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On the Characteristics of Alcohol-Induced Liver Enlargement and Its Possible Hemodynamic Consequences

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ISRAEL, Y. AND H. ORREGO. *On the characteristics of alcohol-induced liver enlargement and its possible hemodynamic consequences.* PHARMACOL BIOCHEM BEHAV **18:** Suppl. 1, 433–437, 1983.—Chronic consumption of alcohol leads to an increase in liver weight, primarily due to an increase in hepatocyte volume. About 50–60% of such an increase is due to an increase in intracellular water. Accumulation of intracellular K^+ osmotically accounts for about one half of the increase in intracellular water, while an increase in soluble proteins plays only a minor role in such an increase in cell volume. The increase in intracellular water is accompanied by a relative reduction in water in the extracellular space, probably due to compression of the extracellular volume by the enlarged hepatocytes. It is suggested that such an increase in hepatocyte size, with an attending reduction of the extracellular volume, results in an increased resistance to blood flow through the liver and thus in an increase in portal pressure. In alcoholics, portal and intrahepatic pressure correlate with cell size both in cirrhotics ($r=0.79$) and in non-cirrhotics ($r=0.74$), thus suggesting that cell enlargement plays a major role in the production of portal hypertension in the alcoholic.

Chronic alcohol consumption Liver weight Hepatocyte volume Portal hypertension Hemodynamics

HEPATOMEGALY appears to be one of the most common clinical findings in alcoholic liver disease; about 90–95% of alcoholics presenting to a liver clinic show liver enlargement [9]. Contrary to what is often stated in the classical literature, that liver size is reduced in alcoholic cirrhosis (see references in [11]), hepatomegaly is now known to be common even in the terminal stages of alcoholic cirrhosis [11]. Average post-mortem liver weight in patients with cirrhosis is about 50% higher than in control subjects [11].

Studies by Baraona *et al.* [1] demonstrated that the hepatomegaly induced by chronic alcohol consumption results from an increase in cell size. Increases in cell size might have important consequences in liver hemodynamics as shown by Leevy *et al.* [6]. These investigators reported that hepatomegalies induced by phenobarbital and oxandrolone were accompanied by increases in hepatic resistance and portal pressures. It should be noted that portal hypertension is one of the most important determinants of mortality in alcoholic liver disease [8]. Because of these observations we have studied further the mechanisms by which alcohol induces liver cell enlargement and the possible role of cell enlargement in the development of portal hypertension in alcoholic liver disease.

Four-week old rats fed alcohol chronically (35% of total calories as ethanol for 30 days) show significant increases in liver weight when compared to controls pair-fed isocaloric carbohydrate [3,7]. In these livers we determined protein, lipid and water content (Table 1). It could be observed that water constitutes the most important component (50–60%) of the increase in liver weight, while total lipids account for 25–30%, and proteins for 15–20% (Fig. 1).

The fact that lipids make up such a small part of the increase in liver weight is of interest since it has been generally assumed that fat accumulation is the most important factor in inducing hepatomegaly after chronic alcohol consumption. An increase in total liver proteins is in agreement with data by Baraona *et al.* [1]. Similarly, we have observed that total hepatic DNA content is not increased but remains constant. On the other hand, DNA content per unit liver weight is significantly reduced (Table 1), in line with the proposal that alcohol-induced hepatomegaly is due to an increase in cell size rather than in cell number [1]. This was further tested by determining hepatocyte surface area in formalin-fixed histological sections (hematotoxolin and eosin stain). Hepatocyte surface area was found to be increased by about 50% in the livers of alcohol-fed animals (Fig. 2).

An interesting observation was made regarding the compartmentation of the excess water in the livers of alcohol-treated animals (Table 1). Using ^{3}H-inulin as a marker of extracellular space, it was found that all the excess water was of intracellular origin, while total extracellular water remained constant. Similar results have been obtained with ^{125}I-albumin (unpublished). This is likely to result from a constraint placed on the total expansion of the organ, which could be produced by the semielastic liver capsule, resulting in a reduction in extracellular space per unit liver weight (Fig. 3).

Since there is no active movement of water per se across

TABLE 1

HEPATIC COMPOSITION AFTER CHRONIC ETHANOL ADMINISTRATION TO RATS

	Control	Ethanol	p
Body weight (g)	148 ± 3	149 ± 5	N.S.
Liver weight (g)	6.64 ± 0.16	9.92 ± 0.40	<0.001
Liver wt/body wt (%)	4.50 ± 0.15	6.68 ± 0.20	<0.001
Total lipids (g)	0.53 ± 0.03	1.44 ± 0.12	<0.001
Total proteins (g)	1.60 ± 0.05	2.18 ± 0.09	<0.001
Total DNA (g)	0.024 ± 0.001	0.025 ± 0.001	N.S.
DNA (mg/g liver)	3.6 ± 0.10	2.6 ± 0.12	<0.001
Total intracellular H_2O	2.58 ± 0.14	4.18 ± 0.18	<0.001
Total Extracellular H_2O	1.87 ± 0.07	2.00 ± 0.13	N.S.

Young male Wistar rats were fed liquid diets containing either ethanol or isocaloric carbohydrate (control) for 4 weeks. Data from [7].

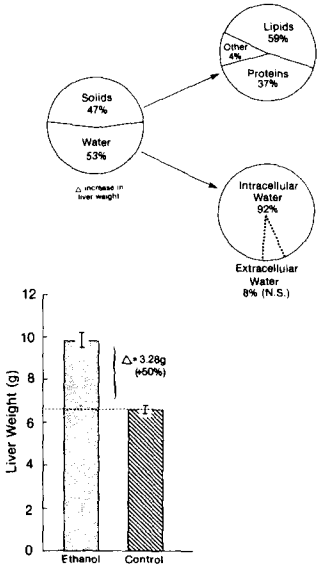

FIG. 1. Components of alcohol-induced liver enlargement in young Wistar rats fed 35% of calories as ethanol for 4 weeks. Controls received isocaloric carbohydrate replacing ethanol (from [7], with permission).

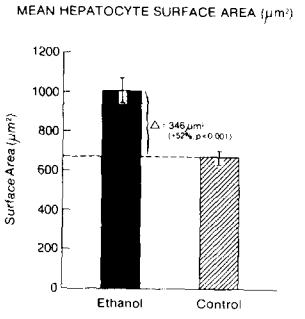

FIG. 2. Hepatocyte surface area in the liver. Young Wistar rats fed alcohol chronically for 4 weeks (from [7], with permission).

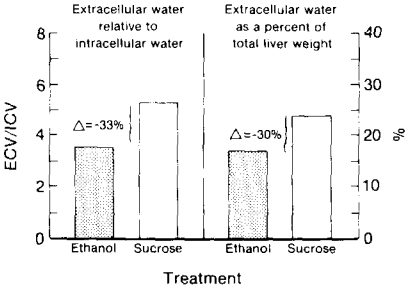

FIG. 3. Reduction in the ratio of hepatic extracellular volume (ECV) to hepatic intracellular volume (ICV) and decrease in the hepatic extracellular volume as a percentage of total liver weight in alcohol-fed rats. Both differences are statistically significant ($p<0.01$).

cell membranes, water accumulation in the cell must be accounted for by an increased total osmotic activity inside the cell. Thus, it was of importance to determine the mechanism that might account for such an increase. We have observed that the major intracellular cation in the livers of alcohol-fed animals is potassium [3,7], with small or non-significant increases in sodium and chloride ions (unpublished data). Total intracellular potassium is markedly increased in the livers of alcohol-fed rats, accounting, on an osmotic basis, for 40–50% of the excess in intracellular water (Fig. 4). An increase in intracellular potassium could result from an increased activity of the sodium pump, a reduction in K^+ leakage, or both. We have previously reported that (Na^+ + K^+)-ATPase is markedly increased in livers of alcohol-treated animals [2]. Ouabain-sensitive rubidium^{86} transport, an analogue of potassium, is also increased in the livers of alcohol-fed animals [2].

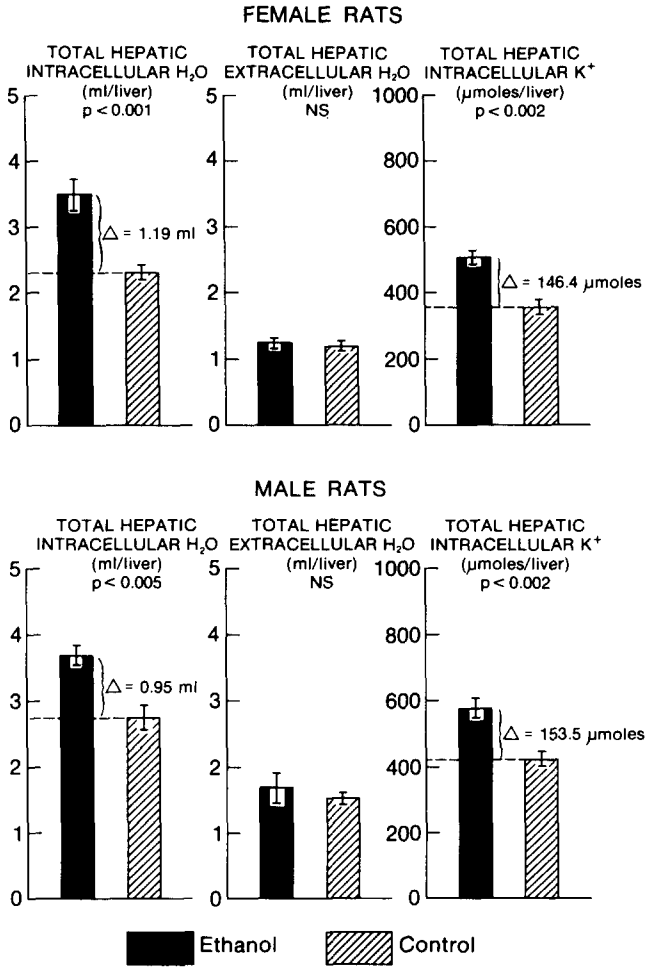

FIG. 4. Increases in intracellular water and intracellular potassium in rat liver after chronic alcohol treatment (data from [4] with permission).

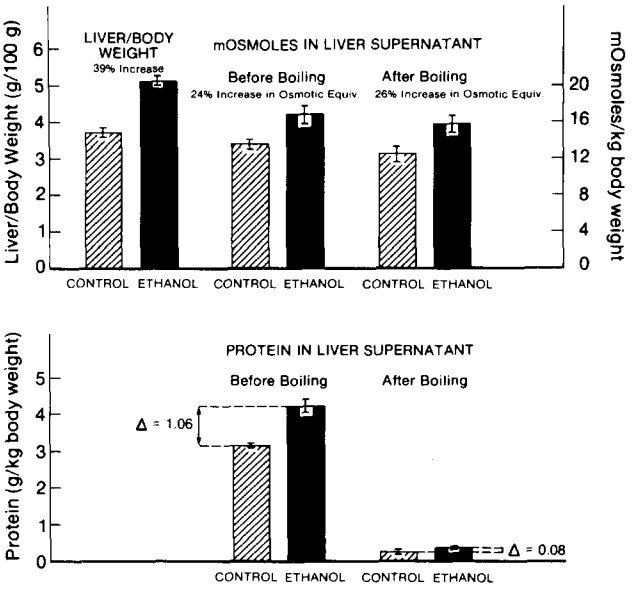

FIG. 5. Osmolality of liver supernatants from rats fed alcohol chronically. Removal of soluble proteins by heat denaturation and centrifugation did not reduce the increase in total osmotic activity observed in the livers of alcohol-fed animals (from [4]), with permission).

On electrochemical grounds it is reasonable to expect that an increase in total intracellular anions should accompany the increase in total intracellular potassium. Liver proteins, which increase following chronic alcohol consumption, might provide negative charges to neutralize the positive charges of the excess in potassium ions. It has been suggested [1] that the increase in hepatic proteins after chronic ethanol administration may result in an osmotic gain of water by the hepatocytes. In order to determine if this was the case, liver supernatants (43,000 × g) were prepared, and soluble protein and osmolality were determined. The supernatants from ethanol-fed rats contained virtually all the excess osmotic activity of the liver; they also showed an increase in total protein content. However, heat denaturation (in sealed tubes) and precipitation of 90–93% of the proteins did not reduce the excess in total osmotic equivalents or K^+ concentration (R. Britton, unpublished data) observed in the liver supernatants from alcohol-fed animals (Fig. 5). This suggests that proteins play only a small role in the osmotic mechanism accounting for the increase in intracellular water in hepatocytes from alcohol-fed animals. Since it is likely that the boiled supernatants contain osmotically active substances of small molecular weight, we determined the pattern of elution of small organic molecules absorbing at 280 nm on Sephadex G-15. As can be seen in Fig. 6, virtually no differences were observed between the alcohol-fed and control animals. Thus, further studies should be directed at the elucidation of the type of molecule(s) other than potassium that accounts for the increase in osmotic activity in the livers of alcohol-fed animals.

Our findings that hepatomegaly in the rat is accompanied by a relative reduction in the extracellular (and most likely in the vascular) compartment per unit liver weight suggests that an increased resistance to blood flow is likely to occur. At a normal blood flow this could result in increased portal pressure. A puzzling, yet unexplained finding is that in our laboratory, under "constant" conditions of housing and feeding, hepatomegalies can vary between 15–60% in different animals or batches of rats. The reasons for this variation are not clear. In some groups of rats fed alcohol chronically [7], but not in others, intrahepatic pressures were found to increase by an average of 80%. In the groups of animals in which increases in intrahepatic pressures were observed, hepatomegalies exceeded 50%. Recent unpublished studies with a large number of animals (n=24) indicated that the increases in intrahepatic pressures are significantly correlated with the degree of hepatomegaly developed after chronic treatment with ethanol ($r=0.63$; $p<0.001$). The need for a substantial degree of hepatomegaly before an increase in portal pressure is to be expected since several factors need to be overcome before an increase in cell size is translated into an increase in resistance to blood flow: (a) the capacity of the capsule (and of the liver infrastructure) to stretch has

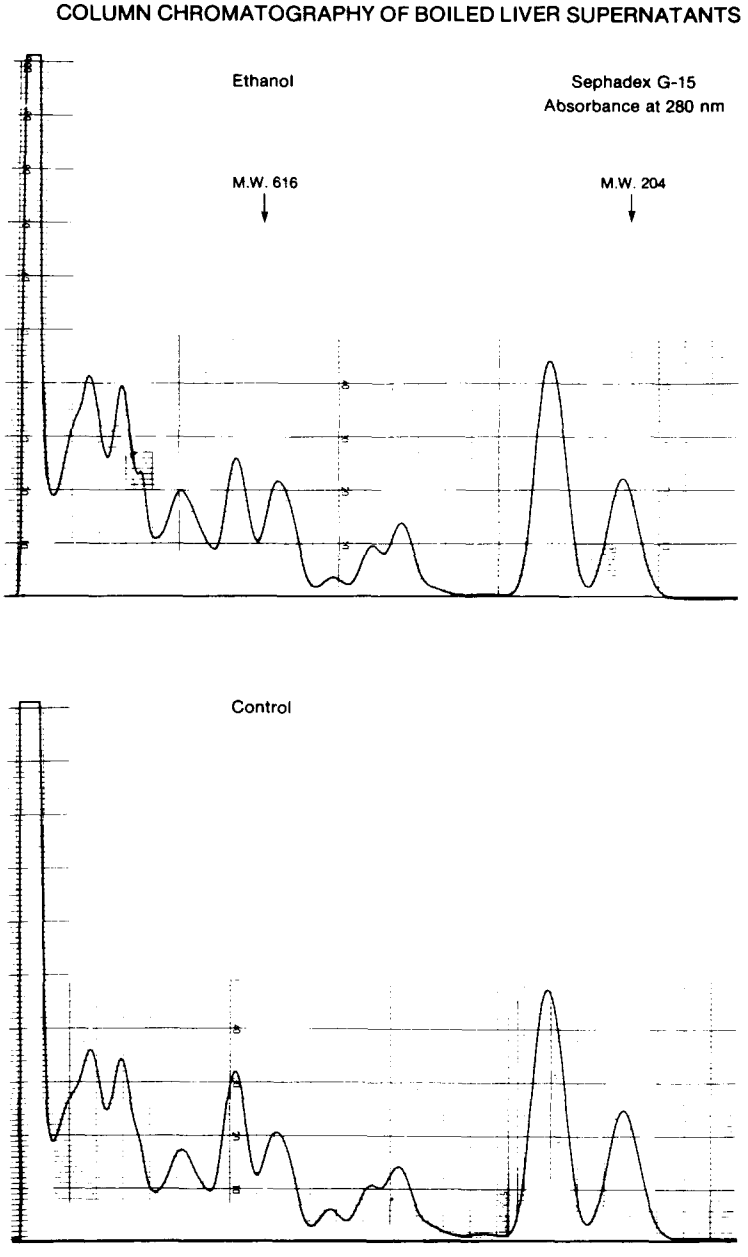

FIG. 6. Elution pattern in Sephadex G-15 of boiled liver supernatants of alcohol-fed and control animals (from [4] with permission).

to be exceeded before the extracellular space is compressed; (b) any shunting must be fully utilized; (c) capacitance (low resistance) vessels should be compressed until they are converted into resistance pathways. Sinusoids are known to be pathways of low resistance because of their large number compared to portal venules [10]. Cell expansion must markedly reduce sinusoid caliber before they become resistance vessels.

In humans, because of the methodological problems in determining actual liver size (or weight), hepatocyte enlargement, as determined from surface areas in needle biopsies, rather than total liver size, was used in correlating liver enlargement and portal pressure. In patients with alcoholic liver disease, the variations in hepatocyte surface areas exceeded that found in animals. In the alcoholic patients, hepatocyte areas varied between 1000 and 4000 μm^2. In these, a cell area of 1600–1700 μm^2 (Fig. 7) appears to constitute the threshold value for cell enlargement, beyond which increases in portal and intrahepatic pressure are observed. An association between cell enlargement and intrahepatic pressure was observed independently of the presence ($r=0.79$; $p<0.001$) or absence ($r=0.74$; $p<0.001$) of

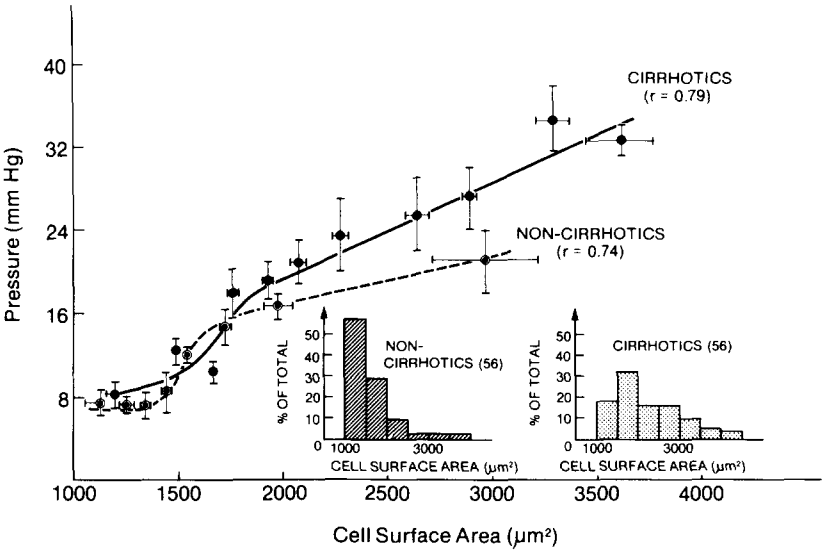

FIG. 7. Relationship between hepatocyte size and intrahepatic pressure in alcoholic patients presenting to a liver clinic. Patients were divided into two groups, cirrhotics (n=56) and non-cirrhotics (n=56) according to liver histology and biopsy specimens. The inserts are histograms of frequency indicating that enlarged hepatocytes occur more often in patients presenting with cirrhosis. Also note that normal pressures are observed in cirrhotic patients presenting with small hepatocyte surface areas (from [5] with permission).

cirrhosis (Fig. 7). Many patients with histologically diagnosed cirrhosis show normal portal pressures. In these patients, hepatocyte surface areas were observed to be substantially smaller than in cirrhotics with elevated portal pressures. Conversely, an important proportion of patients without cirrhosis show elevated portal pressures. The fact that portal hypertension occurs more often in cirrhotics than in non-cirrhotics can be explained by the finding that the proportion of cirrhotics presenting with hepatocyte enlargement is higher than in non-cirrhotics (inset of Fig. 7).

An excellent correlation, such as the one observed between hepatocyte enlargement and portal hypertension in human alcoholics, does not indicate causality in the association between these factors. Thus, it was important to develop an animal model in which hepatocyte size could be varied in a short time, and in which liver perfusion could be controlled. This was accomplished using the isolated perfused rat liver. Different degrees of hepatomegaly and hepatocyte enlargement could be produced in livers from naive animals by perfusing them with hypotonic solutions. With this method, cell enlargement was shown to result in increases in portal pressure which were proportional to the degree of hepatomegaly [5].

In conclusion, we have postulated that hepatocyte enlargement produced by chronic alcohol consumption results in compression of hepatic vascular pathways and therefore may be a contributory mechanism in the production of portal hypertension. Data from alcoholic patients and laboratory animals support this hypothesis.

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