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Hepatic Catalase Is Not Essential for the Hypolipidemic Action of Peroxisome Proliferators[1] (39699)

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Previous studies demonstrated that a number of structurally unrelated compounds that lower serum lipid levels in man (1) and experimental animals (2-4) cause a striking increase in hepatic peroxisome (microbody) profiles in rats and mice (5-10). The increase in peroxisomes was accompanied by a significant elevation of hepatic catalase and carnitine acetyltransferase activities (9, 11, 12). The frequent association between hepatic peroxisome proliferation and hypolipidemia strongly suggested the possibility that either peroxisome catalase or some other peroxisomal enzyme may be involved in lipid metabolism (8, 9).

The present studies were conducted to evaluate the role of hepatic peroxisomal catalase in the hypolipidemic effect exerted by the peroxisomal enzyme inducers. These compounds were administered either in combination with allylisopropylacetamide (AIA), which inhibits the catalase synthesis (13), or with aminotriazole (AT), which combines irreversibly with catalase and inactivates this enzyme (14). We now report that a substantial reduction in serum lipids occurred in rats, although the liver catalase activity was markedly diminished, and that the proliferated peroxisomes lacked cytochemically demonstrable catalase.

Materials and Methods. Inbred male F-344 rats (Simonson Laboratories, Inc., Gilroy, Calif.) weighing between 135-175 g were used in these studies. The animals were force-fed a semisynthetic diet as described previously (15). The hypolipidemic drugs, clofibrate (100 mg/kg body wt), methyl clofenapate (10 mg/kg body wt), or nefenopin {2-methyl-2[*p*-(1, 2, 3, 4-tetrahydro-1-naphthyl)phenoxy]-propionic acid, Su-13,437} (50 mg/kg body wt) were administered by gavage twice daily at 12-hr intervals. Allylisopropylacetamide (AIA) (Hoffman-LaRoche, Inc., Nutley, N.J.), dissolved in saline, was given sc in a dose of 200 mg/kg body wt every 12 hr. 3-Amino-1,2,4-triazole (K & K Laboratories, Plainview, N.Y.) was dissolved in water and injected ip (1 g/kg body wt) twice daily. Each group consisted of five rats (Fig. 1).

The serum total cholesterol and glyceride glycerol were determined by the method of Azarnoff (16). Liver catalase activity was assayed by the spectrophotometric method of Lück (17) as previously described (7). The activity of carnitine acetyl transferase (CAT) was measured on high-speed supernatants of sonified rat liver homogenates, using the thio-acceptor DTNB [5,5′-dithiobis(2-nitrobenzoate)]. The release of CoA from acetyl CoA, in the presence of carnitine, was measured at 412 nm (12). Total protein was measured by the method of Lowry *et al.* (18).

Liver for electron microscopy was fixed in 2.5% glutaraldehyde, buffered with 0.1 *M* sodium cacodylate, pH 7.4, for 4 hr and rinsed overnight in the cacodylate buffer. The finely chopped sections were incubated in the 3,3′-diaminobenzidine medium of Novikoff and Goldfischer (19), postfixed in 2% OsO_4, and processed for electron microscopy (7).

Results. The effect of combined administration of AIA and the peroxisome proliferators on liver catalase and serum lipids. Figure 1 shows the results of AIA administration in combination with the peroxisome proliferator clofibrate, methyl clofenapate, or nafenopin for 7 days. The expected increases in liver weight and catalase activity were observed in rats treated with the hypo-

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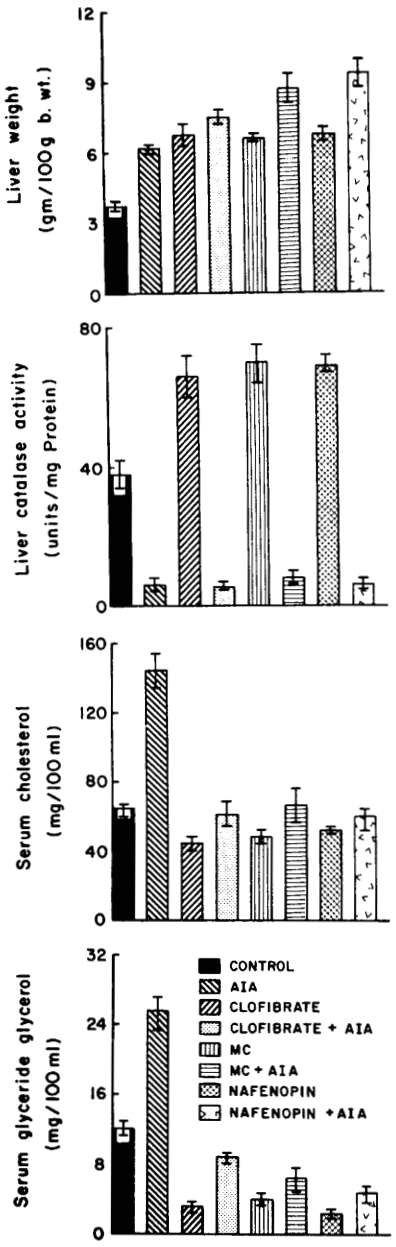

FIG. 1. Changes in liver weight, liver catalase activity, and serum lipids in rats treated with allylisopropylacetamide (AIA) and the hypolipidemic drugs clofibrate, methyl clofenapate (MC) and nafenopin. Rats received the hypolipidemic drug by gavage twice daily; AIA was injected sc also twice daily for 7 days. Doses are given in the text. Each bar represents the mean ± SE of five rats in each group.

lipidemic drugs clofibrate, methyl clofenapate, or nafenopin. In contrast, the liver catalase activity decreased markedly in animals given AIA alone or in combination with the peroxisome proliferators. A significant hypercholesterolemic as well as hypertriglyceridemic effect was observed in rats treated with AIA alone, confirming the results of other workers (20, 21). On the other hand, rats to which AIA was administered with clofibrate, methyl clofenapate, or nafenopin, had significantly lower serum lipids than those which received AIA alone ($p < 0.001$), although the liver catalase activity in these animals was almost completely abolished. A marked proliferation of hepatic peroxisomes, however, occurred in these animals given combined treatment, but these organelles did not contain cytochemically demonstrable catalase (Fig. 2B). As expected, the proliferated peroxisomes in animals treated with only clofibrate, methyl clofenapate, or nafenopin showed intense reaction product, when incubated in the 3,3′-diaminobenzidine medium (Fig. 2A). The peroxisomes in the livers of rats treated with AIA alone for 7 days were smaller than normal and showed no increase in their numbers (15, 22).

Hepatic carnitine acetyltransferase activity (CAT) in rats treated with clofibrate and allylisopropylacetamide (AIA) or with aminotriazole (AT). CAT activity was assayed following the administration of clofibrate with AIA or with AT to ascertain if this enzyme is increased in proliferated peroxisomes which lacked catalase. The CAT activity increased significantly in rats treated with clofibrate alone or in combination with AIA or AT (Table 1). The administration of AIA or AT alone for 7 days did not increase the activity of this enzyme.

Discussion. In an earlier study (15) we have shown that when clofibrate was administered to rats receiving AIA, an agent that blocks the synthesis of catalase (13), the proliferation of peroxisomes occurred even though the liver catalase activity was negligible. The present studies extend this observation with other peroxisome proliferators and demonstrate that peroxisomes proliferated under the influence of AIA lacked cytochemically demonstrable catalase. It is to be noted that the cytochemical staining reaction in peroxisomes is the result of peroxidatic oxidation of 3,3′-diaminobenzidine by the enzyme catalase. The absence of cyto-

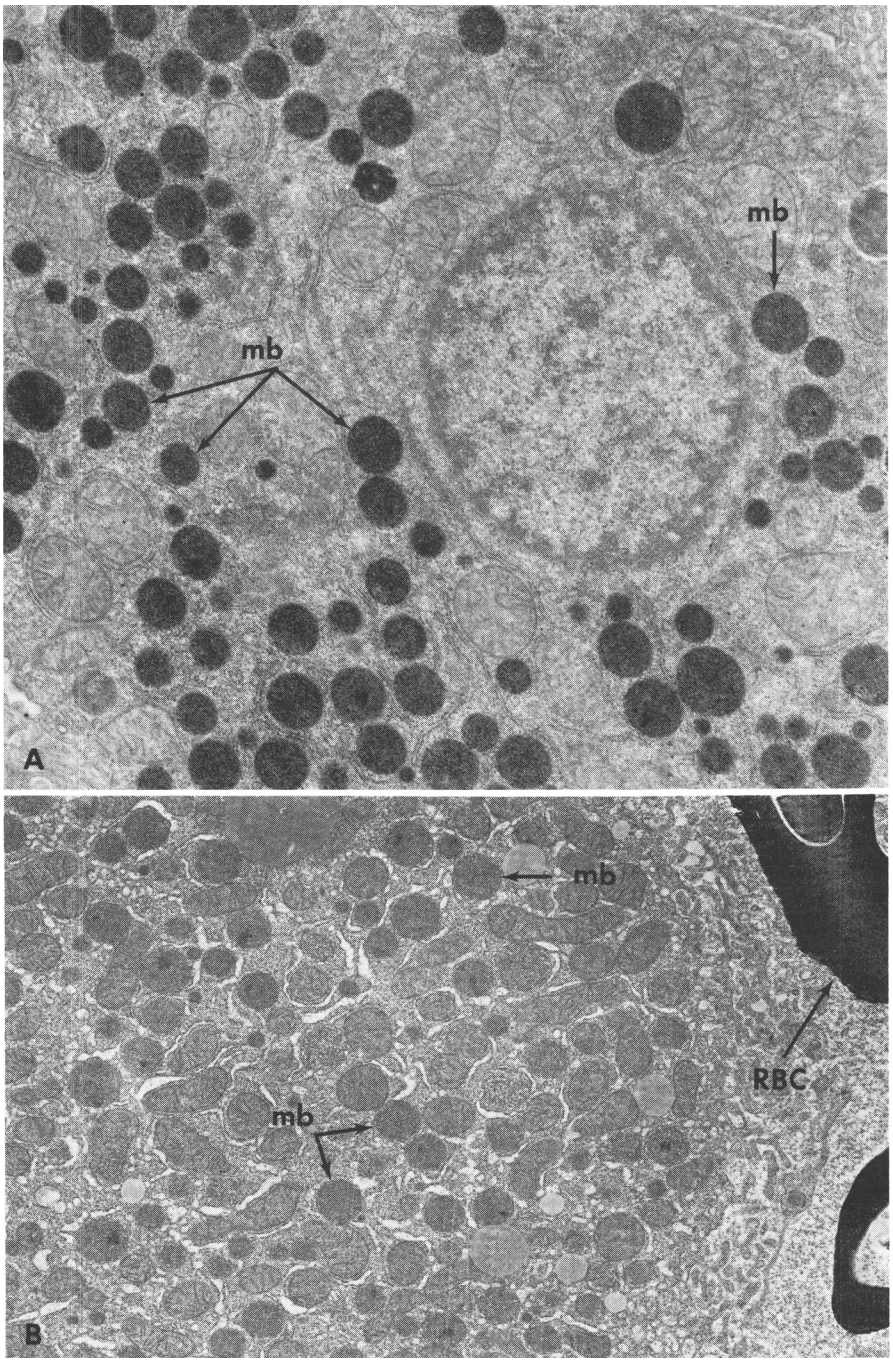

FIG. 2. A. Rat treated with clofibrate for 7 days. The hepatic peroxisomes (microbodies, mb) are increased in number and display intense reaction product after incubation for 30 min at 37° in the alkaline 3,3′-diaminobenzidine medium, pH 9.0. × 14,800. B. Rat treated with AIA and clofibrate for 7 days. Peroxisome (mb) proliferation is evident, but these organelles contain very little reaction product after incubation in the diaminobenzidine medium as in Fig. 2A. The red blood cells (RBC) in the sinusoids stained positively because of the peroxidatic activity of hemoglobin. × 12,500.

TABLE I. EFFECT OF ADMINISTRATION OF CLOFIBRATE WITH ALLYLISOPROPYLACETAMIDE (AIA) OR WITH AMINOTRIAZOLE (AT) ON HEPATIC CARNITINE ACETYLTRANSFERASE ACTIVITY IN F-344 Male Rats.

Group[a]	Number of animals	Carnitine acetyltransferase activity (units/mg of protein)[b]
Control	4	3.3 ± 0.4
Clofibrate	4	128.9 ± 12.6*
AIA	5	4.2 ± 1.4
Clofibrate + AIA	4	43.1 ± 3.3*
AT	5	5.9 ± 1.7
Clofibrate + AT	5	80.9 ± 6.5*

[a] The drugs were administered for 7 days: clofibrate, 100 mg/kg body wt, twice daily by gavage; AIA, 200 mg/kg body wt twice daily by sc injection; and AT, 1000 mg/kg body wt twice daily by ip injection.
[b] Mean ± SE.
* Significantly different from controls or from AIA- or AT-treated animals ($p < 0.001$).

chemical staining reaction in peroxisomes as well as the marked reduction in catalatic activity appear to be the result of low levels of catalase protein in the livers of rats treated with AIA and the hypolipidemic peroxisome proliferators. By immunoprecipitation method, the amount of catalase protein in these animals was found to be markedly diminished (unpublished observation). It is also clear from these studies that there is a significant serum lipid lowering effect in rats treated concurrently with a hypolipidemic agent and AIA, despite the abolition of catalase in proliferated hepatic peroxisomes and the very low activity of this enzyme in liver homogenates.

The studies of Caravaca *et al.* (23) demonstrated that hepatocatalase peroxidase, an active peroxidase-oxidase subunit isolated from beef liver catalase, induced a hypocholesterolemic effect in rabbits. This observation, prompted Svoboda and Azarnoff (6) to suggest that the hypolipidemic effect of clofibrate might be mediated by the endogenous increase of liver catalase resulting from peroxisome proflieration. Furthermore, the lower levels of serum lipids in a strain of acatalasemic mice were attributed to unstable catalase that displayed increased *in vivo* peroxidatic activity (24). In the present studies a substantial serum cholesterol and triglyceride lowering effect was evident in animals treated simultaneously with AIA and peroxisome proliferators, suggesting that peroxisome catalase is not necessary for the mediation of hypolipidemic action. A recent study on the effect of 3-amino-1,2,4-triazole on lipid metabolism also indicates that catalase itself is not directly related to lipid metabolism (25).

The observation that peroxisome catalase is not essential for the hypolipidemic action of these drugs should not be construed as evidence that peroxisome proliferation is not related to lipid metabolism. Because peroxisome proliferation can be induced in the absence of catalase synthesis, Reddy *et al.* (15) suggested that this might be due to the possible induction of yet unidentified proteins that make up between one-half and two-thirds of the total peroxisome proteins (26). The increase in hepatic CAT activity in rats treated simultaneously with AIA and clofibrate, in which the peroxisomes were deficient in catalase, is an indication that other peroxisomal enzymes are induced by these hypolipidemic drugs. The CAT has been identified in rat liver peroxisomes (27), and we have shown that all agents capable of inducing peroxisome proliferation also increase the activity of this enzyme (12). Lazarow and deDuve (28) have recently detected the fatty acyl-CoA oxidizing system in rat liver peroxisomes and showed that this activity increased in rats treated with clofibrate. It appears that synthesis of these and possibly other peroxisomal enzymes, but not catalase, may be responsible for the hypolipidemic effect of these peroxisome proliferators.

Summary. To evaluate the role of peroxisomal catalase in lipid metabolism the hypolipidemic drugs clofibrate, methyl clofenapate, and nafenopin were administered to rats in combination with allylisopropylacetamide, an agent which inhibits catalase synthesis. The number of peroxisomes in liver cells was increased, but these organelles did not contain cytochemically demonstrable catalase. A marked reduction in liver catalase activity was also noted. Although the proliferated peroxisomes were deficient in catalase activity, a substantial hypolipidemic effect was observed in these animals, suggesting that peroxisome catalase is not nec-

essary for the hypolipidemic effect. The increase in carnitine acetyltransferase activity in these animals emphasizes the need to delineate other peroxisomal enzymes which may be involved in lipid metabolism.

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