diates represent a step closely preceding a prototropic shift which subsequently leads to deaminated putrescine. A rapid exchange between the 2 orientations on the enzyme surface would account for the observed intramolecular isotope effect.

Irrespective of the actual mechanism, it remains that in every experiment performed, intramolecular effects were greater than values obtained for intermolecular effects. The intermolecular isotope effect is probably a measure of the influence of deuterium on the overall reaction, while intramolecular effects, which are less influenced by events prior to interaction with the catalytic surface, are an indicator of the process closely associated with bond cleavage.

1 Acknowledgment. This investigation was supported by a research grant from the National Institutes of Health, grant number NS 14017.

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Inducing effect of clofibrate on alkaline phosphatase and histidine-glyoxylate aminotransferase in rat liver

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Summary. The activity of plasma membrane alkaline phosphatase and both mitochondrial and peroxisomal histidine-glyoxylate aminotransferase was significantly increased in the livers of male rats following treatment with the hypolipidemic drug clofibrate. Cycloheximide or puromycin administration to rats inhibited the effects of clofibrate.

The hypolipidemic drug clofibrate (ethyl-p-chlorphenoxy-isobutyrate) induces hepatomegaly and marked proliferation of peroxisomes and smooth endoplasmic reticulum[1]. Treatment with this drug results also in an increase in the activities of some peroxisome-associated enzymes; catalase, carnitine acetyltransferase and the peroxisomal fatty acyl-CoA oxidizing system[1-3]. However, there are few data concerning the effect of clofibrate on the enzyme activity in the other cell compartments[4]. In the present communication we study the effects of clofibrate administration to rats on the activity of liver alkaline phosphatase and histidine-glyoxylate aminotransferase.

Materials and methods. Male Wistar rats (250–300 g) were injected i.p. with saline (control) or clofibrate in a dose 250 or 800 mg/kg once daily in the course of 4–16 days as indicated in the text. Animals were anesthetized with light ether, and killed by decapitation after 16–18 h starvation. Livers were immediately removed, homogenized and fractionated by differential centrifugation as described previously[5,6]. Plasma membranes were isolated from perfused livers by the method of Toda et al.[7]. In some experiments liver subcellular particles (peroxisomes and mitochondria) were fractionated by isopycnic sucrose density gradient centrifugation[5]. Alkaline phosphatase; EC 3.1.3.1[7], D-amino acid oxidase; EC 1.4.3.3[8], acid phosphatase; EC 3.1.3.2[9], glucose-6-phosphatase; EC 3.1.3.9[10] and histidine-glyoxylate aminotransferase; EC 1.6.1[11] were determined at 37 °C. Catalase; EC 1.11.1.6[8], and carnitine acetyltransferase; EC 2.3.1.7[7] were determined at 25 °C. Chlorophenoxy isobutyric acid (sodium salt) was prepared by alkaline hydrolysis of clofibrate[12]. Protein was measured by the method of Lowry et al.[13].

Results and discussion. It was found that clofibrate treatment changes some peroxisomal enzyme activities (table). Thus, the activity of carnitine acetyltransferase was increased more than 20-fold, whereas the activity of D-amino acid oxidase was decreased to 10–20% of the control level. There are no pronounced changes in catalase activity after such treatment. However, the total activity of rat liver catalase in animals treated with clofibrate at a dose of

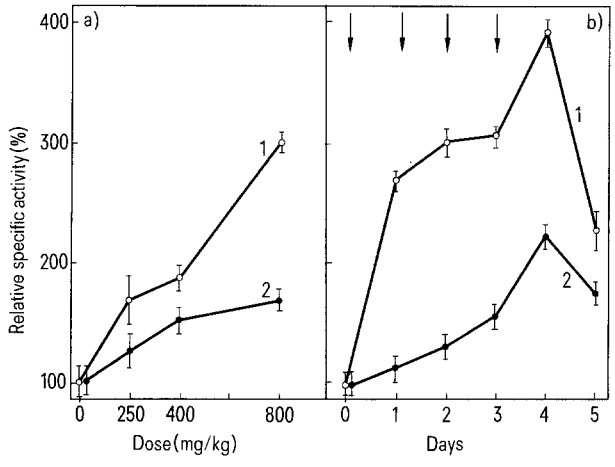
Effect of clofibrate treatment on the activities of hepatic enzymes

Enzyme		Activity (nmole/min/mg protein) Control	Clofibrate
Catalase, units*	(9)	0.298 ± 0.045	0.299 ± 0.058
D-amino acid oxidase	(12)	1.4 ± 0.4	0.4 ± 0.1**
Carnitine acetyltransferase	(5)	2 ± 1	48 ± 12**
Histidine-glyoxylate aminotransferase	(4)	1.0 ± 0.1	2.2 ± 0.2**
Glucose-6-phosphatase	(5)	68 ± 12	53 ± 8
Alkaline phosphatase	(4)	4.8 ± 1.2	16.3 ± 3.0**
Acid phosphatase	(12)	156 ± 18	147 ± 18

* Catalase activity was expressed as described[10]. Mean values ± SE are shown for specific activities, and the number of observations is given in parentheses. Clofibrate (250 mg/kg) was injected for 16 days. ** Increase statistically significant - $p \leq 0.001$.

800 mg/kg for 4 days was more than 1.25-fold ($p \leq 0.01$) greater than in the controls. These results are in a good agreement with previous reports[2,14]. The change in peroxisomal enzyme content was accompanied at the same time by the increase of rat liver alkaline phosphatase and histidine-glyoxylate aminotransferase activities in the clofibrate treated animals (table). The total and the specific activities of acid phosphatase and glucose-6-phosphatase were unchanged. The effect of clofibrate on the alkaline phosphatase and histidine-glyoxylate aminotransferase activities was not observed after incubation (37 °C, 15 min) of rat liver homogenates with 1 mM drug (final concentration) or its pharmacologically active derivative chlorophenoxy isobutyric acid. More pronounced changes of enzyme activities were obtained after treatment with higher doses of clofibrate (fig. a). The time-course of enzyme induction by clofibrate showed (fig. b) that the both activities reached the maximum at 4 days and returned rapidly towards the normal values after the end of clofibrate injections. Cycloheximide (1.5 mg/kg) or puromycin (5 mg/kg) injected i.p. inhibited induction of enzyme activities in rats following a single clofibrate administration (800 mg/kg, animals were killed 28 h after the drug treatment). This result suggests that de novo protein synthesis is essential for the induction of hepatic alkaline phosphatase and histidine-glyoxylate aminotransferase by clofibrate. To clarify the possible effects of clofibrate on the subcellular distribution of both enzymes, plasma membranes, mitochondria, peroxisomes and soluble fraction were isolated from control and treated rat livers. All preparations of plasma membranes showed similar recoveries of the alkaline phosphatase activities (20–23% of the total homogenate activity) and purification ratios of the enzyme (25–28-fold). This indicates that alkaline phosphatase induced by clofibrate was located mainly in the plasma membranes, as observed in intact livers.

After fractionation of liver homogenates by differential centrifugation, the highest specific activity of the histidine-glyoxylate aminotransferase was observed in the 'light' mitochondrial fraction together with catalase, a peroxisomal marker. The soluble fractions from the livers of control and clofibrate-treated animals contained 26.8% and 46.1% of total enzyme activity, respectively. The difference may be a consequence of a greater fragility of peroxisomes in treated rats[14]. In order to confirm the distribution of histidine-glyoxylate aminotransferase in peroxisomes and mitochondria, the 'light' mitochondrial fraction was further fractionated by sucrose density gradient centrifugation. The enzyme was widely distributed in both mitochondria and peroxisomes and its specific activity in both organelles doubled after clofibrate treatment. Thus the clofibrate-induced increase of histidine-glyoxylate aminotransferase in rat liver may be the consequence of the enhancement of the activity in mitochondria and peroxisomes.



Effect of clofibrate on the activity of alkaline phosphatase (1) and histidine-glyoxylate aminotransferase (2) in rat liver homogenate. *a* Effect of various clofibrate doses. Clofibrate (250, 400 and 800 mg/kg) was given once a day for 3 days. *b* Time course of changes in enzyme activities. The arrow indicates the injections of clofibrate (800 mg/kg). Results are expressed as the means ± SD for 3–4 rats.

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Solubilization of prolactin receptor by a Zwitterionic detergent[1]

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Summary. About 60% of the prolactin receptors were solubilized from rabbit mammary gland membranes by Zwittergent 3-12. The use of Zwittergent 3-12 resulted in increased sensitivity of the receptor assay and permitted use of ovine prolactin instead of human growth hormone in the receptor assay.

Purification of protein hormone receptors requires the use of a suitable detergent to remove the receptors from the membrane. The nonionic detergent Triton X-100 has been used to solubilize prolactin receptors[2,3] but has several undesirable properties, including absorbance of UV-light, interference with common protein determination methods,