Functional Compartmentation of Acetaldehyde Oxidation in Rat Liver

(Received for publication, February 15, 1974)

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SUMMARY

Perfused rat liver and suspensions of isolated rat liver cells have been used to study the influence of the transaminase inhibitors aminooxyacetate and DL-cycloserine on the rate of acetaldehyde utilization, and the effects of acetaldehyde on the state of reduction of cytosolic and mitochondrial pyridine nucleotides. In the presence of 4-methylpyrazole to inhibit acetaldehyde reduction to ethanol, acetaldehyde removal was constant over the range from 0.1 to 0.4 mM at a rate of approximately 400 μmoles per g dry weight per hour. Between 0.4 and 10 mM acetaldehyde, the rate of acetaldehyde uptake increased by 60% with increasing acetaldehyde concentrations, with a half-maximum increment of uptake being achieved at about 1 mM acetaldehyde. DL-Cycloserine had no effect on acetaldehyde uptake at low concentrations of acetaldehyde but almost completely inhibited the stimulation of uptake observed at high concentrations. Neither DL-cycloserine nor aminooxyacetate had any inhibitory effect on the reduction of mitochondrial pyridine nucleotides induced by acetaldehyde. At high acetaldehyde concentrations (3 to 4 mM) pyruvate stimulated acetaldehyde uptake, but little effect was observed at low acetaldehyde concentrations (0.3 mM). Acetaldehyde uptake was inhibited by fatty acids, β-hydroxybutyrate, and amobarbital, and stimulated by acetoacetate. Oxidation of endogenous fatty acid was diminished by acetaldehyde.

These data indicate that oxidation of acetaldehyde by rat liver occurs almost entirely in the mitochondrial compartment when the mean arterial-venous acetaldehyde concentration is below 0.4 mM. At higher acetaldehyde concentrations, oxidation occurs also in the cytosol, and reducing equivalents generated in the cytosol by a high Km NADP-linked aldehyde dehydrogenase are transported to the mitochondria mainly by the malate-aspartate cycle. It may be concluded that acetaldehyde generated during ethanol metabolism is oxidized to acetate predominantly in the mitochondria, so that only 1 eq of NADH is generated in the cytosol per mole of ethanol oxidized via alcohol dehydrogenase.

A wide range of enzymes capable of oxidizing acetaldehyde to acetate in liver have been described. One type comprising a variety of flavoprotein oxidases has a low substrate specificity and affinity for aldehydes, and probably plays a minor role in acetaldehyde oxidation under physiological conditions (1-4). Racker (5) described the purification of a nonspecific NAD-linked aldehyde dehydrogenase from beef liver with a high affinity toward acetaldehyde (Michaelis constant less than 10⁻⁵ M). NADP-linked aldehyde dehydrogenases with high affinity toward acetaldehyde have since been isolated and characterized from liver of a number of species (6-13) as well as from other organs such as kidney and brain (6, 14, 15). Subcellular distribution studies of the aldehyde dehydrogenase activity of rat liver have shown that the cell supernatant contains at least two NAD-dependent aldehyde dehydrogenases which differ in their substrate specificities and physical properties (16, 17) in addition to one or more enzymes associated with the mitochondrial fraction (14, 18-22). Clear evidence for the heterogeneity of the cytoplasmic enzymes has been obtained by Deitrich et al. (17, 23), who found that one of the soluble NAD-dependent aldehyde dehydrogenases was induced 10-fold without any change of the kinetic characteristics by treatment of genetically selected rats with phenobarbital. The soluble aldehyde dehydrogenases of rat liver (16, 17) appear to have apparent Km values for acetaldehyde 2 to 3 orders of magnitude higher than those of the corresponding enzymes from beef (5, 9), horse (11), or human (7, 8) liver. In contrast to the earlier studies of Büttner (6) who found that most of the aldehyde dehydrogenase activity of rat liver was extramitochondrial, more recent studies (22-25) have shown that about 80% of the total activity is in the mitochondrial fraction. However, the mitochondrial NADP-dependent aldehyde dehydrogenase activity appears to be heterogeneous. Marjanen (24) reported an apparent Km for acetaldehyde of below 10 μM, while Grunnet (22) found two Km values for acetaldehyde; one below 1 μM and one about 1 mM. Mitochondrial fractionation studies by Tottmar et al. (26) suggest that an enzyme with a relatively high Km for acetaldehyde (1
to 2 mM) similar to that of supernatant aldehyde dehydrogenase is located on the outer membrane while a second enzyme in the matrix has an apparent $K_m$ for acetaldehyde in the region of 1 $\mu$M. These latter authors also found considerable activity of the high $K_m$ enzyme in the microsomal fraction of rat liver, and less than 5% of total activity in the soluble fraction. Isolated liver mitochondria oxidize acetaldehyde and other aldehydes when added at low concentrations, although high concentrations are inhibitory (18–22). Acetaldehyde also inhibited the oxidation of pyruvate by mitochondria from a number of tissues (19).

Previous studies using surface fluorometric techniques to monitor changes in the oxidation-reduction state of pyridine nucleotides in the cytosolic and mitochondrial spaces of blood-free perfused livers (27, 28) have shown that after ethanol addition a rapid reduction of pyridine nucleotides occurred in both spaces. These results were interpreted as indicating a rapid transport of reducing equivalents from cytosol to mitochondria by the malate-aspartate cycle. Furthermore, metabolic balances describing the interaction of ethanol oxidation with glucose-neogenesis from alanine were calculated on the basis of the production of 2 eq of NADH in the cytosol per mole of ethanol utilized (28). The recent studies on the cellular distribution of aldehyde dehydrogenases quoted above, together with further studies of the effects of ethanol and acetaldehyde on cellular pyridine nucleotide oxidation-reduction changes in perfused rat liver (29) suggest that the mitochondrial oxidation of acetaldehyde predominates at low concentrations, and that the cytosolic enzyme is functionally operative only at acetaldehyde concentrations much higher than those of 100 to 200 $\mu$M reported in vivo (30–33) or in the effluent fluid of perfused livers (34) after ethanol administration.

The present study was initiated in order to clarify the respective roles of the cytosolic and mitochondrial aldehyde dehydrogenases for acetaldehyde metabolism in the intact liver cell. Use of DL-cycloserine to inhibit extramitochondrial transaminase reactions (35, 36) associated with the malate-aspartate cycle has established that the reduction of mitochondrial pyridine nucleotides by acetaldehyde was not affected by this inhibitor and that uptake of acetaldehyde by isolated liver cells was also unaffected at acetaldehyde concentrations below 0.4 mM. On the other hand, competition for acetaldehyde oxidation was exerted by compounds such as fatty acids or $\beta$-hydroxybutyrate which generate reducing equivalents directly in the mitochondria. It is evident, therefore, that the various metabolic effects associated with ethanol oxidation by the liver must be interpreted in terms of the subcellular compartmentation of the two reductive steps of ethanol metabolism to acetate.

**EXPERIMENTAL PROCEDURES**

**Animals**—Male albino rats (Holzman) 180 to 220 g in weight were fasted for 20 to 28 hours prior to liver perfusion studies or isolation of hepatocytes.

**Liver Perfusion, Surface Fluorometry, and Oxygen Uptake**—Livers were perfused with Krebs' bicarbonate medium saturated with 95% $O_2$-5% $CO_2$ using a flow-through system as described elsewhere (29). Aliquots of the effluent fluid were collected at 2-min intervals and assayed directly for glucose, lactate, pyruvate, acetocetate, and $\beta$-hydroxybutyrate by standard enzymatic procedures (37). Substrates and inhibitors were added continuously by infusion pumps to fluid immediately prior to the liver to give the desired arterial concentration. Flavin and pyridine nucleotide fluorescence was measured from the surface of the liver as described elsewhere (29, 38), using excitation wavelengths of 475 and 366 nm and emission wavelengths of 580 and 460 nm for flavin and pyridine nucleotides, respectively. Oxygen consumption by the liver was monitored continuously by a small Teflon-covered platinum electrode placed in the effluent fluid. In some experiments the rate of endogenous fatty acid oxidation was estimated by infusion of tracer amounts of [U-'14C]oleate (New England Nuclear, Boston, Mass.) into fluid passing to the liver and measuring acid-labile radioactivity in samples of the effluent fluid collected under toluene. Aliquots (6 to 10 ml) were transferred to sealed vessels containing 0.2 ml of Hyamine in a removable center well, and acidified by injection of 0.2 ml of 5 N HClO$_4$. Radioactive CO$_2$ trapped in Hyamine was counted in 15 ml of Bray's solution (39), using a Packard Tri-Carb liquid scintillation counter.

**Preparation and Incubation of Parenchymal Cells**—Isolated liver cells were prepared from fasted rats according to the procedure of Berry and Friend (40) as modified by Johnson et al. (41). Enzyme digestion with collagenase and hyaluronidase was achieved by recirculation perfusion (42) using 60 ml of calcium-free Hanks' medium containing 2% (w/v) dialyzed fatty acid-free bovine serum albumin (Pentex, Fraction V from Miles Laboratories, Inc.). The cells were incubated at 37°C in sealed 25-ml plastic Erlenmeyer flasks containing 4 to 6 ml of Krebs bicarbonate medium and 4% (w/v) albumin, pH 7.4, equilibrated with 95% $O_2$ and 5% $CO_2$. Aliquots of the cell suspension were removed at intervals by means of calibrated syringes and added to cold perchloric acid (final concentration 0.5% w/v). After removing the denatured protein by centrifugation in the cold, the pH of the supernatant was adjusted to 6 to 6.5 with 6 N K$_2$CO$_3$ containing 0.5 mM piperazine-$N,N'$-bis(2-ethanesulfonic acid) and reconstituted to remove the precipitated KClO$_4$. Acetaldehyde was assayed immediately using acetaldehyde dehydrogenase as described by Lundquist (43). Loss of acetaldehyde by evaporation during cell incubation was negligible since the vessels were sealed and were well mixed before removal of aliquots.

**Materials**—Enzymes and coenzymes were purchased from Worthington Biochemical Corp., Sigma Chemical Co., or Boehringer Mannheim Corp. 4-Methylpyrazole was obtained from Research Plus Laboratory, Denville, N. J. 4-Propionylpyrazole was a gift from Dr. Henry R. Drott of this department. dl-Cycloserine was the generous gift of Doctors A. E. Braunshteln and Y. F. Skulachev, Laboratory of Nitrogenous Metabolism, The Institute of Biological and Medical Chemistry, Chemistry, Academy of Medical Sciences of the U.S.S.R., Moscow. This compound is now available from Regis Chemical Co., Chicago, Ill. Amino-oxyacetic acid hemihydrate chloride was purchased from Eastman Kodak Co., Rochester, N. Y. Sodium amobarbital (Amytal) was obtained from Eli Lilly and Co.

**RESULTS**

A comparison of the flavin and pyridine nucleotide fluorescence responses to brief periods of ethanol and acetaldehyde infusion in perfused rat liver is shown in Fig. 1. Methylpyrazole (50 $\mu$m) was infused prior to acetaldehyde in order to prevent reduc-
Lactate to pyruvate and \( \beta \)-hydroxybutyrate to acetoacetate in the effluent fluid of perfused rat livers after infusion of acetaldehyde. Methylpyrazole (50 \( \mu \)M) and aminooxyacetate (0.2 mM) were added at the times indicated and infusion continued to produce the concentrations shown in the arterial fluid.

**Fig. 2.** Effect of aminooxyacetate on changes of the ratios of lactate to pyruvate and \( \beta \)-hydroxybutyrate to acetoacetate in the effluent fluid of perfused rat livers after infusion of acetaldehyde. Methylpyrazole (0.15 mM) was present throughout the experiment.

A change of the flavin nucleotide fluorescence reflects changes of the oxidation-reduction state of the mitochondrial pyridine nucleotide pool as documented elsewhere (29, 38, 44), while a change of the pyridine nucleotide fluorescence represents the sum of contributions of oxidation-reduction changes of pyridine nucleotides in both the cytosolic and mitochondrial compartments. In the experiments shown in Figs. 1 and 2, lactate (1 mM), pyruvate (0.1 mM), \( \beta \)-hydroxybutyrate (0.1 mM), and acetoacetate (0.2 mM) were infused into the fluid immediately prior to the liver. Methylpyrazole (0.15 mM) was present throughout the experiment.

**Fig. 3.** Effect of \( \delta \)-l-cycloserine on changes of the ratios of lactate to pyruvate and \( \beta \)-hydroxybutyrate to acetoacetate in the effluent fluid of perfused rat livers after infusion of acetaldehyde. Aminooxyacetate (0.2 mM) and aminooxyacetate (0.1 mM) were added at the times indicated and infusion continued to produce the concentrations shown in the arterial fluid.

A change of the flavin nucleotide fluorescence reflects changes of the oxidation-reduction state of the mitochondrial pyridine nucleotide pool as documented elsewhere (29, 38, 44), while a change of the pyridine nucleotide fluorescence represents the sum of contributions of oxidation-reduction changes of pyridine nucleotides in both the cytosolic and mitochondrial compartments. In the experiments shown in Figs. 1 and 2, lactate (1 mM), pyruvate (0.2 mM), \( \beta \)-hydroxybutyrate (0.2 mM), and acetoacetate (0.3 mM) were infused into the arterial fluid flowing to the liver. Studies have shown that at the concentrations used a rapid equilibration of lactate and pyruvate and of \( \beta \)-hydroxybutyrate and acetoacetate occurred with their respective dehydrogenases and the intracellular pyridine nucleotide pools during one passage through the liver (29). Measurements of the ratios of lactate to pyruvate and \( \beta \)-hydroxybutyrate to acetoacetate in the effluent fluid, therefore, provide an ancillary method for estimating the pyridine nucleotide oxidation-reduction potentials in the separate cytosolic and mitochondrial spaces (45). Both ethanol and acetaldehyde caused a prompt reduction of flavin and pyridine nucleotides, but a marked increase in the size of the pyridine nucleotide fluorescence response was observed with acetaldehyde relative to that obtained with ethanol. On the basis of these results alone it would be predicted that acetaldehyde would cause a larger reduction of cytosolic pyridine nucleotides than ethanol. However, direct measurements of the lactate to pyruvate ratio in the effluent fluid from the liver in companion experiments (Fig. 2) failed to confirm this interpretation. The data show that 1 mM acetaldehyde in the presence of methylpyrazole increased the lactate to pyruvate ratio to a value of about 20 compared with an increase to 50 after addition of a similar concentration of ethanol. The lack of effectiveness of low concentrations of acetaldehyde compared with ethanol in raising the lactate to pyruvate ratio in perfused liver has also been reported by Lindros et al. (34). An alternative explanation may be advanced that NADH bound to cytosolic acetaldehyde dehydrogenase exhibits a large fluorescence enhancement. This phenomenon has recently been observed with an acetaldehyde dehydrogenase isozyme purified from human liver (46).

Figs. 1 and 2 show that acetaldehyde infusion in the presence of aminooxyacetate as transaminase inhibitor (47) produced essentially identical changes of flavin and pyridine nucleotide fluorescence and increase of the \( \beta \)-hydroxybutyrate to acetoacetate ratio as in the absence of aminooxyacetate. Addition of aminooxyacetate itself caused an increased state of reduction of total pyridine nucleotides and a slight oxidation of flavin nucleotides, indicating pyridine nucleotide reduction in the cytosol but oxidation in the mitochondria. These changes correlated with an increase of the lactate to pyruvate ratio from 6 to 18 and a decrease of the \( \beta \)-hydroxybutyrate to acetoacetate ratio from 0.48 to 0.42. Since aminooxyacetate largely abolishes the increased reduction of mitochondrial pyridine nucleotides observed after ethanol addition (48-50) it may be concluded that reducing equivalents are not generated at an appreciable rate in the cytosol by oxidation of acetaldehyde at low concentrations (cf. Ref. 29). However, it is evident from the increase of the lactate to pyruvate ratio after acetaldehyde addition that an interaction occurs between cytosolic acetaldehyde dehydrogenase and lactate dehydrogenase to increase the cytosolic NADH/NAD ratio. Further studies using \( \delta \)-l-cycloserine as transaminase inhibitor (Fig. 3), also showed that it was without effect on the increase of the \( \beta \)-hydroxybutyrate to acetoacetate ratio obtained upon addition of 2 mM acetaldehyde to the perfused rat liver.

Aminooxyacetate and cycloserine inhibit transaminases by reacting with the pyridoxal phosphate form of the enzyme to form a relatively stable analogue of the normal enzyme substrate complex (see Ref. 36 for references). \( \alpha \)-Ketoglutarate accelerates the rate of inhibition with aspartate aminotransferase, while glutamate and aspartate protect the enzyme. Kinetic studies with isolated aspartate aminotransferase, measured in the direction of oxalacetate formation in a coupled reaction with malate dehydrogenase, showed that at relatively low aspartate concentrations (1 to 2 mM) aminooxyacetate was a rather better in-
chondria incubated with 1 mM malate and 10 μM glutamate aminotransferase. Aminooxyacetate at concentrations of 0.5 to after correction for ethanol formation (A) and acetaldehyde oxidation, and transport of reducing equivalents from cytosol to mitochondria proceeds mainly by the malate-aspartate cycle. At higher acetaldehyde concentrations, acetaldehyde oxidation proceeds almost entirely via a low

Table I shows that with relatively high acetaldehyde concentrations (3 to 4 mM) added to suspensions of liver cells in the

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Acetaldehyde concentration (mM)</th>
<th>Mean μmoles/g dry weight/h</th>
<th>Control %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td>None</td>
<td>4.0</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>Pyruvate (10 mM)</td>
<td>4.0</td>
<td>2.4</td>
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<tr>
<td></td>
<td>Octanoate (2 mM)</td>
<td>4.0</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>Oleate (1 mM)</td>
<td>4.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>None</td>
<td>3.5</td>
<td>1.9</td>
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<tr>
<td></td>
<td>Oleate (2 mM)</td>
<td>3.5</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>DL-β-Hydroxybutyrate (10 mM)</td>
<td>3.5</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>Acetone (10 mM)</td>
<td>3.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Experiment 3</td>
<td>None</td>
<td>3.4</td>
<td>2.2</td>
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<td></td>
<td>Pyruvate (10 mM)</td>
<td>3.4</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>Oleate (1 mM)</td>
<td>3.4</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>DL-β-Hydroxybutyrate (10 mM)</td>
<td>3.4</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td>Amytal (2 mM)</td>
<td>3.4</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td>Amytal (4 mM)</td>
<td>3.4</td>
<td>2.7</td>
</tr>
</tbody>
</table>

Rat liver cells (4 to 6 mg dry weight per ml) were incubated at 37°C in Krebs bicarbonate medium containing 4% (w/v) bovine serum albumin and 0.2 mM propionylpyrazole. Aliquots (0.8 ml) of the medium were removed at 6- or 12-min intervals over a period of 30 or 60 min. The mean acetaldehyde concentration refers to the average concentration present over the time interval used to calculate the rate of uptake. Rates were usually linear over the 30- or 60-min time interval under investigation.
presence of 0.2 μM propionylpyrazole to inhibit alcohol dehydrogenase activity, the rate of acetaldehyde uptake was strongly increased by addition of pyruvate as a trapping system for cytosolic NADH. On the other hand, addition of substrates which generate reducing equivalents directly in the mitochondria, such as octanoate, oleate, or β-hydroxybutyrate, all caused a substantial inhibition of acetaldehyde uptake, while addition of acetacetate stimulated acetaldehyde uptake. In Experiment 3 of Table I, addition of acetaldehyde decreased ketone body formation from oleate from 384 to 311 μmoles per g dry weight per hour and increased the ratio of β-hydroxybutyrate to acetacetate from 3.0 ± 0.2 to 8.7 ± 1.3. Addition of amobarbital (Amytal) which inhibits electron transport between NADH dehydrogenase and cytochrome b also caused a severe inhibition of acetaldehyde uptake. When low (0.3 to 0.5 mM) initial acetaldehyde concentrations were used (Table II), the control rate of acetaldehyde uptake was lower (cf. Fig. 4), and pyruvate had a much smaller stimulatory effect on uptake, particularly at a mean acetaldehyde concentration of 0.2 mM. Oleate and β-hydroxybutyrate were strongly inhibitory, while acetacetate addition almost doubled acetaldehyde uptake. These results show a competition for mitochondrial NAD between acetaldehyde dehydrogenase and other mitochondrial dehydrogenases. On the other hand, relatively high acetaldehyde concentrations are required to show interactions with cytosolic NAD-linked dehydrogenases.

The effects of acetaldehyde infusion on oxygen uptake and 14CO₂ production from [U-14C]oleate added in tracer amounts was investigated in perfused rat livers supplied with either 0.45 mM pyruvate (Fig. 5) or 0.45 mM L(+)-lactate (Fig. 6) in the arterial fluid. Methylpyrazole (50 μM) was also infused to inhibit reduction of acetaldehyde to ethanol. Addition of substrate stimulated cell respiration and oxidation of endogenous fatty acids, the latter effect being judged from the 2- to 3-fold stimulation of 14CO₂ production. This finding is in accordance with previous indirect estimates based on calculations from metabolic balance studies of the effect of lactate (51) and pyruvate (52) on endogenous fatty acid oxidation. Infusion of 1 mM acetaldehyde for 10 min inhibited the generation of 14CO₂ with both substrates, but had opposite effects on the rate of respiration; this being stimulated in the presence of pyruvate but inhibited transiently in the presence of lactate. Infusion of 0.2 mM aminooxyacetate caused a small increase of 14CO₂ production, probably as compensation for inhibition of endogenous ureogenesis. It produced no effect on oxygen consumption in the presence of pyruvate but an inhibition of oxygen consumption to the endogenous rate in the presence of lactate. These changes

![Table II](http://www.jbc.org/)

<table>
<thead>
<tr>
<th>Additions</th>
<th>Acetaldehyde concentration</th>
<th>Acetaldehyde uptake, μmoles/g dry wt/hr</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.5</td>
<td>430</td>
<td>100</td>
</tr>
<tr>
<td>Pyruvate (10 mM)</td>
<td>0.5</td>
<td>638</td>
<td>148</td>
</tr>
<tr>
<td>Oleate (1 mM)</td>
<td>0.5</td>
<td>276</td>
<td>64</td>
</tr>
<tr>
<td>None</td>
<td>0.3</td>
<td>450</td>
<td>100</td>
</tr>
<tr>
<td>Pyruvate (10 mM)</td>
<td>0.3</td>
<td>487</td>
<td>106</td>
</tr>
<tr>
<td>Oleate (1 mM)</td>
<td>0.3</td>
<td>325</td>
<td>71</td>
</tr>
<tr>
<td>DL-β-Hydroxybutyrate (10 mM)</td>
<td>0.3</td>
<td>304</td>
<td>66</td>
</tr>
<tr>
<td>Acetacetate (10 mM)</td>
<td>0.3</td>
<td>849</td>
<td>185</td>
</tr>
</tbody>
</table>

![Fig. 5](http://www.jbc.org/)

**Fig. 5.** Effects of acetaldehyde infusion with pyruvate as substrate on oxygen uptake and 14CO₂ production from [U-14C]oleate by perfused rat liver. Substrates and inhibitors were added at the times indicated and infused over the interval denoted by the boxes. AOA, aminooxyacetate.

![Fig. 6](http://www.jbc.org/)

**Fig. 6.** Effects of acetaldehyde infusion with lactate as substrate on oxygen uptake and 14CO₂ production from [U-14C]oleate by perfused rat liver. Substrates and inhibitors were added at the times indicated and infused over the interval denoted by the boxes. AOA, aminooxyacetate.
Livers were perfused with Krebs' bicarbonate medium using a flow-through system at a rate of approximately 30 ml per min. Lactate (0.45 mM) or pyruvate (0.45 mM) together with methylpyrazole (50 μM) were added to the arterial fluid by continuous infusion. Acetaldehyde (1 mM) and aminooxyacetate (AOA) (0.2 mM) were also infused for 10-min intervals as indicated in the table. Samples of effluent fluid were collected over 2-min intervals. Uptake of acetaldehyde was determined from the arteriovenous concentration difference and the measured flow rate. Values shown are mean ± S.E. The rate of acetaldehyde uptake in the absence of substrate was 369 ± 37 μmoles per 100 g body weight per hour.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Acetaldehyde uptake</th>
<th>Lactate change</th>
<th>Pyruvate change</th>
<th>Glucose formation</th>
<th>Ratio of lactate to pyruvate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyruvate</td>
<td></td>
<td>62 ± 15</td>
<td>−324 ± 8</td>
<td>104 ± 4</td>
<td>0.26 ± 0.06</td>
</tr>
<tr>
<td>Pyruvate + acetaldehyde</td>
<td>424 ± 13</td>
<td>84 ± 11</td>
<td>−387 ± 9</td>
<td>140 ± 6</td>
<td>0.68 ± 0.08</td>
</tr>
<tr>
<td>Pyruvate + AOA</td>
<td>70 ± 13</td>
<td>−322 ± 5</td>
<td>112 ± 9</td>
<td>0.16 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>Pyruvate + AOA + acetaldehyde</td>
<td>478 ± 20</td>
<td>147 ± 19</td>
<td>−388 ± 2</td>
<td>116 ± 9</td>
<td>1.96 ± 0.25</td>
</tr>
<tr>
<td>Lactate</td>
<td>−153 ± 10</td>
<td>31 ± 2</td>
<td>54 ± 4</td>
<td>6.5 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Lactate + acetaldehyde</td>
<td>−169 ± 10</td>
<td>12 ± 1</td>
<td>57 ± 5</td>
<td>16.4 ± 2</td>
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</tr>
<tr>
<td>Lactate + AOA</td>
<td>−34 ± 11</td>
<td>8 ± 1</td>
<td>21 ± 1</td>
<td>34 ± 4</td>
<td></td>
</tr>
<tr>
<td>Lactate + AOA + acetaldehyde</td>
<td>388 ± 15</td>
<td>−9 ± 2</td>
<td>14 ± 1</td>
<td>300 ± 14</td>
<td></td>
</tr>
</tbody>
</table>

Correlated with a lack of inhibitory effect of aminooxyacetate on gluconeogenesis from pyruvate but an almost complete inhibition of gluconeogenesis from lactate (Ref. 53 and Table III). A second 10-min infusion of 1 mM acetaldehyde in the presence of aminooxyacetate again inhibited 14CO2 production. With pyruvate as substrate, the inhibitory effect of acetaldehyde on 14CO2 production was the same with or without aminooxyacetate also present. However, with lactate as substrate the inhibitory effect of acetaldehyde was smaller in the presence than the absence of aminooxyacetate, and a small stimulation of respiration was observed in contrast to an inhibition in the absence of aminooxyacetate.

Table III shows further metabolic changes induced by acetaldehyde in a series of liver perfusions following the same experimental protocol as those shown in Figs. 5 and 6. Acetaldehyde stimulated gluconeogenesis from pyruvate by about 40%, increased pyruvate uptake by 20%, and the lactate to pyruvate ratio 2.6-fold, but had no significant effect on lactate production. Uptake of acetaldehyde was stimulated about 15% by pyruvate addition. It is evident that acetaldehyde oxidation occurred partly in the mitochondrial compartment, thereby competing with the malate-aspartate cycle to the overall flux of reducing equivalents into mitochondria, while oxidation of acetaldehyde is also present. However, with lactate as substrate the inhibitory effect of acetaldehyde was smaller in the presence than the absence of aminooxyacetate, and a small stimulation of respiration was observed in contrast to an inhibition in the absence of aminooxyacetate.

The increased oxygen consumption (Fig. 5) upon addition of acetaldehyde may be partly accounted for by the increased energy demands for gluconeogenesis. Aminooxyacetate had no effects on pyruvate metabolism. Acetaldehyde added in the presence of pyruvate and aminooxyacetate had no effect on gluconeogenesis, but stimulated lactate formation 2-fold. This latter effect is presumably a response to the inhibition of cytosolic NADH transport into mitochondria. Gluconeogenesis from lactate was relatively small because of the low lactate concentration and was unaffected by acetaldehyde infusion, but was inhibited 60% by aminooxyacetate. Lactate uptake was almost completely suppressed. Addition of acetaldehyde in the presence of aminooxyacetate failed to release the inhibition of gluconeogenesis and caused a 10-fold increase of the lactate to pyruvate ratio, indicating that under these conditions the experiments it was not reversing inhibition of aspartate aminotransferase. Oxidation of acetaldehyde in the mitochondrial compartment is illustrated by the fall of 14CO2 production and small stimulation of respiration (Fig. 6). Uptake of acetaldehyde was 70% higher with pyruvate than with lactate as substrate, presumably as a result of the rate of electron transport was higher with pyruvate as substrate. Aminooxyacetate had little effect on acetaldehyde uptake in the presence of pyruvate, but in the presence of lactate a 50% stimulation of acetaldehyde uptake was observed. This correlated with an increase of respiration (Fig. 6), suggesting that prior to acetaldehyde addition the liver was substrate-depleted.

**DISCUSSION**

Indirect evidence based on the steady state level of reduction of pyridine nucleotides in the mitochondria and cytosolic compartments in the intact rat liver suggested that low acetaldehyde concentrations (e.g. below 0.5 mM) reducing equivalents were generated directly in the mitochondria, while at higher concentrations, acetaldehyde was also reduced by alcohol dehydrogenase to ethanol and oxidized by acetaldehyde dehydrogenase to acetate in the cytosol (29). The present results, made possible by the use of isolated liver cells to measure rates of acetaldehyde uptake over a wide range of concentrations, fully support this conclusion. Further insight into the compartmentation of acetaldehyde oxidation has been gained by the use of aminooxyacetate and NAD-cycloserine to inhibit the transamination steps of the malate-aspartate cycle (54). A number of previous studies with rat kidney cortex and liver (44, 53, 55–61) have established that transamination via aspartate aminotransferase is involved obligatorily in gluconeogenesis from lactate and in the transfer of excess reducing equivalents from the cytosol to mitochondria, although the quantitative contribution of the malate-aspartate cycle to the overall flux of reducing equivalents is in dispute (60, 61).
data using these inhibitors due to the possibility of Schiff-base formation between aminooxyacetate and acetaldehyde, no notable differences could be detected between the effects of aminooxyacetate and dl-cycloserine at concentrations of substrate and inhibitors used in the present experiments. Furthermore, clear evidence of inhibitory effects in the intact liver cell was obtained with both aminooxyacetate (Fig. 2, Fig. 6, and Table III) and dl-cycloserine (Figs. 3 and 4) in the presence of acetaldehyde. It may be concluded, therefore, that no major artifact is introduced by use of either inhibitor under carefully selected conditions.

The finding that dl-cycloserine at acetaldehyde concentrations below about 0.4 mM had no effect on acetaldehyde uptake by isolated liver cells but suppressed the increased uptake observed at higher acetaldehyde concentrations is strongly suggestive of a dual location for acetaldehyde oxidation. The high degree of inhibition by dl-cycloserine at elevated acetaldehyde concentrations indicates that reducing equivalents generated in the cytosol were transferred to the mitochondria for reoxidation largely by the malate-aspartate cycle. Furthermore, when alcohol dehydrogenase is active, ethanol formation and acetaldehyde uptake are greatly stimulated by increasing acetaldehyde concentrations (29, 34). The apparent $K_m$ of 1.1 mM for the cycloserine-inhibited acetaldehyde uptake in the presence of methylpyrazole agrees closely with the apparent $K_m$ for the cytosolic aldehyde dehydrogenase (16, 17, 22, 25, 26). The data in Fig. 4 show that the total activity of the cytosolic aldehyde dehydrogenase appears to be at least as great as that of the mitochondrial enzyme, despite reports that the over-all NAD-linked aldehyde dehydrogenase activity is largely mitochondrial (29–25). Direct mitochondrial oxidation of acetaldehyde is evidenced by the inability of either aminooxyacetate or dl-cycloserine to inhibit mitochondrial NADH generation and acetaldehyde uptake at low acetaldehyde concentrations. Since acetaldehyde levels do not rise above 0.2 mM during ethanol metabolism (30–34), it is clear that when acetaldehyde is generated from ethanol its oxidation is purely a mitochondrial function in rat liver. However, this might be a species peculiarity since the apparent $K_m$ for hepatic cytosolic NAD-linked aldehyde dehydrogenases is much lower in other species than in the rat (5, 7–9, 11).

Direct mitochondrial oxidation of acetaldehyde has important implications with regard to the regulation of ethanol metabolism. Notably, mutual competitive inhibition between acetaldehyde and fatty acids for oxidation appears to be at the level of mitochondrial NAD-linked dehydrogenases rather than at the step of transport of reducing equivalents into mitochondria. Likewise, amobarbital inhibition of ethanol metabolism (27, 48, 62) is probably accounted for by an indirect inhibition of mitochondrial acetaldehyde oxidation as a result of decreased NADH reoxidation by the electron transport chain with secondary feedback via alcohol dehydrogenase to ethanol uptake. The present data also illustrate inhibition of hepatic endogenous fatty acid oxidation by acetaldehyde, which is similar to that observed by ethanol.1 Presumably the suppression of citric acid cycle activity by ethanol (28, 63–67) is partially accounted for by competition between acetaldehyde dehydrogenase and citric acid cycle dehydrogenases for mitochondrial NAD. The very high affinity of the mitochondrial aldehyde dehydrogenase for acetaldehyde indicates that the acetaldehyde concentration is unlikely to limit ethanol oxidation, as previously suggested by Krebs (66). Regulation of the hepatic rate of ethanol uptake appears to be affected both by the rate of translocation of reducing equivalents generated from alcohol dehydrogenase into mitochondria (48), as well as by the rate of NADH reoxidation in the electron transport chain (34, 44).

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