Specificity in the action of hypolipidemic drugs: increase of peroxisomal β-oxidation largely dissociated from hepatomegaly and peroxisome proliferation in the rat

Paul B. Lazarow,[1] Helen Shio, and Marie Anne Leroy-Houyet

The Rockefeller University, New York, NY 10021

Abstract Hypolipidemic drugs increased 3- to 4-fold the activity of the peroxisomal β-oxidation system in rat liver, with modest or no effects on catalase activity, liver weight, or peroxisome abundance. This specificity of action was observed in two experimental models: 1) bezafibrate treatment of male rats (25 mg/kg body wt., p.o.) and 2) clofibrate treatment of female rats (5 g/kg chow). Bezafibrate had no effect on the liver content of protein, catalase, or cytochrome oxidase, and little or no effect on mitochondrial β-oxidation. The results indicate that the hypotriglyceridemic mechanism of action of these drugs involves an induction of the peroxisomal β-oxidation system, but this mechanism does not obligatorily include gross hepatomegaly or other alterations of peroxisomes that are often caused by hypolipidemic compounds. This dissociation of specific biochemical changes from other effects demonstrates a precise regulation of organelle biogenesis. Peroxisomes synthesized under the influence of bezafibrate or clofibrate have a different enzymatic composition than do normal peroxisomes. These results have several implications. 1) Side effects of clofibrate that are of current clinical concern may be unrelated to its lipid-lowering effects. 2) Measurement of peroxisomal β-oxidation should be a sensitive and specific tool for screening for new hypotriglyceridemic compounds. 3) Peroxisome proliferation or lack thereof is not central to efficacy. 4) Other new drugs may be discovered that are highly discriminating in elevating specific enzymes of fatty acid catabolism while causing even less or no hepatomegaly and other side effects.—**Lazarow, P. B., H. Shio, and M. A. Leroy-Houyet.** Specificity in the action of hypolipidemic drugs: increase of peroxisomal β-oxidation largely dissociated from hepatomegaly and peroxisome proliferation in the rat. *J. Lipid Res.* 1982. **23:** 317–326.

Supplementary key words mitochondria • bezafibrate • clofibrate • serum lipids • hepatic fatty acid oxidation • cell fractionation • morphometry

There is considerable interest in finding new drugs capable of specifically and safely lowering serum lipid levels. In evaluating new compounds, it would be helpful to know as much as possible about the mechanism of action of the existing drugs.

There are two separate systems for the β-oxidation of fatty acids in rat liver: the familiar mitochondrial system, and the peroxisomal system that we have described (1, 2). The peroxisomal system has also been detected in human liver (3). The activity of the peroxisomal β-oxidation system in male rats is increased one order of magnitude by the hypolipidemic drugs, clofibrate,[2] tibric acid,[3] and Wy-14,643[4] (1, 4), leading us to infer that increased peroxisomal β-oxidation plays an important role in the hypotriglyceridemic mechanism of action of these compounds (4, 5).

The three drugs mentioned above, as well as many analogs of clofibrate, also cause proliferation of hepatic peroxisomes, an increase in the activity of catalase (a major peroxisomal enzyme (6)), and an increase in liver weight (7–16). These latter effects, which are not necessarily desirable, are generally assumed to be obligatory properties of this class of hypolipidemic drugs. In addition, the possibility has been raised that peroxisome proliferators as a class may be carcinogenic (17, 18).

However, these latter effects are not observed under all conditions. In particular, clofibrate reduces serum lipid levels in both male and female rats, but did not affect peroxisome frequency or catalase activity in females (9, 10). In comparative studies, clofibrate caused peroxisome proliferation in dogs and hamsters, but not in squirrel monkeys and guinea pigs, at the dose tested (9).[5] This raises some doubt concerning our conclusion that the mechanism of action of hypotriglyceridemic drugs involves an effect on peroxisomes.

---

[1] To whom reprint requests should be addressed.
[2] 2-(p-Chlorophenoxy)-2-methylpropionic acid ethyl ester.
[3] 2-Chloro-5-(3,5-dimethylpiperidinosulfonyl) benzoic acid.
[4] (4-Chloro-6-(2,3-xylidino)-2-pyrimidinylthio)acetic acid.
[5] Little information is available concerning the effect of hypolipidemic drugs on the abundance of peroxisomes in human liver. One patient treated with clofibrate had a lower mitochondria/peroxisomes ratio than three controls (15). This was interpreted as most likely being due to an increase in peroxisomes. Several female patients treated with Su-13437 did not show any notable change in the abundance of their hepatic peroxisomes (46).

It is entirely possible, however, that the peroxisomal β-oxidation enzymes could be induced without any effect on other peroxisomal enzymes or on the number of peroxisomes or on liver weight. Such induction would further support the proposed mechanism of action and would have important implications for the evaluation of new hypolipidemic drugs.

In order to test this possibility, we have investigated the effect of clofibrate on female rats. In addition, we have tested the effects of bezafibrate,[6] a clofibrate analog that is active at lower doses (19), and which has recently been introduced into clinical use in West Germany. A dose and protocol (25 mg/kg p.o. to male rats) was selected which has previously been shown to produce 63% of the maximal hypotriglyceridemic effect (19). In these two experiments, we found large elevations of the peroxisomal β-oxidation system, with modest or sometimes no effects on other peroxisome parameters measured and on liver weight.

METHODS

Drug treatments

Three female Fisher F-344 rats (ca. 190 g) were fed ad lib. ground Purina lab chow containing 5 g of clofibrate per kg chow for 1 week. Three control rats received normal ground chow. Water was provided ad lib.

Six male Fisher F-344 rats (ca. 200 g) received 25 mg/kg body wt bezafibrate once daily at 11 AM by stomach tube as a 2.5 mg/ml suspension in 1% methylcellulose. Five control rats received methylcellulose only (10 ml/kg body wt). Treatment was for one week. The rats received pelleted Purina chow and water ad lib.

Preparation of homogenates

Rats were fasted overnight and then decapitated. Individual liver homogenates were prepared in 0.25 M sucrose/0.1% ethanol (1), each at a dilution of 1 g liver per 5 ml homogenate. In the bezafibrate experiment, the rats were anesthetized with ether prior to killing, and 1 ml of blood was withdrawn from the inferior vena cava for determination of plasma triglycerides and cholesterol.

Cell fractionation

Equal amounts (5–8 ml) of the homogenates within each experimental group were pooled. From each pool a postnuclear supernatant was prepared, which was then fractionated by the one-step gradient centrifugation method of Lazarow and de Duve (20) in order to separate the mitochondria, peroxisomes, microsomes, and cell sap.

The organelles' locations in the gradient were determined by assaying their characteristic marker enzymes (21).

Assays

Enzyme assays were carried out on each homogenate and on all the various subcellular fractions that were prepared.

Palmitoyl-CoA oxidation was measured by several methods. Palmitoyl-CoA-dependent reduction of NAD was assayed as described previously (1) in the presence of 1 mM KCN to prevent reoxidation of the NADH formed. Oxidation of [1-^{14}C]palmitoyl-CoA (2 Ci/mol) was determined under similar conditions except that KCN was omitted (4). Under the conditions of these assays (which included Triton X-100), the mitochondrial β-oxidation system was not very active.[7] In the bezafibrate experiment, we also measured the oxidation of [U-^{14}C]palmitoyl-CoA under conditions where both mitochondria and peroxisomes were active (1 mM carnitine, 0.25 M sucrose, no Triton X-100)[7] (22).

Catalase, cytochrome oxidase, and protein were determined according to Leighton et al. (23). Plasma cholesterol and triglycerides were measured according to Block, Jarrett and Levine (24) and Kessler and Lederer (25), respectively, using the Auto Analyzer II (Technicon Instrument Corp., Tarrytown, NY) in the laboratory of Dr. E. H. Ahrens, Jr. These automated procedures are standardized by the Center for Disease Control.

Morphology

Small pieces of liver were prepared for routine electron microscopic examination. The cytochemical reaction for catalase (26) was carried out as described previously (1) on additional liver samples. One-μm Epon sections were photographed under the light microscope; silver sections were photographed under the electron microscope.

Morphometry

Electron micrographs were photographed at random (27) using one or two blocks (one section/block) from each of three rats from each experimental group. The final magnifications were approximately 30,000; the exact magnifications were determined using a grating replica (E. F. Fullam, Inc., Schenectady, NY).

The peroxisome population was characterized by means of the size distribution analysis of Wicksell (28), using a Quantimet 720 (Cambridge Instruments, Cambridge, England) connected to a PDP 11/10 minicomputer, as described by Baudhuin et al. (29). Additional estimations of organelle volume and number were made using multipurpose test systems as described by Weibel, Kistler, and Scherle (27).

---

[6] 2-(4-(2-(4-Chlorbenzamido)ethyl)phenoxy)-2-methyl-propionic acid.

[7] Lazarow, P. B. Unpublished observations.

Computations

Statistical comparisons were made by means of the Student's t-test. Cell fractionation data were computed and plotted according to Leighton et al. (23).

Materials

Fisher F-344 rats were purchased from Microbiological Associates (Bethesda, MD); reagents were from Sigma (St. Louis, MO). Clofibrate and bezafibrate were supplied by Ayerst Laboratories (New York, NY) and by Boehringer Mannheim (Mannheim, Federal Republic of Germany), respectively. [1-^{14}C]- and [U-^{14}C]-palmitoyl-CoA were obtained from New England Nuclear (Boston, MA).

RESULTS

Effects of clofibrate on female rats

Biochemical results. Liver homogenates prepared from clofibrate-treated female rats were about 3 times more active in oxidizing palmitoyl-CoA than were control homogenates, under conditions that mainly measured the peroxisomal β-oxidation system (**Fig. 1**).

Similar results were obtained by means of two independent assay procedures: NAD reduction (Fig. 1A) and [1-^{14}C]palmitoyl-CoA oxidation (Fig. 1B). The mean activity of the controls of 0.8 μmol NAD reduced per min per g liver was similar to that observed previously for male rats (1, 4).

Clofibrate had no significant effect (**Fig. 2**) on the activity of catalase, the principal peroxisomal enzyme, in confirmation of the results of Svoboda, Azarnoff, and Reddy (10). This differs from the 30–140% increase observed in males (1, 4, 7, 9, 10). Clofibrate also had no effect on cytochrome oxidase activity or on protein concentration (Fig. 2).

Liver weight increased 22% in the drug-treated females ($P < 0.05$). Multiplying the rate of palmitoyl-CoA oxidation per g of liver by this increase in liver weight shows that the total liver capacity for palmitoyl-CoA oxidation is 3.6–4.0 times greater in the rats that received clofibrate than in the controls.

Morphological results. Light microscopic examination (**Fig. 3A**) indicated that clofibrate does not cause the striking changes in peroxisomes that are seen in male rats at this dose (Fig. 3B). This is consistent with previous reports by Svoboda et al. (9, 10). However, morphometric analysis revealed that the number and the total volume of peroxisomes did approximately double (not illustrated). This increase in volume is modest in comparison to the 3- to 10-fold increases observed for male rats (8, 9, 16, 30, 31 and confirmed by us for Fig. 3B).
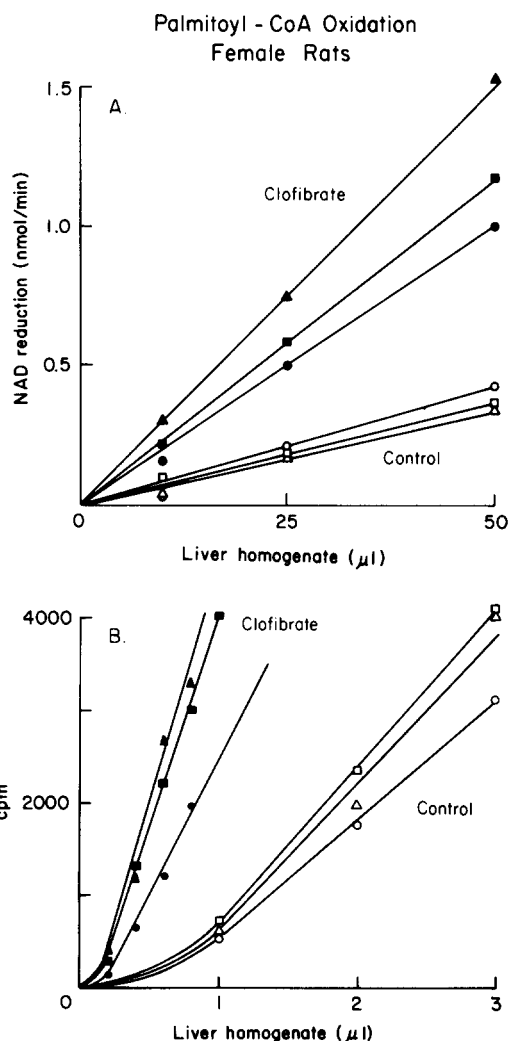

Fig. 1. Palmitoyl-CoA oxidation by liver homogenates from female rats, under conditions that mainly measure peroxisomal β-oxidation. Each symbol represents the results obtained on one animal, either control (open symbols) or clofibrate-treated (closed symbols). One hundred ml of homogenate contains one g of liver. A., NAD reduction assay. Values shown are the means of duplicate determinations. B., [1-^{14}C]palmitoyl-CoA oxidation assay. Each point represents a single determination.

Cell fractionation results. Postnuclear supernatants prepared from pooled homogenates were fractionated by sucrose gradient centrifugation (20). As illustrated in **Fig. 4,** the distributions of protein, catalase, and cytochrome oxidase from the clofibrate-treated rat livers were similar to those of the controls. Protein and these enzymes were not affected by the drug treatment.

From previous work (20) we know that the soluble cell sap proteins largely remain in the top two fractions where the postnuclear supernatant was layered (at the left in Fig. 4). The next two or three fractions were found to consist largely of microsomal protein. These components were not reinvestigated in the present experiments.
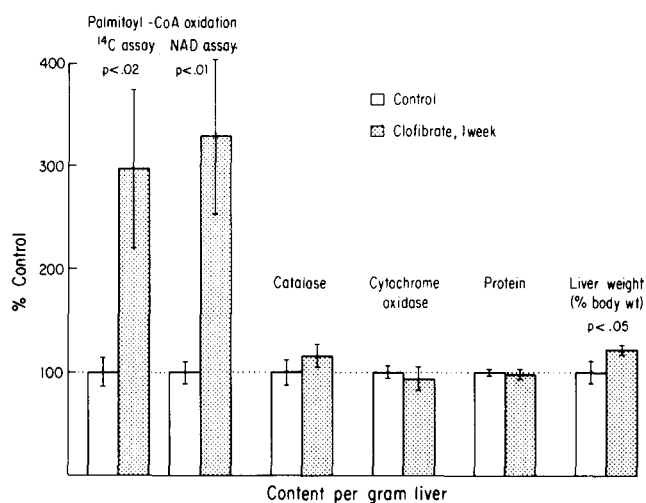
Fig. 2. Effect of clofibrate on hepatic enzyme activities, hepatic protein concentration, and liver weight in female rats. Means and standard deviations are given, expressed as percentages of the control values, which are: palmitoyl-CoA oxidation (0.76 μmol NAD reduced/min per g liver), catalase (56 U/g), cytochrome oxidase (33 U/g), protein (203 mg/g), and liver weight (3.0% body weight). These values are similar to those observed previously (4, 20, 23). Mean rat weight (193 g) was not affected by drug treatment.

The mitochondria, detected by their content of cytochrome oxidase, were in the center of the gradient at density 1.19 g/ml, coincident with a major peak of protein. The peroxisomes, revealed by their catalase content, were at a density of 1.23 g/ml, to the right of the mitochondria in Fig. 4. The peroxisomes, which in normal liver contribute only about 2.5% of the total liver protein, constituted part of the shoulder of the protein distribution, but did not form a noticeable bump. The soluble catalase at the left end of the distribution is believed to have been released from peroxisomes broken during homogenization (20, 23). Similar amounts of soluble catalase were present in the homogenates of control and drug-treated rats.

The distribution of palmitoyl-CoA oxidizing activity in the gradient from the control rats was similar to that of catalase, regardless of which assay was used, indicating that the activity measured was due to peroxisomes. The shape of the palmitoyl-CoA oxidation distributions from the clofibrate-treated rats was also peroxisomal, but the absolute activities were about 3 times greater than in the controls. This demonstrates that the increased activities measured in the homogenates were due to the peroxisomal β-oxidation system.

Effects of bezafibrate on male rats

Biochemical results. Bezafibrate was administered to male rats by stomach tube once daily for a week at a dose of 25 mg/kg, which has been demonstrated to be an effective hypolipidemic dose (19). As shown in **Table 1,** this bezafibrate treatment lowered plasma triglyceride and cholesterol levels significantly, in agreement with the results of Stegmeier et al. (19).

The drug caused an 11% increase in liver weight (Table 1). This is the smallest increase that we have observed to date in our experiments with hypolipidemic compounds, and is much less than the 43–146% increases reported in the literature for six other drugs (7–14).

The bezafibrate treatment had no effect on the hepatic protein concentration or on the activities of catalase and cytochrome oxidase.

The capacity of the liver homogenates of the bezafibrate-treated rats to oxidize palmitoyl-CoA was 3.3- to 3.5-fold greater than the controls (Table 1).

Cell fractionation results. As illustrated in **Fig. 5,** the fractionation of male rat livers gave results similar to those obtained with females (Fig. 4). The peroxisomal β-oxidation system is clearly seen to be elevated by oral administration of bezafibrate (Fig. 5B). In addition, we measured the activity of the mitochondrial β-oxidation system, using an assay procedure in which both peroxisomes and mitochondria are active (Fig. 5A). The mitochondrial activity appeared to be unaffected, or perhaps increased slightly.

Changes in mitochondrial and peroxisomal β-oxidation. These changes were quantitated in two ways. First, we computed the mean ratio of β-oxidation/cytochrome oxidase in the two peak mitochondrial fractions, and the mean ratio of β-oxidation/catalase in the two peak peroxisomal fractions; we used these values to estimate the total mitochondrial and peroxisomal activities per g of liver. As shown in **Table 2,** mitochondrial β-oxidation apparently increased 23%, while peroxisomal β-oxidation increased 321%.

Second, we computed the specific activity of β-oxidation throughout the two gradients. As shown in **Fig. 6,** bezafibrate increased the specific activity in the peroxisomal region about 4-fold, but had little or no effect in the mitochondrial region of the gradient. Because the recoveries on the gradients for the β-oxidation assays were low, we cannot rigorously exclude the possibility that there was a larger change in mitochondrial β-oxidation that we failed to detect for technical reasons. However, we found no evidence for such a change.

Morphological results. Visually, there was no noticeable effect of bezafibrate on the number of peroxisomes. Morphometric analysis (**Fig. 7**) demonstrated that bezafibrate caused the number of peroxisomes and the total peroxisome volume to increase 40% and 60%, respectively (**Table 3**). The size distribution of the peroxisome population was not appreciably affected. These changes are
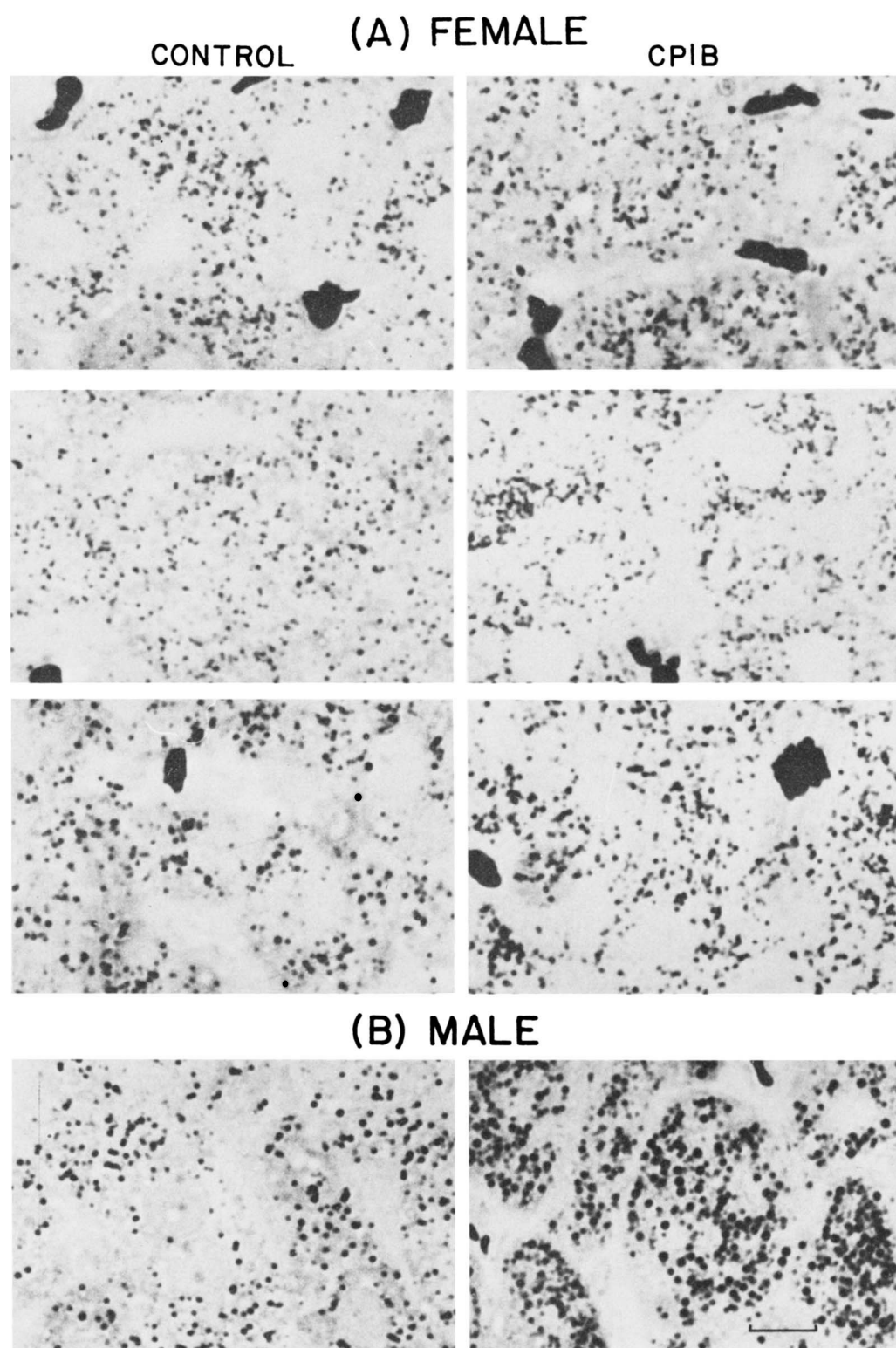
Fig. 3. Peroxisomes in livers of (A) female and (B) male rats. Left: controls. Right: clofibrate-treated. The larger, irregularly shaped, stained structures are red blood cells in sinusoids; the clear round areas are nuclei. The magnifications are the same; scale bar, 10 μm. These light micrographs are typical of a much larger number that were examined.
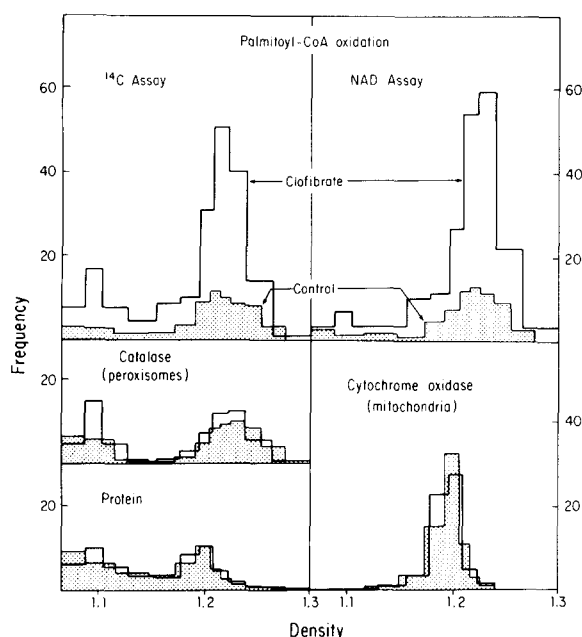
Fig. 4. Fractionation of livers of normal and of clofibrate-treated female rats. Postnuclear supernatants were sedimented into linear sucrose density gradients in order to separate the various organelles, partly on the basis of size and partly on the basis of density; see reference 20 for details. The distributions of the activities from the control rats (stippled areas) are each normalized to the same surface area of 1.0 as described by Leighton et al. (23). The areas under the distributions from the clofibrate-treated rats (white areas) reflect the abundance of these components relative to the controls. Palmitoyl-CoA oxidation assays as in Fig. 1. The starting material was the postnuclear supernatant from 1 g of liver, specifically 0.33 g from each of the three rats in a group. The recoveries of the marker enzymes varied between 92 and 105%. The recoveries of the palmitoyl-CoA oxidation activities were lower, varying between 56 and 76%.

Fig. 5. Fractionation of livers of normal and of bezafibrate-treated (25 mg/kg p.o.) male rats. Absolute enzyme activities are plotted; the ordinate values are chosen such that the surface areas of the control distributions (stippled areas) are all the same, as in Fig. 4. A., Oxidation of [U-^{14}C]palmitoyl-CoA measured under conditions where both mitochondria and peroxisomes are active. 1 mU = 1 nmol palmitoyl-CoA oxidized/min. B., Oxidation of palmitoyl-CoA measured with the NAD reduction assay under conditions that mainly measure peroxisomal β-oxidation (as in Figs. 1, 2, and 4). 1 mU = 1 nmol NADH formed/min. Recoveries were 83–122% for the marker enzymes and 53–78% for palmitoyl-CoA oxidation.

small compared to the 3- to 10-fold increases in peroxisome volume observed with other drugs (14, 16, 30, 31). No change occurred in the volume fraction of mitochondria (not illustrated).
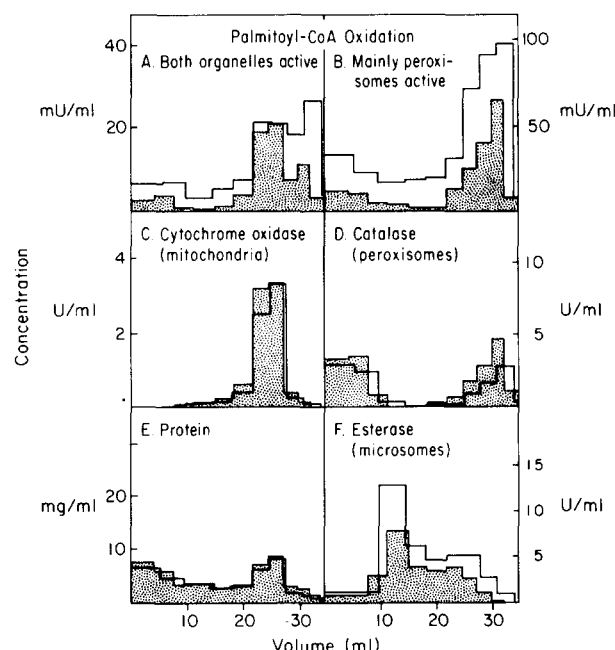
DISCUSSION

The results presented demonstrate that certain hypolipidemic drugs are capable of specifically elevating the activity of the peroxisomal enzyme system catalyzing fatty acid β-oxidation, without affecting the main per-

TABLE 1. Effects of 25 mg/kg oral bezafibrate[a]

	Rat Weight	Plasma		Liver Weight	Liver Activities				
		Triglycerides	Cholesterol		Protein	Catalase	Cytochrome Oxidase	Palmitoyl-CoA Oxidation	
								A[b]	B[c]
	g	mg/100 ml		% rat wt	mg/g liver	U/g liver			
Control (n = 5)									
Mean	205	33	36	2.83	234	90.7	41.0	1.01	0.49
Standard deviation	20	5	3	0.18	5	10.0	3.2	0.12	0.04
Bezafibrate (n = 6)									
Mean	204	26	27	3.15	232	91.1	41.3	3.57	1.64
Standard deviation	14	2	4	0.15	13	8.5	3.0	0.36	0.29
Percent of control	100	79	75	111	99	100	101	353	332
P value		0.02	0.01	0.01				0.001	0.001

[a] Male Fisher F-344 rats were fed Purina chow and water ad lib. Six experimental rats received 25 mg/kg of bezafibrate once daily by stomach tube as a 2.5 mg/ml suspension in 1% methylcellulose. Five control rats received methylcellulose only. Treatment was for 1 week.

[b] NAD reduction assay; optimal conditions for peroxisomal β-oxidation; mitochondria largely inactive.

[c] Oxidation of [U-^{14}C]palmitoyl-CoA under conditions where both peroxisomes and mitochondria are active[7] (22).

TABLE 2. Computation of peroxisomal and mitochondrial β-oxidation activities

	Control	Bezafibrate	Unit	Percent of Control[a]
Peroxisomes				
A. Palmitoyl-CoA oxidation/catalase in the two peak peroxisomal fractions (Fig. 5)	2.48	10.4	mU/U	419%
B. Mean catalase in the homogenates (Table 1)	90.7	91.1	U/g	100%
C. Peroxisomal β-oxidation (Product of A and B)[b]	225	947	mU/g	421%
Mitochondria				
D. Palmitoyl-CoA oxidation/cytochrome oxidase in the two peak mitochondrial fractions (Fig. 5)	6.07	7.41	mU/U	122%
E. Mean cytochrome oxidase in the homogenates (Table 1)	41.0	41.3	U/g	101%
F. Mitochondrial β-oxidation (Product of D and E)	249	306	mU/g	123%
Whole liver				
G. Sum of calculated activities in peroxisomes and mitochondria (C + F)	474	1253	mU/g	264%
H. Percent in peroxisomes	47%	76%	%	
I. Measured (Table 1)	495	1643	mU/g	332%

[a] The accuracy and reproducibility of this type of fractionation experiment are such (20, 21, 23) that we consider the 321% increase in peroxisomal activity to be highly significant, but would not argue that the 23% increase in mitochondrial activity is significant. No statistics are available since this is a single fractionation of pooled livers.

[b] This calculation assumes that all catalase is in peroxisomes and that the distributions of catalase and β-oxidation activity within the peroxisome population are the same.

oxisomal enzyme, catalase, and without causing a striking proliferation of the organelle. It makes good biochemical sense that catalase is not obligatorily increased by hypolipidemic drugs, because it is not involved directly in lipid metabolism, and is present in sufficient quantity to dispose of the H_2O_2 generated by the acyl-CoA oxidase. Modification of its activity appears to be a less specific effect of the drugs. Moreover, the enzymes cat-

alyzing the β-oxidation reactions are not major constituents of normal peroxisomes.[7] Consequently, these enzymes can be increased manyfold without greatly increasing total peroxisomal protein.

Drug dose appears to be involved in differentiating the more specific biochemical effects from the other biochemical or morphological changes. Larger doses of bezafibrate cause increased hepatomegaly (32) as well as elevated catalase and obvious proliferation (33), but the elevation of β-oxidation was similar to that reported here (32). Thus, the dose response for β-oxidation apparently differs from the dose response for proliferation and other changes.
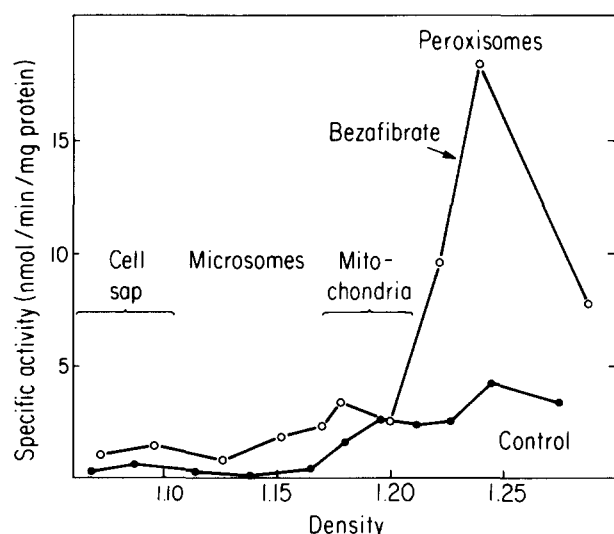

Fig. 6. Effect of bezafibrate on the specific activity of β-oxidation in the two gradients. The plotted values represent the ratios of histograms A and E in Fig. 5.
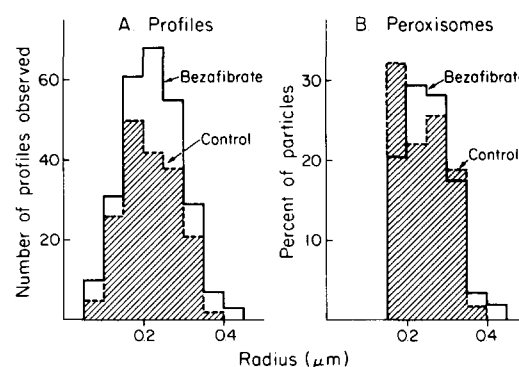

Fig. 7. Effect of bezafibrate (25 mg/kg p.o.) on the size distribution of peroxisomes. A., Profiles measured with a Quantimet 720 and minicomputer (29). B., Peroxisome size distribution computed according to Wicksell (28) from the data in A.

TABLE 3. Morphometric analysis of effects of bezafibrate on peroxisomes[a]

	Control	Bezafibrate	% of Control
Measured values			
Number of electron micrographs	23	23	
Area analyzed (μm^2)	788	762	
Profiles observed	184	264	
Total profile area (μm^2)	29.7	45.9	
Computed values			
Mean radius (μm)	0.243	0.255	105%
Standard deviation	±0.058	±0.060	
Number of peroxisomes/μm^3	0.472	0.659	140%
Volume fraction of peroxisomes	0.0334	0.0535	160%

[a] Complementary data to Fig. 7A

Larger doses of clofibrate to female rats can elevate catalase and cause peroxisome proliferation, as shown by Reddy and Kumar (34). However, lower doses of clofibrate to male rats have not produced so far the dissociation of effects observed here with bezafibrate. For example, at a dose of clofibrate (0.05% in the chow) that increased liver weight 11%, palmitoyl-CoA oxidation was only 28% greater than controls.[7]

The reason for the sex difference in response to clofibrate is not known. Svoboda et al. (10) have shown that the increase in catalase activity depends on, and can be manipulated by changing, sex hormone levels.

The present results support and extend previous observations (1, 2, 7, 13, 31, 35) that the relative activities of the peroxisomal enzymes are not fixed, and the enzymatic composition of peroxisomes is subject to specific and delicate regulation.

These results, together with those of other workers (36–40), further substantiate the conclusion (4, 5) that the hypotriglyceridemic mechanism of action of these drugs involves peroxisomes. A total of seven hypolipidemic compounds has been found to cause increases in peroxisomal β-oxidation (1, 4, 5, 36–40).

In contrast, the mitochondrial β-oxidation system does not appear to be consistently affected by hypolipidemic drugs. We found only a small change (+23%) with bezafibrate (that may not be significant). Studies of the effect of clofibrate have given variable results: mitochondrial oxidation of palmitate has been reported to decrease (41), to increase (36), or not to change (37).

The possibility has been raised that peroxisome proliferators as a class may be carcinogenic in rats (17, 18). Whether this is of practical concern for the use of hypolipidemic drugs in humans deserves careful and cautious consideration. Svoboda and Azarnoff (17) note species differences in toxicity, rates of metabolism, etc. Dose may also be important. It is noteworthy that these peroxisome proliferators are not mutagenic in the *Salmonella*-microsome assay (42). Moreover, acetylsalicylic

acid is a peroxisome proliferator in rats (43, 44), but has been widely used by humans without apparent carcinogenicity.

The finding that the specific elevation of peroxisomal β-oxidation is not obligatorily coupled to increased catalase, gross hepatomegaly, or striking peroxisome proliferations, has several implications for future work on hypolipidemic drugs. First, it is not justified to infer that a drug is inactive as a lipid-lowering agent if it does not cause obvious peroxisome proliferation. Species and sex differences with respect to proliferation do not necessarily imply similar differences with respect to efficacy.

Second, the results suggest that a rapid and sensitive method of screening for new hypolipidemic drugs would be to look for changes in peroxisomal β-oxidation, or in one of the specific β-oxidation enzymes. These parameters increase sufficiently that treatment of three rats for 1 week suffices to detect changes that are statistically highly significant.

Lastly, comparison of these observations of bezafibrate's biochemical effects on male rats with previous studies of clofibrate's effects on male rats (1, 2, 4, 5, 7–11, 15, 16, 31, 33, 34, 36, 37, 41) suggests that clofibrate acts less specifically. Thus the side effects of clofibrate recently noted in the W. H. O. trials (45) may be unrelated to the drug's lipid-lowering effects. If true, it implies that useful clinical results may be obtainable with new drugs of this class and that further research and testing may be warranted.■

REFERENCES

1. 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. USA.* **73:** 2043–2046.
2. Lazarow, P. B. 1978. Rat liver peroxisomes catalyze the β-oxidation of fatty acids. *J. Biol. Chem.* **253:** 1522–1528.
3. Bronfman, M., N. C. Inestrosa, and F. Leighton. 1979. Fatty acid oxidation by human liver peroxisomes. *Biochem. Biophys. Res. Commun.* **88:** 1030–1036.
4. Lazarow, P. B. 1977. Three hypolipidemic drugs increase hepatic palmitoyl-coenzyme A oxidation in the rat. *Science.* **197:** 580–581.
5. Lazarow, P. B. 1978. Rat liver peroxisomal system of fatty acid β-oxidation: elevation by clofibrate and other hypolipidemic drugs. *In* International Conference on Atherosclerosis. L. A. Carlson, R. Paoletti, C. R. Sirtori, and G. Weber, editors. Raven Press, New York. 15–17.
6. de Duve, C., and P. Baudhuin. 1966. Peroxisomes (microbodies and related particles). *Physiol. Rev.* **46:** 323–357.
7. Hess, R., W. Stäubli, and W. Riess. 1965. Nature of hepatomegalic effect produced by ethyl-chlorophenoxyisobutyrate in the rat. *Nature.* **208:** 856–858.
8. Svoboda, D. J., and D. L. Azarnoff. 1966. Response of hepatic microbodies to a hypolipidemic agent, ethyl-chlorophenoxyisobutyrate (CPIB). *J. Cell Biol.* **30:** 442–450.
9. Svoboda, D., H. Grady, and D. Azarnoff. 1967. Microbodies in experimentally altered cells. *J. Cell Biol.* **35:** 127–152.
10. 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.
11. Reddy, J. K., and T. P. Krishnakantha. 1975. Hepatic peroxisome proliferation: induction by two novel compounds structurally unrelated to clofibrate. *Science.* **190:** 787–789.
12. Reddy, J. K., D. L. Azarnoff, D. J. Svoboda, and J. D. Prasad. 1974. Nafenopin-induced hepatic microbody (peroxisome) proliferation and catalase synthesis in rats and mice. *J. Cell Biol.* **61:** 344–358.
13. Leighton, F., L. Coloma, and C. Koenig. 1975. Structure, 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.
14. Moody, D. E., and J. K. Reddy. 1976. Morphometric analysis of the ultrastructural changes in rat liver induced by the peroxisome proliferator SaH 42-348. *J. Cell Biol.* **71:** 768–780.
15. Svoboda, D. J., and D. L. Azarnoff. 1971. Effects of selected hypolipidemic drugs on cell ultrastructure. *Federation Proc.* **30:** 841–847.
16. Stäubli, W., and R. Hess. 1966. Quantitative aspects of hepatomegaly induced by ethyl chlorophenoxyisobutyrate (CPIB). *In* Sixth International Congress for Electron Microscopy, Kyoto. Vol. II. R. Uyeda, editor. Maruzen Co., Ltd., Tokyo. 625–626.
17. Svoboda, D. J., and D. L. Azarnoff. 1979. Tumors in male rats fed ethyl chlorophenoxyisobutyrate, a hypolipidemic drug. *Cancer Res.* **39:** 3419–3428.
18. Reddy, J. K., D. L. Azarnoff, and C. E. Hignite. 1980. Hypolipidaemic hepatic peroxisome proliferators form a novel class of chemical carcinogens. *Nature.* **283:** 397–398.
19. Stegmeier, K., H. Stork, H. Lenz, F. Leuschner, and V. Liede. 1980. Pharmacology and mode of action of bezafibrate in animals. *In* Lipoproteins and Coronary Heart Disease. New Aspects in the Diagnosis and Therapy of Disorders of Lipid Metabolism. H. Greten, P. D. Lang, and G. Schettler, editors. G. Witzstrock, New York. 76–82.
20. Lazarow, P. B., and C. de Duve. 1973. The synthesis and turnover of rat liver peroxisomes. V. Intracellular pathway of catalase synthesis. *J. Cell Biol.* **59:** 507–524.
21. de Duve, C. 1971. Tissue fractionation: past and present. *J. Cell Biol.* **50:** 20D–55D.
22. Lazarow, P. B. 1981. Assay of peroxisomal β-oxidation of fatty acids. *Methods Enzymol.* **72:** 315–319.
23. Leighton, F., B. Poole, H. Beaufay, P. Baudhuin, J. W. 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.
24. Block, W. D., K. J. Jarrett, and J. B. Levine. 1965. Use of a single color reagent to improve the automated determination of serum total cholesterol. *In* Automation in Analytical Chemistry. J. H. Allen, editor. Mediad, Inc., New York. 345–347.
25. Kessler, G., and H. Lederer. 1965. Fluorometric measurement of triglycerides. *In* Automation in Analytical Chemistry. J. H. Allen, editor. Mediad, Inc., New York. 341–344.
26. Novikoff, A. B., P. M. Novikoff, C. Davis, and N. Quintana. 1972. Studies on microperoxisomes. II. A cytochemical method for light and electron microscopy. *J. Histochem. Cytochem.* **20:** 1006–1023.
27. Weibel, E. R., G. S. Kistler, and W. F. Scherle. 1966. Practical stereological methods for morphometric cytology. *J. Cell Biol.* **30:** 23–38.
28. Wicksell, S. D. 1925. The corpuscle problem. A mathematical study of a biometric problem. *Biometrika.* **17:** 84–99.
29. Baudhuin, P., M. A. Leroy-Houyet, J. Quintart, and P. Berthet. 1979. Application of cluster analysis for characterization of spatial distribution of particles by stereological methods. *J. Microsc.* **115:** 1–17.
30. Moody, D. E., and J. K. Reddy. 1976. Morphometric analysis of the ultrastructural changes in rat liver induced by the peroxisome proliferator SaH 42-348. *J. Cell Biol.* **71:** 768–780.
31. Moody, D. E., and J. K. Reddy. 1978. The hepatic effects of hypolipidemic drugs (clofibrate, nefanopin, tibric acid and Wy-14,643) on hepatic peroxisomes and peroxisome-associated enzymes. *Am. J. Pathol.* **90:** 435–437.
32. Lazarow, P. B. 1980. Elevation of peroxisomal β-oxidation by bezafibrate. *In* Lipoproteins and Coronary Heart Disease. New Aspects in the Diagnosis and Therapy of Disorders of Lipid Metabolism. H. Greten, P. D. Lang, and G. Schettler, editors. G. Witzstrock, New York. 96–100.
33. Fahimi, H. D., P. Kalmbach, K. Stegmeier, and H. Stork. 1980. Comparison between the effects of clofibrate and bezafibrate upon the ultrastructure of rat heart and liver. *In* Lipoproteins and Coronary Heart Disease. New Aspects in the Diagnosis and Therapy of Disorders of Lipid Metabolism. H. Greten, P. D. Lang, and G. Schettler, editors. G. Witzstrock, New York. 64–75.
34. Reddy, J. K., and N. S. Kumar. 1979. Stimulation of cat-

alase synthesis and increase of carnitine acetyltransferase activity in the liver of intact female rats fed clofibrate. *J. Biochem.* **85:** 847–856.

35. Reddy, J. K., D. E. Moody, D. L. Azarnoff, and M. S. Rao. 1977. Hepatic catalase is not essential for the hypolipidemic action of peroxisome proliferators. *Proc. Soc. Exp. Biol. Med.* **154:** 483–487.

36. Mannaerts, G. P., L. J. Debeer, J. Thomas, and P. J. de Schepper. 1979. Mitochondrial and peroxisomal fatty acid oxidation in liver homogenates and isolated hepatocytes from control and clofibrate-treated rats. *J. Biol. Chem.* **254:** 4585–4595.

37. Christiansen, R. Z., H. Osmundsen, B. Borrebaek, and J. Bremer. 1978. The effects of clofibrate feeding on the metabolism of palmitate and erucate in isolated hepatocytes. *Lipids.* **13:** 487–491.

38. Osumi, T., and T. Hashimoto. 1978. Enhancement of fatty acyl-CoA oxidizing activity in rat liver peroxisomes by di-(2-ethylhexyl)phthalate. *J. Biochem.* **83:** 1361–1365.

39. Inestrosa, N. C., M. Bronfman, and F. Leighton. 1979. Detection of peroxisomal fatty acyl-coenzyme A oxidase activity. *Biochem. J.* **182:** 779–788.

40. Ishii, H., and T. Suga. 1979. Clofibrate-like effects of acetylsalicylic acid on peroxisomes and on hepatic and serum triglyceride levels. *Biochem. Pharmacol.* **28:** 2829–2833.

41. Cederbaum, A. I., T. V. Madhavan, and E. Rubin. 1976. Effect of chronic clofibrate administration on mitochondrial fatty acid oxidation. *Biochem. Pharmacol.* **25:** 1285–1292.

42. Warren, J. R., V. F. Simmon, and J. K. Reddy. 1980. Properties of hypolipidemic peroxisome proliferators in the lymphocyte [^{3}H]thymidine and *Salmonella* mutagenesis assays. *Cancer Res.* **40:** 36–41.

43. Hruban, Z., H. Swift, and A. Slesers. 1966. Ultrastructural alterations of hepatic microbodies. *Lab. Invest.* **15:** 1884–1901.

44. Hruban, Z., M. Gotoh, A. Slesers, and S-F. Chou. 1974. Structure of hepatic microbodies in rats treated with acetylsalicyclic acid, clofibrate, and dimethrin. *Lab. Invest.* **30:** 64–75.

45. Report of the committee of principal investigators, 1980. W. H. O. cooperative trial on primary prevention of ischaemic heart disease using clofibrate to lower serum cholesterol: mortality follow-up. *Lancet.* **2:** 379–385.

46. Stäubli, W., and R. Hess. 1975. Lipoprotein formation in the liver cell. Ultrastructural and functional aspects relevant to hypolipidemic action. *In* Hypolipidemic Agents. D. Kritschevsky, editor. Springer-Verlag, New York. 229–289.