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Rates of Allyl Alcohol Metabolism in Periportal and Pericentral Regions of the Liver Lobule

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SUMMARY

Rates of allyl alcohol metabolism in periportal and pericentral regions of the liver lobule were measured to determine whether the zonal toxicity due to allyl alcohol results from its selective metabolism in periportal regions. Infusion of allyl alcohol into perfused livers from fed, phenobarbital-treated rats caused an increase in NADH fluorescence (366 → 450 nm) measured with a large-tipped (2-mm) light guide placed on the surface of the liver. A linear increase in NADH fluorescence was observed when 25–150 μM allyl alcohol was infused; however, when allyl alcohol exceeded 200 μM, oxygen uptake by the liver was inhibited 30–40%, and a large increase in NADH fluorescence occurred. Miniature oxygen electrodes were then placed on periportal and pericentral regions of the liver lobule and local rates of oxygen uptake were determined [Matsumura and Thurman, *Am. J. Physiol.* **244**:G656–G659 (1983)]. Allyl alcohol (350 μM) or acrolein (200 μM) inhibited oxygen uptake only in periportal regions. Micro-light guides were placed on periportal and pericentral regions of livers perfused in either the anterograde or retrograde direction. The maximal increase in NADH fluorescence due to allyl alcohol infusion (100 μM) was greater in pericentral than in periportal regions. 4-Methylpyrazole (80 μM), an inhibitor of alcohol dehydrogenase, prevented the fluorescence increase due to allyl alcohol in both regions, indicating that the changes were due entirely to NADH generated from alcohol dehydrogenase-dependent allyl alcohol metabolism. Using the correlation ($r = 0.91$) between rates of allyl alcohol uptake and the increase in NADH fluorescence established for the whole organ, local rates of allyl alcohol metabolism were 23 and 31 μmoles/g/hr in periportal and pericentral regions, respectively. These results indicate that metabolism of allyl alcohol occurs at slightly greater rates in pericentral than in periportal regions of the liver lobule. Thirty minutes after the i.p. injection of a necrogenic dose of allyl alcohol *in vivo*, the concentrations of allyl alcohol in the portal vein and vena cava were 1210 and 530 μM, respectively. Thus, both periportal and pericentral regions of the liver lobule were exposed to concentrations of allyl alcohol (e.g., >200 μM) which were metabolized in the perfused liver. Since allyl alcohol is metabolized in both regions of the liver lobule, the hypothesis that the zone-specific hepatotoxicity results from its exclusive metabolism to acrolein in periportal regions seems unlikely.

INTRODUCTION

Many hepatotoxins exert their toxicity in specific regions of the liver lobule. For example, carbon tetrachloride, bromobenzene, and acetaminophen selectively damage pericentral regions of the liver lobule, whereas allyl alcohol, ferrous sulfate, and phosphorus damage periportal tissue (1). Clear mechanisms for these differences in zonal toxicity have not been elucidated. Although it has been proposed that the damage to periportal regions due to allyl alcohol is due to a higher concentration of alcohol dehydrogenase within periportal cells which metabolizes it to toxic acrolein (2, 3), this concept has not been evaluated critically.

Allyl alcohol has long been known to cause extensive periportal necrosis of rat liver (4). It is oxidized to acrolein by liver alcohol dehydrogenase in an NAD^+-dependent dehydrogenation (2). Pretreatment of animals with 4-methylpyrazole, an inhibitor of alcohol dehydrogenase, blocked the hepatic necrosis due to ^{14}C-allyl alcohol and reduced the amount of radiolabeled material bound in the liver by 80% (2). This study indicated that

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both the binding of ^{14}C-allyl alcohol to macromolecules and the subsequent necrosis were dependent on the oxidation of allyl alcohol to acrolein.

Using histochemistry, several investigators reported that the activity of alcohol dehydrogenase was higher in periportal than in pericentral regions of the liver lobule (5, 6). This observation led to the hypothesis that the hepatotoxicity due to allyl alcohol resulted from the metabolism of allyl alcohol to acrolein exclusively in periportal regions of the liver lobule (2, 3). However, this hypothesis was not supported by the observation that alcohol dehydrogenase activity determined microchemically was greater in pericentral hepatocytes (7). In accord with the idea that alcohol is metabolized in both regions of the liver lobule, Kashiwagi *et al.* (8) observed recently that rates of NAD^+-dependent ethanol metabolism were similar in periportal and pericentral regions of the liver lobule. Local rates of ethanol metabolism were determined by employing techniques to detect NADH fluorescence in distinct periportal and pericentral regions of the liver lobule (9, 10). This involves placement of micro-light guides consisting of two strands of optical fibers on dark spots (pericentral regions) and light areas (periportal regions) on the liver surface. Pyridine nucleotide fluorescence can then be excited via light conducted to the liver surface with one fiber, and fluorescence can be detected with the other fiber.

Now that technology exists to measure rates of allyl alcohol metabolism based on NADH fluorescence in distinct regions of the liver lobule, the question can now be addressed of whether the zonal toxicity due to allyl alcohol results from its selective metabolism in periportal regions of the intact liver. The data from this study indicate that rates of allyl alcohol metabolism to the toxic intermediate acrolein are greater in pericentral than in periportal regions of the liver lobule.

MATERIALS AND METHODS

Animals and liver perfusion. Female Sprague-Dawley rats, 200–300 g, were treated with sodium phenobarbital (1 mg/ml) in drinking water for at least 1 week prior to perfusion experiments to facilitate identification of periportal and pericentral regions (9). Phenobarbital treatment did not affect rates of allyl alcohol uptake by the whole organ (data not shown) or the selective toxicity induced by allyl alcohol (2). Livers were perfused with Krebs-Henseleit bicarbonate buffer (pH 7.4, 37°) saturated with an oxygen-carbon dioxide mixture (95:5) in a nonrecirculating system as described previously (11). The fluid was pumped into the liver via a cannula placed in the portal vein and flowed past a Teflon-shielded, Clark-type oxygen electrode. Rates of oxygen uptake were calculated from the influent minus effluent oxygen concentration difference, the flow rate, and the liver wet weight. Allyl alcohol (Aldrich), acrolein (Aldrich), and 4-methylpyrazole (Sigma) were diluted with buffer and infused into the liver at concentrations described in the text and legends. Samples of effluent perfusate were collected every 2 min for enzymatic determination of allyl alcohol with alcohol dehydrogenase (12). Because relatively low concentrations of allyl alcohol were used in this study, rates of allyl alcohol metabolism (see below) were determined during both anterograde and retrograde perfusions. Allyl alcohol uptake was calculated from the influent minus effluent concentration difference, the liver wet weight, and the flow rate.

Determination of allyl alcohol concentration in blood. Rats received allyl alcohol (42 mg/kg) by i.p. injection of a 300 mM solution in normal saline. Thirty minutes later, rats were anesthesized with sodium pentobarbital (65 mg/kg), and 1.5 ml of blood were withdrawn from the portal vein and vena cava and centrifuged at 6500 × *g* for 15 min to remove red blood cells. The supernatant (0.8 ml) was deproteinized with 0.2 ml of 7% $HClO_4$, centrifuged, and neutralized with 0.1 ml of 1.6 M K_2CO_3. Allyl alcohol was then determined in the supernatant by a standard enzymatic procedure using alcohol dehydrogenase (12).

Micro-light guide detection of NADH fluorescence. Micro-light guides constructed from two strands of 70-μm diameter glass optical fibers have been employed previously for the detection of NADH fluorescence (8, 9). Anterograde (via the portal vein) and retrograde (via the vena cava) perfusions of liver with India ink identified lightly pigmented areas as periportal areas and darkly pigmented spots as pericentral regions (9). Thus, the natural distribution of liver pigments was used to place micro-light guides on periportal and pericentral regions of the liver lobule on the liver surface. One strand of a two-stranded micro-light guide was then connected to a near-ultraviolet light source, and the other strand to a photomultiplier (9). In some experiments, a large-tipped light guide (tip diameter 2 mm) was employed. The liver was illuminated with a 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 (13).

Rates of oxygen uptake in periportal and pericentral regions of the liver lobule. A miniature oxygen electrode was made by inserting a 50-μm diameter platinum wire into a glass capillary tube (Corning No. 770; outer diameter 1 mm, inner diameter 0.5 mm) which was coated with an oxygen-permeable acrylic ester polymer, Rhoplex (14). In order to measure local rates of oxygen uptake, the electrode was placed on periportal or pericentral regions, and flow was stopped by clamping the outflow and stopping the inflow simultaneously (15). Rates of oxygen uptake were then determined by measuring the rate of decrease of oxygen concentration as described elsewhere (15).

RESULTS

Effect of allyl alcohol on oxygen uptake and NADH fluorescence. So that fluorescence could be compared with rates of allyl alcohol uptake by the whole organ, a large-tipped light guide was placed on the surface of the liver. This light guide excites and collects fluorescence from many periportal and pericentral regions in several lobules. In order to determine the sensitivity of this light guide to changes in NADH fluorescence at the liver surface, a cycle of anoxia was performed by saturating the Krebs-Henseleit bicarbonate buffer with a nitrogen-carbon dioxide mixture (95:5). A large increase in NADH fluorescence was observed during the cycle of anoxia (Fig. 1), indicating that changes in the NADH:NAD^+ ratio could be detected from the liver surface with the light guide. After reoxygenation, allyl alcohol was infused in a stepwise fashion into a perfused liver from a fed, phenobarbital-treated rat (Fig. 1). With the infusion of 50 and 100 μM allyl alcohol, NADH fluorescence and allyl alcohol uptake increased in a linear fashion while oxygen uptake remained relatively constant (Fig. 1). When the concentration of allyl alcohol infused into the liver was increased to 200 μM, rates of oxygen uptake began to decline. This inhibition of oxygen uptake was irreversible and was accompanied by a decrease in allyl alcohol metabolism and a large increase in NADH fluorescence (Fig. 1). No further effect on any of these metabolic processes was observed by increasing the concentration of allyl alcohol to 400 μM (Fig. 1). When allyl alcohol infusion was terminated, a small decrease in oxygen uptake was observed (Fig. 1).

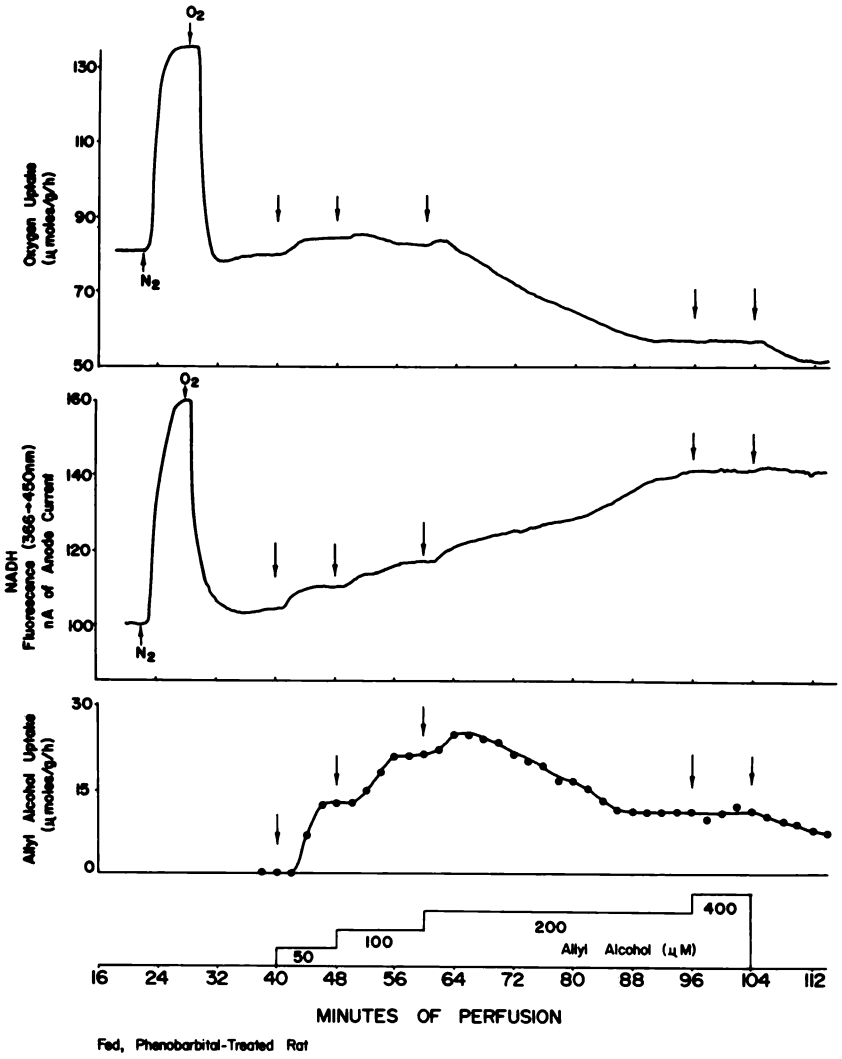

FIG. 1. *Effect of allyl alcohol on oxygen uptake and NADH fluorescence*

A large-tipped light guide (2 mM) was placed on the surface of livers perfused in the anterograde direction. NADH fluorescence, oxygen concentration, and allyl alcohol concentrations were determined as described under Materials and Methods. Rates of oxygen uptake and allyl alcohol uptake were calculated from influent minus effluent concentration differences, the flow rate, and the liver wet weight. A cycle of anoxia was performed by changing the 95% O_2/5% CO_2 gas mixture saturating the Krebs-Henseleit buffer to 95% N_2/5% CO_2 (labeled N_2). Under these conditions, changes in oxygen concentration did not reflect changes in oxygen uptake. Allyl alcohol was infused into the liver in a stepwise fashion as indicated by the *horizontal bar*. A typical experiment is shown.

When allyl alcohol was infused in increasing but low concentrations (25–150 μM), stepwise increases in NADH fluorescence as well as allyl alcohol uptake were observed (Fig. 2). A good correlation (r = 0.91) was observed when rates of allyl alcohol uptake were plotted as a function of NADH fluorescence (Fig. 3).

Rates of allyl alcohol metabolism in periportal and pericentral regions of the liver lobule. Micro-light guides were placed on periportal and pericentral regions of livers perfused in either the anterograde or retrograde direction. When 100 μM allyl alcohol was infused into a liver perfused in the anterograde direction, NADH fluorescence in periportal regions increased by 3.4% of basal fluorescence (Fig. 4; Table 1). During retrograde perfusions, increases of 5.8% were observed in pericentral regions (Fig. 4; Table 1). When 25 and 50 μM allyl alcohol were infused into a liver perfused in the anterograde direction (Fig. 5A), NADH fluorescence increased only in the periportal regions; with 100 μM allyl alcohol, NADH fluorescence increased by approximately 3% in both regions. Further increases in the concentration of allyl alcohol increased NADH fluorescence predominantly in pericentral regions of the liver lobule (Fig. 5A). Similar data were obtained with retrograde perfusions, with increases in NADH fluorescence always greatest in the "upstream" pericentral regions (Fig. 5B). The addition of 4-methylpyrazole (80 μM), an inhibitor of alcohol dehydrogenase, blocked the increase in NADH fluorescence during allyl alcohol infusion (Fig. 6). Under these conditions, all infused allyl alcohol was recovered in the effluent perfusate (data not shown). This indicates that uptake of allyl alcohol by the perfused liver is equivalent to its metabolism via alcohol dehydrogenase. After removal of the inhibitor, the uptake of allyl alcohol and NADH fluorescence increased in both regions of the liver lobule (Fig. 6). Rates of allyl alcohol metabolism in

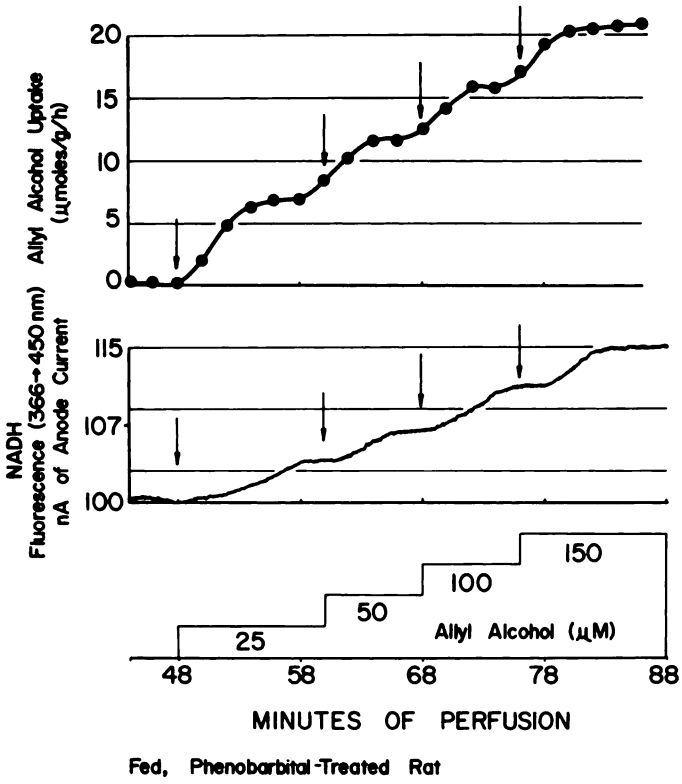

FIG. 2. *Effect of allyl alcohol on hepatic NADH fluorescence and allyl alcohol uptake*

Allyl alcohol was infused into the liver of a fed, phenobarbital-treated rat in a stepwise fashion as indicated by the *horizontal bars* and *vertical arrows*. Allyl alcohol uptake and NADH fluorescence from the tissue were determined as described under Materials and Methods.

periportal and pericentral regions of the liver lobule were calculated by equating the proportion of NADH fluorescence produced in both zones by allyl alcohol to rates of allyl alcohol metabolism by the liver. For this calculation, periportal and pericentral regions of the liver were assumed to be of equal mass based on densitometry of photographs of the liver surface.[4] Rates of allyl alcohol uptake were calculated to be 23 and 31 μmoles/g/hr (Table 1) in periportal and pericentral regions, respectively.

FIG. 3. *Correlation of NADH fluorescence and allyl alcohol uptake*

Fluorescence change was expressed as a percentage of the basal fluorescence. The best-fit linear regression line was obtained using data from eight different livers.

[4] J. J. Lemasters and R. G. Thurman, unpublished data.

Allyl alcohol concentrations in rat blood after administration of allyl alcohol to rats in vivo. Thirty minutes after the injection of a necrogenic dose (42 mg/kg) of allyl alcohol (3), portal and caval blood was collected rapidly and assayed for allyl alcohol. Under these conditions, the concentrations of allyl alcohol in the portal vein and vena cava were 1210 ± 240 and 530 ± 210 μM, respectively. Animals killed 24 hr after this dose of allyl alcohol had extensive periportal necrosis, but the pericentral hepatocytes were unaffected (data not shown).

Effect of allyl alcohol and acrolein on rates of oxygen uptake in periportal and pericentral regions of the liver lobule. Rates of oxygen uptake determined with miniature oxygen electrodes were 125 and 48 μmoles/g/hr in periportal and pericentral regions of the liver, respectively (Table 2). The infusion of 350 μM allyl alcohol inhibited oxygen uptake in periportal regions by 35% but had no effect on oxygen uptake of pericentral areas (Table 2). Similarly, when 200 μM acrolein was infused into the liver, oxygen uptake was inhibited by 43% in periportal regions, while oxygen uptake was unaffected in pericentral areas (Table 2). The infusion of 4-methylpyrazole (80 μM) blocked the inhibition of respiration by allyl alcohol completely (data not shown).

DISCUSSION

Metabolism of allyl alcohol in periportal and pericentral regions of the liver lobule. The oxidation of allyl alcohol has been shown to require NAD^+-linked alcohol dehydrogenase (6). In incubations with liver homogenates and isolated mitochondria, Serrafini-Cessi (3) observed that allyl alcohol was converted into acrolein, an aldehyde which can be subsequently oxidized to acrylic acid by aldehyde dehydrogenase (16). Acrolein has been implicated as the reactive intermediate responsible for the hepatotoxicity observed with allyl alcohol (2, 3). *In vitro*, only 5% of the added allyl alcohol was converted to acrolein (3). Observations that the metabolism of allyl alcohol occurred at very low rates *in vitro* and that activity of alcohol dehydrogenase was greatest in periportal regions of the lobule (4, 5) led to the hypothesis that periportal necrosis was due to the exclusive metabolism of allyl alcohol to acrolein in periportal regions. Quantitative measurements described in this study with newly developed technology do not support this hypothesis.

Since a linear correlation exists between rates of allyl alcohol uptake and increases in NADH fluorescence (Figs. 2 and 3), this correlation was used to determine rates of allyl alcohol metabolism in periportal and pericentral regions calculated from fluorescence changes detected with micro-light guides. The increase in NADH fluorescence due to infusion of 150 μM allyl alcohol was larger in pericentral regions than in periportal regions of the liver lobule during both anterograde and retrograde perfusions (Fig. 5), indicating that the capacity to metabolize allyl alcohol in the intact liver is slightly greater in pericentral regions of the liver lobule. Since allyl alcohol is metabolized in both periportal and pericentral regions of the liver lobule, the hypothesis that the zone-

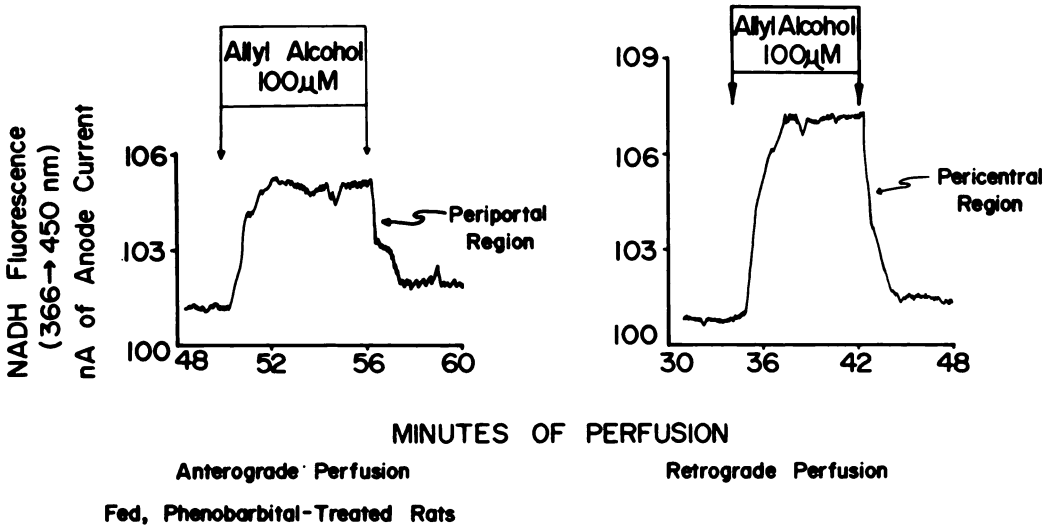

FIG. 4. *Effect of allyl alcohol on NADH fluorescence in periportal and pericentral regions of the liver lobule*

Micro-light guides (tip diameter, 170 μm) were placed on periportal and pericentral regions of a liver perfused in either the anterograde or retrograde direction. NADH fluorescence was determined as described under Materials and Methods. Allyl alcohol (100 μM) was infused at the times designated by the *horizontal bars* and *vertical arrows*.

specific hepatotoxicity of allyl alcohol results from its exclusive metabolism to acrolein only in periportal regions of the liver lobule seems unlikely (2, 3). Thus, some other factor(s) unique to either the periportal or pericentral regions must contribute to mechanisms of zonal toxicity.

One such possibility is that the *in vivo* uptake of allyl alcohol may occur mainly in periportal regions (i.e., the regions of the liver lobule first exposed to flow). This notion also seems unlikely, since the concentration of allyl alcohol in the vena cava was 530 μM following a necrogenic dose; therefore, the concentration in the pericentral regions must have been at least 530 μM. Since allyl alcohol metabolism was greater in pericentral regions than in periportal regions of the liver lobule (Fig. 5) with a lower dose (150 μM), we conclude that metabolism of allyl alcohol to acrolein occurs in both zones whereas necrosis is observed only in the periportal regions.

TABLE 1

Rates of allyl alcohol uptake in periportal and pericentral regions of the perfused liver

Rates of allyl alcohol metabolism in periportal and pericentral regions were calculated as described under Results. Rates of allyl alcohol uptake were determined from anterograde and retrograde perfusions, for periportal and pericentral regions, respectively, in the presence of 100 μM allyl alcohol. Values are means ± standard error of the mean from eight livers per group.

	Lobular region	
	Periportal	Pericentral
Maximal increase of fluorescence (% of basal)	3.4 ± 0.3	5.8 ± 0.7[a]
Local rate of allyl alcohol uptake (μmoles/g/hr)	23.2 ± 1.3	31.1 ± 3.6[b]

[a] $p < 0.01$ with respect to periportal regions (Student's *t*-test).
[b] $p < 0.05$ with respect to periportal regions (Student's *t*-test).

Another possibility is that the toxic intermediate, acrolein, accumulates to a greater extent in periportal than in pericentral regions as a result of differential metabolism of acrolein to acrylic acid via aldehyde dehydrogenase. This also seems unlikely because acetaldehyde is metabolized equally in both regions via aldehyde dehydrogenase (17).

Inhibition of oxygen uptake by allyl alcohol and acrolein. Previous studies have indicated that acrolein, but not allyl alcohol, inhibited succinate-linked oxygen uptake in isolated mitochondria (6). Furthermore, when mitochondria were isolated from rats 1 hr after i.p. injection of allyl alcohol, a marked inhibition of oxygen uptake was observed. The addition of glutathione and dithiothreitol to the incubation mixture protected mitochondrial respiration *in vitro* from inhibition by acrolein completely (6). Since no histological changes were seen in these livers 1 hr after allyl alcohol poisoning, these findings suggest that early biochemical alterations are due to the highly reactive aldehyde, acrolein.

Consistent with these observations, allyl alcohol (200 μM) inhibited oxygen uptake by the perfused liver (Fig. 1), which in turn caused a large increase in NADH fluorescence (Fig. 1). The decrease in allyl alcohol metabolism observed during the inhibition of oxygen uptake is presumably due to inhibition of alcohol dehydrogenase by NADH. When allyl alcohol infusion was terminated, neither oxygen uptake nor NADH fluorescence returned to basal conditions (Fig. 1), suggesting that the respiratory chain had been damaged irreversibly. Furthermore, when local rates of oxygen uptake were measured using miniature oxygen electrodes placed on periportal and pericentral regions of the liver, allyl alcohol diminished oxygen uptake only in periportal regions (Table 2). The infusion of acrolein also decreased oxygen uptake selectively in periportal regions (Table 2). The addition of 4-methylpyrazole blocked the inhibition of respiration observed with allyl alcohol (data not shown), indicating

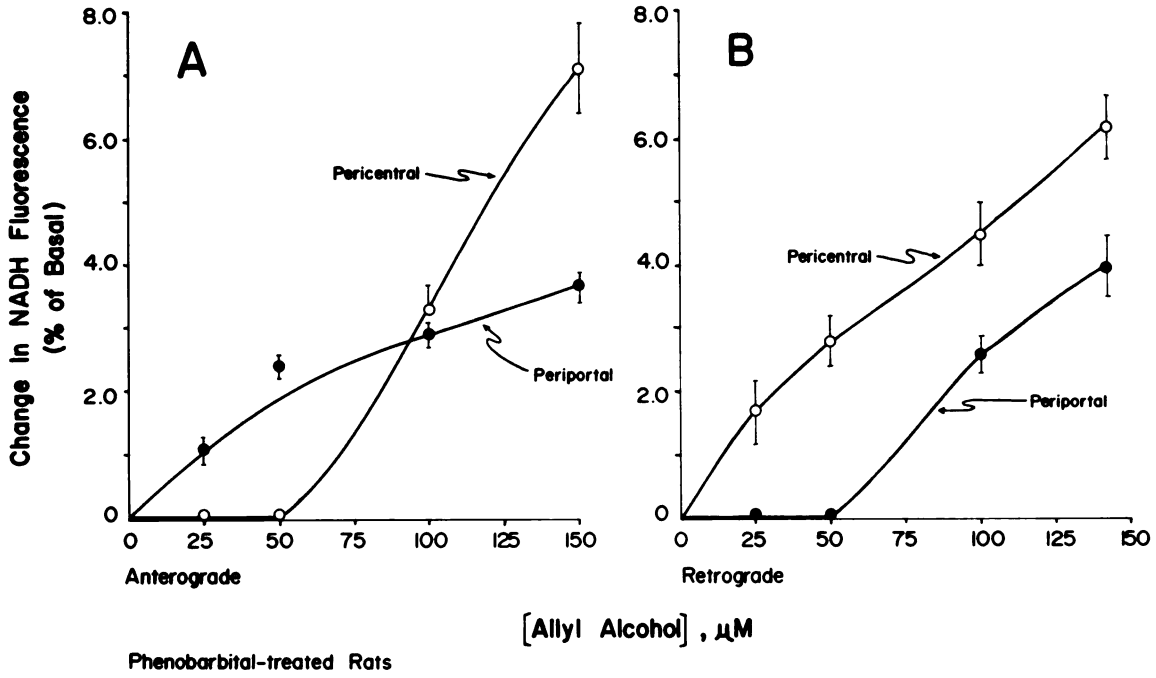

FIG. 5. *Effect of allyl alcohol concentration on NADH fluorescence in periportal and pericentral regions during anterograde and retrograde perfusions*

Micro-light guides were placed on periportal and pericentral regions of livers perfused in either the anterograde (A) or retrograde (B) direction. Four different concentrations of allyl alcohol (25, 50, 100, and 150 μM) were then infused into each liver. NADH fluorescence was determined as described under Materials and Methods. Values are means ± standard error of the mean from four livers perfused in either direction.

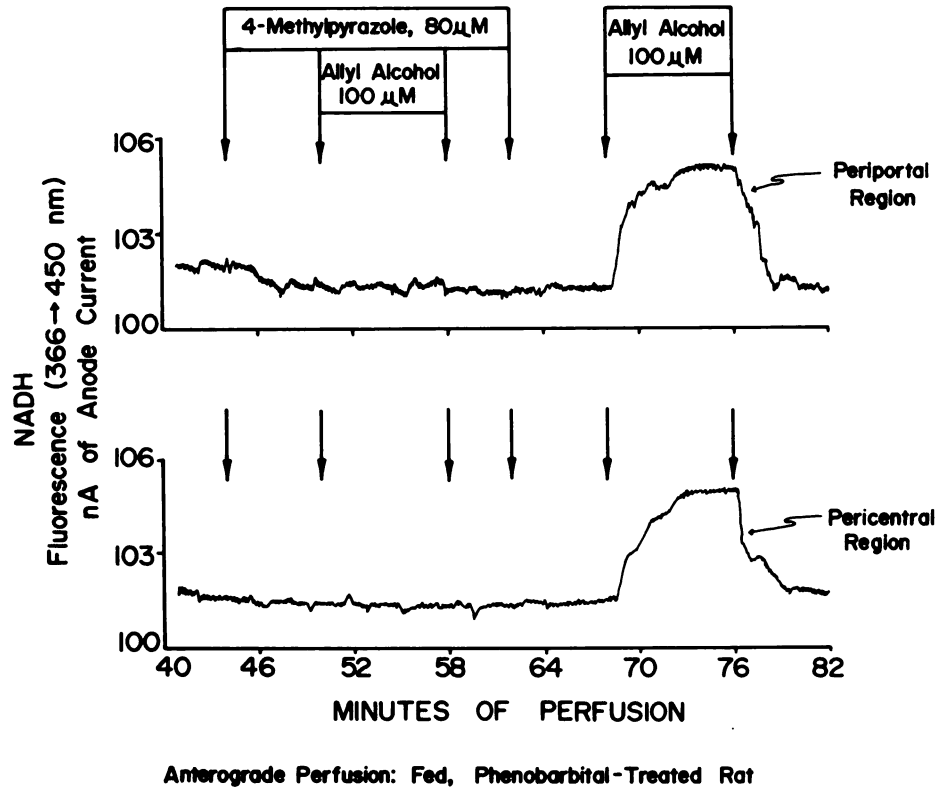

FIG. 6. *Effect of 4-methylpyrazole on allyl alcohol-induced NADH fluorescence increases in periportal (upper panel) and pericentral (lower panel) regions of the liver lobule*

Experimental conditions as in Fig. 4. After 8 min of 4-methylpyrazole infusion (80 μM), allyl alcohol (100 μM) was infused as indicated by the *horizontal bars* and *vertical arrows*. After 4-methylpyrazole infusion was terminated, allyl alcohol was infused 6 min later.

TABLE 2

Effect of allyl alcohol and acrolein on rates of oxygen uptake in periportal and pericentral regions of the liver lobule

Oxygen uptake in distinct areas of the liver lobule was measured by stopping the flow and measuring oxygen concentration changes with a miniature oxygen electrode as described elsewhere (15). 4-methylpyrazole (80 μM) was infused during the addition of acrolein to prevent the metabolism of acrolein to allyl alcohol by alcohol dehydrogenase. Values are means ± standard error of the mean from five livers.

Addition	Oxygen uptake	
	Periportal	Pericentral
	μmoles/g/hr	
None	125 ± 18	48 ± 2
Allyl alcohol (350 μM)	84 ± 9[a]	53 ± 3
None	105 ± 13	47 ± 6
Acrolein (200 μM)	60 ± 7[b]	43 ± 7

[a] $p < 0.05$ as compared with no addition using matched-paired *t*-test.
[b] $p < 0.01$ as compared with no addition using matched-paired *t*-test.

that the decrease in oxygen uptake results from the oxidation of allyl alcohol to acrolein. Therefore, it is concluded that the mitochondrial respiratory chain in periportal cells may be more sensitive to the toxic effects of acrolein. We hypothesize that the selective toxicity of periportal regions of the liver lobule to allyl alcohol results from local alteration of energetics due to inhibition of the mitochondrial respiratory chain by acrolein. Since rates of oxygen uptake were 3 times greater in periportal than pericentral regions (15) (Table 2), possible structural or functional differences in the mitochondria in different regions of the liver lobule may be involved in the mechanism of the hepatotoxicity produced by allyl alcohol.

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