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Increased Hepatic Oxygenation following Ethanol Administration in the Baboon[1] (39968)

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Introduction. Necrosis and sclerosis around the central veins of the hepatic lobule have been observed in alcoholics (1, 2) as well as in experimental models of alcoholic liver injury (3). The centrolobular zone possesses the lowest oxygen tension within the hepatic lobule (4) and is the site of liver injury secondary to hepatic ischemia (5, 6). The observation of enhanced oxygen consumption following ethanol administration (7-10) lead to the hypothesis that increased hepatic oxygen consumption produced by alcohol results in hypoxia of the centrolobular zones and is responsible for the lesions observed in alcoholic liver injury (6). Based upon this assumption, antithyroid medication has been proposed for the treatment of alcoholic hepatitis (6). The administration of ethanol may have a varied effect upon hepatic blood flow depending upon the dose given and the route of administration. Some authors using relatively small amounts of alcohol have reported no effect of ethanol administration on hepatic blood flow (11, 12), while others noted an increase (13, 14); in some of the latter studies, blood alcohol levels were similar to those found after moderate drinking (14). Increased hepatic blood flow would tend to increase hepatic oxygen delivery and thereby offset the effects of increased hepatic oxygen consumption by alcohol. In view of the clinical importance of the problem, we undertook to test directly the net effects of ethanol upon hepatic oxygenation in an experimental model of alcohol-induced liver injury.

Materials and methods. Baboons were pair-fed either an alcohol-containing diet in which ethanol constituted 50% of total calories or an isocaloric diet in which ethanol was replaced by carbohydrate, for a period of 1-4 years. The detailed composition of the diet and the feeding technique have been published previously (15).

The baboons were housed at the Laboratory for Experimental Medicine and Surgery in Primates (LEMSIP), Tuxedo, New York, until the time of study. During their maintenance at LEMSIP none of the animals was observed to have either clinical or laboratory evidence of respiratory disease (cough, excess sputum production, fever, leucocytosis) or blood loss (melena, change in hemoglobin). These tests were performed to exclude other possible causes of hypoxia in these animals. None of the animals in this study had signs of withdrawal from alcohol following an overnight fast.

All baboons were subjected to open liver biopsies within 6 months of the time of study. Tissue fixation and staining were as previously described (3).

Following an overnight fast, anesthesia was obtained using ketamine hydrochloride (up to 40 mg/kg im) and/or Nembutal and nitrous oxide. All animals underwent endotracheal intubation and were ventilated mechanically on a respirator with a mixture of oxygen and nitrous oxide. Blood samples for gas analysis were obtained after ventilation for at least 5 min with room air only.

A No. 7 French polyethylene catheter (Cook Inc., Bloomington, Indiana) was passed via the brachial vein and superior vena cava into the right hepatic vein under fluoroscopic observation. Additional catheters were positioned in the aorta just above the aortic valve and in the femoral vein.

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Following catheterization all animals received 1000 units of Na heparin iv. Anticoagulation was reversed with protamine sulfate at the conclusion of the study. Blood samples were obtained with the hepatic vein catheter in a peripheral branch in the "free" position. Arterial blood samples were collected at the same time. Samples were taken just prior to and at the conclusion of the ethanol infusion and analyzed for pH, pCO_2, and pO_2 on a Radiometer acid-base analyzer (Radiometer, Cophenhagen, Denmark). Oxygen saturation of hemoglobin was measured with an oximeter (American Optical Company, Bedford, Massachusetts).

Hepatic blood flow (EHBF) was estimated using the Fick principle with a sulfobromophthalein infusion (BSP, Westcott and Dunning, Baltimore, Maryland) (16). Plasma volume was determined with the Evans blue (Warner Chilcott, Morris Plains, New Jersey) dilution method (17).

Ethanol in 0.45% saline was infused at a constant rate with a Harvard pump into a peripheral vein; 1.75 g/kg was administered over 1 hr. Blood ethanol was measured by gas-liquid chromatography as previously described (18).

Ethanol infusions were completed in three of the four pairs of animals. In one of these pairs, the pair-fed control did not complete the entire infusion because of a technical breakdown during the catheterization; a chow-fed control was used as a substitute.

Splanchnic oxygen consumption was calculated by multiplying the difference in oxygen content of the arterial and hepatic venous blood by EHBF. Oxygen content was calculated from the pO_2, oxygen saturation, and the hematocrit.

Means (±standard error of the mean) were calculated for all data. Differences between matched pairs or differences before and after ethanol were assessed by Student's paired *t* test.

Results. Hepatic morphology. Liver biopsies were performed on each animal within 6 months preceding study (generally 2–3 months). Biopsies of the four alcohol-fed animals revealed steatosis and sclerosis with inflammation around the central canals. In addition, two of the animals had some fibrous bands spreading from the centrolobular zones, but the lobular architecture had remained intact. By contrast, pair-fed controls had normal liver structure, indistinguishable from that of animals fed chow diet *ad libitum*. Representative biopsies were published previously (3).

Effect of ethanol on hepatic oxygenation. Six of the animals (three alcohol-fed animals and two pair-fed controls and one chow-fed control) were studied before and after ethanol infusion. Peak ethanol concentrations of greater than 170 mg/dl were achieved in each of the animals (mean = 275.5 ± 33.1 mg/dl). Splanchnic oxygen consumption increased from 14.2 ± 3.1 ml/min before ethanol to 19.8 ± 4.9 ml/min following the infusion, but this increment did not reach statistical significance (Table I) and in two of the alcohol-fed animals it actually decreased. Concomitantly, EHBF increased from 280 ± 52 to 505 ± 90 ml/min ($P < 0.02$) (Table I). This increase in hepatic blood flow offset any effects of enhanced oxygen consumption and resulted in a rise of pO_2 in the hepatic vein of all animals (Table I). Mean pO_2 increased from 29.5 ± 5.5 mm Hg before ethanol to 35.2 ± 5.6 mm Hg after ethanol ($P < 0.001$). The ethanol infusion did not affect arterial oxygenation (before alcohol, pO_2 = 109.5 ± 6.3 mm Hg, % saturation = 97.2 ± 1.0%; after alcohol, pO_2 = 109.2 ± 7.2 mm Hg, % saturation = 98.5 ± 0.7%; all N.S.). However, a decrease in both hepatic venous pH (before alcohol, pH = 7.40 ± 0.05; after alcohol, pH = 7.29 ± 0.04; $p < 0.05$) and arterial pH (before alcohol; pH = 7.40 ± 0.04; after alcohol, pH = 7.32 ± 0.04; $P < 0.02$) occurred following the infusion.

In the fasting state when no ethanol was measureable in the blood, four ethanol-fed animals had a lower venous pO_2 compared to pair-fed controls (pO_2 = 23.3 ± 3.1 vs 30.0 ± 4.3 mm Hg; $p < 0.05$). Following ethanol infusion, however, hepatic venous pO_2 increased in each of three ethanol-fed baboons. The mean hepatic venous pO_2 for these animals (26.7 ± 3.5 mm Hg) was not significantly different from that of fasting controls.

No significant differences were noted be-

TABLE I. HEPATIC OXYGENATION, HEPATIC BLOOD FLOW, AND SPLANCHNIC OXYGEN CONSUMPTION BEFORE AND AFTER ETHANOL INFUSION.[a]

Hepatic vein oxygenation		Hepatic blood flow						
		Before alcohol				After alcohol		
Before alcohol Hepatic vein pO_2 (mm Hg)	After alcohol Hepatic vein pO_2 (mm Hg)	Animal	BSP extraction[b] (mg/dl)	Arterial BSP[c] (mg/dl)	Flow[d] (ml/min)	BSP extraction (mg/dl)	Arterial BSP (mg/dl)	Flow (ml/min)
23	32	504 (Alc.)	0.84	7.3	410	0.72	14.8	475
15	20	528 (Alc.)	1.96	6.8	175	0.79	27.5	433
25	28	508 (Alc.)	2.58	7.8	133	2.42	33.2	142
22	30	530 (Cont.)	1.08	2.1	319	0.56	5.7	612
42	50	574 (Cont.)	1.08	7.5	319	0.43	18.3	798
50	59	838 (Cont.)	0.94	5.5	364	0.61	9.8	562
$\bar{X} = 29.5 \pm 5.5$	$\bar{X} = 35.2 \pm 5.6$		$\bar{X} = 280 \pm 52$				$\bar{X} = 505 \pm 90$	
$P < 0.001$					$P < 0.02$			

Splanchnic oxygen consumption

	Before alcohol							After alcohol						
		Arterial		Hepatic venous		Arterial-hepatic venous O_2 content	O_2 consumption		Arterial		Hepatic venous		Arterial-hepatic venous O_2 content	O_2 consumption
Animal	Venous hct.	pH	%Hb. sat.	pH	%Hb. sat	(ml/dl)	(ml/min)	Venous hct.	pH	%Hb. sat.	pH	%Hb sat.	(ml/dl)	(ml/min)
504 (Alc.)	31	7.34	95	7.34	55	5.73	23.5	27	7.23	100	7.23	80	2.56	12.2
528 (Alc.)	26	7.54	100	7.56	71	3.75	6.6	30	7.27	100	7.43	66	4.86	21.1
508 (Alc.)	25	7.44	98	7.47	53	5.24	7.1	24	7.29	100	7.24	67	3.8	5.4
530 (Cont.)	31	7.44	100	7.46	52	6.94	22.1	29	7.35	96	7.28	50	6.22	38.1
574 (Cont.)	35	7.35	95	7.25	64	5.15	16.4	34	7.34	98	7.28	76	3.64	29
838 (Cont.)	35	7.29	95	7.29	77	2.98	10.9	36	7.25	97	7.24	83	2.32	13.0
						$\bar{X} = 14.2 \pm 3.1$								$\bar{X} = 19.8 \pm 4.9$
											N.S.			

[a] Ethanol, 1.75 g/kg, was given intravenously over a period of 1 hr.
[b] Difference in arterial and hepatic vein concentration of BSP (mean of two determinations) corrected for rate of accumulation of BSP.
[c] BSP concentration at the time of BSP extraction determinations.
[d] Flow is the estimated hepatic blood flow based upon the BSP extraction during a continuous infusion of BSP (3.43 mg/min).

tween alcohol-fed animals and controls following an overnight fast with respect to arterial oxygenation (pO_2 = 117.3 ± 15.0 vs 105.5 ± 10.6 mm Hg; % saturation = 98.3 ± 1.2 vs 95.5 ± 1.6%), arterial pH (7.44 ± 0.04 vs 7.40 ± 0.03), venous pH (7.44 ± 0.05 vs 7.34 ± 0.05), EHBF (261 ± 70.8 vs 275 ± 27.8 ml/min), splanchnic oxygen consumption (14.7 ± 3.3 vs 16.5 ± 2.5 ml/min), or hemoglobin (12.9 ± 0.6 vs 13.2 ± 0.3 g/dl). The tendency to a lower hemoglobin and lower EHBF in the alcohol-fed animals may, at least in part, explain the trend to a lower hepatic venous pO_2 observed in these fasting animals.

Discussion. This study reveals an increase in hepatic vein oxygenation after ethanol administration in the baboon. This effect is the net result of two opposite changes. Administration of alcohol produced an increase in splanchnic oxygen consumption. This effect, however, was offset by a rise in EHBF. The net result was an increase in hepatic vein oxygenation. Increased oxygenation was observed not only in control animals with normal hepatic architecture but also in alcohol-fed animals that had developed centrolobular lesions. Thus, the present study does not support the thesis that alcoholic centrolobular liver injury necessarily results from centrolobular ischemia.

A lower hepatic vein pO_2 was observed in alcohol-fed animals compared to controls following a 12-hr fast. However, the lowest hepatic vein pO_2 observed among alcohol-fed animals was 15 mm Hg, which is above the level of oxygen tension in the hepatic vein required to produce anoxia in hepatic parenchyma in the rat (19). It should be noted, however, that the oxygen tension of hepatic venous blood constitutes an average with deviation in two directions: shunted blood and blood from sinusoids of differing lengths. Progressive central sclerosis developed during continuous ethanol feeding in which an overnight fast only rarely occurred as part of experimental procedures and, furthermore, in the presence of ethanol, hepatic vein oxygen tension was not significantly different in animals chronically fed alcohol compared to fasting controls. Thus, the observation of a lower hepatic vein pO_2 among alcohol-fed animals compared to controls following an overnight fast does not appear relevant to the development of central sclerosis in these animals.

In this study 1.75 g/kg of ethanol was given intravenously over a period of 1 hr and resulted in mean peak blood alcohol of 275 mg/dl. Such levels are not uncommon among alcoholics and, in fact, a blood level of 300 mg/dl is a minor criterion for alcoholism used by the National Council on Alcoholism (20). The range of blood alcohol measured in the baboons during their periods of normal dietary intake was from 90 to 376 mg/dl. Thus, the levels of blood alcohol we achieved were within the range found in human alcoholics and in these animals when fed alcohol chronically.

The failure to demonstrate hepatic vein hypoxemia in the alcohol-fed animals in this study does not exclude the possibility of previous episodes of hepatic ischemia. However, none of the alcohol-fed animals had laboratory or clinical evidence of abnormalities such as pulmonary disease or gastrointestinal bleeding which promote hepatic vein hypoxia. Furthermore, in other studies it was observed that the lesions of central sclerosis in these animals were progressive over several years and that some inflammation was present throughout the course (21). Thus, central sclerosis in these animals appears to result from a continuous active process rather than from a single isolated episode of centrolobular hypoxia.

The increase in EHBF after ethanol administration was observed in all animals after relatively high blood ethanol levels had been achieved. Furthermore, significant increases in EHBF have also been noted in humans following blood levels which are found after moderate drinking (14). The results of this study were obtained in anesthetized animals. However, similar effects of alcohol on hepatic blood flow have been observed in human volunteers in the absence of anesthesia (14). The increase in EHBF was most marked in control animals and less so in animals with alcoholic liver disease. In patients with cirrhosis EHBF is decreased (22), and thus in advanced alcoholic liver injury hepatic oxygenation may be impaired by reduced blood flow. However, in our animals the lesion of central sclerosis was already present in animals without cirrhosis or marked lobular fibrosis.

After excessive ethanol consumption plasma lactate may increase (23). Furthermore, chronic alcohol consumption may be associated with increased ketogenesis (24) and even nondiabetic ketoacidosis (25). The decrease in arterial and hepatic vein pH following ethanol administration may be related to one or a combination of these effects. The fall in pH may have, in part, contributed to the rise in hepatic vein pO_2 after ethanol by causing a shift in the oxyhemoglobin dissociation curve.

Intrahepatic arteriovenous shunting could produce an increase in the oxygenation of hepatic venous blood. However, in the normal liver, such shunting occurs at the sinusoidal level (26). In addition, in the alcohol-fed animals lobular architecture was not distorted and in these animals hepatic vein oxygen tension was lower in the fasting state than in controls. Thus, significant shunting in these animals is unlikely.

In summary: (i) Chronic ethanol administration in the baboon produced central vein sclerosis and inflammation; (ii) hepatic vein catheterization of these animals revealed an absence of hepatic vein ischemia in the fasting state; (iii) following the administration of ethanol intravenously, hepatic vein oxygenation increased in all animals regardless of prior exposure to ethanol or the presence of moderate alcoholic liver injury on previous biopsy (including pericentral sclerosis).

Summary. The effects of ethanol on hepatic oxygenation were measured by hepatic vein catheterization in the baboon. Chronic alcohol administration resulted in slightly lower oxygen tension in the hepatic vein, but not ischemia, when measured after an overnight fast when alcohol was no longer present in the blood. Intravenous alcohol administration resulted in an increase in hepatic vein oxygen tension regardless of prior exposure to alcohol or the presence of liver disease. The increased oxygen tension after alcohol was associated with a marked increase in hepatic blood flow. These data in conjunction with the observation of progressive liver injury in the alcohol-fed animals support the concept that alcoholic liver injury can develop despite unimpaired hepatic oxygenation.

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