Decrease of Ethanol-Induced Fatty Liver by Ethyl α-p-Chlorophenoxyisobutyrate.* (30721)

NORTON SPRITZ† AND CHARLES S. LIEBER‡ (Introduced by E. L. Becker)
2nd (Cornell) Medical Division, Bellevue Hospital and Department of Medicine, Cornell University Medical College, New York City

Ethyl α-p-chlorophenoxyisobutyrate (CPIB) has been shown to produce widespread effects on lipid metabolism in the rat. It lowers serum cholesterol and triglyceride concentrations(1,2,3), decreases hepatic cholesterol content(2,3) as well as the rate at which liver tissue synthesizes cholesterol from acetate(3). Liver triglyceride content has been found not to be diminished, however(2), and hepatic triglyceride synthesis from acetate-C^{14}, tritium water and glycerol-C^{14} was unaffected by CPIB in the normal animal(3).

In the present study the effect of CPIB on hepatic triglyceride concentration was studied while liver lipid was increased by the administration of ethanol. Under these circumstances, rats to which CPIB was administered with ethanol had significantly lower liver triglyceride than those which received ethanol alone.

Methods. Eight groups of 2-4 Charles River rat litter mates each were utilized. They weighed 114-176 g at the start of the study. Feedings consisted of a liquid diet. This nutritionally adequate formula has been described previously(4); it provides approximately 1 calorie/ml of which protein is 16%, carbohydrate 41%, and fat 43%. In each litter group one animal received the basic formula (sucrose control), one was given the same formula except that 36% of the calories supplied as sucrose in the controls were isocalorically replaced by ethanol, and a third member of the group received the ethanol-containing formula to which CPIB§ (0.6 mg/ml) had been added (ethanol + CPIB). Two litter groups (F and G) contained animals fed the basic formula to which 0.6 mg/ml CPIB was added (sucrose + CPIB). One litter (H) consisted of only 2 animals, neither receiving ethanol, one receiving CPIB. The animals were group fed; consumption for the entire group was limited to the intake of the member ingesting the smallest quantity. On this program, intake per rat ranged from an average of 31 to 59 ml/day or .25 to .31 ml/g body weight. CPIB intake ranged from an average of .150 to .190 mg/g body weight among the 8 groups.

At the end of the feeding period the animals were sacrificed by decapitation, the liver weighed, a sample homogenized and extracted in ethanol:ethyl ether (3:1). Total lipid was determined by the method of Amenta(5). An aliquot of the extract containing 2-4 mg of lipid was applied to a 2 g silicic acid column‖ and eluted with 50 ml of ethyl ether. This elution contained neutral lipids, while phospholipid remained on the column. Hydrolysis of the neutral lipid and glycerol measurement was carried out by the method of Van Handel and Zilversmit(6).

The results have been analyzed for statistical significance by the paired t-test.

Results. Body weight. Initial body weight ranged from 114 to 171 g in the 14 rats which received ethanol and did not differ between the ethanol and ethanol + CPIB groups, the mean being 150 g in both groups. Weight gain was comparable in the two groups averaging 32 and 30 g in the ethanol and ethanol + CPIB groups respectively ($p>0.5$). Weight gain in the sucrose control group averaged 43 g. This difference in weight gain between rats fed isocaloric ethanol and su-

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‖ Bio Rad Laboratories, Richmond, Calif.

TABLE I. Liver Lipids and Weights in Group-Fed Rat Litter Mates.

Litter	Treatment Alcohol	Treatment CPIB	Liver wt (g)	Total lipid (mg/g)	Triglyceride (mg/g)
A	—	—	5.7	45.3	10.8
	+	+	6.7	73.7	15.5
	+	—	6.1	77.8	48.1
B	—	—	6.6	58.1	10.2
	+	+	7.3	67.8	29.0
	+	—	9.0	117.5	100.4
C	—	—	6.4	39.5	2.4
	+	+	7.0	92.1	44.3
	+	—	8.3	95.1	54.4
D	—	—	5.5	40.9	4.2
	+	+	5.4	73.3	37.2
	+	—	7.1	149.7	100.2
E	—	—	7.2	48.4	12.8
	+	+	8.8	64.1	29.9
	+	—	9.3	152.2	97.2
F	—	—	3.7	42.9	6.1
	—	+	4.6	52.2	10.7
	+	+	4.8	60.4	37.8
	+	—	5.5	112.7	68.7
G	—	—	9.4	43.1	10.1
	—	+	10.5	46.8	11.3
	+	+	11.0	104.0	72.3
	+	—	10.9	179.9	143.7
H	—	—	4.3	38.7	4.5
	—	+	5.3	40.6	4.9

crose diets confirms previous findings(4).

Liver lipids. Total lipid and triglyceride contents were determined in the 8 sets of group-fed litter mates comprising 25 rats (Table I). In all instances the highest values were found in the animals receiving alcohol without CPIB; sucrose controls were the lowest while intermediate values were obtained in the animals receiving alcohol in combination with CPIB. The mean difference between the ethanol and ethanol plus CPIB animals in the 7 group-fed litter mates was 50.4 mg/g for hepatic total lipid and 53.3 mg/g for triglyceride. These differences were both significant ($p<.02$ and $p<.001$, respectively). Total lipid in the CPIB plus ethanol animals averaged 31.3 mg/g higher than that in the sucrose controls; triglyceride was 30.0 mg/g higher, both differences were again significant ($p<.01$).

In 3 groups of litter mates (F, G and H) (Table I), comparison can be made of the lipid content of the livers of rats that received feedings with and without CPIB but without ethanol. In all 3 instances, in contrast to the findings when animals were receiving ethanol, both total lipid and triglyceride were not decreased in animals receiving CPIB with the control diet. Total lipids were 46.8, 52.2 and 40.6 mg/g and triglycerides 11.3, 10.7, and 4.9 mg/g respectively in the 3 sucrose + CPIB animals compared to 43.1, 42.9, and 38.7 for total lipids and 10.1, 6.1, and 4.5 for triglyceride respectively in their litter mates that did not receive CPIB.

Liver weights. The mean weight of the livers of the animals fed CPIB with ethanol was 7.3 ± 1.9 g (4.0% of body weight), compared to 7.9 ± 2.1 g (4.4% of body weight) for those receiving ethanol alone ($p>0.5$). This small difference was largely attributable to the differences in lipid since the mean weights of the non-lipid portions of the livers were 6.8 and 6.7 g respectively ($p>0.5$). Comparison between livers of ethanol-fed and sucrose control animals revealed significant differences in total liver weight (7.6 and 6.4 g, respectively; $p<.05$). This difference also largely reflected changes in lipid content since non-lipid weights were 6.8 and 6.4 respectively ($p>.5$). The 3 animals that received CPIB without ethanol had livers weighing 10.5, 4.6, and 5.3 g respectively compared to 9.4, 3.7, and 4.3 g in the sucrose control animals not receiving CPIB.

Discussion. The present study documents that CPIB, which does not decrease the liver glycerides of normal rats(2), reduced the triglyceride accumulation produced by 24 days of ethanol administration by approximately one-half.

This effect differs from that produced by CPIB in normal rats' livers. It has previously been shown(2), and is confirmed in the present study in 3 sets of animals, that CPIB does not decrease liver total lipid and triglyceride in animals receiving no ethanol. Similarly, while total liver weight was lowered (fat-free weight essentially unchanged) by CPIB in animals that received ethanol, liver weights were somewhat higher in 3 sets of animals when CPIB was administered without ethanol, confirming previous reports(2, 3).

Prevention or amelioration of increased

liver lipid resulting from prolonged ingestion of ethanol with adequate diets has not been previously reported, except for a partial protection with large amounts of lipotropes(7). Antioxidants(8) and asparagine(9) have been reported to prevent fatty liver which followed administration of a large single dose of ethanol, but these compounds were ineffective following prolonged ethanol ingestion as carried out in the present study(7,10). This difference between acute and chronic experiments is consistent with evidence that the mechanism of fatty liver production differs in these two situations(7,11). Although hepatic cholesterol synthesis has been shown to be diminished in the rat(3), less positive evidence is available concerning the effect of CPIB on triglyceride metabolism. Neither rat(3) nor human(12) studies support the contention that synthesis of triglyceride is diminished by CPIB. Uncertainty concerning the mechanism by which CPIB ameliorates ethanol-induced increase in liver triglyceride is further compounded by controversy concerning pathogenesis of the ethanol effect itself(7,13-15).

Whether the incomplete protective effect observed in these studies would be increased at a larger dose remains to be determined. The dose of CPIB used in the present study, 0.6 mg/calorie, is slightly lower than the 0.25 (2) and 0.3 mg/g(3) of commercial rat chow used in previous studies since the caloric content calculated from the composition of commercial rat chow is approximately 3.4 calories/g. Expressed as mg/calorie it is essentially the same as the recommended human dose of 2.0 g/day. On a body weight basis, however, the rat intake of 170 mg/kg body weight greatly exceeds the human dose which approximates 28 mg/kg. Whether similar dosage in humans will likewise ameliorate ethanol-induced fatty liver remains to be determined by future studies.

Summary. The effect of CPIB on ethanol-induced fatty liver was studied in 7 sets of group-fed rat litter mates. Total hepatic lipids averaged 50.4 mg/g higher in the animals fed ethanol alone than in those receiving ethanol plus CPIB ($p<.02$) with a corresponding difference in triglyceride of 53.3 mg/g ($p<.001$). Hepatic total lipid and triglycerides of the CPIB-ethanol group averaged 31.3 and 30.0 mg/g higher respectively ($p<.01$) than the control animals fed isocaloric diets without ethanol. These findings indicate that CPIB significantly reduces triglyceride accumulation produced by prolonged ethanol intake; in the absence of ethanol hepatic triglycerides were not decreased.

1. Thorp, J. M., Waring, W. S., Nature, 1962, v194, 948.

2. Best, M. M., Duncan, C. H., J. Lab. and Clin. Med., 1964, v64, 634.

3. Avoy, D. R., Swyryd, E. A., Gould, R. G., J. Lipid Res., 1965, v6, 369.

4. Lieber, C. S., Jones, D. P., DeCarli, L. M., J. Clin. Invest., 1965, v44, 1009.

5. Amenta, J. S., J. Lipid Res., 1964, v5, 270.

6. Van Handel, E., Zilversmit, D. B., J. Lab. Clin. Med., 1957, v50, 152.

7. Lieber, C. S., Progress in Liver Disease Vol. II. H. Popper, F. Schaffner, Eds., Grune & Stratton, New York, 1965 p134.

8. DiLuzio, N. R., Life Sci., 1964, v3, 113.

9. Lansford, E. M., Hill, I. D., Shive, W., J. Nutr., 1962, v78, 219.

10. Lieber, C. S., DeCarli, L. M., Gastroenterology, 1966, v50, March.

11. Lieber, C. S., Spritz, N., DeCarli, L. M., J. Clin. Invest., 1966, v45, 51.

12. Spritz, N., Circulation, 1965, v32, Suppl. 2, II-201.

13. Lieber, C. S., Davidson, C. S., Am. J. Med., 1962, v33, 319.

14. Lieber, C. S., Gastroenterology, 1966, v50, 119.

15. Isselbacher, K. J., Greenberger, N. J., New England J. Med., 1964, v270, 351.

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