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Ethanol-Induced Changes in the Intralobular Oxygen Gradient of Perfused Rat Liver[1]

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JI, S., J. J. LEMASTERS AND R. G. THURMAN. *Ethanol-induced changes in the intralobular oxygen gradient of perfused rat liver.* PHARMAC. BIOCHEM. BEHAV. 13: Suppl. 1, 41–45, 1980.—A new tissue fluorometric method was developed to estimate the intralobular oxygen gradient in hemoglobin-free perfused rat liver. The method employs a two-branch micro-light guide with a tip diameter of 170 μ. With this light guide, it was possible to measure pyridine nucleotide fluorescence (366 nm → 450 nm) from periportal and pericentral regions of the liver lobule. By measuring inflow PO_2 values at which pyridine nucleotide fluorescence increased in the pericentral regions of the liver lobule, the mean intralobular oxygen gradient was estimated. The measured gradient was approximately 180 torr in livers from sucrose-treated control rats. Chronic treatment with ethanol increased both the mean intralobular oxygen gradient and the rate of hepatic oxygen uptake by 30%. The antithyroid drug, 6-propyl-2-thiouracil, completely reversed the effects of ethanol on both the intralobular oxygen gradient and the rate of oxygen uptake. These data present direct physical evidence that the increased tissue respiration induced by chronic ethanol treatment indeed accentuates the intralobular oxygen gradient and thus support the hypothesis that selective depletion of oxygen in the pericentral region of the liver lobule may underlie ethanol-induced cellular injury confined to this site.

Intralobular oxygen gradient Pericentral and periportal oxygen gradients Pyridine nucleotide fluorescence
Ethanol-induced pericentral cell injury Micro-light guides Perfused liver 6-Propyl-2-thiouracil

CHRONIC treatment with ethanol is known to cause pericentral liver injury in man [18,21] and in experimental animals [5, 6, 13]. In addition, chronic [1, 7, 22] as well as acute [8] treatment of rats with ethanol increases oxygen uptake in the liver. These observations led Israel and coworkers [6,7] to postulate that pericentral liver damage following chronic treatment with ethanol arises from local anoxia confined to the pericentral region of the liver lobule (zone 3 of Rappaport [16]) secondary to increased tissue respiration. However, direct evidence for this interesting hypothesis has been lacking. A test of this hypothesis is now feasible as a result of a recent development which makes it possible to measure pyridine nucleotide fluorescence from periportal and pericentral regions of the liver lobule in the hemoglobin-free perfused rat liver [11]. By measuring inflow PO_2 values at which pyridine nucleotide systems become reduced in pericentral regions of the liver lobule, it is possible to estimate the mean intralobular oxygen gradient (see Discussion). Using this technique, we have found that chronic ethanol treatment of the rat leads to a dramatic increase in the estimated mean intralobular oxygen gradient. This effect of ethanol is reversed completely by treatment with 6-propyl-2-thiouracil (PTU). The results therefore support the hypothesis [6,7] that pericentral hypoxia plays an important role in ethanol-induced liver injury.

METHOD

Sprague-Dawley female rats (200 to 250 g) received 25% (w/v) ethanol in aqueous sucrose solution for 4 to 6 weeks as described by Porta *et al.* [15]. Control rats received sucrose in tap water (25% w/v), and both groups had free access to laboratory chow. Livers were perfused with Krebs-Henseleit bicarbonate buffer, pH 7.6, at 37°C as described elsewhere [19]. Oxygen tensions in the influent and effluent perfusate were measured continuously with Clark-type oxygen electrodes.

Tissue pyridine nucleotide fluorescence (366 nm → 450 nm) was monitored from the surface of the perfused rat liver employing a micro-light guide as described previously [9–11]. Fluorescence was observed from a tissue volume of approximately 3×10^6 μ^3 [11] lying within the first mm of the liver surface.

Periportal and pericentral regions of the liver lobule were identified from the pattern of surface pigmentation of the perfused liver as described previously for livers from phenobarbital-treated rats [11]. Lightly pigmented areas were periportal regions, and darkly pigmented spots were pericentral regions. Retrograde and anterograde infusion of India ink confirmed that surface pigmentation reflected periportal and pericentral regions in livers from both

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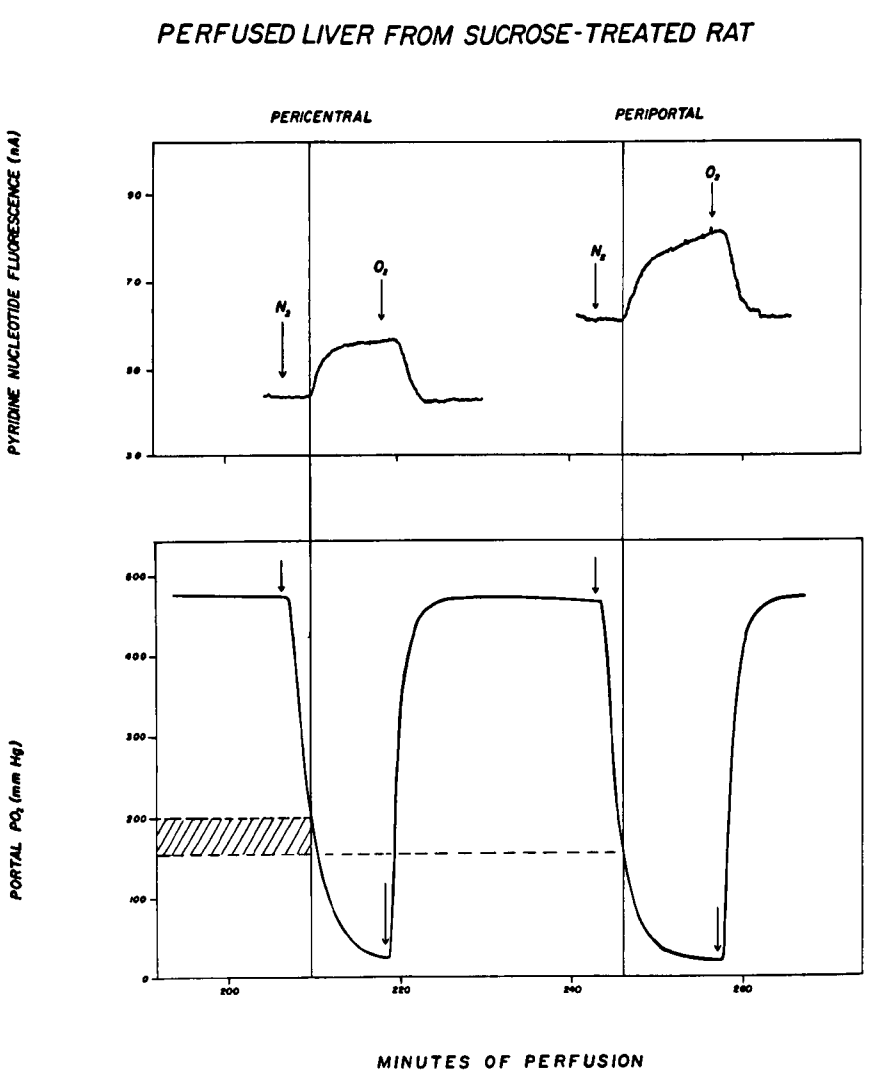

FIG. 1. Dynamic fluorescence responses (upper panel) of the periportal and pericentral areas to anoxic perfusion in liver from sucrose-treated rat. The 170 μ tip micro-light guide [11] was placed on a dark spot (pericentral) or light area (periportal) on the surface of the perfused liver with a flexible mechanical arm. The pressure exerted by the tip of the light guide on the liver surface was minimized by varying the position of the mechanical arm holding the micro-light guide. Arrows indicate that the equilibrating gas mixture was changed either to 95% N_2 and 5% CO_2 (N_2) or to 95% O_2 and 5% CO_2 (O_2). Livers were from female Sprague-Dawley rats which were fed laboratory chow and 25% w/v sucrose in water ad lib prior to perfusion. Influent (lower panel) oxygen tension was monitored continuously with an oxygen electrode. Pyridine nucleotide fluorescence was excited with a mercury arc lamp at 360 ± 50 nm and measured at 450 ± 50 nm [11]. The vertical lines allow the estimation of the input PO_2 value at which pyridine nucleotide reduction begins. A typical representative experiment.

ethanol-treated rats and sucrose-treated controls. Using surface pigmentation as a guide, the micro-light guide was placed in light or dark areas in order to selectively monitor periportal or pericentral pyridine nucleotide fluorescence.

RESULTS

A typical experiment showing the differential responses of pericentral and periportal regions of the liver lobule to a cycle of anoxia is depicted in Fig. 1. The basal fluoresence of pyridine nucleotides was considerably higher in periportal than in pericentral regions of the liver lobule as reported previously [11]. Under normal perfusion conditions, influent PO_2 values were between 400 and 500 torr, and effluent PO_2 values ranged from 100 to 200 torr. When oxygen bubbled into the perfusate was replaced with nitrogen (see arrows in Fig. 1), a rapid decrease in influent PO_2 occurred. The influent PO_2 was 200 torr when pyridine nu-

TABLE 1

EFFECT OF CHRONIC ETHANOL TREATMENT ON INFLOW PO_2 VALUES FOR PYRIDINE NUCLEOTIDE REDUCTION AND ON TISSUE OXYGEN UPTAKE; REVERSAL BY 6-PROPYL-2-THIOURACIL (PTU)

Treatment	Inflow PO_2 at which Pyridine Nucleotide Fluorescence Increased (torr)		Rate of O_2Uptake (μmoles/g/hr)
	Periportal	Pericentral	
Sucrose-treated (7)	181 ± 17	232 ± 16	100 ± 6
Ethanol-treated (14)	179 ± 15	298 ± 18	130 ± 5
(Ethanol + PTU)-treated (7)	131 ± 13	173 ± 10	106 ± 3

Experiments were carried out as described in the legend to Fig. 1. Control rats (5) were treated with sucrose (25% (w/v) in drinking water) for 4 weeks and the ethanol-treated rats (8) with the Porta diet for 5 to 6 weeks. The (ethanol + PTU)-treated rats (3) were first treated with the Porta diet for 6 weeks; PTU (50 mg/kg body weight daily in one ml of corn oil administered by gastric intubation) was then added for 3 to 4 weeks. The numbers in the parentheses in the table represent the number of optical measurements. Means ± SEM.

cleotide fluorescence began to increase in pericentral areas. This means that when the influent PO_2 is 200 torr, a certain population of cells within the field of observation of the micro-light guide became anoxic. After reoxygenation, the anoxic cycle was repeated with the micro-light guide positioned in a periportal area. The first sign of pyridine nucleotide fluorescence increase was detected when the influent PO_2 was about 160 torr in this particular experiment. In livers from sucrose-treated control rats, pericentral areas began to be reduced at the mean influent PO_2 of about 180 torr and periportal areas started to be reduced at the mean influent PO_2 of 230 torr (Table 1).

The experiment depicted in Fig. 1 was repeated in livers from ethanol-treated rats (Fig. 2). The kinetic parameters of the pyridine nucleotide systems were not grossly altered, except that the influent PO_2 at which pyridine nucleotide reduction began in pericentral regions was elevated (Table 1). Chronic ethanol treatment did not significantly affect the influent PO_2 value at which the pyridine nucleotide systems began to be reduced in periportal areas (Table 1). The influent PO_2 at which pericentral pyridine nucleotide systems begin to be reduced represents approximately the oxygen gradient across the length of the sinusoid. We will refer to this gradient as the intralobular oxygen gradient (see Discussion). The chronic ethanol treatment increased the mean estimated intralobular oxygen gradient and the rate of oxygen uptake by 30% (Tables 1 and 2). Treatment of rats with the antithyroid drug, 6-propyl-2-thiouracil, abolished the ethanol-induced increase in the rate of oxygen uptake and decreased the intralobular oxygen gradient (Tables 1 and 2).

DISCUSSION

Estimation of the Intralobular Oxygen Gradient by Pyridine Nucleotide Fluorometry Using the Micro-Light Guide

The pyridine nucleotide fluorescence technique introduced by Chance and coworkers [4] can be employed to monitor tissue oxygen tension indirectly. This technique is based on the fact that intracellular reduced pyridine nucleotides (NADH) are fluorescent near 450 nm when illuminated with light at 360 nm. The pyridine nucleotide fluorescence is dependent on the intracellular oxygen tension, because this coenzyme is oxidized predominantly via the mitochondrial electron transport chain which has an extremely high affinity for oxygen; the concentration of oxygen required for half-maximal respiration in isolated mitochondria is much less than 1 μM [3,14]. Thus, if mitochondria in tissue behave similarly to isolated mitochondria, then an increase in pyridine nucleotide fluorescence at a spot in tissue can be equated with a virtual absence of oxygen there. When we position a micro-light guide in periportal or pericentral regions for measuring pyridine nucleotide fluorescence, the fluorescence signal observed by the micro-light guide originates from either the first portion (toward the arterial end) or the second portion (toward the venous end) of the sinusoid, respectively.

Of the cells in the periportal part of the sinusoid, it is those cells toward the middle of the sinusoid which will become anoxic first when the influent PO_2 is lowered, since these are the periportal cells most distal from oxygen source. Therefore, the PO_2 at which the first sign of pyridine nucleotide reduction occurs in periportal regions can be regarded as an approximate oxygen gradient existing between the arterial end to the midportion of the sinusoid. We will refer to this as the periportal oxygen gradient. In coming to this conclusion, we have assumed that little or no oxygen is extracted from the perfusate as it flows through the portal vein to the terminal portal venule so that the PO_2 at the arterial end of the sinusoid is more or less equal to the influent PO_2. Similarly, the PO_2 at which the first sign of pyridine nucleotide reduction occurs in pericentral regions can be equated with the oxygen gradient spanning across the whole length of the sinusoid, namely the intralobular oxygen gradient. The difference between the intralobular oxygen gradient and the periportal one is the gradient established in pericentral regions. This will be referred to as the pericentral oxygen gradient. These oxygen gradients were estimated from the data in Table 1 and are displayed in Table 2.

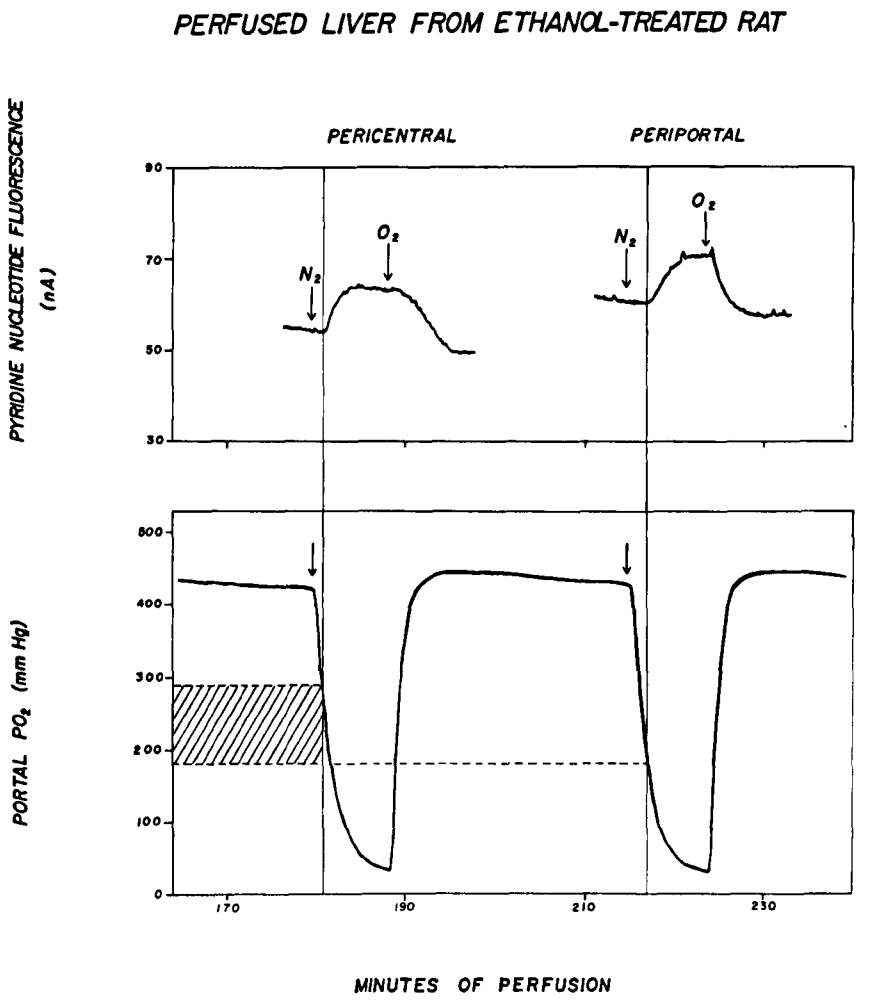

FIG. 2. Measurement of the input PO_2 values corresponding to initial pyridine nucleotide reduction in periportal or pericentral regions of the liver from a rat treated chronically with ethanol. Sprague-Dawley female rats (200 to 250 g) received laboratory chow, 25% w/v sucrose and 25% w/v ethanol in water for 4 to 6 weeks. A typical experiment.

TABLE 2

ESTIMATION OF INTRALOBULAR, PERIPORTAL AND PERICENTRAL OXYGEN GRADIENTS IN THE LIVER

	Oxygen Gradients, torr		
Treatment	Intralobular	Periportal	Pericentral
Sucrose-treated	232 ± 16	181 ± 17	51 ± 7
Ethanol-treated	298 ± 18	179 ± 15	119 ± 10
(Ethanol + PTU)-treated	173 ± 10	131 ± 13	42 ± 8

The oxygen gradients were obtained from the following relations: Intralobular O_2 gradient = Influent PO_2 at which pyridine nucleotide fluorescence began to increase in pericentral regions; Periportal O_2 Gradient = Influent PO_2 at which pyridine nucleotide fluorescence began to increase in periportal regions; Pericentral O_2 Gradient = Intralobular O_2 Gradient − Periportal O_2 Gradient. Data from Table 1.

Effect of Ethanol Treatment on the Intralobular Oxygen Gradient

Israel *et al.* [1,22] demonstrated that chronic treatment with ethanol increased the oxygen uptake of liver slices, a finding which was confirmed in the perfused rat liver [20]. This effect is not observed, however, in older rats (>400 g) [17]. Yuki and Thurman [23] have recently shown that enhanced hepatic oxygen uptake can occur very rapidly (in 2–3 hours) and is due, in part, to diminished ATP synthesis via glycolysis.

Israel *et al.* [6] demonstrated that ethanol-treated rats are more susceptible to liver damage when exposed to hypoxia or ischemic shock than controls. They concluded that the elevated oxygen uptake by hepatocytes resulting from ethanol treatment accentuated the O_2 gradient and rendered the pericentral region of the liver lobule hypoxic.

In the present studies, the average intralobular oxygen gradient was estimated to be about 230 torr in sucrose control livers and 300 torr in ethanol-treated livers (Table 2). Thus, chronic treatment with ethanol increased the mean intralobular oxygen gradient by 30%. This increase in the intralobular oxygen gradient was accompanied by a 30% increase in the rate of oxygen uptake (Table 1). Chronic ethanol treatment did not affect the periportal oxygen gradient but more than doubled the pericentral oxygen gradient (Table 2), suggesting that the ethanol-induced increase in tissue respiration is confined to the pericentral region of the liver lobule. The equal increase in oxygen uptake and the mean intralobular oxygen gradient induced by ethanol is consistent with the theoretical prediction of a linear relationship between the longitudinal oxygen gradient and the rate of tissue respiration [2,12].

Treatment of rats with PTU completely abolished both the enhanced intralobular oxygen gradient and the increased rate of O_2 uptake in perfused livers caused by chronic ethanol treatment (Tables 1 and 2). Also, PTU decreased both the periportal and pericentral oxygen gradients whereas ethanol appears to have a specific effect on pericentral tissue (Table 2).

Thus, the experimental data presented above provide the first physical evidence that the increased oxygen uptake which results from chronic treatment with ethanol indeed causes an increase in the intralobular oxygen gradient as postulated by Israel *et al.* [6,7]. It is possible that the ethanol-induced accentuation of the intralobular oxygen gradient is ultimately responsible for pericentral liver injury due to ethanol.

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