Alterations of Alcohol Dehydrogenase and Other Hepatic Enzymes Following Oral Alcohol Intoxication

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THE PURPOSE of this paper is to report hepatic enzyme abnormalities in adult rats subjected to chronic oral alcohol intoxication under conditions of controlled food intake. Reports in the literature demonstrate that alcohol seems to exert a direct toxic effect on the liver cell.[1,2] Alteration of alcohol dihydrogenase activity in chronic alcohol intoxication has recently been reported.[3] The present study is concerned with the activity of hepatic enzymes in alcohol-fed animals killed after three, six and twelve weeks of chronic intoxication.

MATERIALS AND METHODS

Ninety-six female Sprague-Dawley albino rats initially weighing between 280 and 290 gm. were used. These were divided into four different groups of twenty-four animals each with each animal in a separate cage. All animals in three of the groups received ground Purina Chow rat food. The control group was fed the unground food. Each animal was weighed twice a week.

Group I were normal control animals receiving water and food *ad libitum*. Group II consisted of alcohol-intoxicated animals. Group III were pair-fed isocaloric control animals receiving a caloric equivalent of sucrose in place of the alcohol. Group IV were pair-fed weight control animals with their weights kept the same as the alcoholic animals during the entire experiment. Each group was subdivided into three sub-groups of eight animals each for killing at three, six and twelve weeks.

The animals in group II received ground Purina Chow rat food plus alcohol administered by a stomach tube in 5 ml. doses five days a week. These animals were given no water but instead received a 20 per cent solution of alcohol as their only source of fluid. They drank approximately 3 to 5 ml. of the solution daily averaging a total intake of 2 ml. of pure alcohol each day. Their uneaten food was weighed daily and compared to the previous day's ration providing an accurate weight of the food actually eaten.

In group III the animals received the same type and quantities of food as those in group II with water being substituted for the alcohol. To maintain isocaloricity 3.5 gm. of sucrose were mixed with the food.

The rats in group IV were fed the same as those in group II except that the amount of food was increased or decreased in order to maintain individual weights at the same level as the alcohol-fed animals. Water was administered *ad libitum*.

At the end of each experiment the animals were killed by a blow to the head and exsanguinated by decapitation. The liver was immediately removed and 1 gm. of it was homogenized in deionized water. A 10 per cent solution of this homogenate was then centrifuged at 3,000 r.p.m. The supernatant obtained was again centrifuged at 11,000 × g for thirty minutes resulting in a supernatant solution from which the enzymatic assays were determined. Soluble liver protein as determined by the method of

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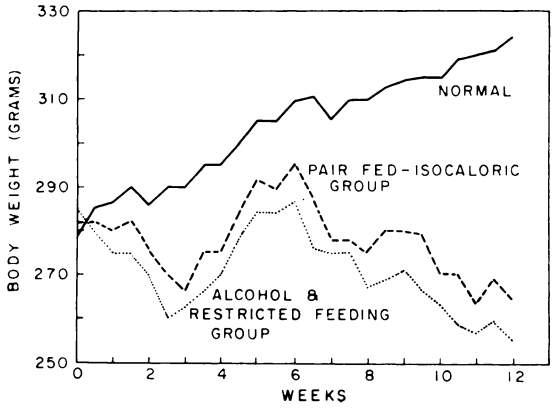

FIG. 1. Mean comparative weights of experimental animals.

Lowry[4] was chosen as the reference base for enzyme activity.

All the enzymes were measured spectrophotometrically at 38°c. Alcohol dehydrogenase activity was assayed by the method of Theorell and Bonnichsen[5] with slight modifications. Isocitric dehydrogenase activity was determined by a modification of the Wolfson and Williams-Ashman method.[6] The supernatant dilution for this was 1:800. Transaminases were studied by the method of Karmen[7] and Wroblewsky[8] using supernatant dilutions of 1:400 for glutamic pyruvic transaminase and 1:800 for glutamic oxalacetic transaminase. All the assays were made in duplicate usually three to five hours after the rats were killed. A corresponding blank was run for each determination.

RESULTS

Animals Killed at Three Weeks

The alcohol-intoxicated animals (group II) as compared to the control animals (group I) lost some weight probably due to a reduced intake of food. The pair-fed isocaloric control animals (group III) and the weight control group (group IV) were not permitted to vary in weight from the alcohol-intoxicated group (Fig. 1). No gross pathologic condition was found in these groups and histologic examination revealed normal livers. Enzyme activities expressed as micromoles per hour per milligram of soluble liver protein are presented in Table I.

A significant increase of alcohol dehydrogenase activity was found in the alcohol-intoxicated animals. Isocitric dehydrogenase activity in this group was significantly decreased and glutamic pyruvic transaminase was decreased but significant variation of glutamic oxalacetic transaminase activity was not demonstrated. These alterations were not observed in the pair-fed isocaloric animals or in the weight control animals except for an increase in glutamic pyruvic transaminase in the pair-fed isocaloric animals.

Animals Killed at Six Weeks

The alcohol intoxicated rats regained some weight (Fig. 1). Final weights, however, were below that of the normal control animals. No significant hepatic changes were evident at histologic examination. Enzyme activities are listed in Table I.

Alcohol dehydrogenase activity was in the normal range in the alcohol-intoxicated and weight control animals. A significant elevation of this enzyme, however, appeared in the sucrose pair-fed, isocaloric animals. Glutamic pyruvic transaminase activity in the alcohol intoxicated animals was again decreased as at three weeks and glutamic oxalacetic transaminase increased. Isocitric dehydrogenase remained normal in all three groups.

Animals Killed at Twelve Weeks

After this period of alcohol intoxication the weight of the animals stayed below that of the control rats. Histologic examination of the liver showed varying degrees of fatty metamorphosis. Enzyme activities are listed in Table I. Significant decreases of alcohol dehydrogenase were found in the alcohol-intoxicated group. The pair-fed isocaloric and weight control animals, however, showed no variation from normal. Isocitric dehydrogenase activity was decreased in the alcohol-intoxicated and weight control animals. Increased glutamic pyruvic transaminase activity above normal occurred in the weight control animals. No significant alteration was encountered in groups II and III. No variation of glutamic oxalacetic transaminase was evident.

COMMENTS

Previous experiments have demonstrated that liver cell injury results in loss of alcohol dehydrogenase activity most readily apparent

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TABLE I

Comparative Enzyme Activities of Normal Rats, Alcohol-Fed Rats, Isocaloric Sucrose-Fed Rats and Matched-Weight Rats

Group*	Micromoles per Hour, per Milligram of Soluble Liver Protein											
	Alcohol Dehydrogenase			Isocitric Dehydrogenase			Glutamic Pyruvic Transaminase			Glutamic Oxalacetic Transaminase		
	Mean	Standard Deviation	p Value	Mean	Standard Deviation	p Value	Mean	Standard Deviation	p Value	Mean	Standard Deviation	p Value
Three Weeks												
I	1.23	0.12	...	57.08	10.85	...	11.99	1.79	...	26.33	3.28	...
II	1.41	0.12	0.01	43.87	3.30	<0.001	8.41	2.30	<0.005	34.16	10.08	N.S.
III	1.24	0.18	N.S.	48.30	10.63	N.S.	16.81	3.49	<0.01	27.13	4.65	N.S.
IV	1.29	0.11	N.S.	45.91	12.14	N.S.	12.84	6.12	N.S.	32.75	7.73	N.S.
Six Weeks												
I	1.21	0.14	...	47.53	9.20	...	15.11	3.27	...	29.43	4.64	...
II	1.28	0.21	N.S.	43.16	4.10	N.S.	7.66	5.10	<0.01	46.08	5.16	<0.001
III	1.52	0.13	0.001	45.71	6.74	N.S.	16.51	5.43	N.S.	21.81	5.76	N.S.
IV	1.35	0.22	N.S.	49.88	11.47	N.S.	13.27	2.63	N.S.	30.09	7.96	N.S.
Twelve Weeks												
I	1.26	0.17	...	50.27	3.90	...	12.50	2.70	...	27.75	1.77	...
II	0.91	0.21	0.001	30.70	4.04	<0.001	12.32	3.89	N.S.	29.95	7.00	N.S.
III	1.12	0.17	N.S.	40.41	8.37	N.S.	18.62	5.65	N.S.	27.17	3.37	N.S.
IV	1.23	0.20	N.S.	39.65	7.31	0.005	18.53	3.60	<0.005	28.88	10.53	N.S.

* Group I, normal control animals; group II, alcohol-fed animals; group III, pair-fed, sucrose isocaloric to alcohol; group IV, weight held to alcohol-fed animals.

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in chronic carbon tetrachloride-intoxicated animals.[9] These experiments also demonstrated that alcohol alters enzyme activity in the liver cell affecting especially isocitric dehydrogenase and glutamic pyruvic transaminase. The present experimental studies confirm the direct toxic effect of alcohol upon hepatic enzymes.

Cirrhosis of the liver found in some alcoholic patients has been explained at times by the reduction of food consumption that often accompanies excessive drinking. This concept emphasizes nutrition as the primary cause of the cirrhosis. In order to study the effect of alcohol alone on the experimental animal the currently described experiments were performed. Biochemical changes of altered enzyme activity in the present experiments are shown to appear before histologic changes become apparent. It is evident that alcohol produces alterations of alcohol dehydrogenase. A significant increase of activity was noted after three weeks of constant intoxication. No change was observed in animals killed six weeks later but a significant decrease of activity was found after twelve weeks of heavy alcohol intake. This suggests that continuous metabolic insult by ethanol ingestion after initial stimulation to alcohol dehydrogenase activity produces metabolic cellular injury within the hepatic cell. This change did not occur in the pair-fed weight control animals (group IV) or in the pair-fed isocaloric group (group III). This suggests that alcohol affects the liver cell and alters the activity of alcohol dehydrogenase independently of the caloric intake and the food intake.

Of great interest also is the increase of alcohol dehydrogenase activity in those animals receiving sucrose for six weeks (group III), an increase not observed in the pair-fed weight control group (group IV). Since sucrose has in its structure fructose and glucose the increase of activity of alcohol dehydrogenase could possibly be due to either one of these carbohydrates. Lundquist and Wolthers[10] studied the influence of fructose on the kinetics of alcohol elimination in man and found that the rate of alcohol elimination from the blood increased significantly in every subject after fructose infusions. This increase was 34 per cent as compared to only 6 per cent for glucose. The studies of Stuhlfanth et al.[11] are in agreement with Lundquist[10] and with Clark and Hulpieu.[12]

The changes of isocitric dehydrogenase are also of great interest. There was a significant decrease of activity in the alcohol-intoxicated animals (group II) killed at three and twelve weeks. No change was experienced in either group after six weeks. Change in isocitric dehydrogenase was independent of the caloric or food intake in the three-week experiment. In the twelve-week experiment isocitric dehydrogenase activity decreased in the pair-fed weight control group (group IV). The change in the alcohol-intoxicated animals, however, was more marked. If one assumes that the animals killed after twelve weeks were malnourished with consequent enzyme alterations then the decrease in isocitric dehydrogenase activity might be expected. Studies by Baron[13] on serum transaminases and isocitric dehydrogenase in kwashiokor support this. He found a marked increase of serum enzyme activity in florid malnutrition in a pattern similar to that found in hepatocellular damage.

The changes of transaminase activities here reported are in agreement with a previously reported study and with other reported studies in the literature.[1,14] Glutamic pyruvic transaminase appears to be a sensitive indicator of liver cell injury. As shown in Table I the activity of this enzyme decreases in those alcohol-fed animals killed at three and six weeks with a corresponding increase of glutamic oxalacetic transaminase at six weeks. Of interest is the increase of activity of glutamic pyruvic transaminase at three weeks in those animals receiving sucrose and in those with reduced intake of food at twelve weeks. No explanation can be given for this. The changes occurring in the transaminases were certainly due to alcohol and were independent of caloric or food intake.

CONCLUSIONS

Chronic oral alcohol intoxication in rats alters hepatic enzyme activities which vary with the length of time of intoxication. These

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alterations are due to the direct toxic effect of ethanol on the liver cell and in part to the reduced food intake. Alcohol dehydrogenase reacted initially by an elevation of its activity and later, after twelve weeks of intoxication, it decreased. This change was not observed in the other two control groups being independent, therefore, of caloric or food intake.

Isocitric dehydrogenase was decreased by a restricted food intake and alcohol enhanced this reduction. The transaminases were also affected in the alcohol-intoxicated (group II) animals with a period of decreasing glutamic pyruvic transminase activity and moderate glutamic oxalacetic transaminase elevation. These changes did not occur in the isocaloric and weight control animals (groups III and IV).

Alcohol dehydrogenase activity increased in those animals receiving sucrose for six weeks. The fructose molecule of the sucrose may play a part in this elevation.

REFERENCES

1. Henley, K. S., Wiggins, H., Hirschowitz, B. and Pollard, H. M. The effect of oral ethanol on glutamic pyruvic and glutamic oxalacetic transaminase activity in the rat liver. *Quart. J. Stud. Alcohol*, 19: 54, 1958.
2. Ashworth, C. T. Production of fatty infiltration of liver in rats by alcohol in spite of adequate diet. *Proc. Soc. Exper. Biol. & Med.*, 66: 382, 1947.
3. Dajani, R. M. and Orten, J. M. The alcohol-acetaldehyde dehydrogenase systems in the livers of alcohol treated rats. *Fed. Proc.*, 19: 47, 1960.
4. Lowry, O. H., Roserbrough, N. T., Farr, A. L. and Randall, Rod J. Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, 193: 265, 1951.
5. Theorell, H. and Bonnichsen, R. K. Liver alcohol dehydrogenase. In: Methods in Enzymology, vol. 1, p. 495. New York, 1955. Colowick and Kaplan.
6. Wolfson, S. K., Jr. and Williams-Ashman, H. G. Isocitric and 6-phosphogluconic dehydrogenase in human blood serum. *Proc. Soc. Exper. Biol. & Med.*, 96: 231, 1957.
7. Karmen, A. A note on the spectrophotometric assay of glutamic-oxalacetic-transaminase in human blood serum. *J. Clin. Invest.*, 34: 131, 1955.
8. Wroblewsky, F. and LaDue, S. J. Serum glutamic pyruvic transaminase in cardiac and hepatic disease. *Proc. Soc. Exper. Biol. & Med.*, 91: 569, 1956.
9. Figueroa, R. B. and Klotz, A. P. Alteration of alcohol dehydrogenase and other hepatic enzymes in experimental chronic liver disease. *Metabolism*, to be published.
10. Lundquist, F. and Wolthers, H. The influence of fructose on the kinetics of alcohol elimination in man. *Acta pharmacol. et toxicol.*, 14: 290, 1958.
11. Stuhlfanth, K., Englhardt-Golkel, A. and Schaffry, J. Untersuchungen uber den enzymatischen alkoholabbau beim menschen unter besonderer besonderer berucksichtigung des intermediarstoffwechsells. *Klin. Wchnschr.*, 33: 888, 1955.
12. Clark, W. C. and Hulpieu, H. R. Comparative effectiveness of fructose, dextrose, pyruvic acid and insulin in accelerating the disappearance of ethanol from dogs. *Quart. J. Alcohol*, 19: 47, 1958.
13. Baron, D. N. Serum transaminases and isocitric dehydrogenase in kwashiorkor. *J. Clin. Path.*, 13: 252, 1960.
14. Sommerville, R. L., Fleisher, G. A., Dearing W. H., Hallenback, G. A. and Dockerty, M. B. Transaminases in hepatic tissue and serum in hepatic disease. *Gastroenterology*, 38: 926, 1960.

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