HEPATIC DAMAGE PRODUCED BY LONG-TERM ALCOHOL CONSUMPTION IN WELL-NOURISHED RATS

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SUMMARY

A study of the effect of long-term alcohol consumption on the liver of well-nourished rats is described. Rats fed for 16 weeks on a semipurified diet supplemented with high levels of vitamins and lipotropic factors and alcohol corresponding to 35% of the total caloric intake developed marked fatty changes of the liver. Mild fatty changes were observed in pair-fed controls receiving as isoenergetic equivalent of sucrose instead of alcohol. Intracellular hyaline bodies, corresponding ultrastructurally to giant mitochondria were abundantly found in the hepatocytes of alcoholic rats, while in the controls they were not seen. Te findings in this investigation are postulated to provide further evidence that the long-term intake of alcohol exerts a direct causative role in the pathogenesis of liver damage.

INTRODUCTION

The association between excessive alcohol consumption and liver desease is well known. However there are still differences of opinion about its pathogenesis. Direct toxic effect of alcohol, additives and congeners in alcoholic beverages, nutritional imbalance,

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and deficiencies in protein, lipotropes and vitamins have been considered to play a role in producing the hepatocellular damage.

Using an experimental model in which alcohol is added into a totally liquid nutritionally adequate diet (1, 2), chronic alcohol intake has been reported to produce hepatic metabolic and morphologic changes in both alcoholic animals and human volunteers (1-9). These results have been questioned by studies in which alcohol was giving in the drinking water of animals fed commercial stock diet or solid semipurified diet (10-17). Such opposite conclusions may be explained by the differences between the two experimental models. The latter model differs from the liquid diet model because of the degree of undernutrition frequently associated with.

In this investigation we report the effects of alcohol on the liver in otherwise well-neurished rats fed a nutrition solid semipurified diet.

MATERIALS AND METHODS

Male rats of the Wistar strain, weighing an average of 50 g were obtained from the breeding colony at the Medical School of Ribeirão Preto. They were allocated into two groups: group A (alcohol diet) —15 rats, and group B (pair-fed controls)— 15 rats. The rats were housed in individual wire cages with raised bottoms, and fed solid food in stainless steel feeding dishes and liquid in Richter graduated drinking tubes. They were weighed thrice weekly and their dietary consumption recorded daily. All rats were fed on a well-balanced semipurified diet similar to that described by Koch, Porta and Hartroft (14). The composition of this diet in weight percent was casein 23, soybean oil 15, sucrose 24, dextrose 18, agar 4, vitamin mixture (Vitamin Diet Fortification Mixture, Nutritional Biochemicals Corporation, Cleveland, Ohio) 10, salt mixture 4 (18), choline, 1.8, and cystine 0.2. The animals in group A received the basic solid diet and a solution of 32% (v/v) ethyl alcohol and 25% (w/v) sucrose in water *ad libitum* according to Porta and Gomen-Dumm (12).

The rats in group B were pair-fed with those in group A and received equal amounts of the basic diet and the same volume of water plus sucrose isoenergetic with the alcohol-ucrose solution consumed by the alcoholic rats. Sucrose was added to the water in the form of a 25% (w/v) solution and also to the solid diet, intending not to desguise the solid food palatability and thus increasing intake.

After 16 weeks the rats were killed under light ether anesthesia by exsanguination from the aorta. Pieces of liver from the median lobe were frozen and sections cut and stained with Sudam III to demonstrate lipid. Additional fragments were placed in neutral formalin, embedded in paraffin and cut at 5-6 micra; sections were stained with hematoxylin and eosin, hematoxylin and phloxine, and Gomori's trichrome. Small pieces to be prepared for electron microscopy were immersed in a 3% glutaraldehyde solution in 0.1M phosphate buffer for 2-4 hours. They were them postfixed in 1% osmium tetroxide in phosphate buffer for 2 hours, dehydrated in ascending concentrations of acetone, and embedded in araldite. Ultrathin sections were cut with glass knifes on a Porter-Blum ultramicrotome and double stained with uranyl acetate and lead citrate. Photomicrographs were taken on a Jeol JEM-100C electron microscope.

RESULTS

Only one rat from the alcoholic group A died early during the experimental period with pneumonia.

The alcohol-fed rats remained in good health and showed no clinical signs of nutritional deficiencies. The mean daily amounts of solid food (expressed in grams and calories) and liquid diet (expressed in milliliters and calories) consumed by them are represented in figure 1. The dietary procedure used allowed the rats in group A to consume 35% of their total calories as alcohol. The lipid content of the final regimen can be considered low and the protein level probably adequate. The final intake of lipotropes (lipotropic value — 184 mg/100kcal) and vitamins can be considered high.

The percentage proportions of dietary energy components of the diets consumed by rats in group A and B are graphically represented in figure 2. The pair-fed controls consumed 94% of total calories consumed by the alcoholic group.

During the 16-week period all rats grew well at a growth rate of about 2 grams of body weight per day. They gained weight continuously and no differences were found at any time between weights of rats in group A and rats in groups B (fig. 3).

On gross examination the liver of rats fed a alcohol were more yellow than those from pair fed controls. Light microscopic examination revealed that hepatic structure was well preserved in all rats. The hematoxylin and eosin stained sections of livers from sucrose control rats disclosed fine vacuolization of periportal hepa-

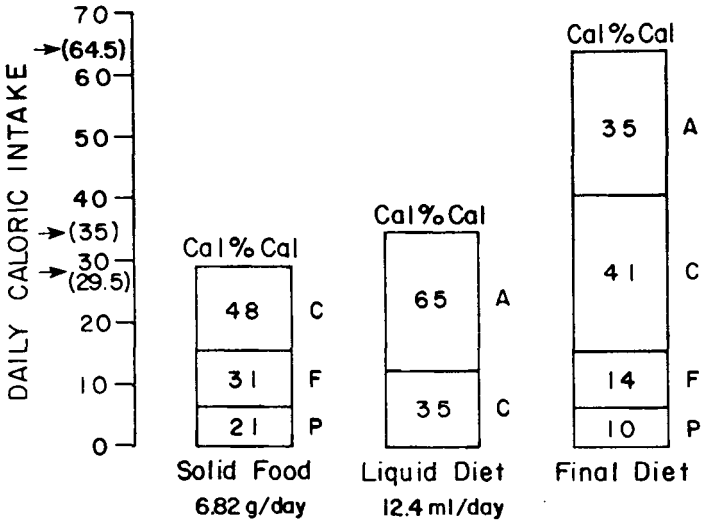

FIGURE 1

Schematic representation of mean daily amounts of food (expressed in grams and calories)and liquid diet (expressed in milliliters and calories) consumed by the alcohol-fed rats. A, alcohol; C, carbohydrate; F, fat; P, protein.

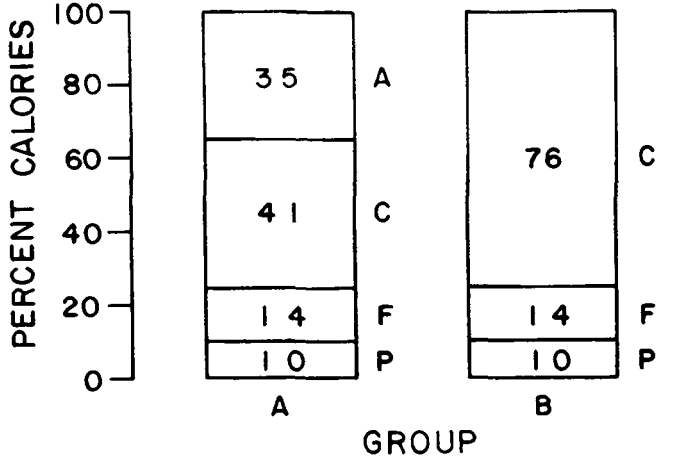

FIGURE 2

Composition of the diets actually consumed by rats in groups A and B, expressed in percent of total calories. A, alcohol; C, carbohytrate; F, fat; P, protein.

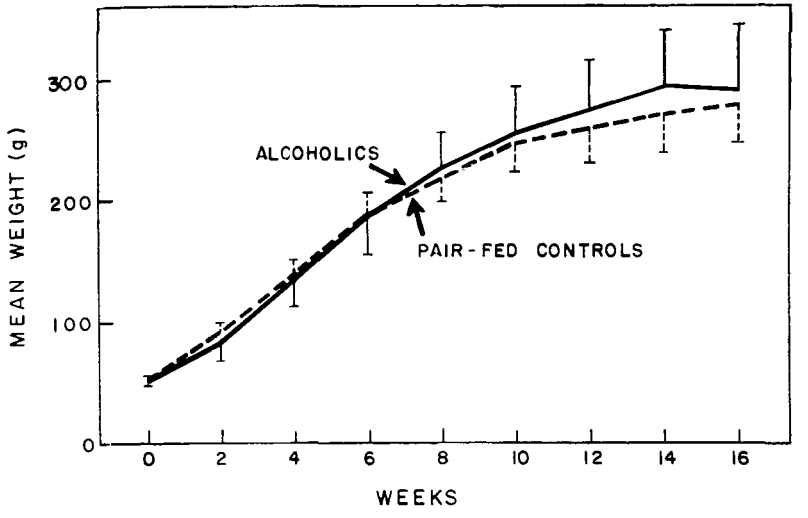

FIGURE 3
Growth curves of alcoholic and pair-fed control rats.

tocytes (fig. 4a). Frozen sections stained with Sudam III exhibited stainable fat in the form of fine droplets located in the cytoplasm of hepatocytes and Kupffer cells (fig. 4b). Livers from alcohol-fed rats displayed pronounced extensive (periportal, midzonal and centrilobular) hepatocytic vacuolization (fig. 4c), which coincided with increased lipid stained by Sudam III (fig. 4d). The stainable fat was found in the form of numerous droplets and globules occassionally displacing nuclei (fig. 4e). Kupffer cells also contained fat in the form of fine droplets.

Hyaline bodies as a regularly outlined, round or elongated cytoplasmic inclusion, typically located around the nucleus, could be abundantly seen in the paraffin-embedded material from alcoholic rats (fig. 4f). These hyaline inclusions were found chiefly in centrilobular areas, particularly around central veins. On the other hand, no intracellular hyaline was found in livers from controls.

At the inspection under the electron microscope the hepatocytes from alcoholic rats exhibitid striking changes in the mitochondia and smooth endoplasmic reticulum (fig. 5). The mitochondrial lesions were characterized by enlargement (giant michondria), irregular forms and disoriented cristae. The smooth endoplasmic reticulum was increased and dilated. No abnormalities were seen by electron microscopy of the hepatocytes of the controls.

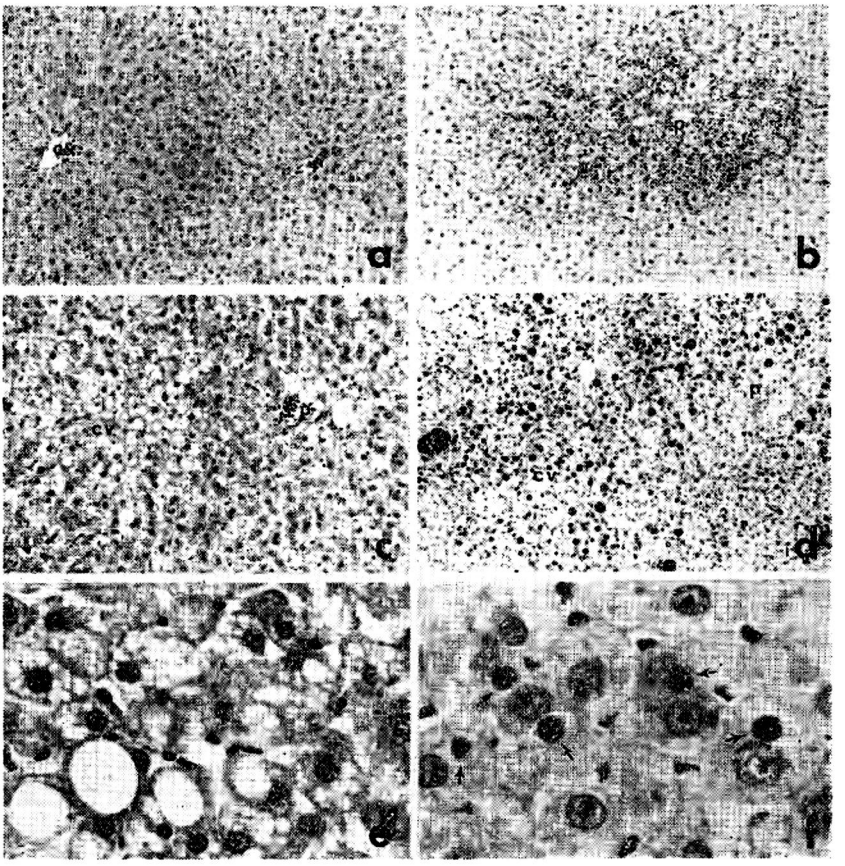

FIGURE 4

(a) Fine vacuolization of the periportal hepatocytes from a pair-fed control rat. H. and E. (x 130); (b) Accumulation of fat droplets in periportal hepatocytes and Kupffer cells of a pair-fed control rat. Sudam III Stain. (x 130); (c) Extensive (periportal, midzonal and centrilobular) hepatocytic vacuolization in alcohol-fed rat. H. and E. (x 130); (d) Extensive fatty accumulation in the hepatocytes and Kupffer cells of an alcohol-fed rat. Sudam III Stain. (x 130); (ee) Hig power view of enlarged vacuolized hepatocytes of an alcoholic rat. H. and E. (x 510); (f) Hyailne bodies (arrows) in hepatocytes of an alcoholic rat. Hematoxylin and phloxine. (x 1200). Abreviations used: p = portal tract. cv = central vein.

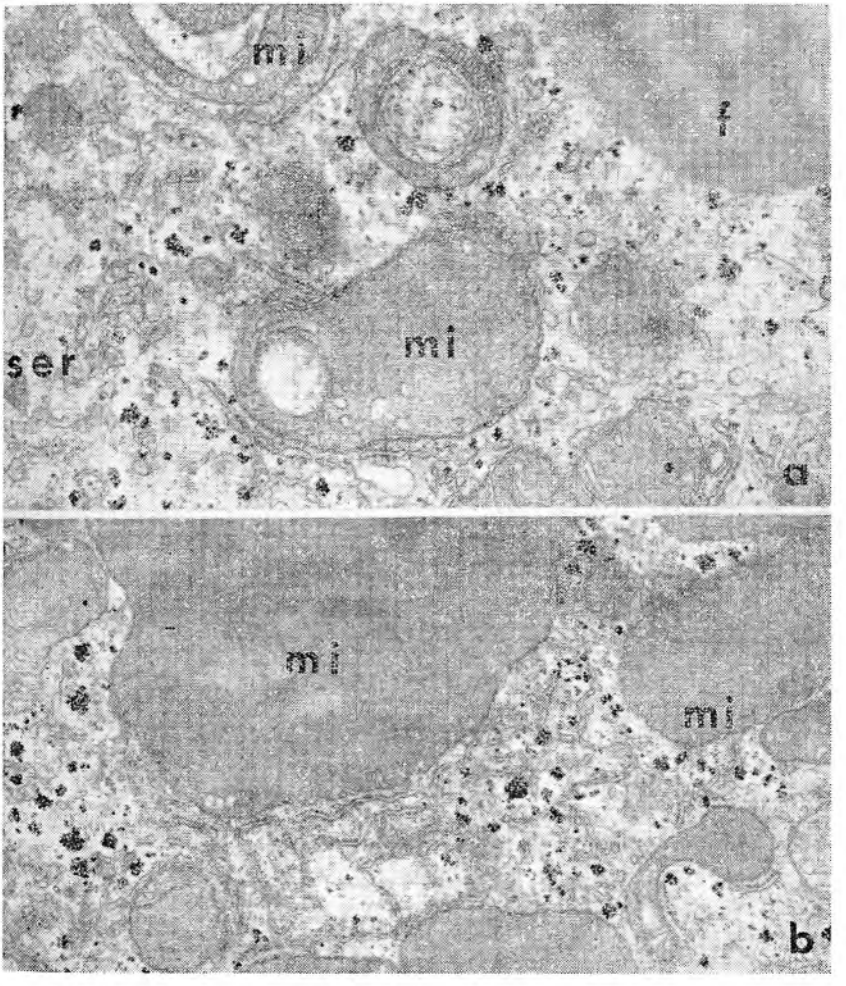

FIGURE 5

Electron micrographs of liver from an alcoholic rat. Mitochondria are enlarged and irregular in forms, exhibiting matrix with increased density, and loss, disorientation and rupture of cristae. Smooth endoplasmic reticulum is dilated mi = mitochondria; ser = smooth endoplasmic reticulum; rer = rough endoplasmic reticulum; f = fat. Uranly acetate and lead citrate (a = x 33.300; b = x 21.600).

DISCUSSION

Our results clearly show that rats fed for 16 weeks on a supplemented solid semipurified diet supplemented with high levels of vitamins and lipotropic factors and alcohol corresponding to 35% of the total caloric intake developed marked fatty changes of the liver. Mild fatty changes were observed in pair-fed controls receiving an isoenergetic equivalent of sucrose instead of alcohol. Intracellular hyalines were found abundantly in the hepatocytes of alcoholic rats, while in the controls they were not seen.

When interpreting the present results it is fundamental to take into consideration not only the amounts of alcohol consumed but also the composition of the diet with respect to protein, fat, lipotropes and vitamins. It is well known from data in the literature that the occurrence and degree of liver damage is affected by these factors.

The total energy consumed by the alcohol fed rats was practically identical to that of the controls, which received the same quality and quantity of diet as the alcoholics, with the only exception of the alcohol replaced isocalorically with sucrose given both as a 25% (w/v) solution and added to the solid diet. The alcohol intake of the rats in the present study was 35% of the total calories, which is in opposition to 46% reported by Porta and Gomez-Dumm (12). The experimental rats consumed 64.5% kcal daily, 35 kcal from the alcohol-sucrose solution and 29.5 kcal from the solid diet. This finding i in contrast to the daily conumption of 49 kcal related by Koch, Porta and Hartroft (14), 35 kcal from the alcohol-sucrose solution and 14 kcal from the solid diet. The growth rate of the alcoholic rats, as evaluated by body weight gain, was not impaired over the 16-week feeding period, as compared with growth of controls, and no signs of nutritional deficiency was recognized. The growth rate of the alcoholic was similar to those reported by other investigators using a well-balanced solid semipurified diet without alcohol in which protein contributed 18.5% of total calories (14) or a liquid diet with alcohol in which protein contributed 25% of total calories (16, 19). Furthermore the growth rate of the alcoholic rats was 2-3 times higher than that reported by Koch, Porta and Hartroft (14) for alcoholic rats.

The protein level in the final regimen of our alcoholic and pair-fed rats was probably adequate, above the minimum requirement for growing rats (20). It is well established that in the syndrome of malnutrion in children or kwashirkor (21), and in

experimental protein deficiency, protein representing 0 to 4% of the total calories (22), there is a marked accumulation of fat in the liver. However, a recent paper by Lau, Flaim and Ritchey (23) relates that mild protein restriction (8% of total calories) with adequate energy intake (about 60 kcal daily) does not cause changes in rat liver lipid content. On the other hand deficiency in dietary protein has been shown to potentiate the hepatotoxic effect of alcohol (5, 24, 25). Lieber, Spritz and DeCarli (5) showed that rats fed a totally liquid deficient diet (protein represented only 4% of the total calories) with 36% of calories as alcohol exhibited a marked increase in hepatic triglycerides as compared to pair-fed controls. Contrarily, Best, Hartroft and coworkers (12, 13, 14, 26) did not observe differences in liver fat content between rats given deficient diets and those receiving deficients diets plus alcohol.

The importance of fat in the diet has been investigated in both men and experimental animals. It has been shown that the fatty liver produced by chronic consumption of alcohol is dependent on the percentage of calories derived from fat in the diet, the amount of liver lipid increasing when fat exceeds 20% of the total calories (27-29). In our studies the fat content of the final diet was 14% of the total calories, and it can be considered low.

The amount of lipotropes and vitamins were adequately high. The lipotropic value of the final regimen of experimental and control rats was 184 mg/100 kcal, which is 4-5 times the minimum requirement for rats (12).

The alcoholic hyaline, first described by Mallory (30), consists of a degenerative change of the liver cell cytoplasm. Intracellular hyaline bodies were found abundantly in the cytoplasm of hepatocytes from our alcoholic rats. At the inspection under the light microscope the usual configuration of the hyaline, inclusions was that of rounded or alongated regularly outlined paranuclear masses. These structures correspond ultrastructurally to giant mitochondria as previously observed by other authors (3, 31, 32). They differ morphologically from true filamentous Mallory bodies (33-35), which have never been observed in animals, except in experiments from primates (8, 9).

Mild accumulation of fat, clearly seen in periportal hepatocytes and Kupffer cells could be found in livers form pair-fed controls. Such alteration can be attributed to the dietary imbalance, with an excess of carbohydrate (76% of the total calories). The increase of liver fat associated with diets rich in carbohydrate has been reported by a great number of authors.

In conclusion, the findings in the present experimental study are postulated to provide further evidence that the long-term consumption of alcohol exerts a direct causative role in the pathogenesis of liver damage in well-nourished rats. Further experiments to get more detailed information on the particular metabolic and structural changes are proceeding.

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SUMARIO

Alteracöes hepáticas produzidas pelo consumo prolongado de álcool em ratos bem nutridos.

Os experimentos relatados no presente trabalho foram realizados para se estudar os efeitos do consumo prolongado de etanol na estrutura morfológica do fígado de ratos bem nutridos. Animais mantidos em uma dieta sólida semi-sintética fortificada e álcool correspondendo a 35% da ingestão calórica total desenvolveram acentuada esteatose hepática. Leve acúmulo de gordura foi observado em ratos controles pareados recebendo um equivalente isocalórico em substituicão ao álcool. Inclusões citoplasmáticas, correspondendo ultraestructuralmente a megamitocondrias, foram também abundantemente encontradas nos hepatócitos de ratos alcoólicos; todavia tais inclusões não puderam ser detectadas em animais controles. Os achados da presente invertigação fornecem evidéncias adicionais que o consumo prolongado de etanol exerce um papel causal direto na patogenese da lesão hepática no alcoolismo cronico.

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