Effect of Ethanol Consumption on Lipid Metabolism in the Buffalo Rat[1] (37090)

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(Introduced by Frederick J. Stare)
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It is known that most strains of rats are capable of utilizing one-carbon units in the presence of dietary vitamin B_{12}, folic acid, and homocystine to synthesize methionine and choline (1–3). However, recent work from this laboratory has shown that the Buffalo strain of rat when maintained on a choline-deficient diet but supplied with vitamin B_{12}, folic acid and homocystine developed a fatty liver and cirrhosis (4). On the same diet the Charles River rat thrives, and does not develop fatty liver. The results suggested that the Buffalo rat has a genetically determined defect in hepatic one-carbon metabolism which under certain dietary conditions predisposes it to the development of fatty liver and cirrhosis. In view of this defect and the known effects of alcohol ingestion on hepatic lipid metabolism (5–7), the present study was undertaken to determine the effect of alcohol ingestion on certain aspects of hepatic lipid synthesis and one-carbon metabolism in the Charles River and Buffalo rats.

Materials and Methods. Weanling male rats of the Buffalo and Charles River CD strains were maintained on a semipurified diet. It contained (%): casein, 6; peanut meal, 25; sucrose, 60; cod liver oil, 1.0; corn oil, 4.0; salts IV (8), 3.0; and choline chloride, 0.3. Vitamins were added so that each 100 g of diet contained 0.4 mg thiamine HCl, 0.8 mg riboflavin, 4 mg niacin, 0.02 mg biotin, 2 mg calcium pantothenate, 0.1 mg menadione, 0.02 mg vitamin B_{12}, 0.4 mg folic acid, 10 mg DL-α-tocopherol and 0.4 mg pyridoxine HCl. Animals were caged individually and fed 10 g of diet/day and water or 20% (v/v) ethanol *ad libitum*.

At the end of 5, 19 and 27 days 6 animals of each group were sacrificed and 10% liver homogenates were prepared in ice-cold Tris–HCl, pH 7.6. These were frozen in dry ice and ethanol and stored at —15°. The total lipids were extracted from 1 g of liver according to the method of Folch, Lees and Stanley (9) quantified by the weight of the dried extract. High speed supernatant fractions (20,000*g* 30 min.) of liver homogenates were used to measure glucose-6-phosphate dehydrogenase (10), malic and citrate cleavage enzymes (11), and formiminotransferase (12).

Animals of both strains, weighing 300–400 g and maintained on lab chow and water *ad libitum* were used for liver perfusion studies. Isolated livers were perfused with Krebs–Ringer bicarbonate buffer containing 4% serum albumin, 100 mg % glucose and 20 μCi each of glucose-1-^{14}C or acetate-1-^{14}C (13). At the end of the 90 min perfusion period livers were extracted according to Folch, Lees and Stanley (9) and radioactivity in evaporated aliquots of the lipid fraction was determined by scintillation counting.

Results. Table I demonstrates the effect of alcohol consumption on body weight, liver weight and extractable liver lipid at 5, 19 and 27 days. No significant changes in body weight were seen on Days 5 and 19, however, both groups showed significant decreases in body weight on Day 27. The alcohol-drinking Buffalo rat showed a marked decrease in growth over the experimental time period when compared to the Charles River animal. The differences in growth between the drinkers and nondrinkers of each strain were not

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TABLE I. The Effect of Ethanol Consumption on Body Weight, Liver Weight and Liver Lipid in Charles River (CR) and Buffalo (BF) rats.[a]

Strain	Ethanol	Days on diet		
		5	19	27
		Change in body wt (g)		
BF	+	13 ± 7.0	63 ± 4.0	−22 ± 5.0
BF	−	19 ± 0.5	59 ± 4.0	66 ± 5.0[b]
CR	+	17 ± 4.0	74 ± 2.0	1.0 ± 3.0
CR	−	15 ± 2.0	68 ± 4.0	90 ± 3.0[b]
		Liver wt (g)		
BF	+	2.9 ± 0.6	5.4 ± 0.4	1.8 ± 0.4
BF	−	3.4 ± 0.3	4.6 ± 0.2	4.6 ± 0.3[b]
CR	+	4.3 ± 0.3	5.1 ± 0.1	3.4 ± 1.0
CR	−	4.2 ± 0.3	5.1 ± 0.6	4.7 ± 0.5
		Liver lipid (mg/g of liver)		
BF	+	54 ± 7.0	47 ± 2.0	56 ± 0.3
BF	−	48 ± 7.0	32 ± 6.0	49 ± 0.8
CR	+	48 ± 4.0	54 ± 13	53 ± 0.6
CR	−	40 ± 2.0	60 ± 14	48 ± 0.7

[a] Values represent mean ± SEM for 6 rats.

[b] Values significantly different from alcohol group, $p < .01$.

due to differences in caloric intake which averaged 47.9 ± 2 cal/animal/day. This is noteworthy since the alcohol-drinking rats consumed less than 10 g of food/day after Day 19 and compensated for the lack of food calories by increasing their alcoholic consumption. The alcohol-drinking groups consumed 30–35% of their calories as ethanol.

Changes in liver weight were not significant with the exception of alcohol-drinking Buffalo rats which on Day 27 showed a significant decrease in liver weight compared to the water-drinking group. This appears to be in accord with the marked weight loss seen in this group. There were no significant differences in the amount of extractable lipid from excised livers among any of the groups and all values were within the normal range (5).

Significant decreases in four liver enzymes accompanying alcohol consumption in the Buffalo rat were not seen in the Charles River group (Table II). Significant decreases in glucose-6-phosphate dehydrogenase were seen over the entire experimental period in the Buffalo alcohol-drinking group. This pattern is markedly different in the Charles River rat which showed no significant differences in enzyme activities as affected by alcohol consumption or period of time on the diet. There was a similar but even more pronounced effect of alcohol on malic enzyme in the alcohol-drinking Buffalo rat as compared to the Charles River strain. Throughout the entire experimental period highly significant decreases in malic enzyme were seen only in the Buffalo alcohol-drinking group. No significant differences in enzyme activity were seen among the alcohol-drinking Charles River group. Citrate cleavage enzyme was markedly depressed by alcohol consumption in the Buffalo rat but was without effect in the Charles River group. Alcohol consumption decreased the activity of formiminotransferase only in the Buffalo rat.

As shown in Table III isolated perfused livers from Buffalo rats were capable of synthesizing more lipid from both labeled glucose and acetate when compared to Charles River rats. A significant difference occurred only with glucose suggesting that the Buffalo rat is capable of increased lipid synthesis from glucose *via* the glycolytic pathway when maintained on an ordinary laboratory regimen without alcohol.

Discussion. It has been shown that isoca-

TABLE II. The Effect of Ethanol Consumption on Liver Enzymes in Charles River (CR) and Buffalo (BF) Rats.[a]

Strain	Ethanol	Days on diet		
		5	19	27
		Glucose-6-phosphate dehydrogenase[b]		
BF	+	5.2 ± 0.6	8.4 ± 0.4	8.0 ± 1.0
BF	−	8.9 ± 0.4[f]	12.0 ± 0.4[f]	12.5 ± 0.5[f]
CR	+	5.2 ± 0.4	10.0 ± 0.5	9.4 ± 1.3
CR	−	6.2 ± 0.5	10.0 ± 1.0	10.4 ± 0.5
		Malic enzyme[c]		
BF	+	0.48 ± 0.05	2.1 ± 0.11	1.5 ± 0.3
BF	−	3.1 ± 0.4[f]	3.0 ± 0.15[f]	3.0 ± 0.2[f]
CR	+	1.7 ± 0.4	3.5 ± 0.5	2.1 ± 0.7
CR	−	2.6 ± 0.5	2.9 ± 0.4	2.8 ± 0.3
		Citrate cleavage enzyme[d]		
BF	+	41.1 ± 6.7	18.6 ± 5.0	5.1 ± 1.0
BF	−	150.0 ± 28[f]	23.1 ± 2.0	17.0 ± 0.3[f]
CR	+	93.0 ± 2.8	16.7 ± 1.2	20.0 ± 1.0
CR	−	65.7 ± 20	16.0 ± 1.9	12.7 ± 2.3[g]
		Formiminotransferase[e]		
BF	+	76 ± 9.1	70 ± 2.2	69 ± 1.4
BF	−	82 ± 10	99 ± 1.1[f]	77 ± 2.4[g]
CR	+	72 ± 6.1	90 ± 5.3	68 ± 4.1
CR	−	74 ± 3.2	84 ± 1.1	69 ± 9.1

[a] Values represents mean ± SEM for 6 rats.
[b] nmoles NADPH/min/mg protein.
[c] μmoles NADPH/hr/mg protein.
[d] μmoles citrate cleaved/hr/mg protein.
[e] μmoles FIGLU converted/hr/mg protein.
[f] Values significantly different from alcohol group: $p < .01$; [g] $p < .05$.

loric replacement of sucrose with ethanol in liquid diets containing 35–43% of total calories as fat will induce fatty metamorphosis in rat livers (5–7). Recently, it has been demonstrated that on a diet containing 15% fat the Buffalo rat when supplied with the precursors for choline biosynthesis will nonetheless develop a fatty liver and subsequently cirrhosis (4). The present study shows that certain aspects of lipid metabolism are affected also by alcohol consumption in the Buffalo rat.

Enzymes involved in fatty acid synthesis, namely glucose-6-phosphate dehydrogenase, malic and citrate cleavage enzyme activities were significantly depressed by alcohol in the Buffalo rat. Formiminotransferase, an enzyme involved in one-carbon metabolism and the synthesis of lipotropes, was also significantly decreased. These observations suggest that the Buffalo rat may be favoring the glycolytic pathway for triglyceride synthesis. The inherent ability of the Buffalo rat for increased hepatic lipogenesis *via* glycolysis (Table III) supports this contention and indicates that the water-drinking Buffalo rat is capable of increased triglyceride synthesis.

The observed changes related to lipid metabolism in alcohol-drinking Buffalo rats cannot be readily explained by our current knowledge of alcohol metabolism and at present are not directly applicable to lipid metabolism and alcoholic liver disease. However, it appears in this study that the decreased activity of enzymes involved in fatty acid synthesis and one-carbon metabolism in alcohol-drinking Buffalo rats may be related to an apparent genetic disorder involving a

TABLE III. Lipogenesis by Perfused Livers of Charles River (CR) and Buffalo (BF) Strain rats.[a]

Substrate	Group (no. of rats)	cpm/g tissue[b]	
Glucose-1-^{14}C	CR (6)	124 ± 19	$p < .01$
	BF (6)	570 ± 20	
Acetate-1-^{14}C	CR (6)	7900 ± 1708	NS
	BF (6)	16,670 ± 8658	

[a] Values represent mean ± SEM.
[b] Counts per minute per gram of tissue.

number of interrelated metabolic pathways. The effect of alcohol ingestion on these enzyme systems and their relationship between overall lipid metabolism and alcoholic liver disease remains to be determined.

Summary. The substitution of 20% alcohol for drinking water of Buffalo rats maintained on an adequate diet demonstrated significant decreases in glucose-6-phosphate dehydrogenase, formiminotransferase, malic and citrate cleavage enzyme activities. This is in contrast to the Charles River and other strains of rats. Liver studies using labeled glucose and acetate indicated that the Buffalo rat is capable of synthesizing approximately twice as much lipid from glucose compared with the Charles River rat. The results suggest that the Buffalo rat has a sensitivity to alcohol related to certain aspects of lipid metabolism not seen in the Charles River rat or other strains.

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