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Metabolic Heterogeneity in the Perfused Rat Liver[1]

R. G. THURMAN,*[2] F. C. KAUFFMAN,† S. JI,*[3] J. J. LEMASTERS,‡
J. G. CONWAY,* S. A. BELINSKY,* T. KASHIWAGI* AND T. MATSAMURA*

*Departments of *Pharmacology and ‡Anatomy, University of North Carolina, Chapel Hill, NC and †Department of Pharmacology, University of Maryland, College Park, MD*

THURMAN, R. G., F. C. KAUFFMAN, S. JI, J. J. LEMASTERS, J. G. CONWAY, S. A. BELINSKY, T. KASHIWAGI AND T. MATSAMURA. *Metabolic heterogeneity in the perfused rat liver.* PHARMACOL BIOCHEM BEHAV **18:** Suppl. 1, 415–419, 1983.—New methods have been developed to monitor metabolic events non-invasively within periportal and pericentral regions of perfused rat livers. These techniques utilize two-fiber micro-light guides and miniature oxygen electrodes positioned on identified lobular regions of the perfused liver based on differential pigmentation of periportal and pericentral areas. Two-fiber micro-light guides detect the fluorescence of native and introduced fluors and are used to monitor redox changes of endogenous pyridine nucleotides and the generation of fluorescent products (e.g., 7-hydroxycoumarin) from exogenous substrates. Changes in fluorescence detected with two-fiber micro-light guides are correlated with changes measured with large, multi-fiber light guides and with whole organ rates of metabolism. Subsequently, local rates are estimated. With these techniques, we show that (a) rates of ethanol and acetaldehyde metabolism are similar in periportal and pericentral regions of the liver lobule; (b) mixed-function oxidation predominantes in pericentral regions in livers from phenobarbital-treated rats; (c) rates of sulfation of 7-hydroxycoumarin are greater in periportal than in pericentral hepatocytes; and (d) oxygen uptake is approximately 3-fold greater in periportal than in pericentral areas of the liver lobule.

Metabolic heterogeneity Perfused liver Rat Redox monitoring system

IT is well known that some enzymes and metabolites are distributed unevenly within the liver lobule [21]. For example, periportal regions have a higher concentration of glycogen, oxygen and mitochondrial enzymes than pericentral areas. In general, key enzymes involved in gluconeogenesis are more abundant in periportal while key enzymes in glycolysis are relatively more concentrated in pericentral regions [14].

The claim that alcohol dehydrogenase activity is higher in periportal than in pericentral tissue is controversial [5,20]. NAD^+ and NADH , important determinants of rates of alcohol dehydrogenase-dependent ethanol metabolism, are equally distributed within the liver lobule [19]. At present, it is not clear whether enzyme concentration or cofactor supply predominates in regulating ethanol metabolism in the liver [4,23].

The question of rates of ethanol metabolism in periportal and pericentral regions of the liver lobule can now be addressed, since techniques have been developed to monitor NADH fluorescence continuously in distinct periportal and pericentral regions of the perfused liver [9,11]. This technique involves the placement of two-fiber micro-light guides on dark (pericentral) and light (periportal) regions of the liver surface. One glass optical fiber of the two-fiber micro-light guide conducts excitation light to the liver surface, and the other fiber collects emitted fluorescence. Identification of periportal and pericentral regions by differences in pigmentation can be used to study oxygen gradients confined within specific zones of the liver lobule [3, 10, 12, 13]. Rates of mixed-function oxidation and conjugation reactions can also be quantitated in the two regions using this approach.

The purpose of the experiments described below was to study rates of ethanol and acetaldehyde metabolism in periportal and pericentral regions of the liver on the basis of changes in NADH fluorescence. The data indicate that rates of NAD^+-linked ethanol and acetaldehyde metabolism are similar in periportal amd pericentral regions of the liver while other metabolic events, e.g., oxygen gradients, mixed-function oxidation and conjugation reactions differ between the regions.

METHOD

Animals and Liver Perfusion

Sprague-Dawley female rats (250–350 g) maintained on laboratory chow ad lib were used in the study. Livers were

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[2]Requests for reprints should be addressed to R. G. Thurman, Department of Pharmacology, University of North Carolina, Chapel Hill, NC 27514.

[3]Present address: Department of Pharmacology and Toxicology, College of Pharmacy, Rutgers University.

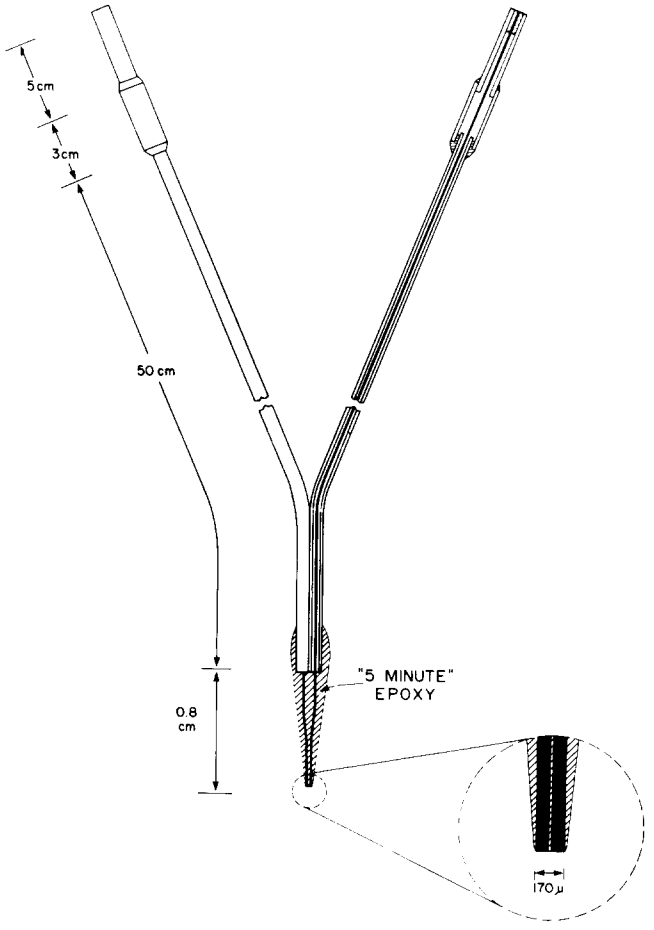

FIG. 1. Schematic representation of the micro-light guide. The micro-light guide was fabricated essentially as in [9], except that the two loose glass fibers were glued together at the tip without any steel tubing. The tip was cut with a sharp razor blade so that the cut cross-sections of the fibers were smooth and parallel.

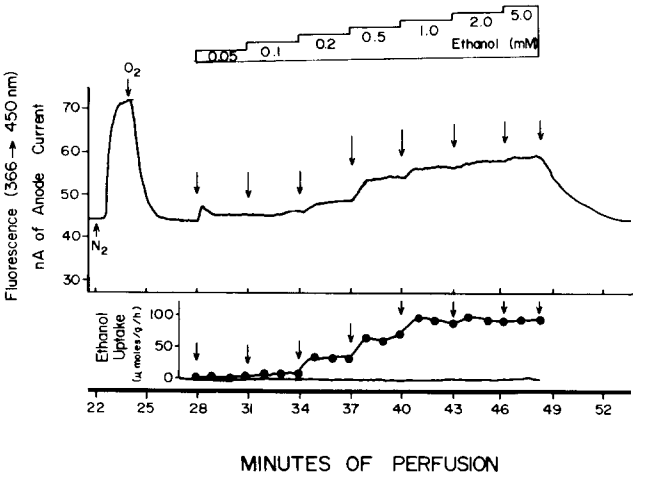

FIG. 2. Effect of ethanol on hepatic NADH fluorescence and ethanol uptake. Livers were perfused with Krebs-Henseleit bicarbonate buffer (pH 7.4, 37°) in a nonrecirculating system. A large-tipped light guide (tip diameter=2 mm) was placed on the surface of the liver, and NADH fluorescence (366→450 nm) 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 [1], and ethanol uptake was calculated from inflow-outflow concentration differences, the flow rate, and the liver wet weight.

perfused with Krebs-Henseleit bicarbonate buffer (pH 7.4, 37°) in a nonrecirculating system as described previously [22]. 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 inferior vena cava. Effluent perfusate flowed past an oxygen electrode before it was discarded.

Micro-Light Guide

A micro-light guide (Fig. 1) has been employed previously for determination of NADH fluorescence [9, 10, 11, 13]. To construct a tip small enough to detect NADH fluorescence selectively on periportal and pericentral regions of the liver lobule, two loose fibers were glued together with epoxy. The resulting two-fiber light guide possessed an excitation-collection tip of 170 μm diameter, smaller than the average diameter of the liver lobule (~1 mm). The measured fluorescence arose from that portion of the tissue which was 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 were present on the surface of the hemoglobin-free perfused liver [11,13]. When India ink was infused into the liver via the portal vein, the light areas surrounding the dark spots were stained first. This identified these light areas as periportal regions. In contrast, retrograde infusion 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 [11,13].

Detection of NADH Fluorescence

One optical fiber of the two-fiber micro-light guide was connected to a near-ultraviolet light source, and the other to a photomultiplier [9]. In some experiments, a large, multi-fiber 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 with a photomultiplier, amplified and recorded as described elsewhere [2].

Ethanol and Acetaldehyde Uptake

Ethanol or acetaldehyde were determined enzymatically in samples of effluent perfusate [1]. 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.

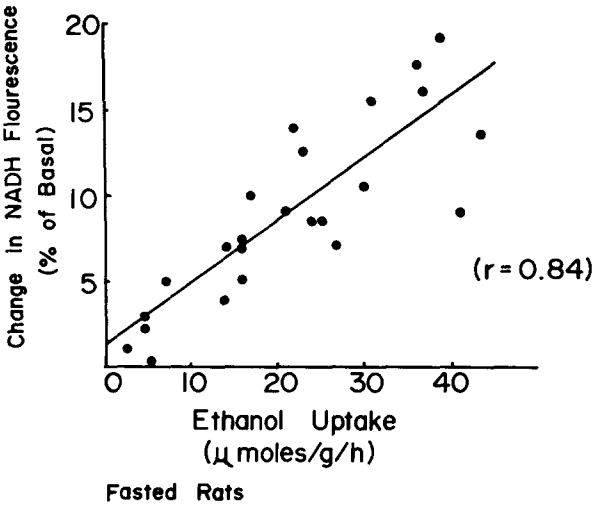

FIG. 3. Relationship between NADH fluorescence and ethanol uptake. Experimental conditions as in Fig. 2. Fluorescence changes were expressed as a percentage of the basal fluorescence. The best-fit linear regression line was obtained from data from eight livers.

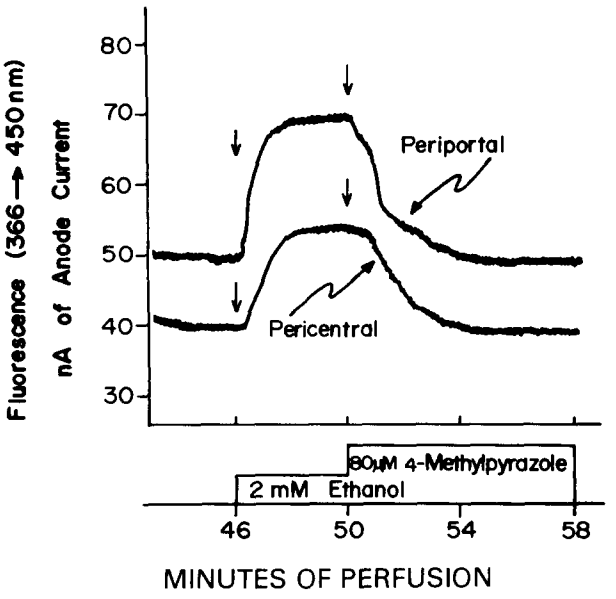

FIG. 4. Effect of 4-methylpyrazole on ethanol-induced NADH fluorescence increase. Micro-light guides were placed on periportal and pericentral regions of a liver perfused in the anterograde direction. NADH fluorescence was determined as described under Methods. After 4 min of ethanol infusion (2 mM),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

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-tipped light guide was placed on the liver surface. The large 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. 2). Under these conditions, stepwise increases in NADH fluorescence and ethanol uptake were observed. When rates of ethanol uptake were plotted as a function of the change in NADH fluorescence, a good correlation was obtained (Fig. 3).

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. The infusion of ethanol (2 mM) produced near-maximal reduction of pyridine nucleotides in periportal as well as pericentral tissues (Fig. 4). The infusion of 4-methylpyrazole (80 μm), an inhibitor of alcohol dehydrogenase, completely abolished the increase in fluorescence due to ethanol (Fig. 4). This indicates that the fluorescence changes observed are 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. When the ethanol infusion was terminated, NADH fluorescence in both regions returned to baseline. Maximal increases in fluorescence produced by ethanol were similar in both periportal and pericentral regions. When fluorescence increases were converted into local rates of ethanol uptake employing the linear correlations between ethanol uptake and fluorescence changes observed with the large, multi-fiber guide (Fig. 3), similar rates of ethanol uptake were calculated for periportal and pericentral regions of the liver (Table 1).

Local Rates of Acetaldehyde Uptake

In an analogous series of experiments where acetaldehyde (up to 4 mM) was infused in the presence of 4-methylpyrazole [16], identical local rates of acetaldehyde uptake were calculated (Table 1).

Local Rates of Mixed-Function Oxidation and Conjugation

By employing micro-light guides, it has also been possible in separate studies for the first time to determine rates of mixed-function oxidation in periportal and pericentral regions of the liver [12]. This method is based on the metabolism of nonfluorescent 7-ethoxycoumarin to the highly fluorescent 7-hydroxycoumarin by hepatic mixed-function oxidases. As above, 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 steps of increasing concentration. 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 [12]. By assuming that this relationship can be used as a calibration curve, local rates were calculated from data obtained with micro-light guides placed on sublobular regions. Mixed-function oxidation was about twice as great in pericentral as in periportal regions of livers from phenobarbital-treated rats. Using a modification of this

TABLE 1

MAXIMAL RATES OF ETHANOL AND ACETALDEHYDE UPTAKE IN PERIPORTAL AND PERICENTRAL REGIONS OF THE LIVER LOBULE

μmoles/g/hr	Periportal	Pericentral
Ethanol Uptake	87 ± 6	89 ± 5
Acetaldehyde Uptake	212 ± 91	198 ± 56

Maximal increase of NADH fluorescence was obtained following the infusion of 2 mM ethanol or 4 mM acetaldehyde using micro-light guides placed on periportal and pericentral tissues. Acetaldehyde was studied in the presence of 0.08 mM 4-methylpyrazole. 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. 3). Each value represents the mean ± standard error of the mean of 16 experiments.

technique, Conway *et al.* [3] defined rates of sulfation and glucuronidation in different regions of the liver lobule.

Local Oxygen Uptake

Micro-light guides and miniature O_2 electrodes have also been used to estimate the O_2 gradient and to determine rates of O_2 uptake in periportal and pericentral regions of the liver lobule [13]. Oxygen gradients in livers from control and ethanol-treated rats are described in more detail in another contribution to this volume.

DISCUSSION

Ethanol Metabolism in Periportal and Pericentral Regions of the Liver Lobule

It is important to know the lobular localization of ethanol metabolism in liver since ethanol-induced liver damage occurs predominantly in pericentral regions of the liver lobule. Because oxygen tension is lower in pericentral hepatocytes than in periportal hepatocytes and because ethanol treatment increases oxygen uptake, Israel *et al.* [8] 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] estimated the lobular oxygen gradients and demonstrated that the intralobular oxygen gradient was increased by chronic treatment with ethanol and diminished by treatment with propylthiouracil. Furthermore, pericentral hypoxia has been shown to produce rapid blebbing of the liver [17].

Local rates of ethanol metabolism were determined from the increases in NADH fluorescence caused by ethanol infusion. A linear correlation between rates of hepatic ethanol uptake and changes in NADH fluorescence was established with a large, multi-fiber light guide (Figs. 2 and 3). This correlation was then used to determine local rates of ethanol uptake from fluorescence changes detected from periportal and pericentral regions with two-fiber micro-light guides (Table 1). The data indicate that rates are similar in both periportal and pericentral regions of the liver lobule (for more details see [15]).

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, whereas considerable evidence has accumulated in support of cofactor limitation. In the liver lobule, alcohol dehydrogenase is either higher in periportal regions than in pericentral areas [5] or vice versa [20]. In contrast, NAD^+ and NADH are equally distributed over the lobule [19]. Thus, rates of ethanol metabolism follow the distribution of NAD^+ over the liver lobule.

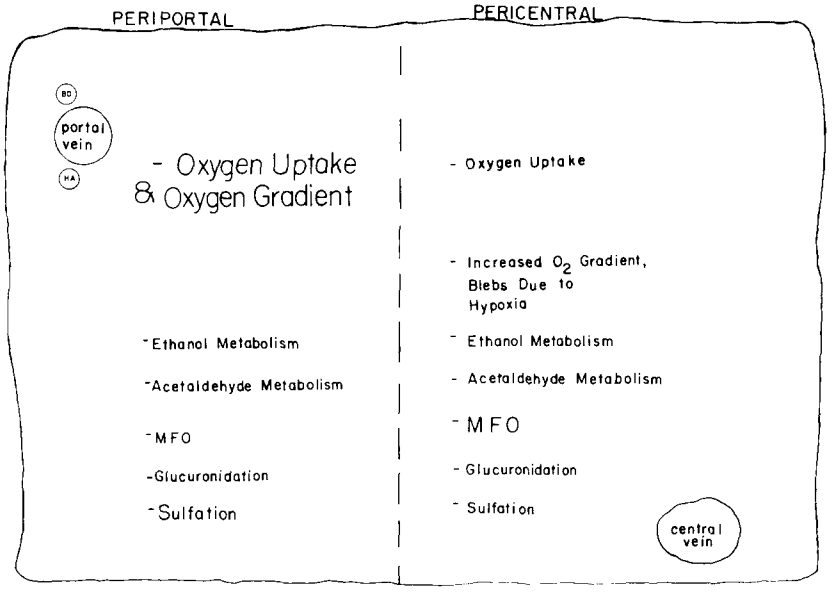

FIG. 5. Scheme depicting relative rates of a number of metabolic processes across the liver lobule. Size of lettering indicates predominance of a specific process. MFO=mixed-function oxidation.

Development of Methods to Study Metabolic Events Noninvasively in Periportal and Pericentral Regions of the Liver Lobule

Results with the new techniques described above are summarized in Fig. 5. The size of the lettering refers to the relative rates of the metabolic process in either pericentral or periportal regions of the liver lobule. For example, oxygen uptake and the oxygen gradients are largely confined (e.g., about 75%) to periportal hepatocytes. In contrast, the increase in O_2 uptake due to ethanol treatment is confined to pericentral regions [13]. This may explain the unique sensitivity of pericentral cells to hypoxia and is consistent with the hypothesis that ethanol causes liver damage by causing pericentral hypoxia [8]. Altered rates of ethanol or acetaldehyde metabolism cannot explain local damage due to ethanol. Rates of glucuronidation, at least at low substrate concentrations, are also similar in both regions of the liver. In contrast, sulfation predominates in periportal and mixed-function oxidation in pericentral regions of the liver lobule, at least in livers from barbiturate-treated rats [3,12].

Thus, metabolism is indeed different in various zones of the liver lobule reflecting a form of biochemical compartmentation. Information concerning intralobular compartmentation may be valuable in explaining the zonal specificity of toxicity to alcohol, drugs and a variety of hepatotoxic chemicals.

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