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Increase in hepatic pyruvate (glyoxylate) aminotransferase activity on administration of clofibrate to the rat

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Administration of hypolipidemic drug, clofibrate has been shown to induce a rapid and marked proliferation of hepatic peroxisomes [1]. The increase in hepatic peroxisomes is accompanied by a significant elevation of peroxisomal enzyme, catalase [1–3]. We have described that pyruvate (glyoxylate) aminotransferase of rat liver is found in the peroxisomal and mitochondrial matrices and in the soluble fraction from broken peroxisomes [4]. The peroxisomal and mitochondrial enzymes have nearly identical enzymatic, physical and immunological properties [5]. However, the two enzymes show different response to glucagon; the mitochondrial enzyme is induced by the injection *in vivo* of glucagon but the peroxisomal enzyme is not [6]. The present report describes the effect of clofibrate feeding on the peroxisomal and mitochondrial pyruvate (glyoxylate) aminotransferase in rat liver.

Materials. Clofibrate (ethylα-*p*-chlorophenoxyisobutyrate) was obtained from Funai General Research Centre (Osaka, Japan). Other materials were obtained as stated previously [4].

Drug administration. Male rats of the Donryu strain weighing 100–160 g were fed 0.5% (w/w) clofibrate in the ground lab. chow *ad lib.* for the period indicated. Control rats were fed the basal diet for the same period.

Determination of enzyme activities. Separate assays of peroxisomal, mitochondrial and supernatant pyruvate (glyoxylate) aminotransferase were carried out as previously described [4]. Pyruvate (glyoxylate) aminotransferase was assayed with phenylalanine (40 mM) and pyruvate (20 mM) as substrates. Transamination between L-amino acids and 2-oxo acids [7], catalase [8], and glutamate dehydrogenase [9] were assayed as described in the cited references. A unit of enzyme activity is defined as the amount of enzyme that catalyses a formation of product or a decrease in substrate of 1 μmole/min at 37°.

Other methods. Protein was determined by the method of Lowry *et al.* [10] with bovine serum albumin as standard. Subfractionation of liver homogenate by sucrose density gradient centrifugation [4], and Sephadex G-150 gel-filtration and sucrose density gradient centrifugation for the determination of approximate molecular weights [11, 12] were carried out as described in the cited references. Statistical significance between means from control and clofibrate-treated groups was estimated by Student's *t*-test.

Results and Discussion. Rat liver pyruvate (glyoxylate) aminotransferase activity associated with peroxisomes and mitochondria was measured during the time–course of clofibrate feeding experiment. The total activity was found to increase rapidly during treatment in the whole homogenate and in the supernatant fraction, reaching maximum values by 10 days (Fig. 1). In contrast, the mitochondrial and peroxisomal activities did not increase significantly. After withdrawal of the drug, pyruvate (glyoxylate) aminotransferase activities in the whole homogenate and in the supernatant fraction decreased rapidly and reached control levels within 10 days.

Leighton *et al.* [13] reported that the treatment by Su-13437, a hypolipidemic drug, resulted in a decrease of catalase activity in particulate-bound activity of rat liver and five-fold increase in the supernatant activity, suggesting that the supernatant activity is from broken peroxisomes. On the basis of previous reports that the supernatant pyruvate (glyoxylate) aminotransferase activity in control rat liver is from broken peroxisomes [4], it is suggested that the increased activity of the supernatant enzyme by clofibrate treatment is presumably from broken peroxisomes. To obtain further evidence, subcellular distribution and properties of hepatic pyruvate (glyoxylate) aminotransferase of clofibrate-treated rats were examined. A representative sedimentation in a sucrose density gradient from clofibrate-treated rat liver homogenate is presented in Fig. 2. The peroxisomes and the mitochondria were separated: the peroxisomal fraction marked by catalase was at a density of 1.25 g ml^{-1} and the mitochondria marked by glutamate dehydrogenase at a density of 1.18 g ml^{-1}. About 32 per cent of phenylalanine : pyruvate aminotransferase [pyruvate (glyoxylate) aminotransferase] activity was distributed in the mitochondria, about 22 per cent in the peroxisomes and the remainder in the supernatant top fraction. About 40 per cent of the catalase activity was in the peroxisomal fraction and the remainder in the supernatant top fraction. Most of the glutamate dehydrogenase activity was found in the mitochondrial fraction with little or no activity in the supernatant fraction. These results suggest that in clofibrate-treated rat liver, supernatant pyruvate (glyoxylate) aminotransferase activity is presumably from broken peroxisomes and not from broken mitochondria.

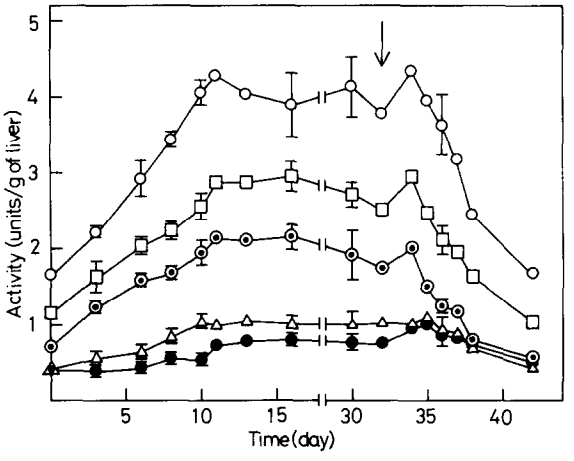

Fig. 1. Time–course of change in pyruvate (glyoxylate) aminotransferase activity during clofibrate treatment in rat liver. Pyruvate (glyoxylate) aminotransferase in each subcellular fraction of rat liver was assayed with L-phenylalanine (40 mM) and sodium pyruvate (20 mM) as substrates during treatment with clofibrate (0.5%, w/w). Whole homogenate (○); supernatant fraction (⊙); mitochondria (△); peroxisomes (●); combined supernatant and peroxisomal fraction (□). Detailed methods of subsecullar fractionation and enzyme assay are as described in the text. After 32 day-clofibrate treatment, rats were replaced on the drug-free diet (indicated by an arrow). Data are expressed as means ± S.D. of three experiments, and points without S.D. are means of two experiments. The activities in the whole homogenate and supernatant and combined supernatant and peroxisomal fractions significantly increased ($P < 0.01$) at the 10th day.

Pyruvate (glyoxylate) aminotransferase was prepared and characterized from the supernatant fraction of clofibrate-treated rat liver as previously described [11]. The final specific activity of the purified enzyme preparation was 3500 units/mg of protein. Substrate specificity, molecular weight (approximately 80,000), pH optimum, isoelectric point and kinetic parameters of the enzyme preparation were nearly identical with supernatant and peroxisomal pyruvate (glyoxylate) aminotransferase from control rat liver [4, 5].

An antibody against the purified pyruvate (glyoxylate) aminotransferase from rat liver mitochondria was prepared in a rabbit as previously described [4]. Upon Ouchterlony double diffusion analysis [14], the anti-mitochondrial pyruvate (glyoxylate) aminotransferase produced a single connecting band of precipitin among three pyruvate (glyoxylate) aminotransferase preparations purified from the peroxisomal and supernatant fractions of control rat liver [4], and from the supernatant fraction of clofibrate-treated rat liver.

These data suggest that the supernatant pyruvate (glyoxylate) aminotransferase activity is presumably from broken peroxisomes in clofibrate-treated rat liver.

The peroxisomal and mitochondrial pyruvate (glyoxylate) aminotransferase of rat liver respond differently to a hormone (glucagon) or a hypolipidemic drug (clofibrate). The mitochondrial enzyme is induced by the injection *in vivo* of glucagon (or cyclic 3′,5′-AMP) but the peroxisomal enzyme is not [6]. In contrast, the peroxisomal enzyme activity increased by clofibrate feeding, but the mitochondrial enzyme activity did not. However, the two enzymes have nearly identical physical, enzymatic and immunological properties, suggesting that the two enzymes are presumably coded by the same nuclear genome. The presice mechanism of the different responsibility of the two enzymes is being investigated.

Reddy *et al.* suggested that hepatic peroxisomal proliferation by hypolipidemic drugs is associated with hepatocarcinogensis in rats and mice [15]. Peroxisomal proliferation is accompanied by the increase in catalase, fatty acyl-CoA-oxidizing system and carnithine acetyltranferase in peroxisomes [1–3, 16–21]. Hepatic pyruvate (glyoxylate) aminotranferase activity may be also used as the marker for hepatic peroxisomal proliferation and carcinogensis.

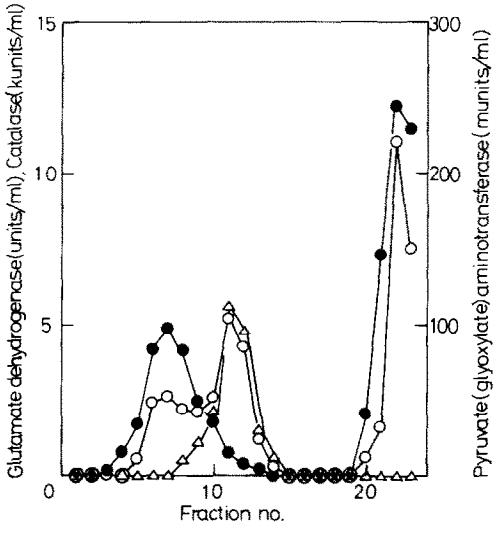

Fig. 2. Subcellular distribution of pyruvate (glyoxylate) aminotransferase in 10 day clofibrate-treated rat liver. The whole homogenate from 0.8 g of clofibrate-treated rat liver, after centrifugation at 300 *g* for 5 min, was placed over a linear sucrose gradient and centrifuged at 58,000 *g* for 150 min in a swingout bucket rotor as described in the text. Fractions (2.5 ml) were collected from the bottom of the tube. Pyruvate (glyoxylate) aminotransferase activity (○) was determined with L-phenylalanine (40 mM) and sodium pyruvate (20 mM) as substrates. Catalase (●) and glutamate dehydrogenase (△).

Summary. Pyruvate (glyoxylate) aminotransferase activity in hepatocytes of male rats was determined during one-month treatment with a hypolipidemic drug, clofibrate. The supernatant activity increased rapidly during treatment, reaching maximum values by 10 days. However, the mitochondrial and peroxisomal activities were not changed significantly. The increase in the supernatant activity was found to be presumably from broken peroxisomes.

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