The Metabolism of Acetone in Rat*

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Intraperitoneal injection of 5 μmol of acetone/g, body weight, into 3 rats previously fed 1% acetone (v/v) in their drinking water resulted in the appearance in blood serum of 16 ± 2 nmol of 1,2-propanediol/ml and 8 ± 1 nmol of 2,3-butanediol/ml. No detectable 1,2-propanediol or 2,3-butanediol was found in the serum of animals after acetone or saline injection without prior addition of acetone to drinking water or in the serum of animals injected with saline after having been maintained on drinking water containing 1% acetone. These data suggest that acetone both acts to induce a critical enzyme or enzymes and serves as a precursor for the production of 1,2-propanediol. It is also clear from these data that chronic acetone feeding plays a role in the 2,3-butanediol production in the rat.

Microsomes isolated from the liver of animals maintained on drinking water supplemented with 1% acetone contained two previously unreported enzymatic activities, acetone monooxygenase which converts acetone to acetol and acetol monooxygenase which converts acetol to methyglyoxal. Both activities require O2 and NADPH. Prior treatment with acetone increased serum D-lactate from 9 nmol/ml ± 9 nmol/ml in control animals to 77 ± 38 nmol/ml in acetone-fed animals after injection with 5 μmol of acetone/g, body weight. This is consistent with methyglyoxal being a by-product of acetone metabolism.

Two pathways for the conversion of acetone to glucose are proposed, the methyglyoxal and the propandiol pathways. The methyglyoxal pathway is responsible for the conversion of acetone to acetol, acetol to methyglyoxal, and the subsequent conversion of methyglyoxal to glucose. The propandiol pathway involves the conversion of acetol to 1,2-propanediol by an as yet unknown process. 1,2-Propandiol is converted to L-lactate by alcohol dehydrogenase, and L-lactaldehyde is converted to L-lactic acid by aldehyde dehydrogenase. Expression of these metabolic pathways in rat appears to be dependent on the induction of acetone monooxygenase and acetol monooxygenase by acetone.

Recent work by Reichard et al. (1) indicates that acetone may be a significant gluconeogenic precursor in fasting humans. Reichard et al. (1) showed that blood acetone, generated by the decarboxylation of acetoacetate, can reach levels of 1.6 mM in the blood of fasted humans. Their data suggest that up to two-thirds of the circulating blood acetone may be converted to glucose. This glucose production could account for 10% of the gluconeogenic demands of humans fasted 21 days (1) and suggests that acetone is an intermediate in the conversion of fat to carbohydrate. While a glyoxylate pathway converting fat to carbohydrate is well documented in plants and certain microorganisms (2), a pathway accomplishing such transformations in mammals has not been described. This paper describes a pathway capable of converting acetone, a product of fatty acid catabolism, to glucose.

The utilization of acetone by living systems has been long known (3,4). Borek and Rittenberg (5) and Price and Rittenberg (6) first reported the utilization of acetone by animals in the late 1940s and early 1950s. A variety of pathways have been suggested (7-9). Sakami et al. suggested that the utilization of acetone involved two different pathways, the degradation of acetone to formate and acetate (8) and the conversion of acetone to a 3-carbon gluconeogenic precursor (9). Rudney (10) reported that isotope from [2,14C]acetone is incorporated into 1,2-propanediol in rat liver homogenates, suggesting that 1,2-propanediol may play a role in acetone metabolism.

Methyglyoxal, one of the intermediates of the pathway proposed in this paper, has been suggested to be a regulator of cell growth (11). Its conversion to d-lactate by the actions of glyoxalase I (EC 4.4.1.5) and glyoxalase II (EC 3.1.2.6) is well established (12). Previously evidence has been presented indicating that some methyglyoxal may be produced in vivo from aminoacetone (13) and glyceraldehyde 3-phosphate (14).

Coleman (15) has recently shown the conversion of [2,14C]acetone to a stereochemically unidentified form of lactate in mitochondria-free liver homogenates from 3-day starved mice. Coleman also indicated that liver homogenates from heterozygous obese mice and heterozygous diabetic mice convert acetone to lactate at a higher rate than do homogenates from wild type mice. In humans with diabetic ketoacidosis, infusion of [2-14C]acetone resulted in incorporation of label into glucose in three out of nine patients and into serum proteins in all nine patients (16).

Clinical studies by Rutstein et al. (17) have recently established that 1,2-propanediol is a normal constituent of human blood and that concentrations of 1,2-propanediol and 2,3-butanediol are elevated in alcoholic drinking ethanol. Neither 1,2-propanediol nor 2,3-butanediol was found to be elevated in nonalcoholics given an acute dosage of ethanol. No other protocol which results in the occurrence of both 1,2-propanediol and 2,3-butanediol in laboratory animals is in the literature. The relationship of the pathways proposed to alcoholism is not clear since alcoholics are not generally thought to have high circulating levels of acetone.

Blood serum obtained from one human subject who had been fasted for 21 days was found to contain approximately 100 nmol/ml of 1,2-propanediol and 16 nmol/ml of acetol, both proposed intermediates of the pathway described here. No 2,3-butanediol was found in this blood sample, but the appearance of 1,2-propanediol and acetol under conditions in which acetone concentrations in excess of 1 mM have been

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G. Cahill and T. Aoki, personal communication.
shown in (1) suggests that 1,2-propanediol is produced from acetol in humans in a manner similar to that shown here for the rat. Acetone metabolism in rat and its relationship to the production of 1,2-propanediol and 2,3-butanediol is the subject of this paper.

EXPERIMENTAL PROCEDURES

Rats—Male Wistar rats from Charles River (Wilmington, MA) weighing between 255 and 390 g were fed the standard NIH rat ration ad libitum. Acetone-treated rats were given 1% acetone (v/v) in their drinking water for at least 3 days prior to death or deprivation of food. Some rats had all food removed 18 h prior to the experiment with the exception that free access to water containing acetone was continued through the period of starvation unless otherwise noted under "Results." All rats used in this study were healthy, vigorous, and indistinguishable from control rats at the time of death. Hema-topcrit was unaffected by chronic acetone feeding.

Reagents—Reagent grade acetone was obtained from J. T. Baker Chemical Co. [2-14C]Acetone (7.5 mCi/mmol) was from New England Nuclear. 1-Hydroxyacetone (acetol), 1,2-propanediol, and β-hydroxyphenylboric acid were purchased from Aldrich. Aqueous methylglyoxal (40% w/v), D-lactate, 4-methylpyrazole, D-threonine, L-threonine, and D- and L-lactaldehyde were from Sigma. D,L- and meso-peaks did not overlap. The resolved diastereomers were from Beckman. 2,3-Butanediol was obtained from the Burdick and Jackson Chemical Company (Muskegon, MI). Stereocchemically unresolved 2,3-butanediol was from the Burdick and Jackson Chemical Company, K and K Chemical Company (Plainview, NY), and Aldrich. Horse liver alcohol dehydrogenase was from Sigma. All other enzymes and pyridine nucleotides were from Boehringer Mannheim. Bovine serum albumin, fraction V, was obtained from Miles Laboratories, Inc. Dowex resins were obtained from Bio-Rad. Porapak 80/100 mesh support was obtained from Applied Science (State College, PA). Hydoflur was purchased from National Diagnostics (Somerville, NJ). 4-Pentylpyrazole was a generous gift from Dr. Neal Cornell. All other chemicals were reagent grade products.

Methods—Most commercially obtained reagents were used without further purification, but both methylglyoxal and 2,3-butanediol were routinely purified before use. Commercially obtained methylglyoxal was diluted to an aqueous 7% (w/v) solution with H2O and distilled under vacuum. The fraction collected between 72-78 °C was stored under N2 and used within 1 week of distillation. The meso- and D,L-diastereomers of 2,3-butanediol were resolved by borate chromatography. 1 g of undiluted 2,3-butanediol was added to a Dowex AG-1X-2 (boreate form) column (1 x 50 cm) and immediately eluted with 200 ml of 1 M NaCl. Fractions of 1 ml were collected. Both the D,L- and meso-diastereomers were eluted. Fractions were selected in which the D,L- and meso-peaks did not overlap. The resolved diastereomers were separated from the salt solution by distillation under N2.

Arterial blood samples from rats were obtained from a cannula inserted into the aorta through the common carotid artery. Heparin was added to the arterial cannula through a cannula inserted through the jugular vein. Substrates were either infused into the atrium or injected under vacuum. The fraction collected between 72-78 °C was stored under N2 and used within 1 week of distillation. The meso- and D,L-diastereomers of 2,3-butanediol were resolved by borate chromatography. 1 g of undiluted 2,3-butanediol was added to a Dowex AG-1X-2 (boreate form) column (1 x 50 cm) and immediately eluted with 200 ml of 1 M NaCl. Fractions of 1 ml were collected. Both the D,L- and meso-diastereomers were eluted. Fractions were selected in which the D,L- and meso-peaks did not overlap. The resolved diastereomers were separated from the salt solution by distillation under N2.

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The Methylglyoxal and Propanediol Pathways

RESULTS

The oxidation of acetone was first investigated in four groups of animals. Intraperitoneal injection of 5 μmol of acetone/g total body weight into rats previously given drinking water containing 1% acetone resulted in the appearance of 16 ± 2 nmol of 1,2-propanediol/ml of serum and 8 ± 1 nmol of 2,3-butanediol/ml of serum (Table I). Diols were not detected in any of the three control groups: saline-injected animals maintained on untreated drinking water, acetone-injected animals maintained on untreated drinking water, or saline-injected animals given drinking water to which 1% acetone had been added. The measurement of 1,2-propanediol and 2,3-butanediol was confirmed by the independent chromatographic quantitation of the p-hydroxyphenylacetic acid derivatives of 1,2-propanediol and 2,3-butanediol. The values obtained by this technique were in good agreement with the data shown in Table I. Derivatization of 2,3-butanediol showed that the predominant diastereomer produced was the meso-isomer. The meso-isomer was also the predominant form found in alcoholic (17). Chronic treatment with acetone also elevated serum D-lactate concentrations greater than fourfold above the levels seen in acutely treated animals. In animals in which acetone-treated drinking water was not removed prior to acetone injection, 1,2-propanediol levels as high as 85 nmol/ml and 2,3-butanediol levels as high as 93 nmol/ml were observed in serum after only 3 days of acetone treatment.

The subcellular location of acetone oxidation in livers from rats given drinking water containing 1% acetone was determined to be microsomal. Incubation of microsomes from the equivalent of 0.056 g of rat liver for 60 min at 38 °C in a 2.0-ml incubation mixture containing 50 mM potassium phosphate, pH 7.3, 5 mM NADPH, atmospheric oxygen, and 5 mM acetone, and atmospheric oxygen produced acetol at a rate of 37 nmol/min/g of liver as calculated from the final acetol concentration. Acetone, a reduced pyridine nucleotide, and oxygen were required for acetol synthesis, and D-lactate synthesis from acetol were linear over the course of the incubations. The production of glucose from the major amount of the acetol used is not accounted for by the rate of acetol synthesis observed with NADPH. The data suggested the following activity for acetone monooxygenase, CH₃-CO-CH₂ + NADPH + H⁺ + O₂ → CH₃-CO-CH₂OH + NADP⁺ + H₂O.

In order to observe linear rates of acetol production with respect to time it was necessary to use an NADPH-regenerating system. When 10 mM glucose 6-phosphate, 5 mM NADP, and 0.5 unit of glucose-6-phosphate dehydrogenase were added to incubations identical with those described above a rate of 130 nmol of acetol production/min/g of liver was observed. Addition of 50 μM pentylenzylazide to incubations resulted in total inhibition of acetone monooxygenase activity. Acetone monooxygenase activity in control rats not previously exposed to acetone was 10-fold less than the rate reported here for acetone-treated rats.

Incubation of microsomes isolated from the equivalent of 0.056 g of rat liver for 60 min at 38 °C in a 2.0-ml reaction mixture containing 50 mM potassium phosphate, pH 7.3, 5 mM NADPH, atmospheric oxygen, and 5 mM acetol, and a methylglyoxal-trapping system consisting of 5 mM glutathione and 2.5 units of glyoxalase 1/ml produced D-lactate at a rate of 174 nmol/min/g of liver as calculated from the final D-lactate concentration in incubations. D-Lactate production was linear with respect to time. The acetol used in these incubations was free of methylglyoxal as measured by enzymatic assay and gas chromatographic assay. No D-lactate production was observed in the absence of glyoxalase I and glutathione. Under these conditions methylglyoxal was produced at a rate of 120 nmol/min/g in microsomal incubations as measured by the gas chromatographic assay. This activity, acetol monooxygenase, required acetol, oxygen, and a reduced pyridine nucleotide. NADPH was the preferred nucleotide with rates of methylglyoxal production more than five times greater than rates observed with NADH. These data suggest the following reaction for acetol monooxygenase,

CH₃-CO-CH₂OH + NADPH + H⁺ + O₂ → CH₃-CO-CHO + NADP⁺ + H₂O.

Addition of 50 μM pentylenzylazide to microsomal incubations resulted in a greater than 90% inhibition of the rate of D-lactate production. Acetol monooxygenase activity in control rats not previously exposed to acetone was more than 4-fold less than the activity observed in microsomes from rats maintained on acetone-treated drinking water for 5 days.

Incubation of hepatocytes with acetol or methylglyoxal resulted in both glucose and D-lactate production (Table II). A maximal rate of 100 nmol of glucose synthesized/min/g, wet weight of tissue, was achieved by incubating hepatocytes with 0.5 mM acetol. Increasing acetol concentrations above 0.5 mM resulted in lower rates of glucose production but higher rates of D-lactate production. The rate of acetol utilization, 280-460 nmol/min/g, wet weight of liver cell, indicates that the major amount of the acetol used is not accounted for by glucose or D-lactate production. Acetol utilization, glucose synthesis, and D-lactate synthesis from acetol were linear over the course of the incubations. The production of glucose from methylglyoxal increased in incubations up to a substrate concentration of 2.5 mM. D-Lactate production from methylglyoxal also increased with increasing substrate concentration. Methylglyoxal utilization was not linear with time. Incubations to which 0.5 mM methylglyoxal was added showed a greater than 50% decrease in methylglyoxal 30 s after initiation and no measurable methylglyoxal after 15 min. No methylglyoxal was detectable at the end of 60-min incubations in any of the incubations shown in Table II.

| Table I |

<table>
<thead>
<tr>
<th>1,2-Propanediol</th>
<th>2,3-Butanediol</th>
<th>D-Lactate</th>
</tr>
</thead>
<tbody>
<tr>
<td>nmol/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control animals</td>
<td>&lt;2, &lt;2, &lt;2</td>
<td>2, &lt;2, &lt;2</td>
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<tr>
<td>Acetone injected</td>
<td>&lt;2, &lt;2, &lt;2</td>
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<tr>
<td>Acetone-treated</td>
<td>&lt;2, &lt;2, &lt;2</td>
<td>2, &lt;2, &lt;2</td>
</tr>
</tbody>
</table>

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Hepatocytes were prepared from a 2-day starved rat given drinking water containing 1% acetone. 0.110 g of hepatocytes was incubated with the additions indicated below for 60 min at 38 °C in Krebs-Henseleit buffer containing 2.5% bovine serum albumin. Total incubation volume was 4.0 ml. The data presented here has been verified by 2 additional experiments. All other experimental details are discussed under “Experimental Procedures.”

<table>
<thead>
<tr>
<th>Table II</th>
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<tr>
<td><strong>Conversion of acetal and methylglyoxal to glucose and D-lactate by isolated hepatocytes</strong></td>
</tr>
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</table>

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### Additions

<table>
<thead>
<tr>
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<th>Glucose synthesis</th>
<th>D-Lactate synthesis</th>
<th>Acetol used</th>
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</thead>
<tbody>
<tr>
<td>None</td>
<td>61</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>0.5 mM methylglyoxal</td>
<td>113</td>
<td>240&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>1.0 mM methylglyoxal</td>
<td>130</td>
<td>500&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>2.5 mM methylglyoxal</td>
<td>195</td>
<td>1390&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>0.5 mM acetol</td>
<td>100</td>
<td>78</td>
<td>280</td>
</tr>
<tr>
<td>1.0 mM acetol</td>
<td>85</td>
<td>108</td>
<td>420</td>
</tr>
<tr>
<td>2.5 mM acetol</td>
<td>76</td>
<td>145</td>
<td>460</td>
</tr>
</tbody>
</table>

<sup>a</sup> None detected.<br><sup>b</sup> Rate of production not linear over time.

Upon addition of 1 mM acetone as the sole substrate for hepatocytes the rate of gluconeogenesis increased between 11 and 18 nmol/min/g of cell, wet weight (Table III). The increased glucose synthesis observed in the incubations containing 1 mM acetone was significantly greater than that observed without added substrate at the 99.9% confidence level as judged by an analysis of variance for balanced data. In incubations to which 1 mM acetone was added acetol concentration was 51.6 ± 12.4 μM (N = 3) at the end of 60 min. Without the addition of acetone, no acetol was detected.

Incubation of 1 mM [2,14C]acetone with hepatocytes resulted in an increase in glucose synthesis of 12 nmol/min/g (Table IV, lines 1 and 2). The rate of incorporation of label from [2,14C]acetone into glucose was 22.4 nmol/min/g (Table IV, line 2). Addition of 0.5 mM acetol to hepatocyte incubations containing 1 μM acetone resulted in a net increase in glucose synthesis, while decreasing the amount of [2,14C] acetone incorporated into glucose to 40% of the value seen in incubations in which 1 mM acetone was the sole substrate (Table IV, lines 2 and 3). The production of glucose from acetol (Table II), the production of acetol in hepatocyte incubations containing 1 mM acetone, and the dilution of 14C label in the glucose synthesized from [2,14C]acetone when unlabeled acetol was added to the incubations all are compatible with acetol being an intermediate in the conversion of acetone to glucose. The production of D-lactate in incubations containing acetone (Table IV) and acetol (Table II and Table IV) and the production of glucose in incubations from methylglyoxal (Table II) strongly suggest that methylglyoxal is an intermediate in the conversion of acetone to glucose by isolated hepatocytes. The inhibition of the conversion of both acetone and acetol to glucose by pentylypyrazole (Table IV, lines 5 and 6) and the inhibition of acetol monooxygenase and acetol monooxygenase by pentylypyrazole suggest that both acetone and acetol monooxygenase are necessary enzymes in the conversion of acetone to glucose by isolated hepatocytes.

While acetol and methylglyoxal appear to be intermediates in the major pathway converting acetone to glucose, our data suggest that the major pathway of methylglyoxal conversion to glucose is not via D-lactate production by glyoxalase I and II. Addition of 0.74 mM D-lactate to cell incubations resulted in an increase in the rate of net glucose synthesis of 96 nmol/min/g of cells (Table IV, line 2 and 4) and a rate of D-lactate utilization of 182 nmol/min/g of cells. The increase in the rate of glucose synthesis observed can be totally accounted for by the rate of D-lactate utilization. While D-lactate is gluconeogenic (32), the failure of the addition of unlabeled D-lactate to significantly decrease the incorporation of [2,14C] acetone into glucose (Table IV, line 4) shows that the major pathway for the incorporation of acetone into glucose does not go through D-lactate.

Intravenous infusion into rat of 5 μmol of acetol/g body weight, resulted in measurable 1,2-propanediol in less than 30 s and a mean 1.2-propanediol in arterial blood of 156 ± 64 nmol/ml (N = 5) after 10 min. Assuming 1,2-propanediol is evenly distributed throughout total body water, which is 72%
of the body mass (33), and that the liver represents 7% of the total body mass of rat, the rate of propanediol production would be 160 nmol/min/g of liver if propanediol production were totally hepatic. Incubation of hepatocytes isolated from 48-h starved rats with 5 mM acetol resulted in the production of only 22 nmol of 1,2-propanediol/min/g of cells, wet weight. The rate of 1,2-propanediol production in perfused liver was 26 nmol/min/g in the presence of 5 mM acetol. No significant difference has been found in 1,2-propanediol concentration between the hepatic artery and vein during infusion of acetol.

In addition, no significant difference in the rate of 1,2-propanediol production in vivo has been observed in the presence of methylopyrazole indicating that alcohol dehydrogenase is not responsible for the production of 1,2-propanediol from acetol in vivo. These data suggest that extrahepatic tissue plays a significant role in the reduction of acetol to propanediol.

1,2-Propanediol was isolated from serum collected 1 h after intraperitoneal injection of 5 μM acetol/g body weight and its chirality determined as described under "Experimental Procedures." In incubations containing alcohol dehydrogenase, aldehyde dehydrogenase, and 100 μM 1,2-propanediol isolated from rat blood, more than 90% of the added 1,2-propanediol was recovered as L-lactic acid. In control incubations containing 100 μM L-1,2-propanediol and 100 μM D-1,2-propanediol, 97 and 80% of the added 1,2-propanediol were recovered as L- and D-lactic acid, respectively. In all reaction mixtures no 1,3-propanediol could be detected at the end of the incubation period. The data indicated that the primary stereoisomer produced in vivo from acetol in rat is L-1,2-propanediol.

Addition of 5 mM L-1,2-propanediol to isolated liver cells from rats previously given 1% acetone drinking water and starved for 48 h resulted in glucose synthesis of 260 nmol/min/g of cells, wet weight (Table V). This rate is almost 40% of the rate of glucose production seen in incubations containing 5 mM L-lactate. Although alcohol dehydrogenase is not responsible for the production of 1,2-propanediol, it does appear to be responsible for its utilization. The complete inhibition of gluconeogenesis from L-1,2-propanediol by pentylpyrazole, an inhibitor of alcohol dehydrogenase (34), and cyanamide, an inhibitor of aldehyde dehydrogenase (35), suggests that L-1,2-propanediol is converted to L-lactaldehyde by alcohol dehydrogenase and that L-lactaldehyde is converted to L-lactate by aldehyde dehydrogenase. The failure of hepatocytes to use more than 10% of the L-1,2-propanediol added to incubations in the presence of cyanamide reflects the unfavorable thermodynamics of the conversion of alcohol to an aldehyde in the absence of the further oxidation of the aldehyde. In data not shown, it has been found that 64 μM cyanamide inhibits the conversion of [2-13C]acetol to glucose by only 20–40% in hepatocytes. The total inhibition of glucose synthesis from L-1,2-propanediol by 64 μM cyanamide (Table V) suggests that the formation of L-1,2-propanediol is not important for the conversion of acetol to glucose in hepatocytes. These data do not address the relative importance of L-1,2-propanediol to gluconeogenesis from acetone in vivo.

D-1,2-Propanediol was utilized at 10% the rate of L-1,2-propanediol. The presence of a small amount of D-lactate in the incubation containing D-1,2-propanediol suggests that D-1,2-propanediol is metabolized like L-1,2-propanediol via alcohol dehydrogenase and aldehyde dehydrogenase to D-lactate, but at a much slower rate.

**DISCUSSION**

The data in this paper suggest that hepatocytes isolated from chronic acetone-fed rats are capable of converting acetone to glucose *in vitro*. In addition, the involvement of at least one extrahepatic enzyme appears to enable the rat to convert acetone to glucose by a second pathway *in vivo*. We propose the pathways illustrated in Fig. 1 and suggest the names of the methylglyoxal and propanediol pathways for the activities described. In the methylglyoxal pathway acetone is converted to acetaldehyde by acetone monoxygenase, acetal is converted to methylglyoxal by acetal monoxygenase, and methylglyoxal is converted to glucose by an as yet undefined mechanism. The propanediol pathway consists of the conversion of acetol to L-1,2-propanediol by an extrahepatic process, the conversion of L-1,2-propanediol to L-lactaldehyde by alcohol dehydrogenase and aldehyde dehydrogenase, and their reduction to L-lactate by alcohol dehydrogenase and aldehyde dehydrogenase. The failure of hepatocytes to use more than 10% of the L-1,2-propanediol added to incubations in the presence of cyanamide reflects the unfavorable thermodynamics of the conversion of alcohol to an aldehyde in the absence of the further oxidation of the aldehyde. In data not shown, it has been found that 64 μM cyanamide inhibits the conversion of [2-13C]acetol to glucose by only 20–40% in hepatocytes. The total inhibition of glucose synthesis from L-1,2-propanediol by 64 μM cyanamide (Table V) suggests that the formation of L-1,2-propanediol is not important for the conversion of acetol to glucose in hepatocytes. These data do not address the relative importance of L-1,2-propanediol to gluconeogenesis from acetone in vivo.

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The data in this paper suggest that hepatocytes isolated from chronic acetone-fed rats are capable of converting acetone to glucose *in vitro*. In addition, the involvement of at least one extrahepatic enzyme appears to enable the rat to convert acetone to glucose by a second pathway *in vivo*. We propose the pathways illustrated in Fig. 1 and suggest the names of the methylglyoxal and propanediol pathways for the activities described. In the methylglyoxal pathway acetone is converted to acetaldehyde by acetone monoxygenase, acetal is converted to methylglyoxal by acetal monoxygenase, and methylglyoxal is converted to glucose by an as yet undefined mechanism. The propanediol pathway consists of the conversion of acetol to L-1,2-propanediol by an extrahepatic process, the conversion of L-1,2-propanediol to L-lactaldehyde by alcohol dehydrogenase and aldehyde dehydrogenase, and their reduction to L-lactate by alcohol dehydrogenase and aldehyde dehydrogenase.

**TABLE V**

Rates of gluconeogenesis from L-1,2-propanediol, D-1,2-propanediol, and L-lactate in isolated hepatocytes

Hepatocytes were prepared from a 2-day starved rat given drinking water containing 1% acetone. 0.166 g of hepatocytes was incubated with the additions noted below for 60 min at 38 °C in Krebs-Henseleit buffer 2.5% in bovine serum albumin. Total incubation volume was 4.0 ml. The data presented here has been verified by two additional experiments. All other experimental details are discussed under "Experimental Procedures."

<table>
<thead>
<tr>
<th>Additions</th>
<th>Glucose</th>
<th>L-Lactate</th>
<th>D-Lactate</th>
<th>L-1,2-Propanediol</th>
<th>Glucose synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nmoI/ml</td>
<td>nmoI/min/g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 mM L-lactate</td>
<td>1130</td>
<td>2180</td>
<td>ND*</td>
<td>ND</td>
<td>655</td>
</tr>
<tr>
<td>5 mM L-lactate, 50 μM pentylopyrazole</td>
<td>1000</td>
<td>2260</td>
<td>ND</td>
<td>ND</td>
<td>611</td>
</tr>
<tr>
<td>5 mM L-lactate, 64 μM cyanamide</td>
<td>590</td>
<td>2340</td>
<td>ND</td>
<td>ND</td>
<td>300</td>
</tr>
<tr>
<td>5 mM L-1,2-propanediol</td>
<td>458</td>
<td>251</td>
<td>ND</td>
<td>3180</td>
<td>260</td>
</tr>
<tr>
<td>5 mM L-1,2-propanediol, 50 μM pentylopyrazole</td>
<td>131</td>
<td>18</td>
<td>ND</td>
<td>4310</td>
<td>59</td>
</tr>
<tr>
<td>5 mM L-1,2-propanediol, 64 μM cyanamide</td>
<td>158</td>
<td>26</td>
<td>ND</td>
<td>4680</td>
<td>76</td>
</tr>
<tr>
<td>5 mM D-1,2-propanediol</td>
<td>242</td>
<td>298</td>
<td>69</td>
<td>4850</td>
<td>129</td>
</tr>
<tr>
<td>No substrates</td>
<td>162</td>
<td>32</td>
<td>ND</td>
<td>ND</td>
<td>79</td>
</tr>
</tbody>
</table>

* ND, none detected.

**FIG. 1.** The methylglyoxal and propanediol pathways.
cohol dehydrogenase, and the conversion of L-lactaldehyde to L-lactic acid by aldehyde dehydrogenase.

From our data on isolated hepatocytes, it is not possible to determine whether the methylglyoxal or the propanediol pathway is the predominant pathway involved in the conversion of acetone to glucose in vivo. The rate of glucose synthesis observed from acetone in isolated hepatocyte incubations, in which only the methylglyoxal pathway is active, was 11–18 nmol/min/g. Although the rate of glucose synthesis reported here may not be maximal, it is still sufficient to supply 7% of the brain's glucose requirement, assuming a rate of glucose utilization for brain of 0.8 μmol/min/g, wet weight (36). It is clear from the data presented (Table II) that at acetol concentrations above 0.5 mM, a significant amount of acetol utilization in isolated hepatocytes is unaccounted for by the methylglyoxal pathway. However, the data presented in Table II suggest at lower levels of acetol, such as may occur in vivo, the per cent conversion to glucose may be greater. In contrast to the situation in isolated hepatocytes in vivo, acetol is rapidly converted to L-1,2-propanediol. Presumably, this conversion is catalyzed by an extrahepatic enzyme. The L-1,2-propanediol produced extrahepatically returns to liver where it is an excellent gluconeogenic substrate. Although we have not addressed how 1,2-propanediol is produced in vivo, others have suggested a mechanism for this reaction (10, 37). In the one serum sample obtained from a human fasted 21 days, 16 nmol of acetol/ml and 100 nmol of 1,2-propanediol/ml were found. These data suggest that the propanediol pathway may be a significant source of glucose production in fasting humans. Acetone monooxygenase activity is elevated 3- to 4-fold in 3-day fasted rats. No change in acetol monooxygenase activity was observed over this period of time. It is unclear whether these data suggest that prolonged fasting in rats is required for the induction of the enzymes required for the methylglyoxal pathway to function or whether the methylglyoxal pathway is unimportant in the fasting animal.

Elevated levels of serum 1,2-propanediol and 2,3-butanediol have been observed in the blood of chronic alcoholics. It has been shown that 2,3-butanediol, but not 1,2-propanediol, can be produced by injection of ethanol into rats in which aldehyde dehydrogenase has been inhibited (38). The production of 1,2-propanediol and 2,3-butanediol reported in this paper certainly occurs by a different mechanism than that reported earlier. Preliminary data indicates that chronic ethanol feeding for a period of 3 weeks results in a 7- to 8-fold increase in the levels of both acetone monooxygenase and acetol monooxygenase in rat. The increased formation of smooth endoplasmic reticulum and drug-metabolizing enzymes in rat liver in response to chronic ethanol feeding is well documented (39–41). Koop et al. (42) have recently purified an ethanol-induced cytochrome P-450 system to homogeneity from rabbit liver. It is not yet clear that acetone monooxygenase and acetol monooxygenase are cytochrome P-450 systems. In preliminary experiments addition of 0.5 mM SKF-525A, a general inhibitor of P-450 enzymes, to acetol monooxygenase assays containing 0.5 mM acetol resulted in a 41% inhibition of β-lactate production. Addition of 0.5 mM SKF-525A to acetone monooxygenase containing 2 mM acetone resulted in an inhibition of acetol production of only slightly more than 10%. The inhibition of acetol monooxygenase observed is consistent with acetol monooxygenase being a P-450 enzyme. The inhibition of acetone monooxygenase is less than would be expected from a cytochrome P-450 system. The relationship of the pathways discussed here to the conversion of 2,3-butanediol and 1,2-propanediol in the blood of human alcoholics is not yet clear. Since alcoholics do not have high blood levels of acetone, this substrate seems unlikely to be the percursor. Preliminary studies indicate that unlike fasting patients, blood acetol is not elevated in alcoholics.

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