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Rates of Alcohol Dehydrogenase-Dependent Ethanol Metaoblism in Periportal and Pericentral Regions of the Perfused Rat Liver

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SUMMARY

Infusion of ethanol into hemoglobin-free perfused rat liver caused an increase in NADH fluorescence (366 → 450 nm) which was measured with a large-tip (2-mm) light guide placed on the surface of the liver. A linear correlation ($r = 0.83$) was observed between the increase in NADH fluorescence and rate of ethanol uptake in the concentration range 0.05–2.0 mM. When a micro-light guide (tip diameter 170 μm) was placed on periportal or pericentral regions of the liver surface, the maximal fluorescence increase due to ethanol (2 mM) was 31.2 ± 2.0 and 31.9 ± 1.7% in periportal and pericentral regions, respectively. The infusion of 4-methylpyrazole (80 μM), an inhibitor of alcohol dehydrogenase, completely abolished the fluorescence increase in both regions, indicating that the changes are entirely attributable to perturbation of cofactor levels due to alcohol dehydrogenase-dependent ethanol metabolism. Using the correlation between the NADH fluorescence increase and rate of ethanol uptake, rates of ethanol metabolism in periportal and pericentral regions were calculated. Values for maximal ethanol uptake were identical in periportal and pericentral regions. Half-maximal ethanol uptake was observed at 0.24 and 0.25 mM ethanol in periportal and pericentral regions, respectively. These results indicate that the rates of alcohol dehydrogenase-dependent ethanol metabolism are similar in periportal and pericentral regions of the liver lobule.

INTRODUCTION

It is well known that some enzymes are distributed unevenly over the liver lobule (1). For example, periportal regions have a higher concentration of glycogen and mitochondria than pericentral areas. Oxygen tension is greater in periportal than in pericentral regions, and key enzymes involved in gluconeogenesis and glycolysis are greatest in periportal and pericentral tissues, respectively (2).

Alcohol dehydrogenase activity has been shown histochemically to be higher in periportal than in pericentral tissue (3). By contrast, NAD^+ and NADH, important determinants of rates of alcohol dehydrogenase-dependent ethanol metabolism, are equally distributed in the liver lobule[4] (4). At present, it is not clear whether enzyme activity or cofactor supply predominates as the regulating factor in ethanol metabolism (5–10) in liver. Furthermore, the question of rates of ethanol metabolism in periportal and pericentral regions of the liver lobule has not been addressed.

Such a question can now be reasonably asked, since techniques have been developed to detect NADH fluorescence in distinct periportal and pericentral regions of the liver (11, 12). This technique involves the placement of micro-light guides consisting of two strands of optical fibers on dark spots (pericentral regions) and light areas (periportal areas) of the liver. Pyridine nucleotide fluorescence can be excited via light conducted to the liver surface with one fiber, and fluorescence can be detected with the other fiber. With this technique, oxygen gradients confined within a liver lobule have been monitored indirectly (13), and rates of mixed-function oxidation and conjugation reactions have been quantitated in the two regions (14, 15).

The purpose of the experiments described below was to assess whether or not ethanol metabolism could be determined in periportal and pericentral regions of the liver on the basis of changes in NADH fluorescence. The data indicate that local rates of ethanol metabolism are similar in periportal and pericentral regions of the liver, consistent with the hypothesis that cofactor supply is a more important determinant of ethanol metabolism than is alcohol dehydrogenase activity. Preliminary accounts of this work have appeared elsewhere (16).

METHODS

Animals and liver perfusion. Sprague-Dawley female rats (250–350 g) maintained on laboratory chow ad libi-

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tum were used in the study. Livers were perfused with Krebs-Henseleit bicarbonate buffer (pH 7.4, 37°) in a nonrecirculating system as described previously (17). Perfusate was pumped into the liver via a cannula inserted in the portal vein, and effluent perfusate was collected with a cannula placed in the interior vena cava. Effluent perfusate flowed past an oxygen electrode before it was discarded.

Micro-light guide. A micro-light guide with a 170-μm tip has been employed previously for determination of NADH fluorescence (11–14). To construct a tip small enough to detect NADH fluorescence selectively in the periportal and pericentral regions of the liver lobule, two loose fibers were held together at the tip with epoxy glue. The resulting two-fiber light guide possessed an excitation-collection tip of ~170 μm diameter, much smaller than the average diameter of the liver lobule (~500 μm). The measured fluorescence arises from that portion of the tissue which is both illuminated by the excitation light and in the field of view of the collection fiber (11).

Light areas and dark spots of 300–500 μm diameter could be detected on the surface of the hemoglobin-free perfused liver with the naked eye or with a low-power dissection microscope (12). When India ink was perfused into the liver via the portal vein, the light areas surrounding the dark spots was stained first. This identified the light areas as periportal regions. In contrast, retrograde perfusion via the vena cava stained the dark spots first and hence identified them as pericentral regions. Thus, the natural distribution of liver pigments could be used to identify periportal and pericentral areas of the liver lobule (12).

Detection of NAD(P)H fluorescence. After the two-strand micro-light guide was placed on a periportal or pericentral region of the liver, one strand was connected to a near-ultraviolet light source, and the other strand to a photomultiplier (12). In some experiments, a large-tipped light guide (tip diameter 2 mm) was employed. The liver was illuminated with the 366-nm mercury arc line, and the NADH fluorescence (450 nm) of the tissue was detected by the photomultiplier, amplified and recorded as described elsewhere (18).

Ethanol uptake. Ethanol was determined in effluent perfusate samples enzymatically (19). Ethanol uptake was calculated from inflow-outflow concentration differences, the flow rate, and the liver wet weight.

Materials. 4-Methylpyrazole was purchased from Sigma Chemical Company (St. Louis, Mo.). All other chemicals were reagent-grade from standard commercial sources.

RESULTS

Correlation between NADH fluorescence and ethanol uptake. In order to correlate changes in NADH fluorescence with rates of ethanol uptake by the perfused rat liver, a large-tip light guide was placed on the liver surface. This light guide excites and collects fluorescence from many liver lobules.

Ethanol was infused in a stepwise fashion from 0.05 to 5.0 mM (Fig. 1). Under these conditions, stepwise increases in NADH fluorescence were observed as well as ethanol uptake by the liver (Fig. 1). When rates of ethanol uptake were plotted as a function of the change in NADH fluorescence, a good correlation ($r = 0.83$) was obtained (Fig. 2).

4-Methylpyrazole sensitivity of fluorescence changes. Micro-light guides were placed on periportal and pericentral regions of a liver perfused in the anterograde (i.e., via the portal vein) direction. When the gas was changed from one containing oxygen to one containing nitrogen, reduction in pyridine nucleotides (e.g., an increase in fluorescence) was observed in both periportal and peri-

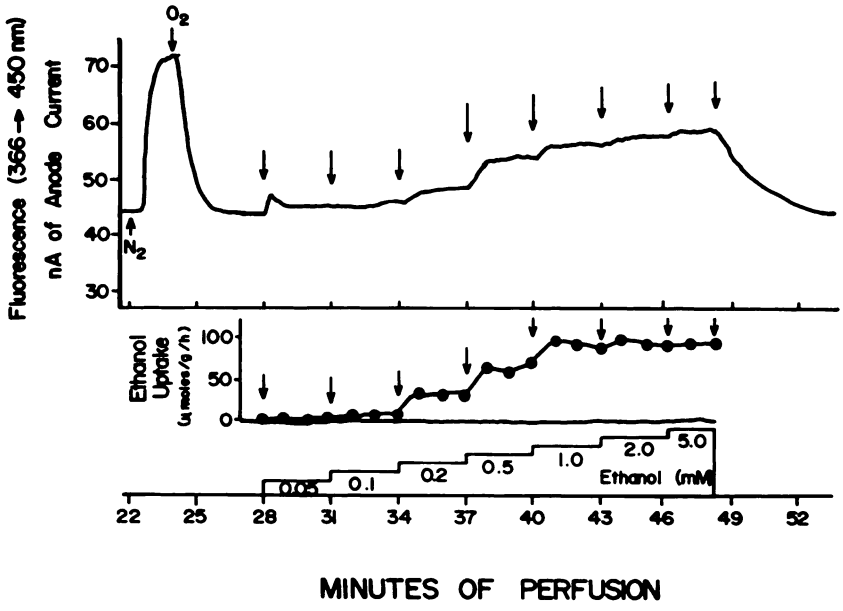

FIG. 1. *Effect of ethanol on hepatic NADH fluorescence and ethanol uptake*

Livers were perfused with Krebs-Henseleit bicarbonate buffer (pH 7.6, 37°) in a nonrecirculating system. A large-tip light guide (tip diameter 2 mm) was placed on the surface of the liver, and NADH fluorescence (366 → 450 nm) was determined. Ethanol was infused in increments from 0.5 to 5.0 mM using a precision infusion pump as indicated by the *horizontal bars*. Ethanol was determined in effluent samples enzymatically (19), and ethanol uptake was calculated from inflow-outflow concentration differences, the flow rate, and the liver wet weight.

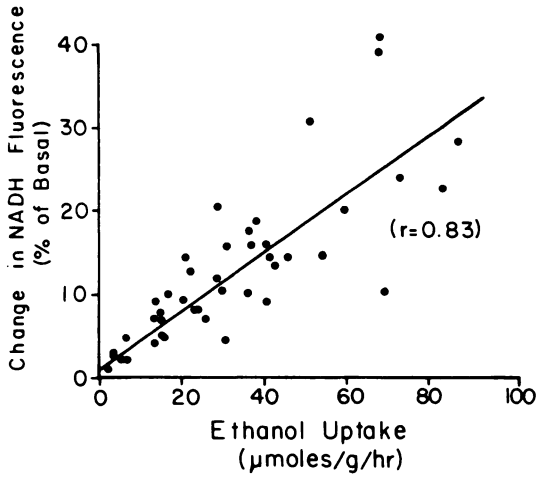

FIG. 2. *Relationship between NADH fluorescence and ethanol uptake*

Experimental conditions as in Fig. 1. Fluorescence change was expressed as a percentage of the basal fluorescence. The best-fit linear regression line was obtained from data from eight livers.

central regions of the liver lobule (Fig. 3). When the oxygen was restored, basal levels of fluorescence were re-established rapidly. The subsequent infusion of ethanol (2 mM) also produced near-maximal reduction of pyridine nucleotides in periportal as well as pericentral tissues. The infusion of 4-methylpyrazole (80 μM), an inhibitor of alcohol dehydrogenase, completely abolished the increase in fluorescence due to ethanol (Fig. 3). This indicates that the fluorescence changes observed are totally due to alcohol dehydrogenase-dependent ethanol metabolism.

Determination of rates of ethanol metabolism in periportal and pericentral tissues. When ethanol was infused in steps (0.05–2.0 mM), NADH fluorescence detected with micro-light guides placed on pericentral and periportal areas began to increase in both regions with 0.1 mM ethanol (Fig. 4). Fluorescence changes were half-maximal at 0.18 mM and 0.38 mM in periportal and pericentral regions, respectively. When the ethanol infusion was terminated, NADH fluorescence in both regions returned to baseline (Fig. 4). Maximal increases in fluorescence produced by ethanol were similar in both periportal and pericentral regions (Table 1). When the fluorescence increases were converted into local rates of ethanol uptake, employing the linear correlations between ethanol uptake and fluorescence change observed with the large-tip light guide (Fig. 2), rates of ethanol uptake were identical in periportal and pericentral regions of the liver (Table 1).

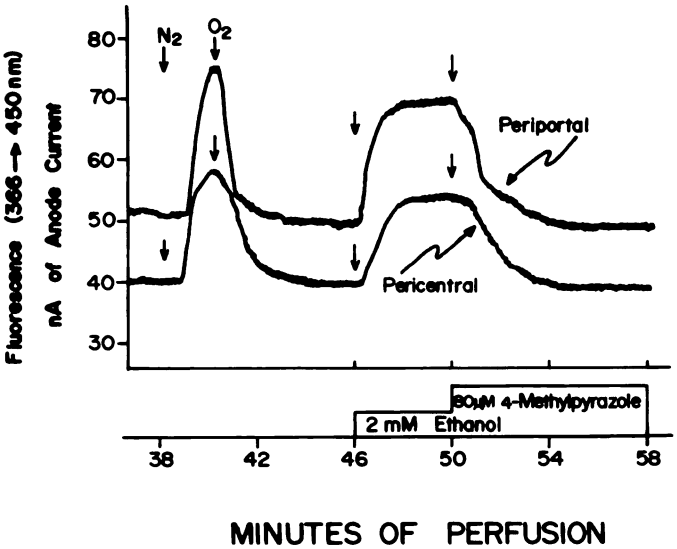

FIG. 3. *Effect of 4-methylpyrazole on ethanol-induced NADH fluorescence increase*

Micro-light guides (tip diameter 170 μm), were placed on periportal and pericentral regions of a liver perfused in the anterograde direction. NADH fluorescence was determined as described under Methods. After 4 min of ethanol infusion (2 mM), 4-methylpyrazole (80 μM) was infused as directed by the *horizontal bars*.

Because ethanol enters the liver via the portal vein and is converted into acetate as it passes the sinusoid, one would not expect pericentral regions to receive the same concentrations of ethanol as the periportal regions during anterograde perfusions. Thus, the result obtained above (Fig. 4; Table 1) could reflect higher rates of ethanol uptake at lower ethanol concentrations by pericentral tissues. To examine this possibility, ethanol was infused in the retrograde (i.e., via the vena cava) direction. Under these conditions, fluorescence began to increase with 0.05 and 0.2 mM ethanol in pericentral and periportal tissue, respectively (Fig. 5). Fluorescence increases were half-maximal with 0.25 and 0.43 mM ethanol in pericentral and periportal regions, respectively. Both regions appeared to be saturated with 2.0 mM ethanol.

When the increase in fluorescence was plotted as a function of ethanol concentration during anterograde perfusion for periportal tissues and retrograde perfusion for pericentral areas, the data in Fig. 6 were obtained. The ethanol concentrations needed for half-maximal fluorescence changes were about 0.25 mM in both regions (Table 2). In addition, similar maximal fluorescence changes were observed in both regions (Table 2), allowing the calculation of similar rates of ethanol uptake in periportal and pericentral regions of the liver lobule.

DISCUSSION

Surface fluorescence of NADH. Chance and co-workers (20) have clearly established in the last two decades that insight into metabolic events can be obtained non-invasively and continuously from the surface of living tissues by monitoring intracellular pigment oxidation-reduction states. One of the most useful pigments is NADH, which fluoresces upon excitation with light at 366 nm. The fluorescence correlates with intracellular changes in NADH content (20), and increases markedly when NAD^+ is converted into NADH during ethanol metabolism via alcohol dehydrogenase (21). This increase in NADH occurs in both the cytosol and mitochondrial spaces. In the present study, a linear relationship between NADH fluorescence increase and ethanol uptake was observed (Figs. 1 and 2).

Metabolic heterogeneity in the liver. There is a growing body of evidence which indicates that the liver is metabolically and functionally inhomogeneous (1). Most previous studies have focused on the histochemical distribution of enzymes or on morphological differences.

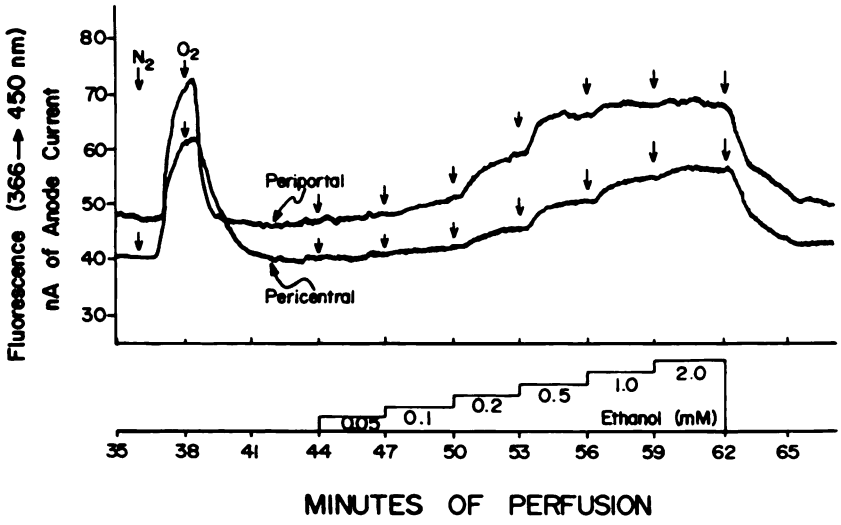

FIG. 4. *Titration of NAD(P)H fluorescence in periportal and pericentral regions with ethanol*
Experimental conditions as in Fig. 3 except that ethanol was infused in concentration steps (0.05–2.0 mM) as indicated by the *horizontal bars*.

TABLE 1

Maximal rates of ethanol uptake in periportal and pericentral regions of the liver lobule

Maximal increase of NADH fluorescence was obtained following the infusion of 2 mM ethanol in anterograde fashion using micro-light guides placed on periportal and pericentral tissues. Maximal rates of ethanol uptake were estimated by converting the fluorescence increase to ethanol uptake using the linear correlation between NADH fluorescence and ethanol uptake (Fig. 2). Each value represents the mean ± standard error of the mean of 16 experiments.

	Periportal	Pericentral
Maximal increase of fluorescence (% of basal)	31.2 ± 2.0	31.9 ± 1.7
Estimated maximal rate of ethanol uptake (μmoles/g/hr)	87.2 ± 5.8	89.2 ± 5.0

Little information exists on possible differences in metabolic rates in periportal and pericentral regions of the liver. For example, Jungermann *et al.* (2), on the basis of microhistochemical distribution of enzymes, have suggested that periportal hepatocytes are preferentially gluconeogenic whereas hepatocytes located near the central vein are primarily glycolytic. However, direct evidence that carbohydrate metabolism in fact differs in periportal and pericentral regions in intact liver is lacking. By employing micro-light guides, it has recently been possible for the first time to determine rates of metabolic processes (e.g., mixed-function oxidation) in periportal and pericentral regions of the liver after making some reasonable assumptions (14). This method is based on the fact that nonfluorescent 7-ethoxycoumarin is metabolized to highly fluorescent 7-hydroxycoumarin by he-

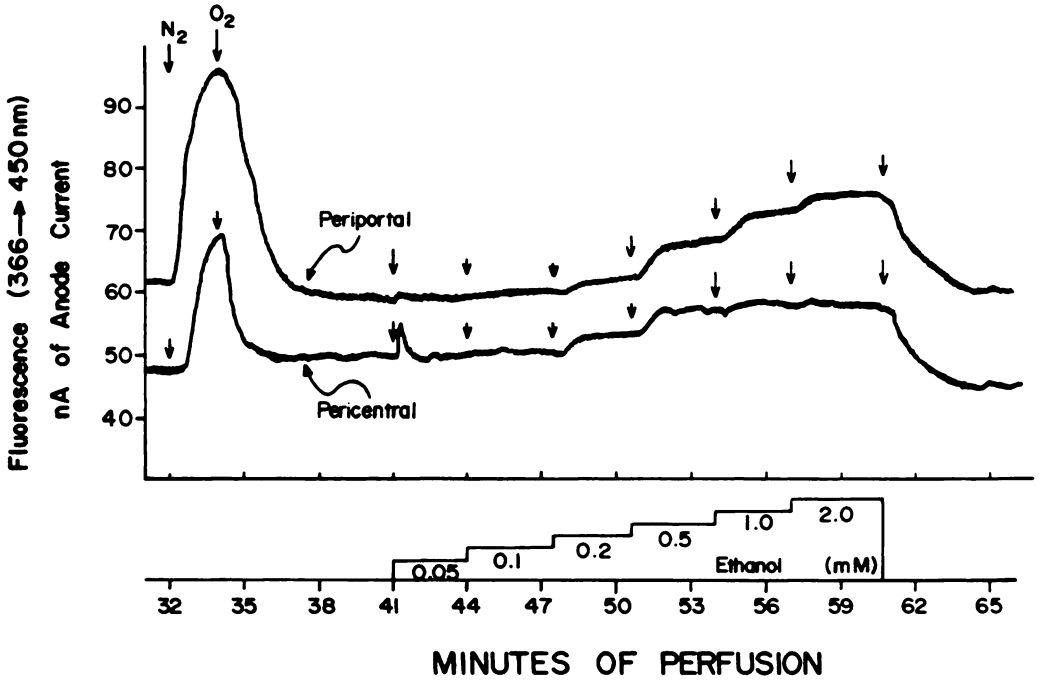

FIG. 5. *Fluorescence of NADH in periportal and pericentral regions of liver during retrograde perfusion of ethanol*
Experimental conditions as in Fig. 4, except that the liver was perfused in retrograde fashion.

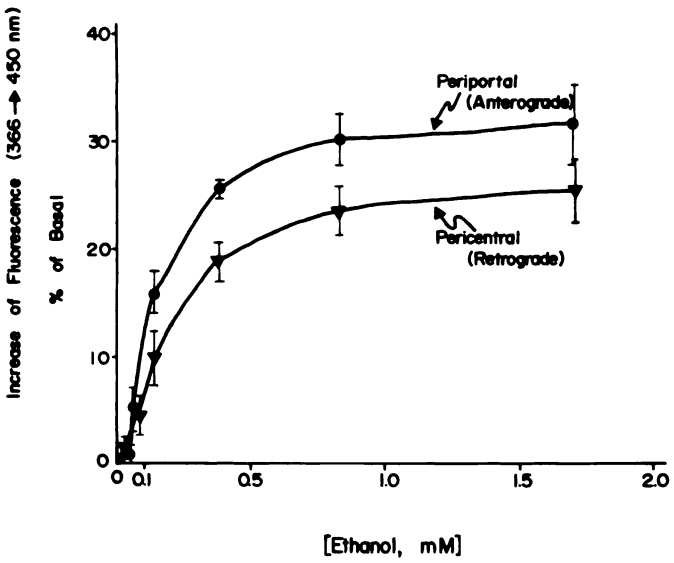

FIG. 6. *Dependence of NADH fluorescence on ethanol concentration in periportal and pericentral regions*

Data from four anterograde perfusions were used to determine ethanol-induced NADH fluorescence changes in periportal regions. Similarly, pericentral data were obtained from retrograde perfusion. The ethanol concentrations in periportal and pericentral regions were calculated by the following equation: [inflow] − ([inflow] − [outflow]/4). This equation estimates the ethanol concentration one-fourth of the way through the liver lobule, assuming a linear distribution of ethanol through the sinusoid. Each point represents the mean ± standard error of the mean.

patic mixed-function oxidases. First, a large-tipped (2 mm) light guide was placed on the surface of the liver, and 7-ethoxycoumarin was infused into the perfused liver in increasing concentration steps. Subsequently, the rate of 7-hydroxycoumarin formed by the liver was correlated with the 7-hydroxycoumarin fluorescence detected from the liver surface. A linear correlation between these parameters was observed (14). The authors then assumed that this relationship could be used as a calibration curve for any periportal or pericentral region on the liver surface. When the experiments were repeated with micro-light guides placed on sublobular regions, it was observed that mixed-function oxidation was about twice as great in pericentral hepatocytes than in periportal hepatocytes. Subsequently, Conway *et al.* (15) used a modification of this technique to define rates of sulfation and glucuronidation in different regions of the liver lobule. Thus, local measurements of rates of metabolic processes are now possible in periportal and pericentral regions of the liver.

TABLE 2

Rates of periportal and pericentral ethanol uptake

Ethanol concentration at half-maximal increase of fluorescence and maximal increase of fluorescence were determined by reciprocal transformation and averaged. Maximal rates of ethanol uptake were estimated as in Table 1. Periportal data are from anterograde perfusions, whereas pericentral data are from retrograde perfusions (see Fig. 6). Each value represents the mean ± standard error of the mean of four experiments.

	Periportal	Pericentral
Ethanol concentration at half-maximal increase of fluorescence (mM)	0.24 ± 0.11	0.25 ± 0.08
Maximal increase of fluorescence (% of basal)	39.4 ± 7.4	30.0 ± 2.7
Estimated maximal rate of ethanol uptake (μmoles/g/h)	111.1 ± 21.6	83.7 ± 7.9

Periportal and pericentral ethanol metabolism. It is very important to know the lobular localization of ethanol metabolism in liver, since ethanol-induced liver damage occurs predominantly in pericentral tissues (22). Because oxygen tension is lower in pericentral hepatocytes than in periportal hepatocytes and because ethanol treatment increases oxygen uptake, Israel *et al.* (23) have implicated pericentral hypoxia in the mechanism of ethanol-induced liver damage. On the basis of increases in NADH fluorescence when oxygen was removed, Ji *et al.* (13) have estimated the lobular oxygen gradient. In support of the hypoxia hypothesis (23), they demonstrated that the intralobular oxygen gradient was increased by chronic treatment with ethanol.

In the present studies, the approach to determining local rates of mixed-function oxidation described above was applied to ethanol metabolism utilizing NADH fluorescence. First, a linear correlation between rates of ethanol uptake and NADH fluorescence increase was established with a large-tip light guide (Figs. 1 and 2). This correlation was then used to determine rates from fluorescence changes detected from periportal and pericentral regions with the micro-light guides (Tables 1 and 2). The data indicate that ethanol metabolism can indeed be measured in this manner, and that rates are similar in both periportal and pericentral regions of the liver lobule (Tables 1 and 2).

Regulation of hepatic alcohol dehydrogenase-dependent ethanol metabolism. Factors governing ethanol metabolism are still controversial. Some laboratories have stressed that alcohol dehydrogenase activity is limiting (5, 6), whereas considerable evidence has accumulated in support of cofactor limitation (7–10). In the liver lobule, alcohol dehydrogenase is in higher concentration in periportal regions than in pericentral regions (3). In contrast, NAD^+ and NADH are equally distributed over the lobule[4] (4). Thus, the fact that rates of ethanol metabolism followed NAD^+ rather than alcohol dehydrogenase distribution over the liver lobules supports the hypothesis that the availability of NAD^+ is a more important regulating factor than is enzyme.

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