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 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 monoxygenase which converts acetone to acetol and acetol monoxygenase 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 ± 36 nmol/ml in acetone-fed animals after injection with 15 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 propenediol 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 propanediol pathway involves the conversion of acetol to 1,2-propanediol by an as yet unknown process. 1,2-Propanediol is converted to D-lactate by alcohol dehydrogenase, and 1-lactaldehyde is converted to L-lactate acid by aldehyde dehydrogenase. Expression of these metabolic pathways in rat appears to be dependent on the induction of acetone monoxygenase and acetol monoxygenase 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). Sakani 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 alcoholics 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 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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1 G. Cahill and T. Aoki, personal communication.
shown in blood (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 throughout 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
tocrit 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 p-bromophenylboric acid were purchased from Aldrich. Aqueous methylglyoxal (40% w/v), D-lactate, 4-methylpyrazole, d-threonine, L-threonine, 2,3-butanediol, and cytosol were from Sigma. 2,3-Butanediol was from the Burdick and Jackson Chemical Company (Muskegon, MI). Stereochemically 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 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 coated with 3% Carbowax and 100 mesh support coated with 3% Carbowax were from Supelco\Hydromatrix. Horse liver alcohol dehydrogenase was purified before use. Commercially obtained methylglyoxal (Maumee, OH) was obtained from Aldrich. 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 1-X2 (borate form) column (1 x 50 cm) and immediately eluted with 100 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 blood before it was transferred into 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 vacuum.

Hemoglobin was extracted from the erythrocytes. Hemoglobin was then homogenized with 6 passes of the Elvehjem homogenizer with 3 ml of an ice-cold 10 mM potassium phosphate, pH 7.0, 0.9% NaCl buffer added for each g of minced liver. The tissue was homogenized with six passes of the motor at 600 rpm. Microsomal and high speed supernatant fractions were obtained from this homogenate as described by Hogeboom (22). 14C]Glucose was isolated from radioactivity in the blood and liver by two different methods. 0.5 ml of a neutralized cell incubation extract was passed through an AG 1-X2 formate column, absorbed to a borate AG 1-X2 column, and eluted with formate as described by Huang and Lardy (23). Alternatively the extract was passed through an AG 1-X2 column, phosphorylated with hexokinase and each fraction was passed through an AG 1-X2 column and eluted as follows. 0.5 ml of neutralized cell extract was passed through a AG 1-X2 column (0.5 x 5 cm) with 1.5 ml of distilled H2O. The neutral fraction was added to a reaction mixture containing 25 mM Tris-HCl, pH 7.8, 1.25 mM MgCl2, 1.25 mM ATP and 2.5 ml of [2-14C]Acetone. The reaction was initiated with the addition of 3.5 units of yeast hexokinase. At the end of 30 min the incubation mixture was added directly to a AG 1-X2 column (0.5 x 5 cm) and washed with 15 ml of H2O. Phosphoryl
ated glucose was eluted with 10 ml of 4 N formic acid. The eluate was evaporated to dryness. The dried sample was solubilized with 1 ml of H2O and counted in 10 ml of Hydrofluor at 28° C in a Beckman LS-350 liquid scintillation spectrometer at a counting efficiency of 94%. Recovery of added glucose was 97% by the borate protocol and 94% by the phosphorylation protocol. Incorporation of [2-14C]acetone into glucose was calculated by dividing the disintegrations/min in isolated glucose by the specific activity of the [2-14C]acetone added to incubations.

ATP (24), glucose (25), L-lactate (26), and D-lactate (27) were assayed as described elsewhere. The concentration of 1,2-propanediol and 2,3-butanediol in perchloric acid-extracted samples was determined by gas chromatography using a column (3 meters x 2 mm) packed with Carbowax 20 M. The column temperature was maintained at 150° C. Carrier gas flow was 30 ml of helium/min. Flame ionization detection was used. Injection volume was 1-3 μl. Acetol and methylglyoxal concentrations in perchloric acid-extracted samples were measured using the same column, but using an oven temperature of 135 and 120°C, respectively. Acetol was not measured by this method because of interfering substances with similar retention times. Methylglyoxal was measured enzymatically by the method of Gawehn and Bergmeyer (28). 1,2-Propanediol and 2,3-butanediol were measured by a second gas chromatographic technique. Both 1,2-propanediol and 2,3-butanediol were extracted as their phenylboronate ester from serum without prior perchloric acid extraction. The esters of both diols were measured by electron capture detection as described by Neetham et al. (29).

D- and L-lactaldehyde was synthesized as described by Raskin and Sokoloff (30). D- and L-lactaldehyde was converted to D- and L-1,2-propanediol by a sodium borohydride reduction. 1 ml of a 2 mM sodium borohydride solution in 0.2 N NaOH was added dropwise to 5.0 ml of 0.5 M D- or L-lactaldehyde with gentle stirring. The reaction was maintained at room temperature for 30 min and then neutralized by the addition of dilute hydrochloric acid. The reaction mixture was passed through an AG 1-X2 column, absorbed to a borate column in the chloride form to remove all borate from the reaction mixture. Yields were consistently in excess of 90%. D- and L-1,2-propanediol synthesized in this manner were stereochemically greater than 90% pure.

The chirality of 1,2-propanediol in sera was determined by isolation of 1,2-propanediol and subsequent conversion to D- or L-lactic acid by the action of horse liver alcohol dehydrogenase and yeast aldehyde dehydrogenase. One-mI aliquots of pooled sera from rats injected with 5 μmol of acetol/g, body weight, were transferred to five glass culture tubes and dried as described by Neetham et al. (29). Tubes were subsequently extracted with 2 ml of an ethyl acetate solution containing 20 mg of p-bromophenylboronate. Each tube was then rinsed with 2 ml of ethyl acetate. Fractions were combined and then dried with a gentle stream of dry N2. The resulting p-bromophenylboronate ester of 1,2-propanediol was hydrolyzed by addition of 1.0 ml of water. This mixture was shaken, centrifuged, and the supernatant added to a AG 1-X2 column (1 x 4 cm) in the chloride form. The column was washed with water. Fractions of 1.0 ml were taken and assayed for 1,2-propanediol. The fraction with the highest 1,2-propanediol content was added to a AG 1-X2 column (1 x 4 cm) in the bisulfite form. This mixture was shaken, centrifuged, and the supernatant added to the fractions containing 1,2-propanediol pooled. At this stage the extracted 1,2-propanediol was greater than 90% pure as determined by gas chromatographic analysis. Incubation media were prepared containing 80 mM sodium carbonate, pH 8.9, 5.0 mM NAD, and 1.1 mM 1,2-propanediol in a total volume of 1.0 ml. To each assay 4.0 units of horse liver alcohol dehydrogenase and 4.25 units of aldehyde dehydrogenase was added. Control tubes were set up in an identical manner except that no 1,2-propanediol was added.
The Methylglyoxal and Propanediol Pathways

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-bromophenylboric 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 livers (17). Chronic treatment with acetone also elevated serum D-lactate concentrations greater than 4-fold 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 no measurable methylglyoxal after 15 min. No methylglyoxal was detectable at the end of 60-min incubations. 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-bromophenylboric 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 livers (17). Chronic treatment with acetone also elevated serum D-lactate concentrations greater than 4-fold 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.

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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 a methylglyoxal-trapping system consisting of 5 mM glutathione and 2.5 units of glyoxalase I/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 acetal 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 acetal, 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,

\[
\text{CH}_3\text{-CO-CH}_3 + \text{NADPH} + \text{H}^+ + \text{O}_2 \rightarrow \text{CH}_3\text{-CO-CH}_2\text{OH} + \text{NADP}^+ + \text{H}_2\text{O}
\]

Addition of 50 μM pentyrrylpyrazole 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 acetal 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 acetal. Increasing acetol concentrations above 0.5 mM resulted in lower rates of glucose production but higher rates of D-lactate production. The rate of acetal utilization, 280-460 nmol/min/g, wet weight of liver cell, indicates that the major amount of the acetal used is not accounted for by glucose or D-lactate production. Acetal utilization, glucose synthesis, and D-lactate synthesis from acetal 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>Saline injected, control animals</td>
<td>&lt;2, &lt;2, &lt;2</td>
<td>6, 2, 20</td>
</tr>
<tr>
<td>Acetone injected, control animals</td>
<td>&lt;2, &lt;2, &lt;2</td>
<td>5, 18, 28</td>
</tr>
<tr>
<td>Saline injected, acetone-treated animals</td>
<td>&lt;2, &lt;2, &lt;2</td>
<td>116, 43, 58</td>
</tr>
<tr>
<td>Acetone injected, acetone-treated animals</td>
<td>14.9, 17.2, 14.3</td>
<td>62, 52, 116</td>
</tr>
</tbody>
</table>

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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 were required for acetol synthesis. Methylglyoxal utilization was not linear with time. Incubations to which 0.5 mM methylglyoxal was added showed a greater than 90% inhibition of the rate of D-lactate production. Acetol monooxygenase activity in control rats not previously exposed to acetone was 10-fold less than the rate reported here for acetone-treated rats.

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The Methylglyoxal and Propanediol Pathways

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>Additions</th>
<th>Glucose synthesis</th>
<th>D-Lactate synthesis</th>
<th>Acetol used</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>61 nmol/min/g</td>
<td>ND*</td>
<td></td>
</tr>
<tr>
<td>0.5 mM methylglyoxal</td>
<td>113</td>
<td>240***</td>
<td></td>
</tr>
<tr>
<td>1.0 mM methylglyoxal</td>
<td>130</td>
<td>500***</td>
<td></td>
</tr>
<tr>
<td>2.5 mM methylglyoxal</td>
<td>195</td>
<td>1300***</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>82</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>

* None detected.

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 acetol to glucose by isolated hepatocytes. The inhibition of the conversion of both acetone and acetol to glucose by pentalpyrazole (Table IV, lines 5 and 6) and the inhibition of acetol monooxygenase and acetol monooxygenase by pentalpyrazole 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 glyoxlase 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, lines 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 mmol/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 methylpyrazole 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-lactaldehyde. 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 pentylnitrazole, 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 [2-14C]acetone to glucose by only 20–40% in hepatocytes. The total inhibition of gluconeogenesis 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 acetone 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 acetyl by acetyl coenzyme A, acetol is converted to methylglyoxal by acetyl coenzyme A, 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-lactate by al-
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 still is 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 is converted to 1,2-propanediol. The production of 1,2-propanediol has been inhibited (38). The production of 1,2-glyoxal pathway is unimportant in the fasting animal. Whether the methylglyoxal pathway to function or whether the methylglyoxal pathway may contribute at lower levels of acetol, such as may occur in vivo, remains to be determined.

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 1,2-propanediol. Presumably, this conversion is catalyzed by a hepatic enzyme. The L-1,2-propanediol produced extrahepatically returns to liver where it is an excellent gluconeogenic substrate. Although we have suggested a mechanism for this reaction (10, 37). In the fasting animal, utilization for brain of 0.8 pmol/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 is converted to 1,2-propanediol. The production of 1,2-propanediol has been inhibited (38). The production of 1,2-glyoxal pathway is unimportant in the fasting animal. Whether the methylglyoxal pathway to function or whether the methylglyoxal pathway may contribute at lower levels of acetol, such as may occur in vivo, remains to be determined.
The metabolism of acetone in rat.
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