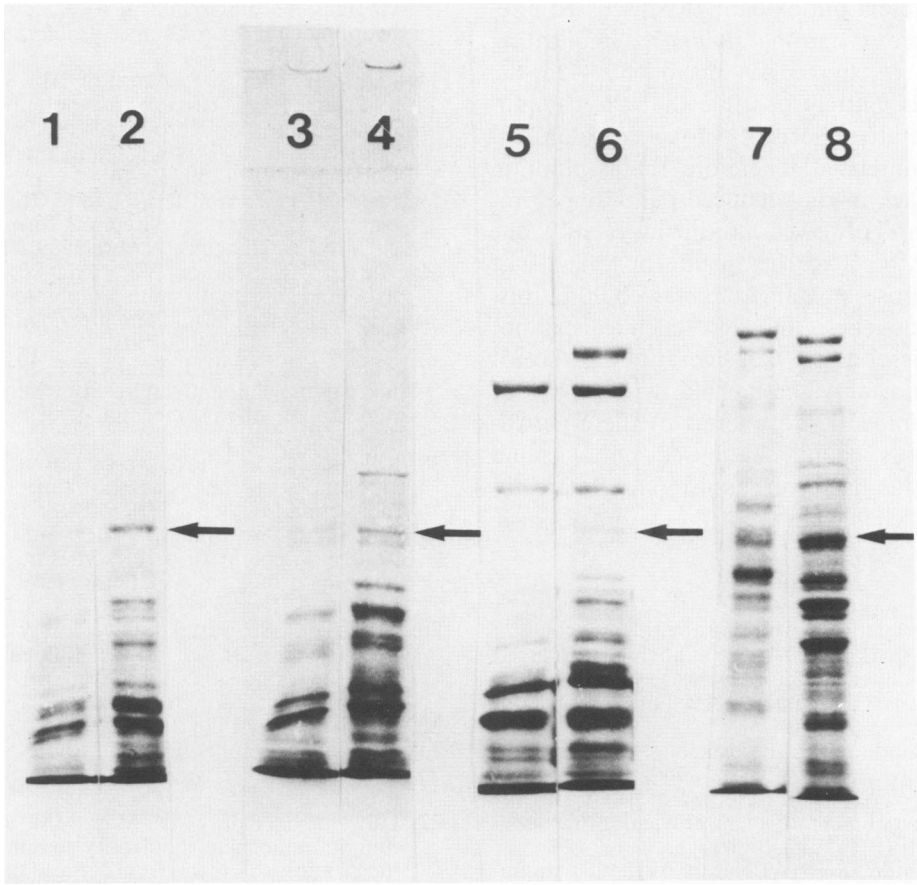


Figure 18—SDS-polyacrylamide slab gel electrophoretic profiles of proteins (~ 20 μg protein/lane) in large-particle fractions obtained from the livers of normal (odd-numbered lanes) and ciprofibrate-treated (even-numbered lanes) cats (lanes 1 and 2), chicken (lanes 3 and 4); rhesus monkey (lanes 5 and 6), and cynomolgus monkey (lanes 7 and 8). Notice the increase in peroxisome-proliferation-associated 80,000 mol wt (*arrow*) polypeptide in the livers of treated animals.

As pointed out earlier, the available evidence indicates that potent hepatic peroxisome proliferators are carcinogenic in rats and mice.[16] However, neither the mechanism of induction of peroxisome proliferation nor the events leading to the development of hepatocellular carcinomas are as yet fully elucidated.[43] The negative evidence in short-term tests of mutagenicity and DNA damage,[16] of these chemicals strongly suggests that we may be dealing with a rather unique situation of microbody (peroxisome)-mediated malignant change in the livers with marked microbody proliferation.[15,44] If that proves to be the case, the risk of carcinogenesis should be correlatable with the extent of hepatic peroxisome proliferation in a given species. This may depend not only upon the hepatotrophic effect of a given compound but the intracellular levels of a possible cytosolic binding protein.[45] Ciprofibrate, like other potent peroxisome proliferators,[6,14] causes a marked enlargement of liver. The induction of peroxisome proliferation only in liver cells[6,24] and the cells of the proximal tubules of kidney,[22] suggests that these cells may possess peroxisome-proliferator-specific receptors. Evidence indicates that these compounds act by increasing the levels in the liver of translatable messenger RNAs for certain peroxisomal proteins.[46] Accordingly, the carcinogenic risk to humans could be predicted with some assurance by quantitative morphometric analysis of the alterations in peroxisome volume and numerical densities and by changes in the levels of H_2O_2 generating peroxisomal oxidases.[43] Proliferated peroxisomes oxidize long-chain and medium-chain fatty acids,[47] and the removal of 2 carbons results in the generation of one molecule of H_2O_2. Hydrogen peroxide is also generated by the other oxidases present in peroxisomes.[2,43] The increase in peroxisomal uricase in the livers of hypolipidemic-drug-fed rodents, and as noted in the present study in cats and rhesus monkeys, will not only lead to increased H_2O_2 generation as a result of catalyzing the decomposition of uric acid[37] but, in addition, cause a reduction in the levels of uric acid, which has been shown by Ames

et al[48] to be a potent antioxidant. As discussed elsewhere[15,43,44] there is strong evidence implicating peroxisomes in carcinogenesis in rodents. On the other hand, it is entirely possible that the carcinogenic and proliferative effects of peroxisome proliferators are totally unrelated. Therefore, the assumption that either the lack of or a reduced peroxisome proliferative response observed in the livers of some animals or man[19,20,49] to therapeutic dose levels of these chemicals poses no danger to man could be misleading if carcinogenesis by these chemicals is not mediated by microbodies. Consideration of risk of cardiovascular mortality and morbidity versus risk of cancer from therapeutic dose levels of these peroxisome proliferators should be based on a sound knowledge of the basic mechanism(s) of carcinogenesis by these chemicals.

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