beled lamb tubulin is inhibited by 50 percent with 120 ng of lamb brain tubulin, 70 ng of chick tubulin, or with 45 ng of mouse tubulin (see Table 2). These results suggest that, while there are antigenic differences between brain tubulin molecules from different species that are discernible by these immunological criteria, this system can be used to quantitate tubulins from different sources. However, it is clear that standard inhibition curves should be generated with the use of competitor tubulin from the same species as the tissue being assayed for tubulin content.

The application of immunological methods to the study of microtubule protein has expanded the repertoire of analytical approaches to this ubiquitous protein. Similarities of tubulin in different species have been shown by cross-reactivity to antiserums produced against microtubule-containing structures (*13*). Antibodies produced to outer doublet tubulin of *Naegleria* flagella have been used to quantitate flagellar tubulin in amoeboid and flagellated *Naegleria*, but fail to cross react with cytoplasmic tubulin (*14*). On the other hand, antibodies to both brain (cytoplasmic) and sea urchin outer doublet (flagellar) tubulins have been used to localize cytoplasmic microtubules in cells by immunofluorescence (*5*, *6*). In addition, antiserum against brain tubulin has been studied for its effect on the colchicine-binding activity of the molecule (*15*), and an immunosorbant made with platelet tubulin antibody has been used for rapid isolation of platelet tubulin (*16*). The RIA described here now provides a sensitive tool to quantitate cytoplasmic tubulin which detects amounts ranging from 20 to 1500 ng, and does so independently of colchicine-binding activity and the ratio of tubulin subunits to microtubules in the assay. Radioimmunoassay thus provides a technique for measuring cytoplasmic tubulin in a variety of systems even when only limited amounts of material are available.

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Three Hypolipidemic Drugs Increase Hepatic Palmitoyl–Coenzyme A Oxidation in the Rat

Abstract. *Male rats treated with clofibrate, tibric acid, or Wy-14,643 show an 11- to 18-fold increase in the capacity of their livers to oxidize palmitoyl-coenzyme A. This provides a plausible biochemical mechanism for the action of these hypolipidemic drugs in reducing lipid concentrations in the serum.*

Clofibrate is an effective hypolipidemic as well as hypocholesterolemic agent (*1*) and is used extensively in the treatment of human hyperlipidemias. Its mechanism of action is not known in detail, however. Recently, we have found that rat liver peroxisomes oxidize palmitoyl–coenzyme A (palmitoyl-CoA), reducing O_2 to H_2O_2 and nicotinamide-adenine dinucleotide (NAD) to NADH (*2*). This peroxisomal system of fatty acid oxidation was found to be increased approximately one order of magnitude by clofibrate (*2*), suggesting that peroxisomes play a role in lowering serum lipid concentrations during clofibrate therapy.

To test further the possibility that hypolipidemic drugs act by increasing hepatic fatty acid oxidation we have measured the rate of palmitoyl-CoA oxidation in the livers of rats treated with two other hypolipidemic drugs that are structurally unrelated to clofibrate. Like clofibrate (*3*), these drugs have been reported to increase the number of hepatic peroxisomes (*4*).

Four groups of three male F-344 rats were fed ad lib with ground lab chow containing Wy-14,643 (1 g/kg of chow), tibric acid (1 g/kg), clofibrate (5 g/kg), or no drug (*5*). After 6 days, individual liver homogenates were prepared in 0.25*M* su-

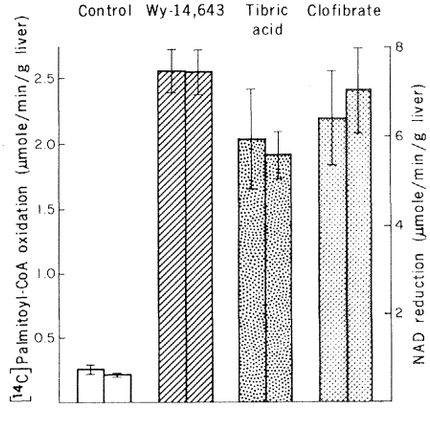

Fig. 1. Palmitoyl-CoA oxidation by liver homogenates of control and drug-treated rats. Assay of the rate of aerobic palmitoyl-CoA–dependent NAD reduction was performed as described (*2*) in the presence of 1 m*M* KCN to prevent reoxidation of the NADH formed (right bar of each pair and right axis). Assay of the oxidation of [1-^{14}C]palmitoyl-CoA was performed under similar conditions except KCN was omitted; the appearance of perchloric acid–soluble products was measured after 10 minutes of incubation at 37°C (left bar and axis). Rates (means and standard deviations) are expressed per gram of liver.

crose. Their ability to oxidize palmitoyl-CoA was measured by the rate of reduction of NAD and directly by the oxidation of [1-^{14}C]palmitoyl-CoA.

Figure 1 illustrates that homogenates of livers from the drug-treated rats oxidize palmitoyl-CoA 8 to 12 times faster than those of the control rats (statistical significance $P < .001$). A similar increase was observed with the two independent assay methods. Figure 1 also shows that under these conditions approximately 3 moles of NAD are reduced per mole of [1-^{14}C]palmitoyl-CoA oxidized, consistent with previous results (*2*).

The activity of catalase, a principal peroxisomal enzyme, is 45 to 55 percent greater in the livers of the drug-treated animals than in the controls (Fig. 2; $P < .01$). Elevation of hepatic catalase activity is characteristic of clofibrate treatment of male rats (*3*, *6*), and was recently reported for tibric acid and Wy-14,643 (*4*). However, the increases in catalase activity are much smaller than the increases in palmitoyl-CoA oxidation (compare Figs. 1 and 2).

The drugs have small and variable effects on the mitochondrial marker enzyme, cytochrome oxidase (Fig. 2). Previously we noted variability in the effects of clofibrate on this enzyme (*2*). The hepatic protein concentration is essentially unchanged in these experiments.

The weight of the liver is increased by 29 to 46 percent in the drug-treated rats [Fig. 2; $P < .005$ except for tibric acid (*7*) where $P < .025$]. If one multiplies the activities of Fig. 1 by these increases in liver weight, one obtains an oxidation rate of palmitoyl-CoA per total liver that is 11 to 18 times greater in the animals that have received a drug than in the controls.

The morphologic appearance of these livers was also examined. Figure 3 shows sections in which the peroxisomes are stained by means of the 3,3′-diaminobenzidine cytochemical reaction for catalase (*8*). Wy-14,643 and tibric acid appear to increase the frequency and size of peroxisomes. The increase in frequency is in agreement with the report of Reddy and Krishnakantha (*4*). Peroxisomal proliferation is a well-documented effect of clofibrate treatment of male rats (*3*, *6*), and has also been observed with several structural analogs of clofibrate (*9*).

These results demonstrate that three structurally diverse drugs with hypolipidemic properties each cause an increase in the rate at which liver homogenates oxidize palmitoyl-CoA. The increases vary between 11 and 18 times. Therefore, these drugs appear to produce their hypolipidemic effects by a common mechanism, namely, increasing fatty acid degradation in the liver.

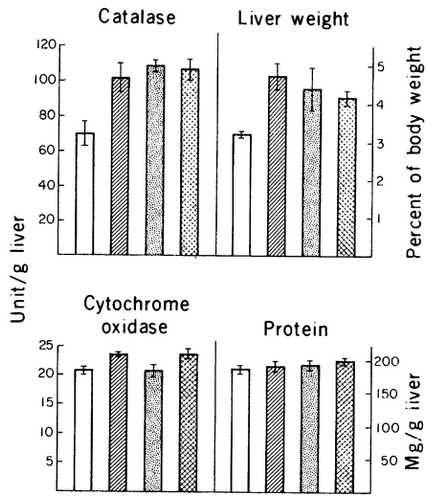

Fig. 2. Other biochemical effects of the drugs. Hepatic catalase, cytochrome oxidase, and protein concentrations were assayed according to Leighton *et al.* (*11*). Liver weights are expressed as percentages of the body weights, which do not change upon drug treatment (317 ± 20 g). Values shown are means and standard deviations. The bars are shaded as in Fig. 1.

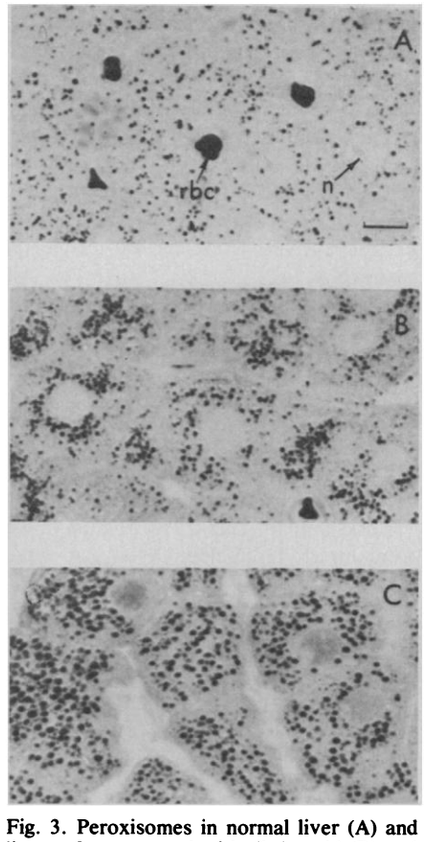

Fig. 3. Peroxisomes in normal liver (A) and livers of rats treated with tibric acid (B) and Wy-14,643 (C). Peroxisomes (the numerous small dark dots) are stained as described (*2*) with the alkaline H_2O_2-3,3′-diaminobenzidine method (*8*). Epon sections (1 μm) were photographed with a light microscope. The larger, irregularly shaped, stained structures are red blood cells (*rbc*) in sinusoids; *n*, nucleus. The magnifications are the same. The scale bar is 10 μm.

Previously we have found that the activity of the peroxisomal system of fatty acid oxidation is increased by approximately one order of magnitude in male rats treated with clofibrate (*2*). Much of the total palmitoyl-CoA oxidation in the homogenates of rats treated with tibric acid or Wy-14,643 also occurs (*10*) in particles with an equilibrium density in sucrose gradients of 1.21 to 1.28, characteristic of peroxisomes (*11*). Furthermore, clofibrate increases hepatic peroxisomal fatty acid oxidation in female rats (*10*), despite the lack of effect of this drug on catalase activity or peroxisome frequency in this sex (*6*). Taken together, these results make it very likely that the mechanism of action of hypolipidemic drugs involves an increase in the peroxisomal system of fatty acid oxidation. Some effect on the mitochondrial system is not excluded.

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