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Behavioral and Biochemical Effects of Chronic Consumption of Ethanol by Hamsters[1]

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HARRIS, R. A., W. KRAUSE, E. GOH AND J. CASE. *Behavioral and biochemical effects of chronic consumption of ethanol by hamsters.* PHARMAC. BIOCHEM. BEHAV. 10(3) 343–347, 1979.—Male Syrian Golden Hamsters consumed about 90% of their fluid as an ethanol-water solution when given a choice between water and ethanol-water solutions. This resulted in a daily alcohol intake of about 11 g/kg. After 53 days of alcohol ingestion there was no evidence of tolerance to the soporific effects of parenterally administered ethanol and removal of the ethanol solutions failed to produce any signs of alcohol withdrawal. However, after cessation of ethanol ingestion, the acquisition of escape responding from footshock was impaired in the alcohol group as compared to a control group. Biochemical and ultrastructural analyses demonstrated that chronic ethanol ingestion increased hepatic glycogen and plasma triglycerides without altering either the hepatic or plasma concentrations of phospholipids, free cholesterol or cholesterol esters. These results demonstrate that the hamster has an unusual metabolic response to ethanol. These findings are contrasted to the biochemical and behavioral effects of the drug in other species.

Ethanol Behavioral effect Biochemical effect Metabolic response

THE SYRIAN Golden Hamster will preferentially consume ethanol solutions rather than water when given a choice between these fluids [2,3]. Voluntary consumption of large amounts of ethanol is unusual among laboratory animals and the possibility that the hamster may serve as a model for the study of alcoholism deserves attention. McMillan *et al.* [10] recently reported that no signs of physical dependence (as evaluated by audiogenic seizure susceptibility and motor activity) could be detected in hamsters consuming ethanol for three months. The present experiments were undertaken to determine the effects of chronic ethanol consumption on shock-avoidance behavior, ethanol sensitivity (tolerance), liver ultrastructure and liver and serum lipid concentrations in order to compare the behavioral and biochemical consequences of ethanol ingestion in the hamster with those observed in other species.

METHOD

Animals

Thirty-two male Syrian Golden Hamsters weighing about 110 g at the beginning of the experiment were obtained from the breeding colony at the Dalton Research Center, University of Missouri at Columbia.

Ethanol Administration

Hamsters were housed four per cage in plastic cages measuring 45 × 24 × 19 cm which were covered by stainless steel lids equipped with depressions where food pellets (Purina lab chow) and bottles were placed. Environmental temperature was maintained between 24 and 26°C and room lights were on between 5 a.m. and 5 p.m. Body weights were determined at the beginning of the experiment and 53 days later. Water and ethanol solutions were provided in calibrated glass bottles fitted with ball bearing tubes (HB71 and BW101, Hoeltge, Inc., Cincinnati, Ohio) to limit spillage. Placing bottles on empty cages for 24 hr indicated a loss of about 1 ml and this amount was subtracted from the amount of fluid apparently consumed. Sixteen hamsters had tap water available as the only drinking fluid while sixteen had one bottle containing tap water and another containing ethanol and tap water. Fluid consumption was determined and the positions of the ethanol and water bottles alternated every second day. Ethanol consumption was monitored during the first 53 days of the experiment. The ethanol concentration was 5% v/v for the first 6 days and 15% v/v for the remainder of the experiment (47 to 61 days).

Behavioral Procedures

After 53 days of exposure to ethanol, the ethanol solutions were removed from two cages for 14 hours (overnight). At this time, six of these eight hamsters, as well as six animals from the control group were trained to avoid footshock. The shock avoidance apparatus consisted of one compartment (24 × 21 × 18 cm) containing a grid floor and a second compartment of identical size which was connected to the

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first compartment by a round hole (8 cm diameter) in the clear Plexiglas panel which separated the two compartments. For each trial a hamster was placed on the grid floor and 10 sec later a bell was sounded for 5 sec followed by 20 sec of footshock (0.5 mA delivered to the grid by a E1064 shock generator-scrambler; Grason Stadler, Boston, MA). Movement of the animal into the other compartment terminated this series of events and the animal was returned to its cage. Nine of these trials were conducted for each animal and each trial was separated by about three minutes. In each trial, the latency for movement across the barrier was determined; latencies less than 15 sec correspond to avoidance responses, while latencies between 15 and 35 sec correspond to escape responses while a latency greater than 35 sec was termed "no escape."

After completion of avoidance training the 15% v/v ethanol solution was returned to the ethanol group. After 19 additional days of ethanol consumption, the ethanol solutions were removed and five hours later the eight hamsters from the ethanol group and eight from the control group were injected IP with 3 g/kg ethanol (20% w/v in saline) and the duration of the loss of righting reflex (sleeping-time) was determind by placing the hamsters on their backs until they could right themselves onto all four paws twice within one minute. Following this test, the ethanol group was given access to 15% v/v ethanol for 5 additional days and both groups were tested with 4 g/kg ethanol using the same protocol as was used with 3 g/kg ethanol. These animals were not used in any further experiments. Our preliminary experiments, as well as those of others [12], indicated that the blood ethanol concentrations were not significant (less than 7 mg/100 ml) five hours after removal of the ethanol solutions.

Biochemical and Ultrastructural Determinations

Six hamsters which had consumed 5% ethanol for six days followed by 15% ethanol for 61 days and six control hamsters were sacrificed and four of each group were used for determination of serum and liver lipid concentrations while two from each group were used for determinations of liver morphology. For the biochemical determinations, animals were anesthetized with ether, the abdomen was opened, blood was obtained by aortic puncture, and the liver removed and homogenized. Lipids from homogenate and plasma were extracted with chloroform and methanol (2:1/v:v) [4]. Lipid extracts were separated by thin layer chromatography and individual classes of lipids analyzed [6].

For determination of liver ultrastructure, hamsters were sacrificed by ether anesthesia and tissue samples were taken from both the central and peripheral regions of the liver. They were fixed by immersion for four hours at 0°C in 3.5% glutaraldehyde buffered in 0.1 M phosphate to a pH of 7.4. The tissues were washed in 0.1 M phosphate buffer and osmicated in 1.0% osmium tetroxide at 0%C for two hours. The specimens were processed routinely, infiltrated with and embedded in Epon 812. Thin sections of this material were mounted on uncoated grids and stained with uranyl acetate and lead citrate. The sections were examined in a RCA EMU-3F electron microscope operated at 50 kv. Additional blocks of tissues, taken for light microscopy, were fixed in 10% buffered neutral Formalin. These tissues were processed routinely, embedded in paraffin, sectioned at about 7 μ and stained with hematoxylin and eosin.

Statistics

Differences in weight gain, duration of loss of righting reflex, avoidance and escape performance and serum and liver lipid concentrations between alcohol and control groups were evaluated by a Students' t test (two-tailed). In all cases the level of significance was taken as $p<0.05$.

RESULTS

Ethanol Consumption and Body Weight

During the first 47 days that both tap water and 15% v/v ethanol were available, the hamsters had an average alcohol intake of 10.8 g/kg/day (Table 1). During this time they displayed a marked preference for the ethanol solutions as they consumed almost 90% of their fluid from the ethanol mixture. During the first week of exposure to 15% ethanol solutions, the hamsters consumed 85% of their fluid as the alcohol solution. Consumption of the alcohol solution increased slightly during the next week and remained stable for the remainder of the experiment. During the first 53 days of alcohol ingestion (6 days at 5% and 47 days at 15%) the weight gain of the alcohol group was almost twice that of the control group (Table 1).

Effects of Chronic Ethanol Consumption on Ethanol Narcosis and Shock-Avoidance Behavior

To evaluate the possibility that chronic ingestion of ethanol might produce tolerance to the soporific effects of the drug, eight animals from each group were injected IP with 3 g/kg ethanol and the duration of loss of righting reflex was determined; 5 days later this was repeated using a dosage of 4 g/kg ethanol. As can be seen in Table 1, at both dosages the duration of narcosis was somewhat less for the alcohol group than for control groups, but this difference was not statistically significant for either dose of ethanol.

The acquisition of the shock-escape response was significantly altered for hamsters which had chronically ingested ethanol (but had been withdrawn from the drug for 14 hr) as compared to hamsters having access to water only. As is shown in Table 1, the hamsters which had ingested ethanol were less successful in escaping from the shock than were control animals. Although the alcohol group displayed fewer escape responses, the frequency of avoidance responses was similar for both groups. No overt signs of alcohol withdrawal (e.g. irritability, tremor, convulsions) were observed during the shock avoidance testing.

Effects of Chronic Ethanol Consumption on Liver and Serum Lipids

Chronic ingestion of ethanol produced few alterations in the lipid content of either plasma or liver tissue. However, the concentration of triglyceride in the plasma was increased by about 3-fold in hamsters given access to ethanol, while the concentration of triglyceride in the liver was not affected by this treatment. In addition, the concentrations of phospholipids, free cholesterol and cholesterol esters in both liver and plasma were not altered by ethanol ingestion. The weights of the livers from hamsters consuming ethanol was increased as was their body weights (Table 1), resulting in a similar ratio of liver weight to body weight for animals from both groups (Table 2).

TABLE 1

EFFECTS OF CHRONIC ETHANOL CONSUMPTION ON BODY WEIGHT, ETHANOL-INDUCED NARCOSIS AND SHOCK AVOIDANCE BEHAVIOR OF HAMSTERS

	Control*	Alcohol
Ethanol Consumption (15% solution)		
g/kg/day (n=6)	—	10.8
% of total fluid as ethanol	—	88.5
Weight Gain†		
g/53 day period (n=16)	25 ± 5.0	49 ± 4.5§
Ethanol Sleeping-Time (min)†		
3 g/kg ethanol (n=8)	21.2 ± 4.9	11.7 ± 2.8ǂ
4 g/kg ethanol (n=8)	185 ± 31	133 ± 16#
Shock Avoidance Behavior†‡		
Avoidance (% of total trials)	32 ± 6.7	37 ± 10
Escape (% of total trials)	57 ± 7.1	31 ± 6.9¶
Failure to Escape (% of total trials)	11 ± 4.5	32 ± 7.3§

*The control group was given only tap water which the ethanol group was given a choice between tap water and 5% v/v ethanol for 6 days followed by 15% ethanol for 47 days.

†Values are Mean ± SEM.

‡Acquisition of shock-avoidance behavior tested 14 hr after withdrawal from ethanol; 6 hamsters/group, 9 trials/hamster.

§Significantly different from control, $p<0.05$.

¶Significantly different from control, $p<0.01$.

ǂNot significantly different from control, $p=0.11$.

#Not significantly different from control, $p=0.16$.

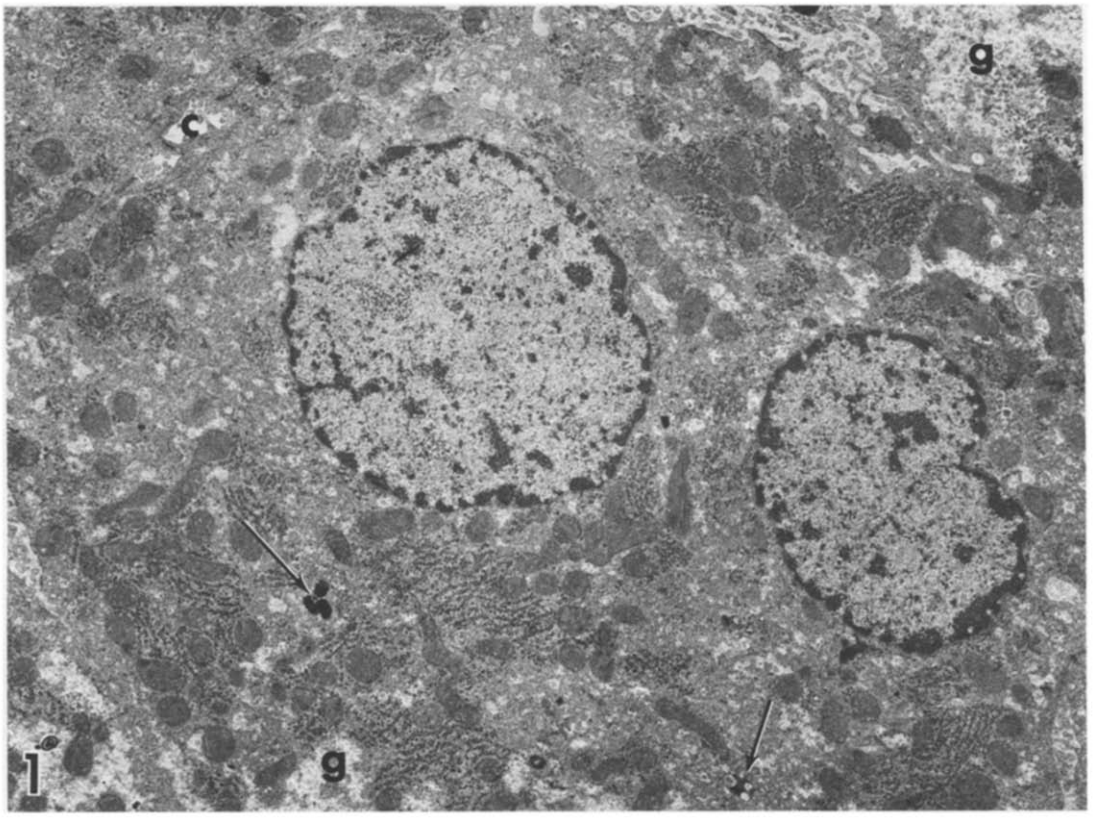

FIG. 1. A hepatocyte taken from a control animal. It is binucleate, shows numerous mitochondria and scattered profiles of rough endoplasmic reticulum. Scattered accumulations of glycogen (g) are in evidence as are lysosome-like structures (arrows). A canaliculus (c) is shown. × 2000.

TABLE 2

EFFECTS OF CHRONIC ETHANOL CONSUMPTION ON LIVER AND SERUM LIPIDS OF HAMSTERS

	Control*	Ethanol
Liver Weight/Body Weight	0.032 ± 0.001	0.036 ± 0.003
Liver Lipids (μmol/g wet wt)		
Triglycerides	3.53 ± 0.40	3.89 ± 0.47
Phospholipids	23.57 ± 0.88	20.22 ± 2.63
Free Cholesterol	6.03 ± 0.31	4.84 ± 0.66
Cholesterol esters	1.04 ± 0.07	1.54 ± 0.28
Plasma Lipids (μmol/ml)		
Triglycerides	1.25 ± 0.12	3.68 ± 0.22†
Phospholipids	1.18 ± 0.16	1.60 ± 0.15
Free Cholesterol	0.71 ± 0.04	0.77 ± 0.07
Cholesterol esters	1.00 ± 0.10	0.89 ± 0.02
Free Fatty Acids	0.23 ± 0.06	0.25 ± 0.01

*Values are Mean ± SEM, n=4. The control groups were given ony tap water while the ethanol group was given a choice between tap water and 5% v/v ethanol for 6 days followed by 15% v/v ethanol for 61 days. See Methods for details.

†Significantly different from control, $p<0.01$.

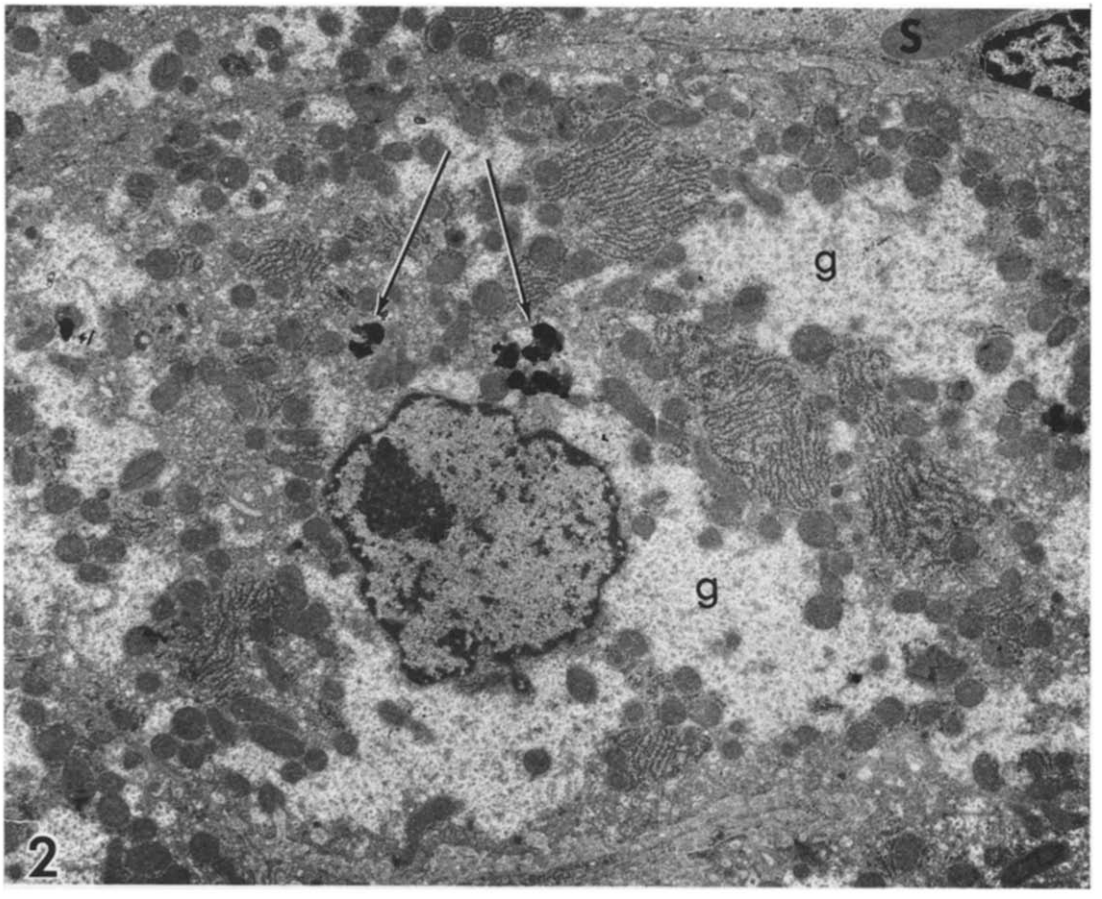

FIG. 2. A hepatocyte taken from an alcohol treated animal. Note the increase in the amount of glycogen (g) and the electron dense lysosome-like structures present (arrows). A portion of a sinusoid (s) also is shown. × 2000.

Effects of Chronic Ethanol Consumption on Liver Ultrastructure

In general, hepatocytes from both control animals and alcohol drinkers exhibit similar ultrastructural features and concentrations of most organelles (Figs. 1 and 2). However, hepatocytes of alcohol treated animals showed a marked increase in the amount of glycogen present as well as what appears be an increase in number of electron dense lysosome-like structures (Fig. 2). The preponderance of glycogen shown in Fig. 2 was consistently observed in tissue obtained from both central and peripheral regions of livers from alcohol treated animals. Abnormal amounts of glycogen were not observed in any of the sections (n=14) obtained from control hamsters. No macroscopic abnormalities were noted in the livers of the ethanol treated animals. Light microscopic examination of the hematoxylin and eosin stained sections from alcohol drinkers and control animals were similar in appearance and showed no evidence of fatty accumulation.

DISCUSSION

These results confirm that the Syrian Golden Hamster will preferentially consume ethanol-water solutions. However, some of the metabolic and behavioral effects of long-term alcohol ingestion are different in the hamster than those reported for other species.

Chronic alcohol consumption appeared to increase the glycogen content of the hamster liver, as determined by ultrastructural evaluation, but the hepatic lipid concentrations were not altered. In the plasma, chronic alcohol ingestion increased the concentration of triglycerides, but failed to alter the concentration of other lipids. These results suggest an increase in gluconeogenesis without marked alterations in lipid metabolism. This is in contrast to results obtained in other species which indicate that chronic alcohol ingestion decreases hepatic glycogen content [7], increases hepatic lipid concentrations [8,9] and generally decreases gluconeogenesis [1]. These results demonstrate that several of the metabolic responses of the hamster to alcohol are quite different than those observed in other species.

After cessation of chronic alcohol ingestion, hamsters displayed a deficit in the acquisition of a shock-escape response. A marked impairment of escape and avoidance responding has been noted in rats and mice after cessation of long-term consumption of ethanol [5,13]. In mice we have found that consumption of a ethanol-containing liquid diet for as few as five days markedly inhibited the acquisition of escape and avoidance behavior [11], while in hamsters the same alcohol treatment did not affect the acquisition of avoidance or escape responding (Snell and Harris, unpublished).

In addition to the biochemical and behavioral differences, we were not able to demonstrate either tolerance or dependence to alcohol in the hamster after prolonged ingestion of the drug. These results are consistent with other evidence suggesting that the hamster has both a rapid metabolism of ethanol and a lack of central nervous system sensitivity to some effects of the drug [12]. Thus, although the hamster does not appear to be a model for human alcoholism, it may be of interest to investigate the processes which allow the hamster to chronically consume substantial quantities of alcohol and yet avoid the development of the tolerance, dependence, marked learning deficits and fatty liver which are found in other species.

REFERENCES

1. Arky, R. A. The effect of alcohol on carbohydrate metabolism: Carbohydrate metabolism in alcoholics. In: *The Biology of Alcoholism, Vol. 1*, edited by B. Kissin and H. Begleiter. New York: Plenum Press, 1971, pp. 197–227.
2. Arvola, A. and O. Forsander. Comparison between water and alcohol consumption in six animal species in free-choice experiments. *Nature* **191**: 819–820, 1961.
3. Emerson, G. A., R. G. Brown, J. B. Nash and W. T. Moore. Species variation in preference for alcohol and in effects of diet or drugs on this preference. *J. Pharmac. exp. Ther.* **106**: 385–386, 1952.
4. Folch, J., M. Lee and G. H. Sloane-Stanley. A simple method for the isolation and purification of total lipids from animal tissues. *J. biol. Chem.* **226**: 497–509, 1957.
5. Freund, G. and D. W. Walker. Impairment of avoidance learning by prolonged ethanol consumption in mice. *J. Pharmac. exp. Ther.* **179**: 284–292, 1971.
6. Heimberg, M. and H. G. Wilcox. The effect of palmitic and oleic acids on the properties and composition of the very low density lipoprotein secreted by the rat liver. *J. biol. Chem.* **247**: 875–880, 1972.
7. Kusewitt, D. F., J. P. Burke and M. E. Tumbleson. Effects of chronic ethanol consumption on liver metabolites in Sinclair (S-1) miniature swine. *Gen. Pharmac.* **8**: 335–339, 1977.
8. Lieber, C. S., D. P. Jones and L. M. DeCarli. Effects of prolonged ethanol intake: Production of fatty liver despite adequate diets. *J. clin. Invest.* **44**: 1009–1021, 1965.
9. Lieber, C. S., E. Rubin and L. M. DeCarli. Effects of ethanol on lipid, uric acid, intermediary, and drug metabolism, including the pathogenesis of the alcohol fatty liver. In: *The Biology of Alcoholism, Vol. 1*, edited by B. Kissin and H. Begleiter. New York: Plenum Press, 1971, pp. 263–305.
10. McMillan, D. E., F. W. Ellis, G. D. Frye and J. R. Pick. Failure of signs of physical dependence to develop in hamsters after prolonged consumption of large doses of ethanol. *Pharmac. Biochem. Behav.* **7**: 55–57, 1977.
11. Snell, D. and R. A. Harris. Impairment of avoidance behavior following short-term ingestion of alcohol. *Psychopharmacology*, in press, 1979.
12. Thurman, R. G., M. A. Bleyman and D. E. McMillan. Rapid blood elimination and withdrawal resistance in the syrian golden hamster. *Pharmacologist* **20**: 160, 1978.
13. Walker, D. W. and G. Freund. Impairment of shuttle box avoidance learning following prolonged alcohol consumption in rats. *Physiol. Behav.* **7**: 773–778, 1971.