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# Redox State and Energy Metabolism during Liver Regeneration

## ALTERATIONS PRODUCED BY ACUTE ETHANOL ADMINISTRATION

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**ABSTRACT.** Ethanol metabolism can induce modifications in liver metabolic pathways that are tightly regulated through the availability of cellular energy and through the redox state. Since partial hepatectomy (PH)-induced liver proliferation requires an oversupply of energy for enhanced syntheses of DNA and proteins, the present study was aimed at evaluating the effect of acute ethanol administration on the PH-induced changes in cellular redox and energy potentials. Ethanol (5 g/kg body weight) was administered to control rats and to two-thirds hepatectomized rats. Quantitation of the liver content of lactate, pyruvate, β-hydroxybutyrate, acetoacetate, and adenine nucleotides led us to estimate the cytosolic and mitochondrial redox potentials and energy parameters. Specific activities in the liver of alcohol-metabolizing enzymes also were measured in these animals. Liver regeneration had no effect on cellular energy availability, but induced a more reduced cytosolic redox state accompanied by an oxidized mitochondrial redox state during the first 48 hr of treatment; the redox state normalized thereafter. Administration of ethanol did not modify energy parameters in PH rats, but this hepatotoxin readily blocked the PH-induced changes in the cellular redox state. In addition, proliferating liver promoted decreases in the activity of alcohol dehydrogenase (ADH) and of cytochrome P4502E1 (CYP2E1); ethanol treatment prevented the PH-induced diminution of ADH activity. In summary, our data suggest that ethanol could minimize the PH-promoted metabolic adjustments mediated by redox reactions, probably leading to an ineffective preparatory event that culminates in compensatory liver growth after PH in the rat. BIOCHEM PHARMACOL **58**;11:1831–1839, 1999. © 1999 Elsevier Science Inc.

**KEY WORDS.** cellular metabolism; energy charge; redox potential; alcohol; cell proliferation

Human and rodent livers have the remarkable capacity of regenerating in response to various stimuli, including massive destruction of hepatic tissue by toxins, viral agents, or surgical resection [1, 2]. Liver regeneration depends on the ability of hepatocytes to undergo cell division, which is controlled tightly by intra- and extrahepatic factors [1, 2]. The proliferative process requires an oversupply of metabolic energy to match increased DNA synthesis, which occurs during liver regeneration [1–4]. In addition, cellular redox potential, another metabolic control system, is subjected to modifications (as the $NAD^+$/NADH redox pair) in both cytosolic and mitochondrial compartments in the regenerating liver [4, 5]. Both cellular parameters are closely linked, and energy parameters (i.e. ATP/ADP ratio and phosphorylation potential) and the free $NAD^+$/NADH ratio depend on activities of the major liver metabolic pathways, in turn reflecting the metabolic integrity of the liver cell [6, 7].

Acute ethanol administration to animals subjected to (PH‡) readily inhibits the resultant liver regeneration after surgery, as assessed by a reliable diminution of parameters of cell proliferation in the remnant liver [8, 9]. Although the exact mechanisms underlying ethanol-induced inhibition of liver regeneration are not well understood, it is reasonable to assume that this hepatotoxin could alter the overall metabolism of the regenerating liver. Indeed, it has been reported that ethanol causes disturbances in the redox state, energy charge, and mitochondrial oxidative capacity of the remnant liver early after PH [10–12].

The effects of ethanol on the regenerating liver could be attributed to its hepatic metabolism, which comprises cytoplasmic ethanol oxidation to acetaldehyde, catalyzed

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‡*Abbreviations*: PH, partial hepatectomy; ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; CYP2E1, cytochrome P450 2E1; $K_{eq}$, equilibrium constant; $E_h$, redox potential; $E_{hc}$, cytosolic redox potential; and $E_{hm}$, mitochondrial redox potential.

by ADH, and further conversion of acetaldehyde to acetate by mitochondrial ALDH. Both enzymatic reactions implicate the reduction of a molecule of $NAD^+$. The excess of NADH production in both cytosol and mitochondria readily modifies the cellular redox state and, consequently, energy availability, leading to alterations of metabolic pathways such as glycolysis, and oxidative mitochondrial function in liver tissue [13–15]. Ethanol administration can affect the regenerating liver at the metabolic level [14, 15]; however, little information is available about the effects of this drug on cellular metabolic systems, such as the redox state and energy availability in the proliferating liver.

Therefore, the present study was aimed at assessing whether energy availability and redox state are major controlling factors in the onset of liver regeneration and possible targets for the deleterious actions of acute ethanol on rat liver regeneration. Results show that liver regeneration *per se* induces modifications in the liver redox state, without significantly changing the energy status. Acute ethanol administration largely shifted the PH-induced modifications in the hepatic redox state, whereas liver energy availability was unaffected by this hepatotoxin.

## MATERIALS AND METHODS

### *Reagents*

Enzymes, coenzymes, and substrates were obtained from the Sigma Chemical Co. Standard analytical grade reagents were purchased from Merck.

### *Animals and Treatments*

Male Wistar rats weighing 220–260 g were maintained on a 12-hr light–dark cycle, with standard rat pellet chow and access to water *ad lib*. All procedures were conducted following our Institutional Animal Care, Selection and User's Guide for Laboratory Animals. Rats were subjected to two-thirds PH under light diethyl ether anesthesia, according to a technique reported by Higgins and Anderson [16]. Sham-operated animals were used as controls.

Animals were grouped according to their surgical status and treated as previously reported [15]. They received a single dose of ethanol (5 g/kg body weight; 63% solution in water) given immediately after surgery through an oral cannula, or an equivalent volume of saline solution. At the times indicated, animals were killed by a blow to the back of the head; around 300 mg of liver tissue was removed as rapidly as possible (less than 10 sec) and frozen in liquid nitrogen. Frozen samples were extracted in 4 vol. of cold perchloric acid for determination of redox metabolites (0.6 N $HClO_4$) and adenine nucleotides (0.8 N $HClO_4$) as indicated previously [17]. The extracts were centrifuged to get rid of denatured protein, and the supernatant was aliquoted in plastic tubes and frozen at −70° until processing.

### *Determination of Metabolites*

Perchloric acid extracts were neutralized with 5 mol/L of $K_2CO_3$ and used for metabolite determinations by enzymatic methods: lactate [18], pyruvate [19], β-hydroxybutyrate [20], and acetoacetate [21]. Adenine nucleotides (ATP, ADP, and AMP) were quantified by HPLC, according to the method of Hoffman and Liao [22].

### *Definition and Calculation of Redox and Energy State*

Cytosolic and mitochondrial redox states were calculated from lactate/pyruvate and β-hydroxybutyrate/acetoacetate ratios, respectively, in accordance with Stubbs *et al*. [23]. Estimation of the $NAD^+$/NADH ratio from both subcellular compartments was done using the following equation: $NAD^+/NADH = [\text{oxidized substrate}]/[\text{reduced substrate}] \times 1/K_{eq}$, taking into account the $K_{eq}$ of lactate dehydrogenase ($1.11 \times 10^{-4}$ M) for the cytosolic fraction, and the $K_{eq}$ of β-hydroxybutyrate dehydrogenase ($4.93 \times 10^{-2}$ M) for the mitochondrial compartment [23, 24]. Redox potential ($E_h$), defined as the ability to dissect an overall electron transfer into two half-reactions [24, 25], was calculated based on the Nernst equation, as follows: $E_h = E'_0 + 0.03 \log NAD^+/NADH$, where $E'_0 = 0.314$ V [25]. The difference between cytosolic and mitochondrial redox potentials ($\Delta E$) was calculated as $\Delta E = E_{hm} - E_{hc}$.

Liver energy charge (E.C.) was calculated from the adenine nucleotide concentrations by the method reported by Atkinson [26], as follows: E.C. = (ATP + 0.5 ADP)/(ATP + ADP + AMP).

### *Enzyme Assays*

Liver samples were homogenized in a buffer containing 0.25 mol/L of sucrose, 10 mmol/L of Tris–HCl, 0.3 mmol/L of EGTA, and 0.2% BSA; pH 7.4. The homogenate was centrifuged at 800 *g* for 10 min, at 4°, and the resulting supernatant was spun for 15 min at 8500 *g* to pellet the mitochondrial fraction. The second supernatant was centrifuged at 25,000 *g* for 60 min to obtain the peroxisomal fraction (pellet). The supernatant was spun further at 100,000 *g* for 60 min, to obtain the cytosolic and microsomal fractions. The specific activities of ADH (EC 1.1.1.1) and ALDH (EC 1.2.1.5) were measured in the cytosolic and mitochondrial fractions, respectively, by standard procedures [27, 28], and expressed as nanomoles per minute per milligram of protein. The specific activity of catalase (EC 1.11.1.6) was determined in the peroxisomal fraction and expressed as nanokatals per gram of liver, as reported by Aebi [29]. In addition, the specific activity of CYP2E1 was measured using the method reported by Salmela *et al.* [30]. Protein was quantitated by the method of Lowry *et al*. [31].

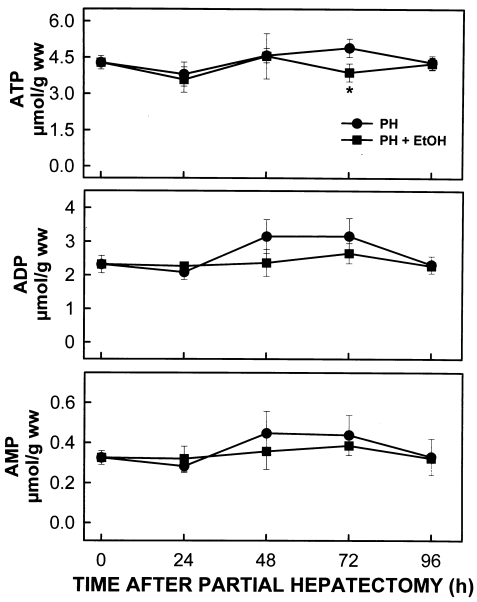


**FIG. 1. Concentration of adenine nucleotides in the regenerating liver after ethanol administration. Rats were partially hepatectomized, and a single intragastric ethanol dose was administered immediately. Adenine nucleotides were determined in the remnant liver by HPLC. Each point is the mean ± SEM of 6–15 independent experiments. Key: (*) indicates statistical significance ($P < 0.01$) vs control.**

### *Statistical Analysis*

All results are expressed as means ± SEM, and statistical significance of differences among groups was assessed by Student's *t*-test.

## RESULTS

### *Liver Energy Parameters*

Figure 1 shows the time–course profile of liver adenine nucleotides (ATP, ADP, and AMP) from animals subjected to PH, with or without acute ethanol administration. Hepatic adenine nucleotides were not modified significantly in sham-operated and intact rats that received ethanol (not shown). Moreover, neither PH nor ethanol administration elicited changes in these parameters, except for ATP, which was lower in the PH + EtOH group at 72 hr post-surgery. Despite the ethanol-induced diminution of liver ATP in the animals subjected to PH, control energy parameters such as total nucleotides (6.93 ± 0.59 µmol/g liver), the ATP/ADP ratio (3.69 ± 0.15), and energy charge (0.78 ± 0.02) were not changed significantly by the treatments tested.

### *Effect of PH and Acute Ethanol Treatment on Redox State of Liver Cells*

While neither PH nor acute ethanol administration promoted significant changes in liver energy parameters, the redox state was modified profoundly under these experimental conditions. Determinations of liver levels of lactate and pyruvate, as well as β-hydroxybutyrate and acetoacetate, were used as redox-pair metabolites for calculating cytosolic and mitochondrial ($NAD^+$/NADH) redox potentials, respectively.

### *Cytosolic Redox-Pair Metabolites*

Liver lactate and pyruvate levels from experimental animals are shown in Table 1. Sham-operated rats receiving ethanol did not present any significant change in the liver lactate level 24 hr post-treatment (3.20 ± 0.12 vs 3.30 ± 0.15 µmol/g liver in controls). An increased level of hepatic lactate was found in animals subjected to PH at 24 hr post-surgery, which was followed by a decrease in this metabolite at 72 hr post-PH and normalized thereafter. Ethanol administration to PH animals showed the same initial peak of liver lactate, but the subsequent decrease of this metabolite found in animals undergoing PH was earlier and lasted longer under ethanol treatment (Table 1). Hepatic pyruvate in controls (0.32 ± 0.02 µmol/g) was not modified significantly in sham-operated animals after ethanol administration (at 24 hr, 0.31 ± 0.03 µmol/g). PH induced a significant decrease of liver pyruvate during the first 48 hr after surgery, returning to the control level thereafter. Ethanol administration to PH rats practically abolished the effect of the partial removal of the liver on the pyruvate concentration, at all times tested (Table 1). The changes elicited by PH and ethanol administration in liver concentrations of lactate and pyruvate induced modifications of the lactate/pyruvate ratio and of the liver redox $NAD^+$/NADH potential (Fig. 2). In both groups, PH and PH + ethanol animals, the lactate/pyruvate ratio increased significantly at 24 hr after surgery. Animals subjected to PH showed a gradual diminution in this ratio starting at 48 hr after PH and normalizing thereafter, while PH rats receiving ethanol showed a sudden decrease of this ratio at 48 hr post-surgery, returning the lactate/pyruvate ratio within normal range at 96 hr after surgery (Fig. 2A). Partial removal of the liver promoted a decrease in the cytosolic $NAD^+$/NADH ratio, indicating a reduced cytosolic redox state, which lasted for 48 hr and normalized thereafter. Acute ethanol administration to this group modified the effects of PH on the cytosolic $NAD^+$/NADH ratio, since at 48–72 hr post-PH, ethanol promoted a significantly more oxidized cytosolic redox state (Fig. 2C). This action was not associated with the presence of ethanol, since ethanol is cleared 10 hr after its administration [15]. At later times post-surgery (96 hr), both experimental groups presented a normal cytosolic redox state (Fig. 2C).

### *Mitochondrial Redox-Pair Metabolites*

Table 1 also presents the hepatic levels of β-hydroxybutyrate and acetoacetate as redox-pair metabolites indicative of the mitochondrial redox state. Again, sham-operated animals receiving acute ethanol administration did not have significant changes in the liver content of acetoace-

**TABLE 1. Concentration of metabolites from livers of partial hepatectomized (PH) rats with or without a single ethanol dose**

| Metabolite (μmol/g liver wet weight) | Time after partial hepatectomy (hr) | | | |
|---|---|---|---|---|
| | 24 | 48 | 72 | 96 |
| Lactate (3.30 ± 0.15) | | | | |
| PH | 4.40 ± 0.38* | 2.80 ± 0.26 | 1.85 ± 0.15* | 3.15 ± 0.16 |
| PH + EtOH | 5.26 ± 0.42* | 1.37 ± 0.25*† | 1.79 ± 0.16* | 3.20 ± 0.18 |
| Pyruvate (0.32 ± 0.021) | | | | |
| PH | 0.24 ± 0.038* | 0.20 ± 0.020* | 0.25 ± 0.030 | 0.35 ± 0.025 |
| PH + EtOH | 0.31 ± 0.022 | 0.31 ± 0.021 | 0.25 ± 0.031 | 0.32 ± 0.025 |
| Acetoacetate (0.13 ± 0.009) | | | | |
| PH | 0.19 ± 0.010* | 0.17 ± 0.009* | 0.14 ± 0.013 | 0.13 ± 0.016 |
| PH + EtOH | 0.17 ± 0.008 | 0.16 ± 0.010* | 0.16 ± 0.010 | 0.13 ± 0.020 |
| β-Hydroxybutyrate (0.22 ± 0.005) | | | | |
| PH | 0.17 ± 0.007* | 0.18 ± 0.008* | 0.20 ± 0.009 | 0.22 ± 0.018 |
| PH + EtOH | 0.21 ± 0.008*† | 0.19 ± 0.009* | 0.17 ± 0.009*† | 0.21 ± 0.007 |

Metabolites were determined according to Materials and Methods. Each point is the mean ± SEM of 5–10 independent experiments. Mean control concentrations ± SEM of each metabolite are shown in parentheses.

* $P < 0.01$ vs controls.

† $P < 0.01$ vs the PH group.

tate at any of the times tested (0.12 ± 0.01 vs 0.13 ± 0.01 μmol/g in controls). PH induced a significant increase of acetoacetate, which lasted until 48 hr after surgery and declined to normal values thereafter; ethanol administration to these animals did not modify the PH-induced changes in liver acetoacetate concentration significantly. In regard to the liver β-hydroxybutyrate level, PH also elicited a significant decrease within the first 48 hr post-surgery, an effect that was initially blocked by ethanol administration (24 hr post-PH), and indeed was shifted to later times post-surgery (48–72 hr; Table 1). Again, ethanol administration to sham-operated rats did not change the hepatic β-hydroxybutyrate level significantly (0.21 ± 0.02 μmol/g) after 24–96 hr of treatment.

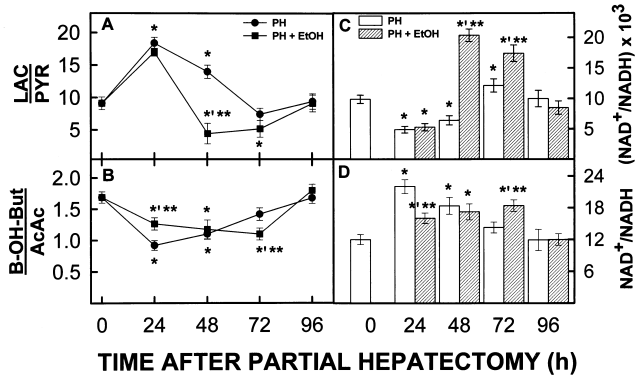


**FIG. 2. Redox parameters in cytosol and mitochondria from the regenerating liver. Panel A shows the lactate/pyruvate ratio, and panel B shows the β-hydroxybutyrate/acetoacetate ratio. Panels C and D depict the calculated $NAD^+$/NADH ratio in cytosol and mitochondria, respectively. The relationships were calculated as described in Materials and Methods. Each point is the mean ± SEM of 6–15 independent experiments. Key: (*) indicates statistical significance ($P < 0.01$) vs control; and (**) indicates statistical significance ($P < 0.01$) vs PH rats.**

Thus, derived parameters of the mitochondrial redox state were modified significantly by the treatments tested. PH promoted a diminution in the β-hydroxybutyrate/acetoacetate ratio in the first 48 hr after surgery, followed by a complete recovery of this ratio. Ethanol administration partially blocked the PH-induced diminution of the liver β-hydroxybutyrate/acetoacetate ratio, but a decrease of this ratio was noted at a later time post-PH (72 hr) in the PH + ethanol group (Fig. 2B). Hence, PH animals presented a more oxidized mitochondrial redox state, as indicated by an increased $NAD^+$/NADH ratio in the first 48 hr after surgery, whereas ethanol treatment of PH rats shifted the highest mitochondrial $NAD^+$/NADH ratio to 72 hr post-surgery (Fig. 2D). Again, at 96 hr after PH no modifications in the mitochondrial redox state were recorded in the different experimental groups (Fig. 2D).

### *Effect of PH and Acute Ethanol Administration on Cytosolic and Mitochondrial Redox Potentials*

Table 2 shows the calculated redox potential ($E_h$; expressed in mV) from the cytosolic and mitochondrial $NAD^+$/NADH ratios, and the difference ($\Delta E$) between the two subcellular compartments. No significant changes were found in the cytosolic redox potential ($E_{hc}$) as a consequence of PH, but ethanol co-administration elicited a more positive redox potential after 48 hr post-surgery. However, the mitochondrial redox potential ($E_{hm}$) was virtually unaffected by the experimental conditions (Table 2). Although $\Delta E$ was a constant

**TABLE 2. Redox potential ($E_h$) in cytosol and mitochondria and the differences between mitochondrial and cytosolic redox potential ($\Delta E$) from livers of rats after partial hepatectomy (PH) with or without a single ethanol dose**

| Parameter | Time after partial hepatectomy (hr) | | | |
|---|---|---|---|---|
| | 24 | 48 | 72 | 96 |
| $E_{hc}$ (224.1 ± 4.6) | | | | |
| PH | −233.3 ± 3.3 | −229.7 ± 4.2 | −221.5 ± 3.3 | −224.3 ± 5.6 |
| PH + EtOH | −232.3 ± 4.5 | −214.7 ± 3.1* | −216.8 ± 1.6 | −224.1 ± 5.6 |
| $E_{hm}$ (281.1 ± 6.6) | | | | |
| PH | −273.7 ± 6.6 | −276.1 ± 6.3 | −279.4 ± 5.6 | −281.7 ± 6.8 |
| PH + EtOH | −277.8 ± 3.5 | −276.9 ± 7.2 | −276.0 ± 2.3 | −281.6 ± 8.6 |
| $\Delta E$ (57.5) | | | | |
| PH | 40.4† | 46.4† | 57.9 | 57.4 |
| PH + EtOH | 45.5† | 62.2† | 59.2 | 57.5 |

Parameters were calculated from metabolites shown in Fig. 1 according to Materials and Methods. Each point is the mean ± SEM of 5–10 independent experiments. The control values ± SEM for $E_h$ (mV) and $\Delta E$ are shown in parentheses.

* $P < 0.01$ vs PH group.

† $P < 0.01$ vs control.

value maintained within a narrow range in control livers (52–58 mV), there were significant changes due to PH. In these animals, a decrease in $\Delta E$ was found during the first 48 hr after surgery, mainly attributed to a more negative (reduced) cytosolic redox potential, which was transient and was readily normalized thereafter. Ethanol treatment of rats undergoing PH promptly shortened the PH-induced effect on $\Delta E$, which was noticeable 24 hr after surgery in the PH + ethanol group (Table 2).

### *Effect of PH and Acute Ethanol Administration on the Activities of Ethanol-Metabolizing Enzymes*

To correlate the changes in both cytosolic and mitochondrial redox states induced by PH and PH + ethanol with liver ethanol metabolism, activities of ethanol-metabolizing enzymes were measured in several subcellular compartments. Figure 3 shows the changes elicited by PH and ethanol in $NAD^+$-dependent enzymes (cytosolic ADH and mitochondrial ALDH), as well as in the $NAD^+$-independent peroxisomal enzyme catalase, which are involved in ethanol catabolism [32].

Acute ethanol administration to sham-operated rats did not modify significantly the activity of the enzymes tested. However, surgical removal of the liver promoted a gradual inhibition of the ADH activity, reaching a minimal activity at 24 hr post-PH and recovering its activity thereafter. Ethanol treatment reduced the PH-induced inhibition of liver ADH activity, readily increasing ADH activity with a peak after 48 hr post-surgery (Fig. 3A).

Mitochondrial ALDH activity was readily increased by PH (starting from 48 up to 96 hr). Ethanol administration promoted an earlier peak of ALDH activity (12 hr), without further modification of the PH-induced pattern of ALDH activity (Fig. 3B).

On the other hand, peroxisomal catalase activity greatly responded to the loss of liver mass. PH induced an early decrease in catalase activity (3–12 hr after surgery), which was recovered at 24 hr post-PH and followed by a further diminution of its activity up to 72 hr post-surgery (Fig. 3C). Acute ethanol co-administration elicited minor changes in the PH-induced liver pattern of NAD-independent catalase

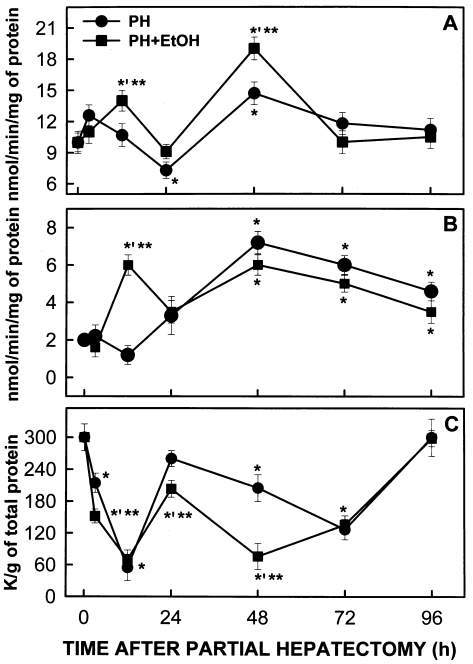


**FIG. 3. Specific activities of ADH, ALDH, and catalase after PH and ethanol administration. The activities of ADH (A), ALDH (B), and catalase (C) were determined in the remnant livers of PH rats receiving ethanol. Results are expressed as the means ± SEM of 6–15 independent experiments. Statistical significance is as indicated in the legend of Fig. 2.**

activity, except for a more pronounced inhibition of catalase activity after 48 hr post-surgery (Fig. 3C).

Finally, the specific activity of the microsomal CYP2E1 also was measured in our experimental groups. CYP2E1 activity in sham-operated rats (0.65 ± 0.02 nmol/min/mg microsomal protein) was not affected significantly by acute ethanol treatment (0.61 ± 0.02). However, PH readily elicited an important diminution of CYP2E1 activity at 24 hr post-surgery (0.37 ± 0.03 nmol/min/mg), which lasted up to 48 hr after PH and returned to within the control range at 72 hr after surgery (0.67 ± 0.04). Interestingly, ethanol administration did not affect the PH-induced profile of liver CYP2E1 activity (not shown).

## DISCUSSION

PH-induced liver regeneration is a good experimental model for studying cellular processes related to cell proliferation. This model has been useful to identify factors that can alter the overall proliferative process in the liver of the experimental animal. Liver regeneration is accompanied by changes in the major metabolic pathways, which must be directed to maintain both (a) adequate energy availability (in the form of ATP) for DNA replication, and (b) the regulatory functions needed for cell division and restitution of the liver mass [1, 2].

In addition, the cellular $NAD^+$/NADH redox state constitutes another major regulatory factor for several metabolic fluxes [13]. The balance between oxidizing and reducing reactions linked to the $NAD^+$/NADH redox pair, as distributed in several subcellular compartments, interacts with energy parameters (i.e. with the ATP/ADP ratio) to regulate enzyme activities in both cytoplasmic and mitochondrial compartments of the liver [10]. Indeed, the specific activities of enzymes tightly regulated by the ATP/ADP and $NAD^+$/NADH ratios (involved in carbohydrate metabolism) are modified readily at the onset of liver regeneration. After PH, the activities of phosphofructokinase and fructose 2,6-biphosphatase [33], aspartate aminotransferase [34], and hexokinase [35] are subjected to coordinated changes. These changes seem to be associated with enhancement of mitochondrial function [5] and a net increase in the flux of metabolic pathways, such as the rate of gluconeogenesis in the regenerating liver [36].

It is well known that liver ethanol metabolism elicits drastic disturbances in the $NAD^+$/NADH redox potential, which can compromise cellular energy availability [13, 14]. These alterations might seriously compromise the overall metabolism of the remnant liver post-PH, and participate in the ethanol-induced inhibition of liver regeneration.

The present data indicate that acute ethanol administration did not modify significantly the PH-induced changes in liver adenine nucleotides, or their energetic relationships. Our results agree with those reported previously [11], where ethanol did not change the liver content of adenine nucleotides and inorganic phosphate or their energetic relationships at 24 hr post-surgery in animals subjected to PH; however, this hepatotoxin did modify the PH-induced changes in the cellular redox state.

In the regenerating liver, we found an important diminution of the cytosolic $NAD^+$/NADH ratio, an effect accompanied by a mirror-image in the ratio corresponding to the mitochondrial compartment, at the onset of DNA synthesis (24 hr after surgery). The PH-induced shift to a more reduced cytosolic redox state can be considered as an indicator of high metabolic activity in the liver cell [37, 38], resulting from energy-dependent processes that are influenced by the transient increases in both portal and arterial blood flows that are present during PH-induced liver regeneration [39]. In agreement with the latter, a more oxidized mitochondrial redox state was found in the remnant liver after PH, probably related to enhanced substrate oxidation by mitochondria in the regenerating liver [12].

Acute ethanol administration profoundly alters the hepatic redox state as a consequence of its cytoplasmic catabolism in the normal liver [13, 32]. Whereas ethanol had no effect on the redox state of liver cells when administered to sham-operated rats, it did induce alterations in the profile of cellular redox changes found in the regenerating liver. In animals subjected to PH and treated with ethanol, the expected changes in cytosolic redox potential ($E_{hc}$) were not accompanied by major modifications in the redox potential of mitochondria ($E_{hm}$). This finding would suggest that the hepatotoxin ethanol readily blocked PH-induced early modifications of parameters of the redox state, such as $\Delta E$. For instance, the difference in redox potential ($\Delta E$) is the result of the transport of reducing equivalents from the cytosol to the mitochondria, which maintains a 100-fold difference between the cytoplasm and the intra-mitochondrial $NAD^+$/NADH ratio in the hepatocyte [40]. This important parameter of the cellular redox state was strongly modified during liver regeneration, whereas the presence of ethanol blocked the PH-induced effect, rather than imposing the characteristic ethanol effect on the hepatic redox state as seen in livers from normal rats.

The role of the cellular redox state in the metabolic adjustments that proceed during most of the time needed for complete restoration of liver mass has not been elucidated. However, we have reported that important changes in the redox state of liver cells are involved in the regulation of mitochondrial function and collagen metabolism, thus being relevant for the physiopathology of experimental cirrhosis [41]. Moreover, changes in the cellular redox state or imbalance of its parameters also seem to be associated with situations involving stimulated cell proliferation linked to metabolic stress. This has been proven in tumor-bearing tissue [42], in liver tissue after portal branch ligation [5], and as a result of physiological adaptations, such as occur during the metabolic burst induced by glucose in isolated pancreatic cells [43]. The aforementioned observations strongly suggest a role for the redox state in the metabolic adjustments that take place in either physiological or pathological conditions of tissue growth.

The effects of ethanol on the $NAD^+$/NADH ratio occur during the time that it remains detectable in the blood [10, 11]. In the PH-induced regenerating liver, modifications of the redox state pattern by ethanol administration lasted up to 72 hr post-surgery, indicating that ethanol metabolism is not directly implicated in the changes in the cellular redox state during liver regeneration. These findings lead us to propose that acute ethanol administration might induce inhibition of liver regeneration by blocking adaptive changes in metabolic pathways occurring in the proliferating liver. Hence, ethanol seems to minimize the metabolic adjustments mediated by redox reactions, probably leading to a decreased preparatory event culminating in the proliferative period that characterizes PH-induced liver regeneration.

In the same context, the process of liver regeneration was accompanied by changes in the specific activity of some enzymes [33–35]. Here, our interest was focused on the activity pattern of ethanol-metabolizing enzymes during liver regeneration, and the putative action of acutely administered ethanol. We recently reported that regenerating liver shows an enhanced capacity to oxidize ethanol, which increases as long as hepatic mass is being restored [44]. In the present study, we found that ADH activity was decreased early after PH; hence, it is difficult to explain the enhanced ethanol oxidation by the regenerating liver when the main oxidative pathway for catabolizing ethanol is partially inhibited in PH animals.

However, ethanol administration early after PH readily protects ADH activity against PH-induced inhibition. Although the underlying mechanism of the PH-induced inhibition of ADH activity is still not elucidated, the decreased ADH activity found in regenerating liver has been reported previously [10]. Thus, the present data showing that ethanol protected against the PH-induced decrease of ADH, as well as against the decrease of cytosolic $NAD^+$ availability, will support the statement that the proliferating liver can actively oxidize the administered ethanol mainly through the participation of cytoplasmic ADH. This statement is supported further by the decreased activity found in microsomal CYP2E1, which is another ethanol-metabolizing system, mainly after chronic alcohol consumption. In our conditions, PH promoted a drastic reduction of CYP2E1 activity from 24 to 72 hr post-surgery, which closely agrees with that reported recently [45], an effect that was unchanged by the administration of ethanol.

Mitochondrial ALDH activity was increased readily at the post-PH times (48–72 hr) when a higher mitochondrial $NAD^+$/NADH ratio was found in the regenerating liver. This enzyme also is involved in ethanol metabolism by removing acetaldehyde, which in turn is converted to acetate. Ethanol administration to PH rats did not significantly modify the mitochondrial profile of ALDH activity, despite the fact that treatment with this hepatotoxin promoted a long-lasting enhancement of the oxidized redox state in mitochondria.

The PH-induced changes already described for the two NAD-dependent enzymes (ADH and ALDH) that are involved in ethanol metabolism could be related to utilization of endogenous substrates potentially involved in liver proliferation. Thus, ethanol administration could modify the activities of these enzymes toward their putative endogenous substrates, altering the metabolic adjustments for cell proliferation.

Catalase is another enzyme involved in ethanol catabolism; this NAD-independent enzyme is limited by the endogenous amount of hydrogen peroxide required to carry out ethanol oxidation to acetaldehyde [46]. This enzymatic activity was also modified by PH, but in a different manner than ADH and ALDH. The role of catalase in ethanol oxidation by the regenerating liver has not been evaluated; however, the presence of high levels of hydroperoxides, found in the cytoplasm of the proliferating liver during the first 36 hr after surgery [47], might favor the participation of this enzyme in the oxidation of ethanol through a non-ADH pathway. The involvement of each metabolic pathway for ethanol catabolism in the regenerating liver is currently being studied in our laboratory.

The effects of ethanol on the PH-induced changes in cellular redox state and activity of cytoplasmic ADH resulted in a blockade in the adaptive modifications of the redox state in the proliferating liver, whose pathophysiological significance remains to be elucidated.

In summary, partial removal of the liver mass did not affect energy availability in the remnant liver, but did modify considerably the cellular redox state in a transient manner, which would suggest its involvement in the preparatory period for cell proliferation. Administration of ethanol, known to readily alter the cellular redox state through its catabolism, in turn blocked the PH-induced pattern of cytosolic and mitochondrial redox reactions without modifying energy parameters. The specific activity of ethanol-metabolizing enzymes also changed during liver regeneration, and ethanol prevented the PH-induced effect mainly on ADH activity. The data suggest that ethanol could minimize the PH-promoted metabolic adjustments mediated by redox reactions, probably leading to a decreased preparatory event that culminates in the compensatory liver growth after PH in the rat.

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