Abstract—The hypolipidemic effect of alfibrate has been studied in normocholesterolemic rats with clofibrate as a reference. At the dosage of 150 mg/kg, 29% increase of liver weight was observed in the alfibrate group and 87% increase in the clofibrate group. Daily administration of alfibrate (150 mg/kg) produced a significant decrease in serum cholesterol (5%) and triglycerides (15%), and liver cholesterol (12%) and triglycerides (23%) with no change in phospholipids and free fatty acids. Like clofibrate, alfibrate exerted a greater effect on lowering the triglyceride level than cholesterol in normocholesterolemic rats. The liver glycogen concentration and glucose tolerance in the alfibrate group were similar to that of control.

Since susceptibility to coronary heart disease is closely related to an increased plasma cholesterol level (1), one of the most effective ways to prevent atherosclerosis is to lower cholesterol and other lipid levels in the serum. The following three mechanisms contribute to a decreasing serum cholesterol level either independently or cooperatively,

1. Decrease in the rate of hepatic cholesterol biosynthesis
2. Increase in the rate of hepatic cholesterol oxidation to bile acids
3. Decrease in cholesterol uptake in the intestine.

Various drugs influencing cholesterol metabolism at specific sites have been synthesized for clinical application. Among the hypolipidemic drugs, ethyl chlorophenoxyisobutyrate (clofibrate) has been most widely used due to the facts of its strong hypocholesterolemic effect through the all mechanisms mentioned above (2–7) and also to the hypolipidemic effect presumably due to the decrease in free fatty acid mobilization from adipose tissue (8), the increase in the peripheral uptake of lipoprotein (9, 10), the decrease in triglyceride and fatty acid synthesis of liver and in hepatic secretion of very low density lipoprotein (11, 12) etc. In spite of its strong hypolipidemic effect, clofibrate has the disadvantages of unpleasant odour, the property as a liquid and frequent occurrence of gastrointestinal. Hydroxy bis-[2-(p-chlorophenoxy)-isobutyric acid] aluminium (alfibrate was recently synthesized in order to overcome these disadvantages.

In this laboratory we found alfibrate to have a much lower acute and chronic
tixicity than clofibrate \( \text{LD}_{50} = 3,300 \text{ mg/kg for alfibrate, 1,625 mg/kg for clofibrate, i.p. in rats, more than 10,000 mg/kg for alfibrate, 1,950 mg/kg for clofibrate, orally in mice, and the excretion into urine was faster than that of clofibrate when 20 mg/kg was administered orally to rats.} \) Alfibrate was present in rat blood as 2-(p-chlorophenoxy)-isobutyric acid and was excreted as its glucuronide. The present studies have been undertaken to investigate the hypolipidemic effect of alfibrate in normo- and hypercholesterolemic rats and to compare it with that of clofibrate. In this paper, results on the hypolipidemic effect of alfibrate in normal rats, and the effects on glucose and glycogen metabolism as related to plasma lipid level and hence atherosclerosis are reported.

**MATERIALS AND METHODS**

*Experimental animals*

Male albino rats of Wistar strain weighing approx. 100 g were divided into three groups. To the first group, 150 or 300 mg/kg alfibrate suspended in 0.5% tragacanth solution was given orally every day for two weeks. To the second, 150 mg/kg clofibrate in 1% methylcellulose solution was given as a reference and to the third, saline as the control. During the experimental period the rats were given free access to commercial rat chow, and at the end of two weeks were fasted for 20 hr before being sacrificed. Blood was withdrawn from the retinal vessels of the rats lightly anethetized with ether. The livers for glycogen assay were obtained without a previous fasting period, chilled immediately by immersion into liquid nitrogen and kept at a temperature of \(-20^\circ\) until assayed. For glucose tolerance tests, the rats were injected with 600 mg/kg of glucose (25% glucose solution) i.v. through the tail vein after a 3 hr fast and 0.02 ml of blood sample was obtained from tail vein at the time specified.

*Measurement*

Liver lipids was extracted by the method of Folch (13). For serum and liver lipid analysis the following methods were used; cholesterol-Zack and Dickenman (14), triglycerides-Block and Jarret (15), free fatty acids-Ducombe (16) and phospholipids-Harris and Popat (18). Serum phospholipids was extracted by the method of Bloor (17) before analysis. Liver glycogen was extracted with 5% trichloroacetic acid and measured by the method of Carroll, Longley and Roe (19), and blood glucose by Boehringer's blood sugar test.

*RESULTS*

**Effect of alfibrate on growth rate and liver weight**

Growth rate of the groups on alfibrate 150 or 300 mg/kg orally for two weeks was similar to that of the control and clofibrate groups. The livers were, however, remarkably enlarged by alfibrate administration as reported by many authors regarding clofibrate. The results presented in Table 1 show that daily administration of alfibrate 150 mg/kg and 300 mg/kg for two weeks produced 29% and 37% increase in liver size respectively and at the dosage of 150 mg/kg clofibrate administration resulted in an 87%
increase, which indicates that the hepatomegalic effect of clofibrate is much greater than that of alfibrate.

**Effect on serum and liver lipid levels**

Tables 2 and 3 show the concentration of cholesterol, triglycerides, free fatty acids and phospholipids in serum and livers of rats treated with alfibrate or clofibrate for two weeks. Alfibrate administration lowered serum cholesterol by 5% and 14% significantly at the low and high dosages and decreased liver cholesterol concentration by approx. 11% at both dosages. Reduction rate of cholesterol at the low dosage was greater in the liver than in the serum. To compensate the dilution due to the hepatomegaly, the total quantity of liver cholesterol was calculated for each group. They were 20.3 mg for the control, 24.5 mg and 25.9 mg for the low and high groups of alfibrate, and 31.7 mg for the low dosage group of clofibrate, corresponding to 21, 28 and 56% increase to the control respectively.

Both alfibrate and clofibrate exerted a greater effect on lowering serum triglycerides than cholesterol, and a 15.4 and 23.0% decrease in serum triglycerides was observed in low alfibrate and clofibrate groups. Although some papers (20, 21) have reported no decrease of liver triglyceride content by clofibrate, a 23 and 19% decrease in liver triglyceride concentration was produced by alfibrate and clofibrate respectively in our experimental condition. Expressed in terms of total liver triglyceride content as in the case of cholesterol, however, it increased by 5.5% (28.7 mg/liver) in alfibrate group and by 56.6% (42.6 mg/liver) in clofibrate group.

The concentration of phospholipids and free fatty acids in the liver and serum was not been significantly changed except for the serum fatty acid level in the clofibrate group. At present, there is no adequate explanation. Our findings on the changes of serum and liver cholesterol, triglyceride and phospholipid levels by clofibrate administration are compatible with the report of Azarnoff, Tucker and Barr (3).
### Table 2. Effect of oral administration of alfibrate and clofibrate on serum lipid levels in normal rats.

| Drug administered (mg/kg daily) | Cholesterol | Triglycerides | Free fatty acids | Phospholipids |
|--------------------------------|-------------|---------------|-----------------|--------------|
|                                | mg% mean ± S.E. | % change | mg% mean ± S.E. | % change | µmole/100 ml mean ± S.E. | % change | mg% mean ± S.E. | % change |
| None                           | 61.7 ± 1.31 (14) |          | 92.0 ± 2.64 (8) |          | 38.6 ± 2.86 (8) |          | 163.2 ± 9.27 (9) |          |
| Alfibrate                      | 57.8 ± 1.50 (9)* | -5      | 77.8 ± 2.53 (10)** | -15.4  | 36.7 ± 1.13 (10) | -4.9    | 161.8 ± 11.49 (10) | -0.9   |
| 300                             | 53.1 ± 2.0 (15)** | -14     |                    |         |                   |         |                   |         |
| Clofibrate                     | 55.1 ± 2.07 (10)** | -10  | 70.8 ± 2.93 (10)** | -23.0  | 51.4 ± 1.54 (10)** | -33.2  | 150.4 ± 11.3 (9) | -7.8   |

Duration of treatment: 2 weeks
Numbers in parentheses indicate numbers of rats employed.
* significant P<0.1
** significant P<0.05
*** significant P<0.01

### Table 3. Effect of oral administration of alfibrate and clofibrate on liver lipids in normal rats.

| Drug administered (mg/kg daily) | Cholesterol | Triglycerides | Free fatty acids | Phospholipids |
|--------------------------------|-------------|---------------|-----------------|--------------|
|                                | mg/g mean ± S.E. (No. of rats) | % change | mg/g mean ± S.E. (No. of rats) | % change | µmole/g mean ± S.E. (No. of rats) | % change | mg/g mean ± S.E. (No. of rats) | % change |
| None                           | 3.86 ± 0.09 (14) |          | 5.16 ± 0.384 (8) |          | 49.1 ± 4.22 (8) |          | 26.2 ± 1.58 (10) |          |
| Alfibrate                      | 3.40 ± 0.148 (10)* | -11.9 | 3.98 ± 0.286 (10)* | -22.9 | 49.3 ± 2.01 (10) | +0.4 | 27.8 ± 1.54 (10) | +6.1   |
| 300                             | 3.45 ± 0.04 (16)** | -10.6 |                    |         |                   |         |                   |         |
| Clofibrate                     | 3.11 ± 0.101 (10)** | -19.4 | 4.18 ± 0.273 (8)** | -19.0 | 46.7 ± 1.34 (10) | -4.9 | 27.3 ± 0.731 (10) | +4.2   |

Duration of treatment: 2 weeks
* significant P<0.1
** significant P<0.05
**Effect on liver glycogen level**

Our preliminary experiment which showed an abnormal accumulation of glycogen in the liver of rabbits successively treated with alfibrate for one month, prompted us to investigate change of liver glycogen concentration in the drug-treated rats as related to the hepatomegalic and hypolipidemic action. Liver glycogen of the rats treated with the drugs for two weeks was determined without a previous fasting and the result is shown in Table 4. Contrary to our preliminary experiment with rabbits, the glycogen concentration did not change at all under the drug administration.

| Drug administered (mg/kg daily) | Liver glycogen (mg/g wet tissue) mean ± S.E. (No. of rats) |
|---------------------------------|----------------------------------------------------------|
| None                            | 33.1 ± 2.50 (8)                                          |
| Alfibrate 150                   | 29.1 ± 1.53 (8)                                          |
| Clofibrate 150                  | 31.9 ± 3.17 (8)                                          |

Rats were given free access to commercial rat chow during the experimental period and the liver for glycogen assay was obtained without a previous fasting period.

**Effect on glucose tolerance**

It is well known that an elevated blood sugar level gives an increased supply of reduced pyridine nucleotides necessary for biosynthesis of cholesterol and fatty acids, and often causes hyperlipidemia. Since long term administration of alfibrate to rats showed a tendency to lower the blood sugar level, effect of alfibrate on glucose metabolism has been studied in relation to the hypolipidemic action. In this experiment 600 mg/kg glucose was injected i.v. into the drug-treated rats through the tail vein and blood glucose disappearance rate was measured at the time specified. Results are shown in Table 5 and Fig. 1. Clofibrate group always revealed a higher blood sugar level than those of alfibrate and control groups without statistical significance. Taking the log concentration of blood glucose in abscissa and the time in the ordinate a declining linear relation was obtained within 45 min. Regression curves for the respective groups

| Drug administered* (150 mg/kg daily) | No. of rats | Time after glucose loaded** (min) |
|--------------------------------------|-------------|-----------------------------------|
|                                      | Before      | 10      | 20      | 30      | 46      | 60      | 90      |
|                                       | Blood glucose (mg%) mean ± S.E. |
| None                                  | 8           | 82 ± 2.2 | 281 ± 61.1 | 181 ± 31.7 | 130 ± 13.6 | 113 ± 5.6 | 94 ± 7.1 | 77 ± 7.8 |
| Alfibrate                             | 7           | 83 ± 3.3 | 267 ± 35.8 | 175 ± 27.1 | 135 ± 19.0 | 95 ± 4.8 | 81 ± 2.8 | 71 ± 3.3 |
| Clofibrate                            | 6           | 71 ± 2.5 | 382 ± 86.8 | 257 ± 62.5 | 186 ± 46.2 | 127 ± 10.2 | 117 ± 7.7 | 101 ± 3.8 |

* Duration of treatment: 2 weeks
** 60 mg/kg glucose injected i.v. through the tail vein following a 3 hr fasting period.
were calculated by the least square method and these are compared in Fig. 2. The rate of glucose disappearance ($K_g$) was calculated according to the following equation (22).

$$K_g = \frac{\log 200 - \log 100}{t_{1/2}} \times 100$$

$t_{1/2}$: half time required to lower blood glucose from 200 to 100 mg\%/dL

$K_g$ values tended to be larger with alfibrate and smaller with clofibrate which implied that glucose metabolism was delayed somewhat in the latter group.

**DISCUSSION**

The concentration of 2-(p-chlorophenoxy)-isobutyric acid in blood was found to be slightly higher in the clofibrate group than in the alfibrate when an equivalent amount of the drugs was administered to rats, while at 150 mg/kg daily dose clofibrate...
caused a much greater hepatomegaly than alfibrate.

Histological and biochemical studies of the liver in clofibrate fed animals (23) showed a marked increase in the intracellular organelles, lysosomes and mitochondria which contained the enzymes involved in hypolipidemic action (24). The hepatomegalic effect, however, appears to be irrelevant to the hypocholesterolemic effect in our experiment with normo- and hypercholesterolemic rats (25). This problem will be discussed further in a following paper.

If the hepatomegaly had been due at least in part to an increase of glycogen in the liver, the hypolipidemic effect could have been more easily explained by a change in carbon flow towards glycogen from lipid synthesis. Contrary to our assumption, glycogen metabolism appears to be unchanged under these drug administration.

Glucose disappearance rate decreases in diabetes due to a decreased uptake of glucose into peripheral tissues, decreased metabolism of glucose in the liver etc. and the rate also decreases in hepatitis due to disorders in glucose metabolism (22). The tendency of Kg decrease by clofibrate is presumably result of the secondary effect of the severe hepatomegaly. In this respect, alfibrate appears to be a more effective than clofibrate on controlling serum lipids.

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