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The interaction between chronic ethanol consumption and oxygen tension in influencing the energy state of rat liver

Priscilla I. Spach, James S. Herbert and Carol C. Cunningham

Department of Biochemistry, Wake Forest University Medical Center, Winston-Salem, NC (U.S.A)

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Hepatocytes were isolated from chow-fed and liquid-diet control rats, and animals fed ethanol chronically for 31 days. These preparations were analyzed for adenine nucleotide and inorganic phosphate concentrations after being maintained under various conditions of oxygenation and nutrient availability. Hepatocytes from ethanol-fed animals resuspended at high cell density (oxygen tensions near zero) demonstrated a greater depression in cellular energy state as indicated by decreases in phosphorylation potential and energy charge. If, however, these hepatocytes were restored to high oxygen tension their energy state was equivalent to that observed with preparations from liquid-diet control animals. Moreover, their rate of oxygen consumption was equivalent to that of control hepatocytes. Analyses of livers from chow-fed, liquid diet control, and ethanol-fed rats which were freeze-clamped while being perfused by the animal's blood revealed that there were no significant differences in the energy states of the hepatic tissue from these three animal groups. These results indicate that (1) the hepatic energy state in rats fed ethanol chronically is maintained under conditions of normal oxygen tension and (2) that hepatic tissue from these animals experiences a much more dramatic depression in energy state than tissue from control rats when subjected to oxygen deprivation.

Introduction

Previous studies have indicated that chronic ethanol consumption decreases the hepatic energy state, as indicated by shifts in adenine nucleotide distribution and increases in inorganic phosphate concentrations [1–4]. These earlier observations suggest that the lowered capacity of hepatic mitochondria from ethanol-fed animals to catalyze the synthesis of ATP (see Refs. 5 and 6 for summaries) may result in an overall depression of the energy state of livers in individuals consuming ethanol chronically. A more recent investigation [7] did not confirm, however, that chronic ethanol consumption causes a decrease in the hepatic energy state. Moreover, the study of Miyamoto and French [8] indicated that the energy state of the liver was decreased more dramatically by a hypoxic episode in an ethanol-fed rat than in a control animal. This latter investigation emphasized the importance of determining the energy state of hepatic tissue from ethanol-fed animals under conditions where the tissue was adequately oxygenated before being sampled for metabolite analyses. Measurements utilizing these sampling conditions are necessary before any conclusions can be reached concerning the relationship between the decreased capacity of the mitochondrion to catalyze the synthesis of ATP and the hepatic energy state.

In the present study hepatocyte preparations, maintained under various incubation conditions, and liver, freeze-clamped in situ while being perfused with the animal's blood, were analyzed for adenine nucleotides and P_i. These determinations were used to evaluate the energy states of the hepatic tissue preparations. The oxygen content in the incubation mixtures and rates of oxygen uptake by the incubated hepatocytes were also measured. Evidence is presented which indicates that hepatic tissue from ethanol-fed animals can maintain an adequate energy state when oxygen tension is maintained at high levels. Moreover, data are provided which demonstrate that the energy state of hepatocytes from an ethanol-fed rat is much more sensitive to oxygen tension than is the case with cells from a control animal.

Abbreviations: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; DMEM, Dulbecco's modification of Eagle's medium.

Correspondence: C.C. Cunningham, Department of Biochemistry, Wake Forest University Medical Center, 300 South Hawthorne Road, Winston-Salem, NC 27103, U.S.A.

Experimental procedures

Materials

Male Sprague-Dawley rats, rat diet, HPLC supplies and most enzymes and reagents were obtained from sources cited previously [9]. Dow Corning silicone oils were purchased from William F. Nye, New Bedford, MA. The high sensitivity membranes for use with the oxygen electrode were purchased from Yellow Springs Instruments, Yellow Springs, OH.

Methods

Male Sprague-Dawley rats (150–250 g) were fed for 31 days on a nutritionally adequate liquid diet in which ethanol provided 36% of the total calories [10]. Pair-fed control rats received the same diet, but with maltose-dextrin isocalorically substituted for ethanol.

For preparation of hepatocytes, animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (0.1 g/100 g body weight). Hepatocytes were prepared according to the two-step procedure of Seglen [11] using Gey's balanced salts supplemented with 25 mM sodium bicarbonate. The pH of the perfusate was maintained at 7.4 throughout the procedure by continuous gassing with 95% O_2/5% CO_2. As a final step in the preparation, hepatocytes were resuspended in DMEM supplemented with 20 mM Hepes and 10 mM $NaHCO_3$ at a cell density of approx. $2 \cdot 10^7$ cells per ml of medium. Viability, measured by Trypan blue exclusion, was normally above 90%. In some experiments aliquots of freshly prepared hepatocyte suspensions were quick-frozen in liquid nitrogen for subsequent metabolite analyses. In other studies the freshly prepared hepatocytes were maintained at 0°C in the medium and at the cell density mentioned above for the intervals required to set up the incubation protocols. This time period never exceeded 40 min. The hepatocytes were in the presence of added glucose during their preparation and while being maintained in suspension. The Gey's balanced salts and the DMEM were 5.5 and 25 mM in glucose, respectively. Immediately before beginning the incubations aliquots of the hepatocytes were quick-frozen in liquid nitrogen for subsequent metabolite analyses.

Hepatocytes were incubated employing two separate protocols to evaluate the effects of nutrient availability and oxygen tension on the levels of energy metabolites. In the first procedure hepatocytes were incubated in 50 ml Erlenmeyer flasks warmed in a 37°C shaking water bath and were gassed continuously with 95%O_2/5% CO_2. The incubation mixture consisted of Krebs-Ringer bicarbonate solution, 2.0 mM glutamate, 2.5% BSA (< 25 μg free fatty acid per ml) and $1 \cdot 10^6$ cells/ml in a total volume of 4 ml. Addition of glutamate resulted in an elevation of the energy state of hepatocytes slightly above that observed with other carbon sources such as glucose, glutamine or alanine. It was established with hepatocytes from chow-fed, liquid diet control rats and ethanol-fed animals that the adenine nucleotide and P_i levels were at steady state after 20 min under these incubation conditions (data not shown). Therefore, after 20 min of incubation aliquots were quick-frozen and stored in liquid nitrogen until adenine nucleotide analyses were performed. Another aliquot was centrifuged through oil as described by Williamson and Corkey [12] to separate the hepatocytes from the medium. These cells were then extracted utilizing perchloric acid [12] and analyzed for inorganic phosphate by the method of Chalvardjian and Rudnicki [13].

In the second protocol, incubations were carried out as described by Andersson and Jones [14] in siliconized round bottom flasks attached to a rotary evaporator. Prior to initiation of each incubation, an aliquot of the hepatocyte suspension was quick-frozen for subsequent metabolite analysis. The flasks were rotated at 45 cycles/min and continuously aerated with breathing air. The incubation mixture contained $1 \cdot 10^6$ cells/ml and was supplemented with 2.0 mM glutamate, 2.5% BSA and 25 mM Hepes in a total volume of 10 ml. The incubation buffer was also 2.4 mM in bicarbonate. The adenine nucleotide and P_i levels reached steady state within a 20 min incubation period under the conditions employed. Therefore, samples for adenine nucleotide determinations were quick-frozen after a 20 min incubation period. The inorganic phosphate levels in incubated cells were determined by the method of Chalvardjian and Rudnicki following separation of the cells from the incubation medium as described above.

The oxygen content of the incubation mixtures and the oxygen uptake of the hepatocyte suspensions was measured for both sets of assay conditions described above. At the end of the 20 min incubation periods an aliquot of assay mixture was rapidly transferred to a water-jacketed cell maintained at 37°C. Oxygen concentration and uptake were measured polarographically using a Clark oxygen electrode. The cell suspensions maintained at 0°C and at high density were also monitored for oxygen content. For these latter measurements the cell was equilibrated at 0–1°C and high-sensitivity membranes were used with the oxygen electrode.

For measurements of metabolites in freeze-clamped livers the animals were anesthetized as described above. The peritoneal cavity was opened by a midline incision to expose the liver. The liver, still being perfused by the animals' blood supply, was freeze-clamped using Wollenberger tongs cooled in liquid nitrogen as described by Hess and Brand [15]. The freeze-clamped tissue was stored in liquid nitrogen until analyzed for metabolite concentrations.

Neutralized perchloric acid extracts of hepatocytes and freeze-clamped liver were analyzed for adenine nucleotide concentrations by reverse phase HPLC on a

C-18 column using a modification of the elution procedure described by Jones [16]. Two solutions were utilized: 0.1 M KH_2PO_4 (pH 6.0) (A); 0.1 M KH_2PO_4 (pH 6.0)/5% methanol (B). The flow rate was 1 ml per min. The nucleotides were separated by eluting first with A for 10 min. A concave gradient was then applied over 5 min which progressed from 100% A to 80% A and 20% B. A linear gradient progressing from 80% A and 20% B to 100% B was then applied over a 5 min period; separation was then completed by eluting with 100% B for 20 min. This elution procedure improved the separation of the adenine nucleotides from other metabolites and further resolved ATP from ADP. The wavelength of detection was 254 nm. The concentrations were determined by comparison of peak areas with those from known amounts of authentic standards.

Inorganic phosphate concentrations in freeze-clamped liver and in hepatocytes not subjected to the incubation procedures described above, as well as lactate and pyruvate concentrations, were measured by methods cited in Malloy et al. [17]. The metabolite concentrations are reported in the tables as nmol/10^6 cells. For calculating phosphorylation potential ATP, ADP and P_i concentrations were converted to their molar equivalents using a volume of 11000 μm^3 for the hepatocyte, as reported in Arias et al. [18]. Phosphorylation potential and energy charge were calculated as described previously [19]. We report phosphorylation potentials for cells at 0° and 37°C, but recognize that the values obtained for hepatocytes at two different temperatures may not be directly comparable if cell volumes are altered by temperature.

Results

Use of the hepatocyte in the following studies allowed us to evaluate the effects of oxygen tension on the energy state of hepatic tissue from ethanol-fed and control animals. This was done by varying the incubation conditions under which hepatocytes were maintained before they were sampled for subsequent metabolite analyses. The concentrations of adenine nucleotides and inorganic phosphate were measured in hepatocytes which were suspended at a cell density of $2 \cdot 10^7$/ml and then immediately quick-frozen. These cell suspensions were essentially anaerobic; no oxygen was detected using a Clark electrode with a high sensitivity membrane optimal for measurements at 0°C. The data, reported in Table I, demonstrate that the hepatocytes from ethanol-fed animals were in a lower energy state as compared with cells from control rats. This is illustrated by significantly lower concentrations of ATP and increased concentrations of AMP and P_i. These alterations result in dramatic ethanol-related decreases in the phosphorylation potential and energy charge.

The energy state of hepatocytes from ethanol-fed and control animals was measured with an added carbon source and at two different oxygen tensions. Glutamate (2 mM) and a small amount of free fatty acid (≤ 25 μg/ml) from the BSA added were available to hepatocytes incubated at a cell density of $1 \cdot 10^6$ cells per ml.

TABLE I

Energy state of hepatocytes unincubated and quick-frozen after preparation

Sample preparation and analyses are described in Experimental procedures.

Energy-related parameter	Source of hepatocytes		*P* value for difference [b]
	control	ethanol-fed	
Metabolite concentrations [a]			
ATP	11.5 ± 1.3	7.6 ± 1.0	0.01
ADP	5.0 ± 0.3	6.1 ± 0.4	n.s. [c]
AMP	1.3 ± 0.2	3.6 ± 0.7	0.01
Total adenine nucleotide	17.8 ± 1.6	17.3 ± 1.3	n.s.
P_i	49 ± 2	79 ± 4	0.0004
Phosphorylation potential	572 ± 58	212 ± 29	0.0001
Energy charge	0.77 ± 0.02	0.61 ± 0.04	0.0013

[a] nmol metabolite per 10^6 viable cells.
[b] $n = 9$ pairs of animals.
[c] n.s. = not significant.

TABLE II

Oxygen tension and utilization of hepatocytes incubated in air and 95% O_2/5% CO_2

Source of hepatocytes [a]	Air incubations [b]		95% O_2/5% CO_2 incubations [b]	
	oxygen concentration [c]	oxygen uptake [d]	oxygen concentration [c]	oxygen uptake [d]
Liquid diet controls	102 ± 3	0.056 ± 0.004	689 ± 6	0.098 ± 0.016
Ethanol-fed	101 ± 2 [e]	0.061 ± 0.003	691 ± 8	0.100 ± 0.006

[a] $n = 6$ pairs of animals.
[b] Incubation conditions, oxygen concentration and oxygen utilization measurements are described in Experimental procedures.
[c] The unit of oxygen concentration is torr (mmHg).
[d] The unit of oxygen utilization is μgatoms O/min per 10^6 cells.
[e] No significant differences were observed between hepatocyte preparations from control and ethanol-fed animals with respect to either oxygen concentration or oxygen utilization using the paired *t*-test.

The hepatocytes were incubated either in the presence of an air atmosphere or 95% O_2/5% CO_2. The oxygen concentrations in the media and the rates of oxygen utilization by cells at the two oxygen tension levels are reported in Table II. As expected, the oxygen content of the incubation medium was much higher with the suspensions maintained in the O_2/CO_2 atmosphere than with those in the presence of breathing air. Furthermore, there was an almost 2-fold increase in the rate of oxygen utilization by hepatocytes in the O_2/CO_2 atmosphere compared with those incubated in the presence of air. Notably, there were no significant differences between ethanol and control preparations with respect to either oxygen content in the incubation media or in their oxygen utilization rates.

The effects of incubating hepatocytes with a carbon source and in an air atmosphere on the cellular energy state are reported in Table III. The metabolite analyses reported in Table III demonstrate that the source of the hepatocytes and the conditions under which the cells are maintained influence the phosphorus metabolite concentrations. In this and the following study (Table IV) the unincubated hepatocytes were not quick-frozen immediately after preparation, but were maintained on ice at a cell density of $2 \cdot 10^7$/ml until the incubation procedures were initiated. At that point (30–40 min after preparation) aliquots were quick-frozen. These are designated in both Table III and Table IV as unincubated. This interval between preparation and quick-freezing resulted in even lower energy states in hepatocytes from ethanol-fed animals, as a comparison of metabolite distributions, phosphorylation potentials and energy charge values reveal (Table I values vs. those in Tables III and IV). These unincubated cells were essentially anaerobic, as determined with a Clark electrode.

When hepatocytes were incubated in an air atmosphere at 37°C and in the presence of an oxidizable carbon source there were significant shifts in metabolite distribution, resulting in increases in the energy states of the hepatocyte preparations from both the liquid diet control and ethanol-fed animals. The shifts were most apparent with hepatocytes from ethanol-fed rats where there were significant increases in ATP and ADP, and dramatic decreases in AMP and P_i. The alteration in the adenine nucleotide distribution in hepatocytes from

TABLE III

Effect of incubation in air on the energy state of hepatocytes

The incubation procedure and the metabolite analyses are described in Experimental procedures. Samples of unincubated hepatocytes were quick-frozen in liquid N_2 immediately before the incubation procedure was initiated with aliquots of the same preparation.

Energy-related parameter	Source of hepatocytes	Unincubated	Incubated
Metabolite concentrations [b]			
ATP	liq. diet control [a]	9.7 ± 0.9	10.2 ± 0.5
	ethanol-fed [a]	4.65 ± 0.35 *	8.6 ± 1.0 †
ADP	liq. diet control	4.7 ± 0.4	6.9 ± 0.5 †
	ethanol-fed	5.65 ± 0.6	8.95 ± 0.75 *,†
AMP	liq. diet control	1.5 ± 0.4	2.2 ± 0.3
	ethanol-fed	5.9 ± 0.9 *	3.5 ± 0.4 *,†
Total adenine nucleotide	liq. diet control	15.9 ± 0.8	19.3 ± 1.0 †
	ethanol-fed	16.2 ± 1.4	21.0 ± 1.9 †
P_i	liq. diet control	68 ± 7	22.2 ± 2.7 †
	ethanol-fed	101 ± 18	21.7 ± 1.9 †
Phosphorylation potential	liq. diet control	364 ± 68	813 ± 146 †
	ethanol-fed	115 ± 28 *	508 ± 54 †
Energy charge	liq. diet control	0.76 ± 0.03	0.71 ± 0.02
	ethanol-fed	0.47 ± 0.03 *	0.59 ± 0.04 *,†

[a] Hepatocytes from six pairs of pair-fed animals.
[b] nmol per 10^6 viable cells.
* $P < 0.05$ or lower for a difference from liquid diet control samples; paired t-test.
† $P < 0.05$ or lower for a difference due to the incubation procedure; paired t-test.

TABLE IV

Effect of incubation in 95% oxygen on the energy state of hepatocytes

The incubation procedure and the metabolite analyses are described in Experimental procedures. Samples of unincubated hepatocytes were quick-frozen in liquid N_2 immediately before the incubation procedure was initiated with aliquots of the same preparation.

Energy-related parameter	Source of hepatocytes [a]	Unincubated	Incubated
Metabolite concentrations [b]			
ATP	liq. diet controls	7.9 ± 0.9	11.1 ± 0.8 †
	ethanol-fed	6.1 ± 0.8	12.3 ± 1.2 †
ADP	liq. diet controls	4.1 ± 0.4	4.2 ± 0.3
	ethanol-fed	4.8 ± 0.4	5.1 ± 0.4 *
AMP	liq. diet controls	2.8 ± 0.6	2.0 ± 0.2
	ethanol-fed	5.8 ± 1.2 *	2.8 ± 0.3 *,†
Total adenine nucleotide	liq. diet controls	14.8 ± 0.9	17.4 ± 1.0 †
	ethanol-fed	16.7 ± 1.4	20.4 ± 1.8 †
P_i	liq. diet controls	74 ± 5	17.3 ± 1.9 †
	ethanol-fed	104 ± 12 *	17.0 ± 2.1 †
Phosphorylation potential	liq. diet controls	340 ± 63	1894 ± 276 †
	ethanol-fed	165 ± 36 *	1817 ± 328 †
Energy charge	liq. diet controls	0.67 ± 0.05	0.76 ± 0.01
	ethanol-fed	0.51 ± 0.05 *	0.73 ± 0.01 †

[a] $n = 9$ pairs of animals.
[b] nmol per 10^6 viable cells.
* $P \leq 0.05$ for a difference between control and ethanol-fed animals.
† $P \leq 0.05$ for a difference between unincubated and incubated hepatocytes.

ethanol-fed animals resulted in a significant increase in the energy charge. The decreases in P_i concentrations in hepatocytes from liquid-diet control and ethanol-fed animals were 3.1- and 4.7-fold, respectively, and resulted in significant increases in the phosphorylation potentials. The total adenine nucleotide concentrations were not significantly different in the hepatocytes from the two animal groups. However, upon incubation in the air atmosphere the total adenine nucleotide levels increased 21 and 30%, respectively, in cells from liquid diet and ethanol-fed animals. The hepatocytes from chow-fed animals also demonstrated a significant elevation in energy state, as indicated by the increases in ATP content and energy charge upon incubation in an air atmosphere (data not shown). The magnitude of the changes with cells from chow-fed rats were similar to those observed with hepatocytes from liquid-diet control animals.

The data in Table III demonstrate that the distribution of energy metabolites was influenced significantly by the conditions under which the hepatocytes were maintained. In order to assess more rigorously the effects of oxygen tension, hepatocytes were incubated in a 95% O_2/5% CO_2 atmosphere. The energy states of the hepatocytes under these conditions are shown in Table IV. Preparations from both liquid-diet control and ethanol-fed animals demonstrated dramatic increases in their energy states. With cells from liquid-diet control animals this is illustrated by a 4.3-fold decrease in the concentration of P_i, a 41% increase in ATP content and a 5.6-fold increase in the phosphorylation potential. Hepatocytes from ethanol-fed rats when incubated in the presence of 95% O_2 demonstrated a 6.1-fold decrease in P_i, a 203% increase in ATP and an 11-fold increase in phosphorylation potential. Moreover, the differences in the energy states of hepatocytes from control and ethanol-fed animals disappeared upon incubation under high oxygen tension as is illustrated by phosphorylation potential and energy charge values that were nearly identical. The total adenine nucleotide content increased 18% and 23%, respectively, in preparations from control and ethanol-fed rats.

The above data with aerated or oxygenated hepatocytes illustrate that the adenine nucleotide distribution was influenced by the oxygen environment of the cells. Livers were therefore freeze-clamped in situ while being perfused continuously by the animals' blood supply in order to maintain normal oxygen levels in the organ. The metabolite analyses are shown in Table V. The lactate/pyruvate ratios are lower than those reported in earlier studies [20,21] and only slightly higher than the values reported using exactly the same technique with chow-fed rats [17]. With the exception of an increase in the AMP concentration in the livers of ethanol-fed animals and the P_i concentration in hepatic tissue of chow-fed rats, there were no significant differences noted in the metabolite concentrations reported (Table V). Moreover, the differences observed in phosphorylation potential and energy charge values between the three groups did not prove to be statistically significant. These observations indicate that in ethanol-fed animals there is no decrease in the energy state of the liver when normal oxygen tension is maintained. The results in Table V also demonstrate that the liquid-diets utilized in this study had no adverse effects on the metabolite distribution, as is indicated by a comparison of the data with that from chow-fed rats.

TABLE V

Effect of ethanol consumption on the energy state of whole liver

The freeze-clamp procedure and metabolite analyses are described in Experimental procedures.

Energy-related parameter	Liver source		
	chow-fed	liquid diet control	ethanol-fed
Lactate/pyruvate ratio	–	4.68 ± 1.11	3.91 ± 0.78
Metabolite concentrations [a]			
ATP	2.45 ± 0.09	2.23 ± 0.21	2.30 ± 0.24
ADP	0.67 ± 0.04	0.70 ± 0.07	0.78 ± 0.15
AMP	0.18 ± 0.07	0.16 ± 0.03	0.27 ± 0.03 [b]
Total adenine nucleotide	3.30 ± 0.19	3.09 ± 0.29	3.35 ± 0.39
P_i	3.87 ± 0.20 [c]	2.75 ± 0.33	2.97 ± 0.45
Phosphorylation potential	765 ± 54	1014 ± 108	969 ± 184
Energy charge	0.85 ± 0.02	0.84 ± 0.01	0.80 ± 0.01

[a] μmol metabolite per g wet weight of liver.

[b] $P = 0.038$ for a significant increase over the liquid diet control value; $n = 6$ pairs of animals, paired t-test.

[c] $P = 0.042$ for a significant increase over the liquid diet control value; $n = 10$ animals, t-test for two means.

Discussion

This investigation demonstrates increased sensitivity of hepatocytes from ethanol-fed rats to the oxygen content of their environment. This was demonstrated in hepatocytes from ethanol-fed animals that were maintained for various time periods in an essentially anaerobic environment, which resulted in dramatically lowered energy states as compared with control preparations under identical conditions (Tables I, III and IV). However, the hepatocytes from ethanol-fed rats demonstrated the ability to recover to normal energy states when incubated in the presence of an additional energy source and increased oxygen tension. While

hepatocytes from all three animal sources (chow-fed, liquid diet control, ethanol fed) demonstrated increases in their energy state, the most dramatic recovery was observed with cells from ethanol-fed rats (Tables III and IV).

The oxygen content of the incubation media had a significant effect on the energy state of the hepatocytes, as indicated by a comparison of the effects of incubations in air vs. 95% O_2. With hepatocytes from ethanol-fed animals the phosphorylation potential was 1817 when incubations were carried out in the presence of 95% O_2 vs. 508 in the air atmosphere. Notably, the phosphorylation potential achieved by hepatocytes from ethanol-fed animals in 95% O_2 was comparable to that observed with cells from liquid diet control rats, even though the energy state of the unincubated hepatocytes from ethanol-fed animals was only half that of the unincubated control hepatocytes (Table IV).

The similar energy states of hepatocytes from ethanol-fed and control animals observed after incubation in either the air (Table III) or 95% O_2 (Table IV) environments were paralleled by similar rates of oxygen utilization. Furthermore, there was no evidence for a differential rate of respiration in hepatocytes as a result of chronic ethanol administration. Also, the oxygen tensions were identical in the incubation mixtures containing the two different hepatocyte groups. In the air atmosphere the O_2 concentration in the incubation mixture is maintained at a level similar to that observed in arterial blood (90 torr) of anaesthetized rats which are breathing air [22]. Thus, the oxygen tension of the air incubations approximates that observed in the circulation. The oxygen tension of the incubation medium saturated with 95% O_2/5% CO_2 is about the same as that reported in the periportal region of the lobule of livers perfused with media saturated with the same gas mixture [23]. Thus, the oxygen environment of the hepatocytes in the highly oxygenated media appears to be similar to those used previously with perfused liver. In another study where anaesthesized animals were breathing 95% O_2/5% CO_2 the arterial oxygen concentration, while high, was somewhat lower at 480 torr [22] than the oxygen tension to which the hepatocytes were exposed in these studies.

The observations made with the hepatocytes suggested that the energy state of in liver cells from ethanol-fed animals would be influenced more by the oxygen tension of the tissue than is the case in liver cells from control animals. This has also been emphasized by the studies of Miyamoto and French [8], which demonstrated that the energy state of livers decreased much more dramatically in rats fed ethanol chronically than in liquid-diet control animals when the rats were made hypoxic by 10% O_2/90% N_2 inhalation. One possible explanation for the decreased energy state observed at low oxygen tensions (Ref. 8, this study) may relate to the level of endogenous substrate available for energy metabolism. Glycogen levels are depleted as a result of chronic ethanol administration [2] and this may be the preferred energy source under conditions of lowered oxygen tension, even with cells that are prepared and maintained in the presence of added glucose. If this is the case anaerobic glycolysis may not be as efficient in hepatocytes from ethanol-fed rats due to lowered hepatic levels of glycogen.

The results obtained in the present study with hepatocytes and those previously reported [8] emphasize the importance of measuring the energy state of livers from ethanol-fed and control animals under normal oxygenation conditions. For this reason livers were freeze-clamped while being perfused by the animal's fully oxygenated blood supply. This procedure was utilized successfully in previous studies [17,24]. The relatively low lactate/pyruvate ratio in livers from both ethanol-fed and liquid diet control animals confirmed that the tissue was adequately oxygenated (see Ref. 17). Under these conditions there was no significant ethanol-related alteration in the energy state of the tissue.

The observations made in this study with freeze-clamped livers are in contrast with earlier reports which indicated that chronic ethanol consumption decreased the energy state of liver. Table VI lists the ATP concentrations measured in those studies and the conditions under which the liver samples were obtained. The ATP concentrations reported in the table reflect the energy state of the tissues as indicated either by energy charge or phosphorylation potential [19]. In these earlier investigations the liver tissue was excised such that the oxygen supply was terminated before the tissue was frozen. In each case [1–4,8] the ATP concentrations in

TABLE VI

Summary of measurements of the effect of chronic ethanol consumption on rat liver ATP concentrations

The data below were obtained from previously published reports.

ATP concentration (μmol/g wet wt. liver)		Tissue fixation procedure (manipulation before extraction)	Ref.
liquid diet control	ethanol-fed		
2.43 ± 0.27	1.70 ± 0.20 *	animal killed; section excised and frozen in liquid nitrogen	1
2.44 ± 0.14	1.42 ± 0.07 *	animal killed; lobe excised and frozen in liquid nitrogen	2
2.62 ± 0.13	1.37 ± 0.08 *	animal killed; section excised and frozen or frozen in situ	3
1.4 ± 0.1	0.9 ± 0.1 *	animal anesthetized; liver excised and frozen in liquid nitrogen	4
2.56 ± 0.58	1.86 ± 0.45 *	animal anesthetized; liver biopsy section frozen in liquid nitrogen	8

* Statistically significant with a P value of ≤ 0.05.

rats fed ethanol chronically were decreased significantly (Table VI). In a more recent investigation [7], where livers were freeze-clamped in a manner similar to the procedure used in our studies (Refs. 17,24; present study), the differences observed between ethanol-fed and control samples were not significantly different. These reports [1–4,7,8] support our conclusion that chronic ethanol consumption renders the energy state of liver very responsive to the oxygen environment of the tissue. They also emphasize that in future studies it will be necessary to maintain normal oxygen tension when ethanol-related effects on the energy states of tissues are measured.

As mentioned above, our measurements of metabolites in freeze-clamped liver demonstrate that when physiological oxygen concentrations are maintained the energy state of the liver in the ethanol-fed rat is normal. Moreover, our observations with hepatocytes, and those of Miyamoto and French [8], emphasize that liver cells from ethanol-fed animals have a lesion in energy metabolism manifested when oxygen tension is below normal. If under conditions of chronic ethanol consumption a steeper oxygen gradient is established across the liver lobule such that the pericentral (centrilobular) hepatocytes are relatively oxygen-deficient [25], it is likely that the pericentral cells are energy-deficient in individuals consuming ethanol. In future studies it will be important to relate the ethanol-induced alterations in mitochondrial function [5,6] with the decreased energy state induced by hypoxia. It is likely that such relationships will be more apparent in the pericentral portion of the liver lobule.

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