Metabolism of Putrescine to 5-Hydroxy-2-pyrrolidone via 2-Pyrrolidone*

David W. Lundgren† and Henry M. Fales‡

From the Pediatric Metabolism Branch, National Institute of Arthritis, Metabolism and Digestive Diseases and the Laboratory of Chemistry, National Heart, Lung, and Blood Institute, Bethesda, Maryland 20205

Incubation of 2-[14C]pyrrolidone with sliced rat liver and analysis of the incubation medium by silica gel chromatography revealed that 2-[14C]pyrrolidone is metabolized to an unknown. It was previously shown by Lundgren and Hankins (Lundgren, D. W. and Hankins, J. (1978) J. Biol. Chem. 253, 7130-7133) that slices of rat liver readily synthesized 2-pyrrolidone from putrescine. The unknown metabolite was partially purified by methanol/chloroform extraction, activated charcoal column chromatography, and two-dimensional thin layer chromatography on silica gel plates. The 2-pyrrolidone metabolite was derivatized with bis(trimethylsilyl)trifluoroacetamide and analyzed by gas chromatography-mass spectrometry. The mass of the molecular ion (245) and fragment ions suggests that the 2-pyrrolidone metabolite is 5-hydroxy-2-pyrrolidone. The mass spectrum of synthetic 5-hydroxy-2-pyrrolidone was identical to that of the unknown metabolite utilized in the synthesis of spermidine which, in turn, is metabolized to spermine. The latter compounds are important in growth and differentiation and are susceptible to rapid modulation by various hormones in a number of target tissues (1-3). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed: Bldg. 10/8N250, Pediatric Metabolism Branch, National Institute of Arthritis, Metabolism, and Digestive Diseases, Bethesda, Md. 20205.

The abbreviation used is: bis-Tris propane, 1,3-bis[tris(hydroxymethyl)methylamino]propane.

It is well established in eukaryotes that putrescine (1,4-diaminobutane) is synthesized from ornithine via decarboxylase (EC 1.4.1.7). Putrescine is one of two metabolites utilized in the synthesis of spermidine which, in turn, is metabolized to spermine. The latter compounds are important in growth and differentiation and are susceptible to rapid modulation by various hormones in a number of target tissues (1-3). The enzymatic steps in spermidine and spermine formation have been examined in detail and numerous studies have been carried out attempting to elucidate the manner in which they are regulated (1-4).

Conceivably less, however, is known about the catabolism of putrescine, and very little is understood with respect to the distribution, function, and regulation of previously described putrescine metabolites. Putrescine is oxidized by diamine oxidase (EC 1.4.3.6) to γ-aminobutyraldehyde, which is spontaneously cyclized to Δ1-pyrrole (5). Δ1-Pyrrole may be further oxidized to γ-aminobutyric acid (6, 7) or it may be reduced to 1-amino-4-butanol (8). Alternatively, putrescine can be acetylated to monoacetylpolyamine and is oxidatively deaminated to N-acetyl-γ-aminobutyric acid, which is deacetylated to γ-aminobutyric acid (9).

Recently, this laboratory reported (10) that rat liver is capable of converting putrescine to 2-pyrrolidone, a lactam not previously identified in any biological system. Concurrent with our communication was the report that 2-pyrrolidone is a normal constituent of rat brain (11). Acid hydrolysis of 2-pyrrolidone yields γ-aminobutyric acid, an important compound in neurohumoral transmission (12). Earlier it was shown that Pseudomonas aeruginosa can hydrolize 2-pyrrolidone to γ-aminobutyric acid and can utilize 2-pyrrolidone as its sole carbon and nitrogen source (13). It was of interest, therefore, to determine if in mammalian systems 2-pyrrolidone is a metabolic end product or an intermediate in an alternative metabolic pathway of putrescine (e.g. γ-aminobutyric acid formation).

This communication reports the unique finding that 2-pyrrolidone is converted *in vitro* by rat liver to 5-hydroxy-2-pyrrolidone, a metabolite which, to our knowledge, has not been identified in any biological system to date. The rates of synthesis of 2-pyrrolidone and 5-hydroxy-2-pyrrolidone from putrescine and 2-pyrrolidone are altered by several compounds known to inhibit or enhance, or both, the activity of a variety of oxidative enzymes.

**MATERIALS AND METHODS**

*Tissue*—Rat livers were obtained from decapitated female Sprague-Dawley rats (80 to 100 days old). The livers were washed with ice-cold phosphate-buffered saline and sliced (0.5 mm) with a McIlwain tissue chopper, and the slices were placed on gauze saturated with cold buffer. The slices were weighed and transferred to a 20.0-ml incubation vial. Incubations were performed in a shaking water bath at 37°C for the desired length of time.

*Medium*—Incubation medium was a modified version (F12K) of Ham’s F12 (14) minus phenol red; sodium bicarbonate was replaced with cold buffer. The slices were weighed and transferred to a 20.0-ml incubation vial. Incubations were performed in a shaking water bath at 37°C for the desired length of time.

*Chemicals*—2-Pyrrolidone and silica gel sheets (No. 6061) were purchased from Eastman Kodak Co. 4-Amino-6-(U-14C)butyric acid (224 mCi/mmol) and L-[3,4-3H]glutamine (42.5 Ci/mmol) were obtained from New England Nuclear, while [1,4-14C]putrescine (116 mCi/mmol) was purchased from Sigma Chemical Co. Silica gel plates (20 × 20 cm, 1000 μm) were obtained from Analtech.

The abbreviation used is: bis-Tris propane, 1,3-bis[tris(hydroxymethyl)methylamino]propane.

4481
Synthesis of 2-[14C]Pyrrrolidone

4482 Biosynthesis of 5-Hydroxy-2-pyrrolidone

The open end of the tube was covered with several layers of filter paper and the lower one-half of the tube was heated at 212°C for 1 h. After cooling, the radioactive compounds were dissolved in approximately 2.0 ml of distilled water and loaded on a column (1.0 x 6.0 cm) of Dowex 50-H+. The column was washed with 20 ml of water. The water wash was taken to dryness and the "C-labeled sample was dissolved in 2.0 ml of water and rerun a second time through a column of Dowex 50-H+. The effluent (20 ml) was taken to dryness and dissolved in the desired medium. Based on recrystallization studies with authentic 2-pyrrolidone (10), the radioactive material from the final step was pure 2-[14C]pyrrolidone. A typical synthesis yielded 46% conversion of 2-[14C]aminobutyric acid to 2-[14C]pyrrolidone.

Synthesis of 5-Hydroxy-2-pyrrolidone—5-Hydroxy-2-pyrrolidone was synthesized by two methods. The first method as described by De Mayo et al. (15), involving the photo-oxidation of pyrrole and hydrogenation of the intermediate to 5-hydroxy-2-pyrrolidone, was utilized to obtain small amounts for identification of 5-hydroxy-2-pyrrolidone as its silyl derivative using gas chromatography-mass spectrometry. A second method was used to obtain larger amounts and 5 hydroxy 2-[14C]pyrrolidone. The latter involved the enzymatic conversion of L-glutamine to α-ketoglutaric acid followed by acid hydrolysis to 5-hydroxy-2-pyrrolidone as described by Duffy et al. (16), with several modifications; L-[3,4-14H]glutamine (100 µCi) in water/HCl was taken to dryness and resuspended in 60 ml of 50 mM Tris (pH 7.5) containing 5 units of L-amino acid oxidase and 22000 units of catalase. The sample was incubated at 37°C for 5 h in a shaking water bath. The reaction mixture was filtered through a UM2 Amicon ultrafiltration membrane and loaded on a Dowex 50-NH4+ column (0.9 x 10 cm). The column was washed with 25 ml of water and the wash was taken to dryness by lyophilization. The residue was resuspended in 5.0 ml of water, and the pH was adjusted to 2.0 with 4 N HCl and heated in an oil bath at 100°C. Alquons (5 µl) were added at specific times and chromatographed on silica gel sheets as described in Fig. 1. After 4 h of hydrolysis approximately 80% of the radioactivity migrated to an Rf value of about 0.6 (α-ketoglutaric acid remained near the origin). The pH of the sample was adjusted to 7 with NH4OH and loaded on a Dowex 1 acetate column (0.9 x 10 cm). The column was washed with 25 ml of water and the water wash was concentrated to 2.0 ml with a rotary evaporator. Utilizing the above procedure on a larger scale with carrier L-glutamine as the starting material, pure 5-hydroxy-2-pyrrolidone was obtained as determined by gas chromatography-mass spectrometry and 14C NMR spectroscopy.

RESULTS AND DISCUSSION

Metabolism of 2-[14C]Pyrrolidone—Incubating sliced rat liver with 2-[14C]pyrrolidone in F12K-3 medium for 4 h and subsequently chromatographing the incubation medium revealed that over 50% of the 2-[14C]pyrrolidone added was converted to an unknown metabolite (Fig. 1A). The unknown 14C-labeled metabolite was not detected in media containing 2-[14C]pyrrolidone spotted at 0 time, incubated for 4 h without liver, or incubated for 4 h with boiled liver (Fig. 1B). This metabolite was not detected in our original studies (10), at which time it was shown that the major product of putrescine metabolism by sliced rat liver was 2-pyrrolidone.

Since rat liver readily metabolizes both putrescine to 2-pyrrolidone and 2-pyrrolidone to an unknown metabolic product, an apparent contradiction existed between present and past observations. However, past and present assay conditions differ in two respects. In earlier studies (10), incubation medium was treated either with 10% trichloroacetic acid or chromatographed on Dowex 50-H+ before application to silica gel sheets; incubation medium in previous studies did not contain bis-Tris propane, but was buffered with sodium phosphate. Medium in our earlier experiments was not gassed with CO2 and a pH shift from 7.4 to approximately 8.8 occurred during the 4-h incubation period. Examination of these two modifications in greater detail revealed the following. Although all the expected radioactivity could be accounted for after chromatography on gel sheets, the unknown metabolite could not be detected if incubation medium was treated with 10% trichloroacetic acid or chromatographed on Dowex 50-H+ but not Dowex 50-Na+, prior to chromatography (not shown). These results suggested that the unknown metabolite is chemically modified by both trichloroacetic acid and Dowex 50-H+. With respect to pH, Fig. 2A shows the optimum appearance of the unknown metabolite in incubation medium as a function of pH as well as providing a pH profile for the conversion of [14C]putrescine to 2-[14C]pyrrolidone by sliced rat liver. The optimum pH for the appearance of the metabolite from 2-[14C]pyrrolidone is approximately 7.4, whereas the rate of conversion of [14C]putrescine to 2-[14C]pyrrolidone is markedly increased at elevated pH values. Enhanced 2-[14C]pyrrolidone production at higher pH values is probably a reflection of the dissociation constants for putrescine which are 10.3 and 9.3 (5). Thus, at pH 7.4, putrescine is fully protonated, its rate of uptake into rat liver cells is reduced, and the amount of [14C]putrescine metabolized and the formation of 2-[14C]pyrrolidone are limited. As might be expected from the foregoing, the optimum pH for the conversion of putrescine to the unknown metabolite (via 2-pyrrolidone) is near 8.5 (Fig. 2B), the point at which the reaction curves cross (Fig. 2A).

Separation of 2-Pyrrolidone from Its Metabolite—Thirty vials each containing 100 mg of sliced rat liver in 1.0 ml of F12K-3 were routinely incubated for 3 h at 37°C. Each vial contained 1.94 µM 2-pyrrolidone and some vials additionally contained a tracer amount of 2-[14C]pyrrolidone. After incubating, 5 µl of medium from all vials containing 2-[14C]pyrrolidone were chromatographed on silica gel sheets. A total of eight such experiments were performed. The conversion of 2-[14C]pyrrolidone to the unknown metabolite, in 22 assays, was estimated to be 27.0 ± 1.0% (mean ± S.E.).

The pooled medium (194 ml) was added to 5 volumes of chloroform/methanol (2:1), shaken for 20 min, and centrifuged at 1000 x g for 5 min. The aqueous methanol phase (363 ml) was concentrated to 25 ml, utilizing a rotary evaporator maintained at 30°C. The sample was fractionated on a column of purified activated charcoal (17). Those fractions (Fig. 3) containing the 2-[14C]pyrrolidone metabolite (Peak I) were pooled.
Fig. 2. Conversion of putrescine to 2-pyrrolidone, 2-pyrrolidone to the unknown metabolite (Panel A), and putrescine to the unknown metabolite (Panel B) by sliced rat liver as a function of pH. Panel A, sliced rat liver (100 mg) incubated in F12K-3 (1.0 ml) with either 7.14 μCi of [14C]putrescine and determination of 2-[14C]pyrrolidone after 4 h (L) or incubated with 0.35 μCi of 2-[14C]pyrrolidone and the determination of the 14C-labeled unknown metabolite after 30 min of incubation (D). Incubation media (5 μl) were chromatographed and 14C products were determined as described in Fig. 1. The buffer utilized in each case was 40 mM bis-Tris propane and the desired pH was obtained with HCl. Each assay was done in triplicate and the mean ± standard error is provided. Panel B, appearance of the 14C-labeled unknown from [14C]putrescine. In order to detect low levels of the 14C-labeled unknown metabolite formed from [14C]putrescine as well as better resolve 2-[14C]pyrrolidone from the 14C-labeled unknown metabolite, the following procedure was utilized. Sliced rat liver (100 mg) was incubated with 2.5 μCi of [14C]putrescine for 4 h, after which 0.5 ml of medium from each sample was added to 0.32 ml of packed Dowex 50-Na+ (0.5 ml of a Dowex 50-Na+ suspension centrifuged for 30 s at 10,000 × g) and the samples were mixed for 15 s and centrifuged for 1 min at 10,000 × g. The supernatant was removed and 10 ml were chromatographed and the amount of radioactivity corresponding to the unknown determined as in Fig. 1. Each point represents the mean ± standard error of six individual assay. Final values for the 14C-labeled unknown metabolite are adjusted for the dilution contributed by the water in 0.32 ml of packed Dowex 50-Na+. All by the following method: F12K-3 medium (0.5 ml) containing a known amount of [14C]glucose was added to 0.32 ml of packed Dowex 50-Na+, and the sample was mixed for 15 s and centrifuged for 1 min at 10,000 × g. The amount of [14C] glucose remaining in the supernatant was determined. The per cent of total disintegrations per min lost to the water associated with 0.32 ml of packed Dowex 50-Na+ was used to correct for the total amount of the 14C-labeled unknown metabolite for each sample before treating with Dowex 50-Na+.

Table I

<table>
<thead>
<tr>
<th>Steps</th>
<th>Unknown cpm × 10^13</th>
<th>Yield cpm × 10^13</th>
<th>2-Pyrrolidone cpm × 10^13</th>
<th>Yield cpm × 10^13</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Medium</td>
<td>2.69</td>
<td>100</td>
<td>9.00</td>
<td>100</td>
</tr>
<tr>
<td>2. Extraction</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Water phase</td>
<td>2.00</td>
<td>74.3</td>
<td>3.11</td>
<td>34.6</td>
</tr>
<tr>
<td>b. Chloroform phase</td>
<td>0.89</td>
<td>33.0</td>
<td>6.04</td>
<td>67.1</td>
</tr>
<tr>
<td>3. Charcoal columnb</td>
<td>1.88</td>
<td>69.9</td>
<td>0.03</td>
<td>0.3</td>
</tr>
<tr>
<td>4. TLC</td>
<td>1.90</td>
<td>59.3</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

*Total counts per min (initial volume, 194 ml).

b Pooled concentrated Fractions 157 to 232 (Fig. 3).

ND, none detected.

Fig. 3. Activated charcoal elution profile of extracted-concentrated incubation medium. The aqueous phase (25 ml) from extracted-concentrated medium was loaded on an activated charcoal column (5.0 × 30 cm). The column was washed with 100 ml of distilled water (no radioactivity was detected in the wash) and the 14C-labeled unknown metabolite (Peak I) and 2-[14C]pyrrolidone (Peak II) were eluted with 4% ethanol. The flow rate was 30 ml/h and the volume of each fraction was 3.5 ml. Radioactivity was determined by assaying 50 μl of each fraction. 2-[14C]Pyrrolidone was localized by chromatographing 5 μl from several of those fractions containing radioactivity. The radioactive compound in Peak II co-chromatographed with the authentic 2-[14C]pyrrolidone. The counts per min provided are for 3.5 ml.

(258 ml), taken to dryness, and immediately resuspended in 1.2 ml of methanol. About 3% of the radioactivity was present as 2-pyrrolidone (Table I). Equal volumes (300 μl) were spotted on four 1-mm thick Silica Gel G plates (20 × 20 cm) and chromatographed in two dimensions with the following solvents: (A, chloroform/2-propanol (4:1); B, methylene chloride/methanol (9:1). The 2:[14C]pyrrolidone metabolite was localized with a Geiger counter, the area containing the bulk of radioactivity was scraped, and the samples from each plate were pooled. The metabolite was eluted from the silica gel with methanol (65 ml), reduced to 10 ml with a rotary evaporator, and further concentrated to approximately 400 μl with nitrogen. A portion of the sample was assayed by gas chromatography-mass spectrometry as described below.

After each purification step 5- or 10-μl aliquots were chromatographed on silica gel sheets (as described in Fig. 1) and the relative amounts of the 2-[14C]pyrrolidone metabolite to 2-[14C]pyrrolidone were determined (Table I). No 2-[14C]pyrrolidone was detected in the unknown 14C-metabolite after the elution from silica gel.

Identification of the 14C-Metabolite by Gas Chromatography and Mass Spectrometry—A portion (20 μl) of the above partially purified sample was evaporated to dryness under nitrogen and silylated with 40 μl of bis(trimethylsilyl)trifluoroacetamide (Supelco Inc.). Analysis of the silylated sample by gas chromatography-mass spectrometry revealed several prominent peaks (not shown). However, all but one of the major peaks were also present in a blank prepared by washing an equivalent amount of silica gel with methanol, drying the
methanol extract under nitrogen, and silylating the residue as described above. Excluding silylated glycerin, which was present in both the unknown and blank samples, the unique peak associated with the partially purified sample, but not the blank, accounted for approximately 60% of the total material that was detected from the gas chromatography column between 70 and 250°C.

The unique peak eluted from the gas chromatography column at 112°C (see Fig. 4 for details) and its mass spectrum is shown in Fig. 4. A molecular ion appears at m/z 245 along with the M – CH₃ ion (m/z 230) expected from trimethylsilyl ethers (18), and an ion at M – 1 suggests the presence of an aldehyde or acetal function (19). The intense ion at m/z 147 is ubiquitous in polytrimethylsilylated compounds, particularly in cases where the two groups are in close proximity (18), and its structure [(CH₃)₃SiO⁺Si(CH₃)₃] shows that the compound contains two trimethylsilyl groups (three would be impossible for a compound of molecular weight 245). The molecular weight of the original compound is therefore 245 – (2 x 72) = 101, i.e. 16 more than pyrrolidone. Confirmation that this mass increase is due to the presence of a hydroxyl group is provided by the ion at m/z 156, representing loss of 89 mass units [OSi(CH₃)₃]. Placing this group at the 5-position facilitates explanation of the intense m/z 147 ion.

To confirm the structure, 5-hydroxy-2-pyrrolidone was synthesized by two methods (15, 16), silylated, and analyzed by gas chromatography-mass spectrometry. Chemically synthesized silylated 5-hydroxy-2-pyrrolidone eluted from the gas chromatography column at 112°C and had a mass spectrum (not shown) identical to that of the 2-pyrrolidone metabolite. The unknown 2-[¹⁴C]pyrrolidone metabolite co-chromatographed with 5-hydroxy-2-[³H]pyrrolidone (see “Materials and Methods”) on silica gel sheets (Fig. 5). It is concluded that 2-pyrrolidone is metabolized to 5-hydroxy-2-pyrrolidone by sliced rat liver.

**Fig. 5.** Chromatography of the unknown 2-[¹⁴C]pyrrolidone metabolite in the presence of 5-hydroxy-2-[³H]pyrrolidone. After incubating sliced rat liver for 4 h, 0.5 ml of F12K-3 containing 2-[³H]pyrrolidone (0.74 µCi/ml) was diluted with 0.5 ml of F12K-3 containing 0.2 µCi of 5-hydroxy-2-[¹⁴C]pyrrolidone (see “Materials and Methods”). 5 µl were chromatographed, and radioactivity was determined as stated in Fig. 1. Radioactivity is corrected for crossover of ¹⁴C into the ³H counting channel (no crossover of the ³H into the ¹⁴C counting channel was observed) as well as for efficiency of counting. Peak II: O, 5-hydroxy-2-[³H]pyrrolidone; 1, 2-[¹⁴C]pyrrolidone metabolite. Peak I, 2-[¹⁴C]pyrrolidone.

**Table II**

Effect of various compounds on the formation of 2-[¹⁴C]pyrrolidone from [¹⁴C]putrescine and of 5-hydroxy-2-[¹⁴C]pyrrolidone from 2-[¹⁴C]pyrrolidone by sliced rat liver

<table>
<thead>
<tr>
<th>Effector compound</th>
<th>Putrescine to 2-pyrrolidone</th>
<th>2-Pyrrolidone to 5-hydroxy-2-pyrrolidone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aminoguanidine</td>
<td>91</td>
<td>72²</td>
</tr>
<tr>
<td>Agmatine</td>
<td>31⁴</td>
<td>149⁴</td>
</tr>
<tr>
<td>Benzaldehyde</td>
<td>97</td>
<td>71¹</td>
</tr>
<tr>
<td>Cadaverine</td>
<td>29⁴</td>
<td>102</td>
</tr>
<tr>
<td>Idoxocetate</td>
<td>100</td>
<td>36⁸</td>
</tr>
<tr>
<td>Methylene blue</td>
<td>10⁴</td>
<td>21⁴</td>
</tr>
<tr>
<td>Methylglyoxyl bis-(guanhydrzone)</td>
<td>62⁴</td>
<td>108</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Effector compound</th>
<th>Per cent of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Oxosuccinate</td>
<td>74</td>
</tr>
<tr>
<td>Fargyline</td>
<td>17⁴</td>
</tr>
<tr>
<td>Quinacrine</td>
<td>58⁵</td>
</tr>
<tr>
<td>Sodium azide</td>
<td>161</td>
</tr>
</tbody>
</table>

² p < 0.01.
⁴ p < 0.001.
⁸ p < 0.05.
⁵ p < 0.005.

Biosynthesis of 5-Hydroxy-2-pyrrolidone

Kinetics of 5-hydroxy-2-pyrrolidone Appearance in Incubation Medium—The appearance of 5-hydroxy-2-[14C]pyrrolidone from 2-[14C]pyrrolidone as a function of time is depicted in Fig. 6A. The formation of 5-hydroxy-2-pyrrolidone was linear for 50 min.

Fig. 6B provides the rate of 5-hydroxy-2-[14C]pyrrolidone formation as a function of the amount of sliced rat liver present. The dependence of rate on the amount of tissue was linear to 100 mg, the greatest weight examined.

The effect of 2-pyrrolidone concentration on the level of 5-hydroxy-2-pyrrolidone appearance in incubation medium is provided in Fig. 6C. The 2-pyrrolidone concentration required to saturate the system is approximately 2 \times 10^{-5} M. At lower 2-pyrrolidone concentrations the appearance of 5-hydroxy-2-pyrrolidone followed first order kinetics (not shown).

Pathway Effector Compounds—The appearance of 2-pyrrolidone and 5-hydroxy-2-pyrrolidone from putrescine and 2-pyrrolidone, respectively, was determined in the presence of several potential effector compounds (Table I). Although definitive mechanisms cannot be provided until each metabolic step is studied in greater detail, a number of these compounds altered the appearance of 2-pyrrolidone and 5-hydroxy-2-pyrrolidone in media after incubating sliced rat liver with the respective [14C]-labeled substrates.

Diamine oxidase (EC 1.4.3.6) is inhibited by aminoguanidine (20), agmatine (21), and methylglyoxyl bis-(guanyldrazone) (22) and activated by oxosuccinate (23). Of the above four compounds, agmatine and methylglyoxyl bis-(guanyldrazone) significantly inhibited the formation of 2-pyrrolidone from putrescine. In contrast, the amount of 5-hydroxy-2-pyrrolidone formed from 2-pyrrolidone was enhanced by agmatine and was not inhibited by methylglyoxyl bis-(guanyldrazone). Methylene blue (24) and parglyine (25), potent inhibitors of monoamine oxidase, significantly inhibited the level of both 2-pyrrolidone formed from putrescine and of 5-hydroxy-2-pyrrolidone formed from 2-pyrrolidone. Quinacrine, a known effector of some flavoproteins in addition to polyamine oxidase (26) significantly inhibited both 2-pyrrolidone and 5-hydroxy-2-pyrrolidone formation. On the other hand, both pyruvate and sodium azide inhibited only the formation of 5-hydroxy-2-pyrrolidone from 2-pyrrolidone. As might be expected, cadaverine (1,5-diaminopentane) inhibited the conversion of putrescine (1,4-diaminobutane) to 2-pyrrolidone but did not effect the formation of 5-hydroxy-2-pyrrolidone from 2-pyrrolidone.

These results lend credence to the contention that the synthesis of 5-hydroxy-2-pyrrolidone from putrescine (via 2-pyrrolidone) is under independent control, due to a metabolic pathway characteristic of rat liver and are not reflections of a nonbiological phenomenon.

The present data do not indicate the precise metabolic steps from putrescine to 5-hydroxy-2-pyrrolidone; these will be clarified only when the appropriate enzymes are isolated and characterized. Other investigators have observed the formation of at least one unknown putrescine metabolite after incubating [14C]putrescine with various tissue homogenates including guinea pig liver (7). These investigators suggested that the unknown putrescine metabolite(s) was synthesized from \( \gamma \)-aminobutyraldehyde, the direct product of putrescine metabolism via diamine oxidase. Although it remains to be

![Fig. 6. Kinetics of 5-hydroxy-2-pyrrolidone appearance in incubation medium. Panel A, time course appearance of 5-hydroxy-2-pyrrolidone. Sliced rat liver (100 mg) was incubated in 1.0 ml of F12K-3 containing 0.22 \( \mu \)Ci (9.9 \times 10^{-4} M) of 2-[14C]pyrrolidone. The medium (5 \( \mu \)l) was removed at specified times and chromatographed, and \(^{14}C\) was determined as described in Fig. 1. Panel B, the appearance of 5-hydroxy-2-pyrrolidone as a function of tissue weight. The methods were as described above except the incubation time was 30 min and F12K-3 medium contained 0.37 \( \mu \)Ci (1.7 \times 10^{-4} M) of 2-[14C]pyrrolidone. Panel C, effect of 2-pyrrolidone concentrations on the rate of 5-hydroxy-2-pyrrolidone appearance in incubation medium. The methods were as described above except 0.93 \( \mu \)Ci of 2-[14C]pyrrolidone were added to carrier 2-pyrrolidone to obtain the concentrations indicated.](image-url)
tested if liver can synthesize 2-pyrrolidone from γ-aminobutyraldehyde or its spontaneously cyclized product (Δ1-pyrroline), our studies with effector compounds suggest that this is a possibility. Both agmatine and methylglyoxal bis-(guanylhydrazone), potent inhibitors of diamine oxidase, inhibit the appearance of 2-pyrrolidone from putrescine. On the other hand, aminoguanidine, also a potent inhibitor of diamine oxidase, did not inhibit the formation of 2-pyrrolidone. Caution must, however, be utilized in equating our data with previous studies since previous studies utilized tissue homogenates, while our results are based on intact tissue.

Putrescine has been shown to be metabolized in mammalian systems by several pathways. The best described, and considered to be the most important physiologically, is its function as a substrate in spermidine biosynthesis (1–3). A second, but less well defined, pathway involves the metabolism of putrescine to γ-aminobutyric acid via acetylated intermediates (9). It now appears, at least in rat liver, that a third pathway involving 2-pyrrolidone and 5-hydroxy-2-pyrrolidone also exists. In view of the important role putrescine plays in growth and development, further studies designed to elucidate the interrelationship of these pathways with respect to precise metabolic steps and their regulation will be of interest.

Acknowledgments—We wish to acknowledge the excellent technical skills of Ms. Jeanne Hankins. We thank Dr. Paul A. di Sant'Agnese for his continued support throughout these studies. Dr. Sidney S. Chernick for his many helpful suggestions, and Mrs. Pat Biggar for her excellent secretarial help.

REFERENCES

Biosynthesis of 5-Hydroxy-2-pyrrolidione