Impaired Oxygen Utilization

A New Mechanism for the Hepatotoxicity of Ethanol in Sub-human Primates

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Abstract

The role of oxygenation in the pathogenesis of alcoholic liver injury was investigated in six baboons fed alcohol chronically and in six pair-fed controls. All animals fed alcohol developed fatty liver with, in addition, fibrosis in three. No evidence for hypoxia was found, both in the basal state and after ethanol at moderate (30 mM) or high (55 mM) levels, as shown by unchanged or even increased hepatic venous partial pressure of O_2 and O_2 saturation of hemoglobin in the tissue. In controls, ethanol administration resulted in enhanced O_2 consumption (offset by a commitant increase in splanchnic blood flow), whereas in alcohol fed animals, there was no increase. At the moderate ethanol dose, the flow-independent O_2 extraction, measured by reflectance spectroscopy on the liver surface, tended to increase in control animals only, whereas a significant decrease was observed after the high ethanol dose in the alcohol-treated baboons. This was associated with a marked shift in the mitochondrial redox level in the alcohol-fed (but not in control) baboons, with striking rises in splanchnic output of glutamic dehydrogenase and acetaldehyde, reflecting mitochondrial injury. Increased acetaldehyde, in turn, may aggravate the mitochondrial damage and exacerbate defective O_2 utilization. Thus impaired O_2 consumption rather than lack of O_2 supply characterizes liver injury produced by high ethanol levels in baboons fed alcohol chronically.

Introduction

Two to three decades ago, the concept prevailed that liver disease in the alcoholic was due exclusively to associated dietary deficiencies. Subsequently, it was clearly established that both for the early fatty liver stage in man (1) and cirrhosis in the baboon (2) alcohol[1] itself can be incriminated, independent of dietary inadequacies. The mechanism of the hepatotoxic effects of alcohol, however, is still the subject of debate. Many of the hepatic and metabolic effects of alcohol have been linked to the metabolism of ethanol in the liver (3). Oxidation of ethanol via the alcohol dehydrogenase (ADH)[2] pathway is associated with a striking redox change to which several alterations of hepatic lipid, protein, and carbohydrate metabolism have been attributed (3). After chronic ethanol consumption, however, an attenuation of these redox changes was observed in the total liver (4, 5). By contrast, the ethanol induced redox changes were found to be exacerbated in the perivenular zone of the liver (6). The question therefore remained to what extent the perivenular redox changes may be associated with liver injury, particularly after chronic ethanol consumption. This question is of special relevance with regards to the mitochondria, in view of the ultrastructural evidence of mitochondrial damage after chronic ethanol consumption (7–9) and the preponderance of these changes in the perivenular zone (8, 9). Furthermore, the perivenular zone of the liver is the area with physiologically the lowest oxygen tension. On the one hand, this relatively low oxygen tension has been shown to potentiate the ethanol induced redox change (6, 10). On the other hand, it had also been postulated that ethanol, by increasing oxygen consumption, might exacerbate the relative hypoxia prevailing in the perivenular zone and might thereby precipitate cellular injury (11). Evidence for decreased oxygenation, however, was provided primarily in animals or patients withdrawn from alcohol (12). Under those conditions, a hyperadrenergic state (13) may contribute to the "hypermetabolic" state and increased oxygen utilization. Whether the latter occurs only in the withdrawal state or whether it also happens in the presence of alcohol was not clearly established, particularly since the ethanol-induced increase in oxygen consumption might be offset by an associated increase in splanchnic blood flow (6, 14–20). The present investigation was undertaken to address these various questions, particularly the issue whether decreased oxygen availability plays a significant role in alcohol-induced liver injury.

Methods

Animals. 12 Papio hamadryas baboons were used in this study. They had been imported from East Africa or were born in the United States. These baboons were pair-fed a nutritionally adequate liquid diet containing 50% of total energy either as ethanol or as additional carbohydrate for 6–18 mo (6 animals) and 3–7 yr (6 animals). The feeding techniques and the dietary constituents (purchased from Dyets, Bethlehem, PA) have been reported previously in detail (21). All animals entered the study only after repeated hematological and stool examinations indicated the absence of preexisting disease. The day before the experiment, animals were given diets (10 ml/kg body wt) at noon. At 4:00 p.m. all diets were removed. The animals received water overnight and the experiment was performed the next day at 9:00 a.m.

Experimental procedures. Animals were anesthesized with ketamine hydrochloride (10 mg/kg body wt) for the introduction of one

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1. The terms alcohol and ethanol (ethyl alcohol) are used interchangeably in this paper.

2. *Abbreviations used in this paper:* ADH, alcohol dehydrogenase; ALT, alanine amino-transferase; AST, aspartate aminotransferase; GDH, glutamic dehydrogenase; IHb, index of hemoglobin concentration; ISO_2, index of oxygen saturation of hemoglobin in tissue; $\dot{V}O_2$, flow-independent tissue oxygen consumption.

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arterial and two peripheral venous cannulas and a catheter into the hepatic vein. The polyurethane catheter (cobra type I, size 7F; USCI Cardiology and Radiology Products, Billerica, MA) was introduced percutaneously into the femoral vein and advanced into the hepatic vein under fluoroscopy. A level of 2–3 μg of indocyanine green (Cardio-Green; Hanson, Westcott & Dunning, Inc., Baltimore, MD) per ml of plasma was established by intravenous injection of the dye (0.15 mg/kg) dissolved in autologous serum and maintained by constant infusion (0.015 mg/kg per min). Two pediatric peritoneoscopes (Olympus Co., Tokyo, Japan) were introduced in the abdominal cavity (using the standard procedure) for spectrophotometric measurements (through optic fibers) and for insertion of a hydrogen electrode under the liver capsule. Reflectance spectroscopy was not carried out in all animals because in some, the liver surface could not be visualized, due to adhesions secondary to prior surgery, carried out to obtain surgical liver biopsies (9). After the start of the alcohol infusion, ketamine was not required any more.

The stabilization period (~ 90 min during which the above listed procedures were carried out) was followed by a 1-h basal period during which hepatic venous and arterial blood samples were taken simultaneously for measurements of flow and related parameters. After this basal period, ethanol was administered intravenously for 30 min at a loading dose calculated to create a blood ethanol concentration of about 30 mM (moderate level), which was maintained constant for 1 h by continuous infusion of ethanol at a rate equal to the rate of ethanol oxidation previously determined in such animals (22). The blood level was then raised to ~ 55 mM (high dose) with a second loading dose, and maintained by continuous infusion.

On a separate day, percutaneous liver biopsy was obtained under ketamine anesthesia after a 6-h food withdrawal. Specimens were prepared for light microscopy and examined as described before (2, 23).

Analytical methods. Blood ethanol concentrations were measured as reported previously (24) utilizing a headspace gas chromatograph (model F-45; Perkin-Elmer Corp., Norwalk, CT) equipped with an automatic sampling device. Acetaldehyde was also measured by headspace chromatography as previously described from this laboratory (25, 26).

The index of hemoglobin concentration (IHb) and oxygen saturation of hemoglobin (ISO_2) and flow-independent tissue oxygen consumption ($\dot{V}O_2$) were estimated by reflectance spectrophotometry (TS-200; Sumitomo Electronic Co. Ltd., Osaka, Japan) during peritoneoscopy in seven baboons as described before (27–29). The regional blood flow was measured by hydrogen gas clearance methods in four animals (28, 29). The methods used here were similar to those applied to human studies before (28, 30). Hepatic blood flow was estimated by the Fick principle (31) in all animals. Aortic and hepatic venous samples for the estimation of hepatic blood flow were taken every 15 min during intravenous infusion of indocyanine green, and the flow was calculated from the extraction of the dye. Plasma volume was determined with Evans Blue dye (Harvey Labs, Inc. Philadelphia, PA) by the dilution method (32). Splanchnic oxygen consumption was calculated from the arteriovenous difference in oxygen content and the hepatic blood flow. Flow and oxygen consumption were expressed per gram of liver using the liver/body weight ratios of 0.025 and 0.030 determined previously for control and alcohol-fed baboons, respectively (33). The oxygen content as well as pH, P_{O_2}, hemoglobin and its saturation with oxygen were measured with a pH/blood gas analyzer and oximeter (Corning Medical, Corning Glass Works, Medfield, MA).

To assess liver cytoplasmic and mitochondrial redox states, acid-extracts of blood (0.6 N $HClO_4$, final concentration) were obtained; after neutralization with 5 M K_2CO_3, lactate and pyruvate were enzymatically measured according to Hohorst (34) and Bücher et al. (35), respectively, β-hydroxybutyrate by the method of Williamson and Mellamby (36) and acetoacetate according to Mellamby and Williamson (37). Activities of alanine-aminotransferase (ALT, EC 2.6.1.2.) and aspartate-aminotransferase (AST, EC 2.6.1.1.) were quantified in serum by the colorimetric assay of Reitman and Frankel, as described by Bergmeyer and Bernt (38, 39). Glutamic dehydrogenase (GDH, EC 1.4.1.3.) was measured by the method of Ellis and Goldberg (40) at 37°C.

Statistics. All values are expressed as means±SEM. The significance of the difference was assessed by Student's *t* test applied to paired comparisons and by two-way analysis of variance (ANOVA) for the simultaneous analysis of the acute and chronic effects of ethanol.

Results

Histology. Whereas all 6 control baboons had normal appearing livers (Fig. 1 *a*), the three animals fed alcohol chronically for 6–18 mo had developed steatosis (Fig. 1 *b*). The three other animals fed alcohol for 3–7 yr developed septal fibrosis in addition to the steatosis (Fig. 1 *c*). Except when indicated, no trend for a difference in hemodynamic or biochemical parameters was noted in the alcohol-fed baboons between the animals with fibrosis and those with fatty liver only; therefore, these animals have been treated as a single group.

Blood ethanol and indocyanine green uptake. In the basal (withdrawal) state, blood levels of alcohol were undetectable; after alcohol infusion, the values achieved the expected level of about 30 mM after the moderate and 55 mM after the higher dose (Table I). Splanchnic indocyanine green uptake was not affected by acute ethanol administration, either in alcohol-fed or in control animals, but there was a trend toward lower values in the former as compared to the latter, particularly in the animals with septal fibrosis (Table I).

Splanchnic oxygen consumption, blood flow, and hepatic venous P_{O_2}. Before alcohol administration, arterial and hepatic venous P_{O_2} were not significantly different in the control and alcohol fed baboons whereas splanchnic oxygen consumption was higher in the latter; there was also a trend (statistically not significant), for the blood flow to be higher in the alcohol-fed animals (Table II). In control animals, acute ethanol administration resulted in an increase in splanchnic oxygen consumption. This was offset, however, by an even greater increase in splanchnic blood flow (Table II). As a consequence, there was no drop, but rather a trend towards increased hepatic venous P_{O_2} in the control baboons given alcohol (Table II). In the animals fed alcohol chronically, the acute effects of ethanol were different. A decrease rather than an increase in splanchnic oxygen consumption was observed after moderate ethanol administration (Table II). A similar effect was observed after the high dose, at least in the animals with fatty liver. As for the controls, after acute alcohol, there was again a trend towards increased splanchnic blood flow. As a net result, there was no decrease, but rather a trend towards increased hepatic venous P_{O_2} (Table II). In four baboons, regional hepatic blood flow was determined and was found to change in parallel with the splanchnic blood flow measured at the same time, with an increase after ethanol in control baboons, but a variable response in the alcohol-fed animals (Fig. 2).

Tissue oxygenation and flow-independent tissue oxygen consumption. After an acute dose of ethanol, the regional changes measured on the surface of the liver generally paralleled the findings reported above in the splanchnic circulation. In control animals, the index of hepatic tissue hemoglobin concentration (IHb) and the capacity of the liver to remove oxygen from the blood after stoppage of the flow ($\dot{V}O_2$) appeared to be increased at the moderate ethanol blood level (Table III), although the results (obtained in three animals) did

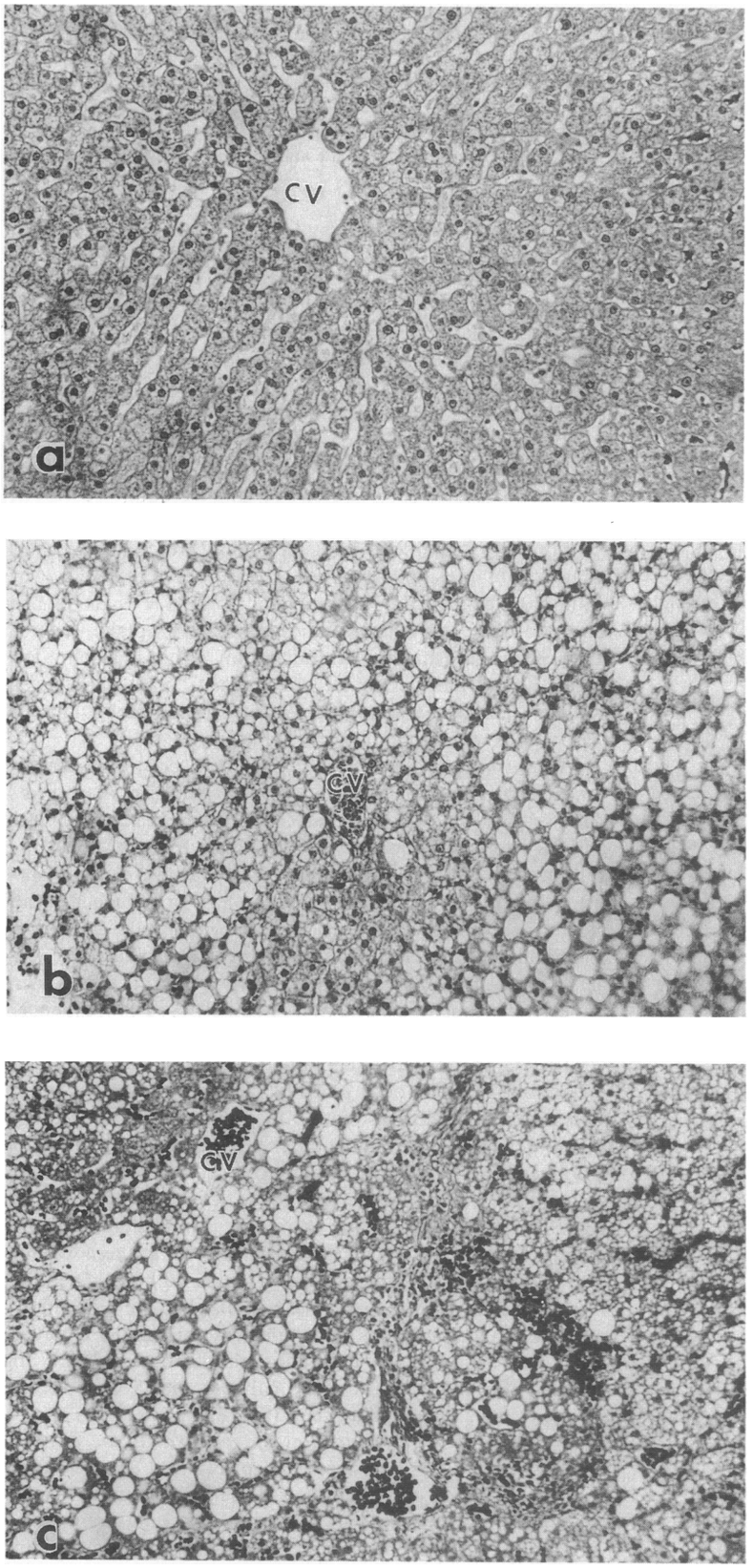

Figure 1. Livers of a control baboon, showing normal histology (*a*) (H & E, ×150), of an animal fed ethanol for 1 yr, with obvious fat accumulation (*b*) (H & E, ×150), and a baboon fed alcohol for 3 yr, with septal fibrosis (*c*) (chromotrope aniline blue stain, ×150) (C.V., central vein).

Table I. Blood Ethanol Levels and Removal Rates of Indocyanine Green

	Basal	Moderate ethanol	High ethanol
Blood ethanol level			
Control baboons (n = 6)	—	28.3±1.3	51.9±1.2
Alcohol-fed baboons (n = 6)	—	31.8±4.5	56.2±4.3
Fatty liver (n = 3)	—	27.7±4.5	56.1±7.2
Septal fibrosis (n = 3)	—	35.9±7.9	56.3±6.5
Removal rate of indocyanine green			
Control baboons (n = 6)	0.278±0.032	0.277±0.031	0.278±0.032
Alcohol-fed baboons (n = 6)	0.209±0.015	0.213±0.013	0.205±0.015
Fatty liver (n = 3)	0.232±0.023	0.232±0.023	0.229±0.024
Septal fibrosis (n = 3)	0.187±0.008	0.193±0.002	0.181±0.006

Blood ethanol levels and removal rates of indocyanine green are expressed as mM and mg/min, respectively.
Values represent means±SEM.

not reach statistical significance. An opposite trend was observed in the four alcohol-fed animals, with a decrease of IHb after the high ethanol dose in the alcohol-fed baboons, and a significant reduction of oxygen removal (Table III). The decrease in oxygen removal was particularly prominent in the animals with septal fibrosis. Oxygen saturation of hemoglobin in the tissue (ISO_2) (Table III) paralleled the results in hepatic venous P_{O_2} (Table II), with trends toward increases (Table III).

Table II. Acute and Chronic Effects of Ethanol on Splanchnic Blood Flow, Oxygen Consumption and Oxygen Tension (PO_2) in Arterial and Hepatic Venous Blood in Baboons

	Basal	Moderate ethanol	High ethanol
Splanchnic blood flow			
Control baboons (n = 6)	0.88±0.14	1.32±0.16*	1.49±0.19*
Alcohol-fed baboons (n = 6)	1.14±0.09	1.29±0.15	1.39±0.10
Fatty liver (n = 3)	1.12±0.11	1.13±0.20	1.23±0.09
Septal fibrosis (n = 3)	1.17±0.17	1.44±0.21	1.54±0.14
Splanchnic oxygen consumption			
Control baboons (n = 6)	2.10±0.15	2.80±0.26*	2.66±0.31
Alcohol-fed baboons (n = 6)	2.67±0.14‡	2.17±0.22*	2.25±0.29
Fatty liver (n = 3)	2.54±0.23	1.93±0.39	1.67±0.17
Septal fibrosis (n = 3)	2.79±0.19	2.41±0.15	2.83±0.26
Arterial PO_2			
Control baboons (n = 6)	84.5±6.7	83.2±8.1	74.3±7.2
Alcohol-fed baboons (n = 6)	79.5±6.4	84.6±7.2	87.5±7.4
Fatty liver (n = 3)	69.6±9.0	79.3±9.5	83.6±13.6
Septal fibrosis (n = 3)	89.3±5.2	90.0±11.9	91.7±8.4
Hepatic venous PO_2			
Control baboons (n = 6)	36.6±3.6	45.7±4.3	43.4±2.5
Alcohol-fed baboons (n = 6)	36.7±3.2	45.0±3.9	43.8±4.3
Fatty liver (n = 3)	35.4±5.9	45.0±5.9	44.3±8.8
Septal fibrosis (n = 3)	38.0±4.0	45.0±3.9	43.3±4.0

Splanchnic blood flow, oxygen consumption, and PO_2 are expressed as ml/min per g liver, μmol O_2/min/g liver and mmHg, respectively.
Values represent means±SEM.
* $P < 0.05$ vs. basal.
‡ $P < 0.05$ vs. control values (basal).

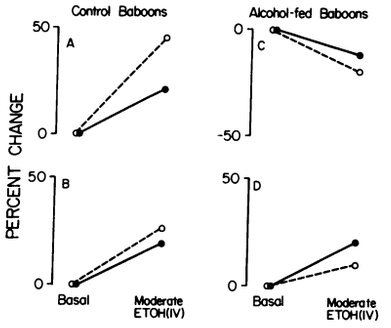

Figure 2. Effect of ethanol on estimated splanchnic blood flow (●) and regional hepatic blood flow (○) in control (*A, B*) and alcohol-fed (*C, D*) baboons. In representative pairs of control and alcohol-fed baboons, effects of ethanol on flow were documented by both the Fick principle (indocyanine green technique) as well as by estimated regional hepatic blood flow (hydrogen technique). When compared to base line level, parallel changes were observed with both procedures, with increases in blood flow after the moderate ethanol dose in both control baboons, whereas in the alcohol-fed animals, administration of the moderate dose of ethanol resulted in increased flow in one and a decrease in the other.

Redox changes. Ethanol administration produced the expected cytosolic redox change as assessed by the hepatic venous lactate/pyruvate ratio (Fig. 3). The corresponding changes in arterial blood were similar but somewhat less striking (in the controls; from 29±4 in the basal state to 47±8 and 73±13 after moderate and high ethanol dose, respectively; the corresponding values in alcohol-fed baboons were 53±14, 58±16, and 101±38). Parameters of the mitochondrial redox

Table III. Acute and Chronic Effects of Ethanol on the Index of Hepatic Tissue Hemoglobin Concentration and Oxygen Saturation of Hemoglobin and Flow-independent Tissue Oxygen Consumption

	Basal	Moderate ethanol	High ethanol
Index of hepatic tissue hemoglobin concentration (IHb)			
Control baboons (n = 3)	127±23	155±31	146±31
Alcohol-fed baboons (n = 4)	134±7	111±6	101±5*
Fatty liver (n = 2)	124±1	110±1	102±10
Septal fibrosis (n = 2)	145±6	112±15	100±6
Flow-independent tissue oxygen consumption ($\dot{V}O_2$)			
Control baboons (n = 3)	1.84±0.08	2.53±0.32	1.20±0.32‡
Alcohol-fed baboons (n = 4)	2.38±0.38	2.08±0.48	1.33±0.16*
Fatty liver (n = 2)	2.78±0.72	2.71±0.72	1.57±0.16
Septal fibrosis (n = 2)	1.98±0.09	1.45±0.24	1.10±0.15
Index of oxygen saturation of hemoglobin (ISO_2)			
Control baboons (n = 3)	31.8±2.8	35.9±1.9	36.5±2.0
Alcohol-fed baboons (n = 4)	35.8±3.7	39.4±5.1	43.2±6.3
Fatty liver (n = 2)	41.3±0.1	46.6±3.6	51.3±6.5
Septal fibrosis (n = 2)	30.3±4.9	32.3±6.5	35.2±8.1

IHb, $\dot{V}O_2$, and ISO_2 are expressed as μmol/g liver, μmol O_2/min per g liver and %, respectively.
Values represent means±SEM.
Statistical significance: * $P < 0.05$ vs. basal.
‡ This apparently low value was due to an unexplained unusually small number in one animal; in the others, the values were the same as for the basal period.

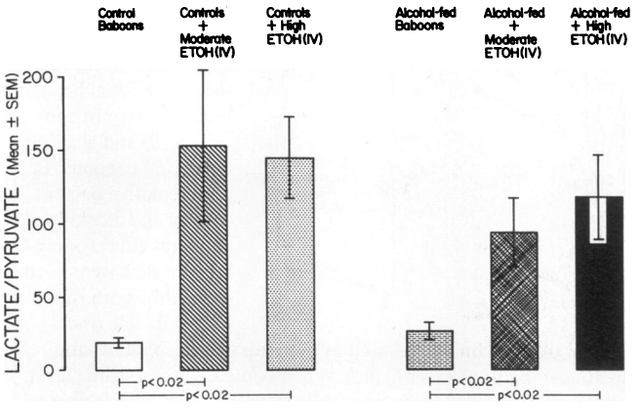

Figure 3. Acute and chronic effects of ethanol on hepatic venous lactate/pyruvate ratio. Ethanol administration was associated with a significant change in the redox state, reflected by the lactate/pyruvate ratio.

state (β-hydroxybutyrate/acetoacetate ratio), which were hardly affected in the control animals, were strikingly and significantly changed in the hepatic venous blood of alcohol-fed baboons (Fig. 4), but not in the arterial blood. The values in arterial blood were, in the control baboons, 2.5±0.5 in the basal state and 1.8±0.6 and 1.8±0.1 after the moderate and high ethanol dose, respectively. The corresponding values in alcohol-fed baboons were 3.6±0.8, 3.2±0.7, and 4.2±1.2. This lack of change after ethanol in the arterial blood contrasts with the striking alterations in hepatic venous blood of the alcohol-fed baboons (Fig. 4).

Release of hepatic enzymes. Both after the moderate and the high dose of ethanol, in controls as well as in the alcohol-fed animals, levels of ALT, AST, and GDH significantly increased in arterial blood (Table IV). Comparable increases in ALT and AST after ethanol were observed in hepatic venous blood (Table IV). There were similar or even greater increases in hepatic venous GDH levels, particularly in alcohol-fed animals (Table IV). Accordingly, this was associated with a striking increase in splanchnic GDH output; the latter acute effect of ethanol (calculated from the data in Tables I and IV) was significantly higher in the animals fed alcohol chronically than in the corresponding controls, both after the moderate ($P < 0.05$) as well as the high ($P < 0.025$) dose of alcohol.

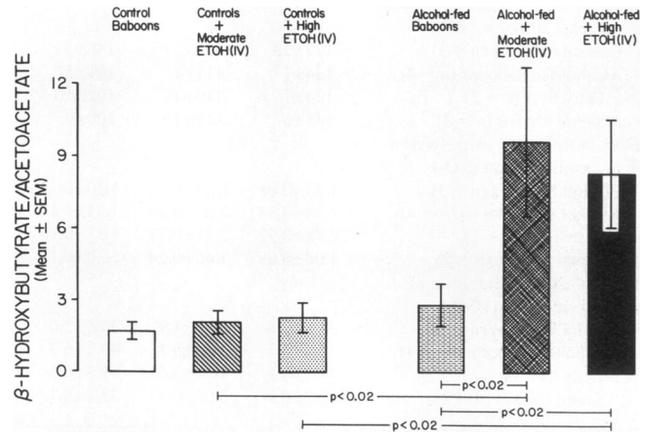

Figure 4. Acute and chronic effects of ethanol on the hepatic venous β-hydroxybutyrate/acetoacetate ratio. Whereas acute alcohol administration in control animals had a negligible effect on this marker of the mitochondrial redox state, a striking alteration was observed in animals fed alcohol chronically.

Acetaldehyde output. The splanchnic output of acetaldehyde was much greater in alcohol-fed baboons than in the controls, particularly at the high dose of ethanol (Fig. 5). The increase was significant for red blood cell acetaldehyde both at moderate and at high ($P < 0.05$) blood ethanol levels and for plasma acetaldehyde at the high blood ethanol dose ($P < 0.05$).

Discussion

The present study revealed that administration of ethanol, particularly at high levels and in animals fed alcohol chronically, is associated with an inappropriately low oxygen utilization by the liver. This was accompanied by increased acetaldehyde release and evidence of mitochondrial injury, probably causally related. There was no indication for a role of decreased oxygen supply in the pathogenesis of acute or chronic alcohol induced liver damage in this baboon model in which both alcohol-induced steatosis (Fig. 1 *b*) and fibrosis (Fig. 1 *c*) were reproduced.

One of the mechanisms invoked to explain alcohol induced liver disease is a decreased oxygen supply sufficient to produce tissular hypoxia (particularly in perivenular zones), the lack of oxygen being attributed to the alcohol-induced increase in oxygen consumption (11). The latter has been postulated to increase the gradient of oxygen tension along the sinusoids to the extent of producing anoxic injury of perivenular hepatocytes (41). Both in alcoholics (42) and in animals fed alcohol chronically (6, 43), decreases in either hepatic venous oxygen saturation (42) or Po_2 (6) and in tissue oxygen tensions (43) have been found during the withdrawal state. However, this decrease was within the range of values found in normal subjects and hepatic venous oxygen content, determined as either oxygen saturation or oxygen tension, was found to be normal in patients with chronic alcoholic liver disease (19). Moreover, the differences in hepatic oxygenation found during the withdrawal state disappeared (6, 16) or decreased (43) when alcohol was present in the blood. Similar results were obtained in the present study. In the basal (withdrawal) state, splanchnic oxygen consumption of the animals fed alcohol chronically was increased compared to controls, but splanchnic blood flow (and thereby the supply of oxygen) was also elevated; consequently there was no significant alteration in hepatic venous Po_2 (Table II). Acute ethanol administration increased splanchnic oxygen consumption in control baboons, but this effect again was offset by increased blood flow resulting in unchanged hepatic venous oxygen Po_2 (Table II). In the alcohol-fed baboons, there was a decreased splanchnic oxygen consumption at the moderate ethanol dose (and unchanged splanchnic flow) with a similar trend at the high ethanol dose, and a tendency for hepatic venous Po_2 to increase in either case (Table II).

There has been controversy concerning the action of ethanol on splanchnic blood flow with some showing no effect (44–46) or even a decrease (47), whereas most studies reported an increase (6, 14–20). In general, unchanged or decreased flow was observed with low blood ethanol levels. In the present study, the increase in splanchnic blood flow was shown in

Table IV. Acute and Chronic Effects of Ethanol on Arterial (A) and Hepatic Venous (HV) Blood Enzyme Activities

	Basal		Moderate ethanol		High ethanol	
	A	HV	A	HV	A	HV
ALT						
Control baboons (n = 6)	19±3	18±1	30±4	36±7‡	39±6‡	38±4§
Alcohol-fed baboons (n = 6)	23±6	27±7	39±8	38±6‡	52±14*	48±9§
AST						
Control baboons (n = 6)	25±3	22±4	38±2§	39±6*	45±6§	45±3‡
Alcohol-fed baboons (n = 6)	35±4	37±5	60±10‡	48±9	52±9	51±8
GDH						
Control baboons (n = 6)	18.2±5.6	25.9±5.6	29.4±6.3	36.4±7.7	30.8±4.9	43.4±6.3
Alcohol-fed baboons (n = 6)	26.6±4.2	51.8±16.1	46.2±7.0‡	98.7±23.8	53.9±8.4‡,‖	97.3±18.9‡,‖

ALT, AST and GDH activities are expressed as International Units per liter. Values represent means±SEM. * $P < 0.05$ vs. basal. ‡ $P < 0.025$ vs. basal. § $P < 0.01$ vs. basal. ‖ $P < 0.025$ vs. control values (high ethanol).

control animals with both moderate (30 mM) and high (55 mM) ethanol concentrations (Table II) and it was found not only when flow was calculated with the Fick principle using indocyanine green, but also, in some of the experiments, by the determination of hydrogen clearance (Fig. 2). In the animals fed alcohol chronically, at the moderate ethanol dose, the results were inconsistant (Fig. 2) and, on the average, there was no significant change (Table II). IHb measurements showed a decrease after the high ethanol dose in the alcohol-fed baboons, but not in the controls (Table III). The decrease in IHb after ethanol administration to alcohol-fed baboons (when high blood concentrations were reached) suggests a possible diversion of blood through intrahepatic shunts (see below).

In control animals, because of the increase in flow, the enhanced oxygen consumption after acute ethanol administration had no apparent effect either on the hepatic venous P_{O_2} (Table II) or on the oxygenation of sinusoidal hemoglobin on the surface of the liver (ISO_2) (Table III). In the alcohol-fed animals, there was also a parallelism in oxygenation of hepatic venous and sinusoidal blood with a lack of effect of ethanol on hepatic venous P_{O_2} and ISO_2 in the liver tissue (Tables II and III). There was a trend towards decreased IHb, a measurement of regional blood volume, which has been found before to correlate with regional blood flow (28). However, IHb reflects changes close to the surface of the liver, whereas the hydrogen gas methods measures regional flow via electrodes inserted inside of the liver and the Fick principle assesses total splanchnic flow. Thus, theoretically, differences in results obtained with these methods might be possible. In any event, the IHb changes appeared to be offset by an opposite trend for the alterations in ISO_2 which tended to be increased (Table III). It is conceivable that functional intralobular shunting may alter hepatic microcirculation and mask changes in O_2 status as measured by either hepatic venous P_{O_2} or tissue ISO_2. However, the effects of ethanol on oxygen utilization were dissociated from possible local flow changes by measuring the capacity of the liver cells to take up oxygen from the blood in situ after stoppage of regional blood flow, through compression of the liver surface ($\dot{V}O_2$). Under these conditions, in alcohol-fed animals, there was a decreased oxygen consumption after ethanol; a similar trend was observed in some control animals given the high dose (Table III) but, because of the small number of animals, it was not possible to determine whether the decrease in $\dot{V}O_2$ was greater in the animals fed alcohol chronically than in the controls. Decreased $\dot{V}O_2$ was not associated with any decrease in ISO_2 or in hepatic venous P_{O_2}, indicating impaired capacity of the hepatocytes to take up O_2, even in the presence of an ample O_2 supply. Thus, measurements of hepatic venous P_{O_2}, tissue ISO_2 and flow independent $\dot{V}O_2$, all indicate that defective O_2 utilization rather than lack of blood O_2 supply characterizes ethanol-induced liver injury in this baboon model.

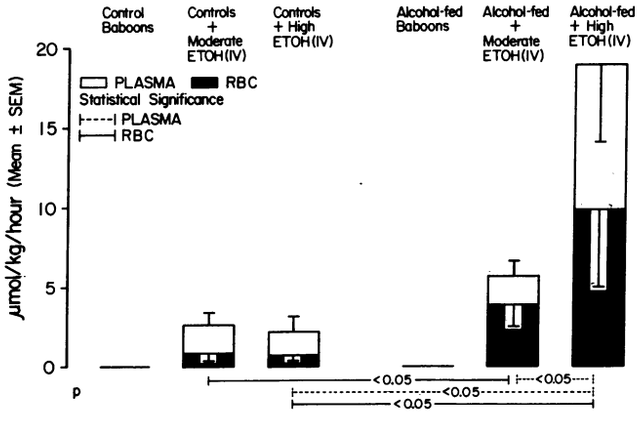

Figure 5. Acute and chronic effects of ethanol on splanchnic acetaldehyde output in the plasma and RBC. The increased release of both red blood cell and plasma acetaldehyde after an acute dose of ethanol was much greater in alcohol-fed animals than in controls, particularly at the high ethanol dose.

The impaired O_2 utilization after acute ethanol administration to the animals fed alcohol chronically was associated with a striking accumulation of reducing equivalents, especially in the mitochondria, as measured by the β-hydroxybutyrate/acetoacetate ratio (Fig. 4), which reflects the redox state in that organelle. Contrasting with the striking changes in hepatic venous blood (Fig. 4), the arterial ratio was unaffected by ethanol, suggesting that the main organ responsible for the ketone changes is the liver rather than peripheral tissues such as muscles. Of course, hepatic venous ketone bodies may also reflect

other factors such as insulin availability and precursor supply, not assessed in this study. In any event, in the presence of the extraordinary load of reducing equivalents produced by the oxidation of ethanol, the lack of increase, or even a decrease (Tables II and III) in oxygen consumption upon ethanol administration to alcohol-fed baboons is obviously inappropriate and denotes a decompensated state. By contrast, in control baboons, the mitochondrial redox level appeared little altered (Fig. 4) after ethanol administration which was associated predominantly with the expected change in the cytosolic redox level (lactate/pyruvate ratio) (Fig. 3), confirming our previous observations (5, 6).

The mechanism for the impaired oxygen utilization after acute ethanol administration in animals fed alcohol chronically has not been clarified; it is our hypothesis that this is linked, at least in part, to cellular injury, particularly at the mitochondrial level. Evidence for injury was provided by alteration in mitochondrial redox state (see above) and the release of enzymes from the liver, in particular the mitochondrial enzyme GDH (Table IV), the output of which was strikingly increased in the alcohol-fed animals. Release of this mitochondrial enzyme can be interpreted as evidence for mitochondrial injury, which is consistent with our previous studies that revealed striking biochemical and morphologic alterations of the mitochondria in similarly treated baboons (9). The mechanism for the mitochondrial injury has not been fully elucidated and different processes may be involved in the various toxic manifestations recorded in the present study; one of the factors is probably acetaldehyde, the toxicity of which has been reviewed elsewhere (3, 48). Electronmicroscopic studies of liver mitochondria in alcoholics have revealed striking morphologic alterations, including swelling and abnormal cristae. Controlled studies in animals and man (8, 9, 49, 50) have shown that these changes are caused by alcohol itself, rather than other factors, such as poor diets. In rats and baboons, these structural abnormalities were associated with functional impairments, especially decreased oxidation of fatty acids and of a variety of other substrates, including acetaldehyde (9, 51), as well as a reduction in cytochrome aa_3 and b content, respiratory capacity and oxidative phosphorylation (9). It is noteworthy that at high concentrations, acetaldehyde mimics the defects of oxidative phosphorylation produced by chronic ethanol consumption (52). Thus, one may wonder to what extent chronic exposure to acetaldehyde is the cause for the impairment observed after chronic ethanol consumption. Alcoholics exhibit higher acetaldehyde levels than nonalcoholics for a given ethanol load and blood level (24, 53). Similarly, in the present study, splanchnic acetaldehyde output was much greater in the alcohol-fed baboons than in the controls (Fig. 5). The increased acetaldehyde output could be due, as least in part, to "induction" of ethanol metabolism in these baboons (22, 33). In addition, it has been shown before that decreased acetaldehyde utilization contributes to the increased blood level (54), the latter being secondary to the injury of the mitochondria and the associated decreased capacity to oxidize a variety of substrates, including acetaldehyde (51). A "vicious cycle" may thereby develop with chronic ethanol consumption inducing increased acetaldehyde formation, which produces mitochondrial toxicity, including an associated decreased capacity of the mitochondria to oxidize acetaldehyde; as a result, the level of acetaldehyde rises even further and perpetuates and aggravates the toxicity. In normal liver, acetaldehyde concentrations higher than those usually achieved after alcohol ingestion are required to produce toxicity. However, after chronic alcohol consumption, the liver mitochondria become unusually susceptible to the toxic effects of acetaldehyde, and important mitochondrial functions (such as fatty acid oxidation) are depressed, even in the presence of relatively moderate acetaldehyde concentrations (55).

Although the theory of alcohol-induced liver necrosis secondary to a "hypermetabolic state" is still controversial, it has led to therapeutic trials with agents that decrease tissue metabolism, such as propylthiouracil. Previous short-term treatment efforts have yielded conflicting results (56, 57), but a recent long-term study reported a lowered mortality in the treated group, at least in those patients in whom alcohol intake was moderate (58). The mechanism of the beneficial effect is not clear. Centrilobular liver cell necrosis could be produced by hypoxia in chronic ethanol-fed rats (41, 59) and experimentally, propylthiouracil has been shown to protect against this effect (41, 60) but such hypoxic conditions were not documented in the type of patients in whom the beneficial effects were obtained. Furthermore, the present study indicates that, in the presence of ethanol, there is no evidence that the primary lesion is hypoxia secondary to increased oxygen consumption. It is possible that propylthiouracil may be beneficial in some nonspecific way: by decreasing hepatic metabolism, it may reduce the need for oxygen at the cellular level and offset some consequences of liver injury, particularly those resulting from impaired oxygen utilization. Tissular hypoxia, however, cannot be considered as a primary mechanism and as a *sine qua non* of alcohol-induced liver injury since, experimentally, not only fatty liver but also fibrosis can be produced with ethanol in the baboon (2, 23) without decreased availability of blood oxygen, as shown in the present study. The problem appears to be that despite an ample oxygen supply, the liver of the baboons fed alcohol chronically, when confronted with a high level of ethanol, is unable to consume oxygen appropriately. This newly recognized defect has obvious implications in terms of the impairment of the functioning of the hepatocyte and the pathogenesis of the ultimate necrosis and associated fibrosis.

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