The Dynamics of Synthesis and Degradation of Polyamines in Normal and Regenerating Rat Liver and Brain*

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SUMMARY

Putrescine (1,4-diaminobutane), the product of the decarboxylation of ornithine, is a precursor of the polyamines spermidine and spermine. After administration in vivo of radiolabeled ornithine or putrescine, changes in specific activity of putrescine, spermidine, and spermine have been assessed in normal and regenerating liver and in brain. In both normal liver and in brain, after labeling with exogenous putrescine, the specific activity of spermidine declines with a half-life of about 4 days. Following partial hepatectomy, marked and characteristic changes occur in the endogenous levels of putrescine, spermidine, and spermine in the regenerating liver of the rat. Putrescine concentration is increased 5-fold as early as 4 hours after the operation, whereas spermidine levels rise more slowly and attain a maximum which is double that of control values within the first 5 days. Spermine concentration declines initially but becomes elevated by 11 days after hepatectomy. In regenerating rat liver, the rate of decline of the labeling of spermidine after administration of 14C-putrescine or 14C-ornithine is the same as in normal liver. There is a very rapid fall (t₁/₂ = 2 hours) of the labeling of putrescine in regenerating rat liver after the administration of 14C-ornithine.

Polyamine synthesis in bacteria (1) and in mammals (4–6) involves the decarboxylation of ornithine to putrescine, which is then linked to a propylamine moiety derived from S-adenosylmethionine to form spermidine. Putrescine is incorporated into spermine molecules suggesting that spermidine and putrescine also are involved in the biosynthesis of spermine (1). Although enzymes that deaminate diamines and polyamines are well known (1), it is not clear whether these reactions comprise the major route for polyamine catabolism in vivo, particularly in mammalian systems.

Attempts to discern the biological function of the polyamines have focussed on the effects in vitro of polyamines on nucleic acids, membranes, and enzymes, on attempts to determine their intracellular localization, and on measurement of their concentrations and the activity of their synthesizing enzymes under a variety of physiological conditions. Polyamines stabilize nucleic acids, membrane fractions, and polynucleosomes, and these effects can be attributed partially to the polybasic nature of the polyamines (1).

Attempts to study the intracellular distribution of polyamines in animal tissues have met with limited success since homogenization of tissues may cause redistribution of these polycationic substances (1). However, polyamine levels in the nuclei of rat liver and calf thymus gland appear to approximate their concentration in the cytoplasm (7), and certain studies suggest a possible association of spermidine with cytoplasmic ribosomes (8, 9).

In animal systems, increased spermidine concentrations paralleling increased RNA content have been demonstrated in rapidly growing tissues such as regenerating rat liver (2, 10) and chick embryo (11, 12).

A close relationship has been observed between the activity of ornithine decarboxylase, an enzyme which may be rate-limiting in spermidine synthesis, and the initiation of rapid growth in several tissues including regenerating rat liver, chick embryo, and certain tumors (13, 14). In regenerating rat liver, ornithine decarboxylase activity triples 1 hour after partial hepatectomy, and it is 10 times control levels by 4 hours (13, 14), although DNA synthesis and cell mitosis do not begin to increase until 16 to 18 hours after operation (15). In the chick embryo, ornithine decarboxylase activity is greatly elevated in all tissues during certain phases of development with a peak activity at the 5 to 6 day state (13), and then declines to negligible levels near hatching. In some rapidly growing sarcomas, very high levels of ornithine decarboxylase activity were detected (13). The activities of both ornithine decarboxylase and S-adenosyl-
methionine decarboxylase were enhanced in rat ventral prostate in response to the administration of testosterone to orchieto-

mized rats (16).

Growth hormone has marked effects on hepatic polyamine levels (17, 18) and on the regenerative response of rat liver (19). Hypophysectomy produced a delayed increase and an early decline of ornithine decarboxylase activity after partial hepatec-
tomy. These effects of hypophysectomy could be restored by administration of growth hormone (20). Increased hepatic ornithine decarboxylase activity has also been observed in normal rats receiving growth hormone (20-22).

The increases in ornithine decarboxylase activity after hepatec-
tomy were prevented by treatment with puromycin, cyclo-

heximide, or actinomycin D (23). When cycloheximide or puromycin were used to block enzyme synthesis, ornithine decarboxylase activity declined rapidly with a half-life of about 11 min in both normal and regenerating rat liver. This extraor-
dinarily high turnover of ornithine decarboxylase suggests a possible role in the rapid and fine control of putrescine production (23).

In studies on the disposition of 14C-spermidine and 14C-spermine in rats, Siimes (24) observed that there was interconversion of these amines. However the rate of decline of 14C-spermidine synthesized from exogenous spermine differed from the rate of decline of exogenous spermidine itself. In chick embryos, Raina (12) found that 14C-putrescine and 14C-ornithine were converted to spermidine which declined with a half-life of about 30 hours when the label was derived from 14C-putrescine, but with a half-life of about 15 hours when the radioactivity origi-
nated from 14C-ornithine. The difference in rates of decline of exogenous and of endogenously synthesized polyamines sug-
gests that exogenous polyamines may not reliably label the endogenous stores. Jänne (6) investigated the formation of spermidine from 14C-putrescine and 14C-ornithine in rat liver, but did not study the rate of decline of spermidine synthesized from these precursors. He also observed that exogenous putres-
cine disappeared from the liver with a half-life of about 90 min but did not study the disappearance rate of putrescine synthe-
sized from ornithine.

Since the polyamines are polycationic substances which bind to a variety of cell constituents, exogenously administered amine might not localize in the same tissue component as the endoge-

nous amine. Accordingly, we have attempted to compare the turnover of putrescine, spermidine, and spermine in normal and regenerating rat liver and rat brain after the administration of labeled precursors, either ornithine or putrescine.

**Experimental Procedure**

### Materials

- dl-Ornithine-5-14C hydrochloride (4.5 mCi per mmole), spermi-
dine-14C trihydrochloride (10.7 mCi per mmole), spermine-14C tetrahydrochloride (9.9 mCi per mmole), putrescine-1,4-14C dihydrochloride (11.3 mCi per mmole), and putrescine-2,3,5-3H dihydrochloride (102 mCi per mmole) obtained from New England Nuclear.

### Procedures

14C-Ornithine or 14C-putrescine was administered intraperi-

toneally in 0.9% NaCl to hepatectomized or normal rats. One hour after 14C ornithine or 14C putrescine administration, no radioactivity was detectable in 1-ml samples of whole blood or plasma. Sprague-Dawley male rats (150 to 200 g) were used in all experiments. For the brain studies, cisternal punctures with a 29 gauge ½ inch needle were performed on rats under light ether anesthesia. 3H-Putrescine was dissolved in a 0.9% NaCl solution and 20 µl were injected into each rat with a Hamilton microsyringe. To ensure localization of the needle within the ventricular system, a small amount of ventricular fluid (about 2 µl) was withdrawn prior to the injection. In a few experiments, 25-µl samples of ventricular fluid withdrawn 1 hour after 3H-putrescine administration were assayed for radioactivity and only negligible amounts were detected. Rats appeared to be normal in behavior within 5 min of intracisternal injection.

Partial hepatectomy was performed by the method of Higgins and Anderson (25). Sham-operated animals were anesthetized and laparotomized.

### Determination of Putrescine, Spermidine, and Spermine

These amines were extracted from tissues with butanol, sepa-

rated by electrophoresis, and quantitated by modifications of the method of McIntire, Roth, and Shaw (26) similar to those described by Raina (12) and by Jänne (6).

#### Butanol Extraction

Rats were killed by decapitation and 1.5 g of the liver remnants (caudate and right lateral lobes) were removed, chilled on ice, and homogenized in 6 ml of 0.1 N HCl. To the homogenates, 6 ml of 19% trichloracetic acid were added, the tubes were stirred thoroughly, allowed to stand for 1 hour with occasional stirring, and centrifuged for 20 min at 20,000 × g. To 10 ml of the supernatant fluid were added 1 ml of 5 N NaOH, 2 g of a salt mixture (62.5 g of anhydrous sodium sulfate and 9 g of trisodium phosphate ground together and desiccated), and 10 ml of 1-butanol (12). The mixture was shaken for 30 min and centrifuged. An 8-ml aliquot of the butanol phase was evaporated to dryness in a Buchler Evapo-

#### Electrophoresis

An aliquot (5 to 25 µl) of the dissolved residue after evaporating the butanol extracts was applied to Whatman No. 3MM chromatography paper and subjected to electrophore-
sis at 80 volts per cm for 1½ hours in a 0.1 N citric acid-NaOH buffer, pH 4.3 (6). Corrections for recovery of putrescine, spermidine, and spermine were made by determining recoveries from tissue samples to which 0.15 µCi of the appropriate 14C-amine had been added prior to homogenization. Recovery rates (90 to 95%) were comparable to those obtained by Raina (12) and Jänne (6). In this system, putrescine typically migrates 34 cm, spermidine 22 cm. The three amines were separated with no overlap and negligible streaking.

### Determination of Endogenous Amine Concentrations

The chromatography sheets were dipped in a tray containing a mixture of 1 g of ninhydrin, 100 ml of acetic acid, 5 ml of concentrated acetic acid, and 100 mg of cadmium acetate, dried for 90 min at 60°C, and the colored areas cut out and eluted for 30 min in 5 ml of water-ethanol-glacial acetic acid mixture (1:4:5, by volume) that contained 2 mg of cadmium acetate per ml (12). Blank readings for each electrophoretic experiment were prepared by eluting a square of the electrophoresis paper equal in size to the colored sample area in the same manner as the experimental samples. The spectrophotometer was always zeroed against such a blank sample to correct for any background color that might be present on the paper after staining. Background color
Formation and Disappearance of \(^{14}C\)-Polyamines in Normal and Regenerating Rat Liver after Administration of \(^{14}C\)-Putrescine

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If the decline of radioactivity of the amine is to reflect its turnover, it is important that the precursor no longer be present when the decline rate of the product is studied. Jānne (6) examined the formation of spermidine in rat liver in vivo at time intervals up to 4 hours after exogenous putrescine administration. He found that hepatic \(^{14}C\)-putrescine levels decreased rapidly and that there was a gradual increase in \(^{14}C\)-spermidine so that by 4 hours its levels markedly exceeded those of \(^{14}C\)-putrescine. Accordingly, we felt that \(^{14}C\)-putrescine might serve as a valid precursor for studies of the turnover of spermidine and spermine in rat liver.

We administered \(^{14}C\)-putrescine intraperitoneally to rats, killed the animals at intervals ranging from 15 min to 2 weeks, and assayed their livers for \(^{14}C\)-putrescine, \(^{14}C\)-spermidine, and \(^{14}C\)-spermine as well as for the endogenous levels of these amines. As had been found earlier by Jānne (6) \(^{14}C\)-putrescine declined rapidly with a half-life of about 90 min. Twenty-four hours after its administration, no \(^{14}C\)-putrescine was detectable in the liver. \(^{14}C\)-Spermidine was measurable at 1 hour and its specific activity at 4 hours was 4 times that of \(^{14}C\)-putrescine (Fig. 1). Peak specific activity of \(^{14}C\)-spermidine was reached in 4 hours and thereafter declined slowly in a logarithmic manner with a half-life of about 4 days. Under the same conditions \(^{14}C\)-spermine was not detectable until 2 days after putrescine administration, when its specific activity was only 20% that of spermidine. The specific activity of spermine, however, remained constant for at least 14 days, when it was almost double that of spermidine.

In order to examine the turnover of polyamines in regenerating rat liver, rats received injections of \(^{14}C\)-putrescine 24 hours prior to partial hepatectomy. They were killed at intervals between 4 hours and 2 weeks after operation, and their livers were assayed for \(^{14}C\)-spermine and \(^{14}C\)-spermidine, as well as for endogenous amines.

Our observations of changes in endogenous putrescine and polyamines after partial hepatectomy resembled those of Jānne (6) who followed levels until 5 days after operation, as well as those of Dykstra and Herbst (10) who measured spermidine and spermine between 24 and 96 hours after hepatectomy. Between 4 and 24 hours after partial hepatectomy, endogenous putrescine levels were 5 times control values (Table I), and, thereafter, declined gradually, and in 7 days reached control values. Spermidine concentration was not significantly increased until 10 to 24 hours after operation when levels were \(\times 2\) to \(\times 4\) times control values and remained elevated for at least 14 days. The endogenous spermine concentration initially decreased gradually to almost one-third of control values 2 days after hepatectomy and then gradually increased to about \(\times 2\) times control values at 11 to 14 days. The labeling patterns of spermine and spermidine from \(^{14}C\)-putrescine were almost...
Formation and Disappearance of \(^{14}\text{C}\) Amines in Regenerating Rat Liver after Administration of \(^{14}\text{C}\) Ornithine—Measurements with putrescine as a precursor may not provide a valid estimate of the turnover of spermidine and spermine. For instance, because of its cationic character putrescine may bind to tissue components other than those concerned with polyamine synthesis. Accordingly, we examined the specific activities of polyamines after administration of \(^{14}\text{C}\) ornithine. These studies could not be performed in normal rats, since no \(^{14}\text{C}\) polyamines were detected after the administration of as much as 50 to 100 \(\mu\text{Ci}\) of \(^{14}\text{C}\)-ornithine. Such experiments were feasible in partially hepatectomized rats in which there is an increased formation of \(^{14}\text{C}\)-putrescine and \(^{14}\text{C}\)-polyamines from \(^{14}\text{C}\)-ornithine (6). Partially hepatectomized rats received \(^{14}\text{C}\)-ornithine 6 hours after operation, and were killed at intervals between 15 min and 7 days after receiving \(^{14}\text{C}\)-ornithine. The livers were assayed for endogenous levels and specific activities of \(^{14}\text{C}\)-putrescine, \(^{14}\text{C}\)-spermidine, and \(^{14}\text{C}\)-spermine.

**Table I**

<table>
<thead>
<tr>
<th>Time intervals</th>
<th>Putrescine</th>
<th>Spermidine</th>
<th>Spermine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\text{mMoles/g, wet wt})</td>
<td>(%) of control</td>
<td>(\text{mMoles/g, wet wt})</td>
</tr>
<tr>
<td>4 hr regenerating</td>
<td>300 ± 35*</td>
<td>506</td>
<td>1000 ± 100</td>
</tr>
<tr>
<td>4 hr sham-operated</td>
<td>60 ± 11</td>
<td></td>
<td>800 ± 72</td>
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<tr>
<td>10 hr regenerating</td>
<td>310 ± 32a</td>
<td>441</td>
<td>1220 ± 120a</td>
</tr>
<tr>
<td>10 hr sham-operated</td>
<td>71 ± 7</td>
<td></td>
<td>810 ± 52</td>
</tr>
<tr>
<td>1 day regenerating</td>
<td>330 ± 42a</td>
<td>490</td>
<td>1670 ± 100a</td>
</tr>
<tr>
<td>1 day sham-operated</td>
<td>68 ± 7</td>
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<td>810 ± 106</td>
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<tr>
<td>2 day regenerating</td>
<td>200 ± 27a</td>
<td>273</td>
<td>1600 ± 70a</td>
</tr>
<tr>
<td>2 day sham-operated</td>
<td>73 ± 8</td>
<td></td>
<td>800 ± 48</td>
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<tr>
<td>5 day regenerating</td>
<td>110 ± 8a</td>
<td>140</td>
<td>1800 ± 80a</td>
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<tr>
<td>5 day sham-operated</td>
<td>76 ± 13</td>
<td></td>
<td>1050 ± 62</td>
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<tr>
<td>7 day regenerating</td>
<td>73 ± 11</td>
<td>122</td>
<td>1680 ± 95a</td>
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<td>7 day sham-operated</td>
<td>60 ± 14</td>
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<td>890 ± 68</td>
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<td>11 day regenerating</td>
<td>66 ± 7</td>
<td>80</td>
<td>1700 ± 110a</td>
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<tr>
<td>11 day sham-operated</td>
<td>82 ± 12</td>
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<td>1040 ± 110</td>
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<tr>
<td>14 day regenerating</td>
<td>91 ± 8</td>
<td>108</td>
<td>1840 ± 80a</td>
</tr>
<tr>
<td>14 day sham-operated</td>
<td>84 ± 8</td>
<td></td>
<td>920 ± 65</td>
</tr>
</tbody>
</table>

* Denotes the results differ from sham-operated controls (\(p < 0.001\)).

**TABLE II**

<table>
<thead>
<tr>
<th>Time intervals</th>
<th>Putrescine</th>
<th>Spermidine</th>
<th>Spermine</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>(\text{mMoles/g, wet wt})</td>
<td>(%) of control</td>
<td>(\text{mMoles/g, wet wt})</td>
</tr>
<tr>
<td>24 hr regenerating</td>
<td>90.6 ± 9.9</td>
<td></td>
<td>408 ± 24</td>
</tr>
<tr>
<td>24 hr sham-operated</td>
<td>90.6 ± 9.9</td>
<td></td>
<td>408 ± 24</td>
</tr>
</tbody>
</table>

\(^{14}\text{C}\)-Spermidine was present in measurable amounts at 30 min and its specific activity peaked at 4 hours (Fig. 2). The specific activity of spermidine fell precipitously between 4 and 24 hours (Fig. 3), coincident with the increase in endogenous levels of spermidine. Thereafter its specific activity declined with a half-life of 34\(\frac{1}{2}\) days, similar to that found after \(^{14}\text{C}\)-putrescine administration, and suggesting that both precursors labeled the same pool of spermidine. \(^{14}\text{C}\)-Spermine could not be detected until 4 hours after ornithine administration. Its specific activity peaked 24 hours after \(^{14}\text{C}\)-ornithine, after which it fell to values one-third of the peak levels by 7 days.

**Formation and Disappearance of \(^{3}\text{H}\) Polyamines in Rat Brain after Administration of \(^{3}\text{H}\)-Putrescine—Rats received intracisternal injections of small doses (28 \(\text{mMoles}\)) of \(^{3}\text{H}\)-putrescine, which were killed at time intervals between 15 min and 14 days after injection, and their brains assayed for endogenous levels and specific activities of putrescine, spermidine, and spermine.**

<table>
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<th>Putrescine</th>
<th>Spermidine</th>
<th>Spermine</th>
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<tr>
<td></td>
<td>(\text{mMoles/g, wet wt})</td>
<td>(%) of control</td>
<td>(\text{mMoles/g, wet wt})</td>
</tr>
<tr>
<td>20 min</td>
<td>90.6 ± 9.9</td>
<td></td>
<td>408 ± 24</td>
</tr>
<tr>
<td>40 min</td>
<td>90.6 ± 9.9</td>
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<td>408 ± 24</td>
</tr>
<tr>
<td>1 hr</td>
<td>90.6 ± 9.9</td>
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<td>408 ± 24</td>
</tr>
<tr>
<td>2 hr</td>
<td>90.6 ± 9.9</td>
<td></td>
<td>408 ± 24</td>
</tr>
<tr>
<td>4 hr</td>
<td>90.6 ± 9.9</td>
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<td>6 hr</td>
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<td>8 hr</td>
<td>90.6 ± 9.9</td>
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<td>10 hr</td>
<td>90.6 ± 9.9</td>
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<td>408 ± 24</td>
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</tr>
<tr>
<td>24 hr</td>
<td>90.6 ± 9.9</td>
<td></td>
<td>408 ± 24</td>
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</tbody>
</table>

**TABLE III**

<table>
<thead>
<tr>
<th>Time intervals</th>
<th>Putrescine</th>
<th>Spermidine</th>
<th>Spermine</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>(\text{mMoles/g, wet wt})</td>
<td>(%) of control</td>
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</tr>
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<tr>
<td>24 hr sham-operated</td>
<td>90.6 ± 9.9</td>
<td></td>
<td>408 ± 24</td>
</tr>
</tbody>
</table>

**Formation and Disappearance of \(^{3}\text{H}\) Polyamines in Rat Brain after Administration of \(^{3}\text{H}\)-Putrescine—It was not detected 24 hours after injection (Fig. 4).**

Labeled putrescine disappeared very rapidly from the brain and was not detectable 24 hours after injection. 

\(^{3}\text{H}\)-Spermidine was present in considerable quantities 4 hours after putrescine injection and the specific activity reached peak levels at 24 hours. 

Identical in normal and operated animals. Except for a more rapid initial decline in the specific activity of spermidine because of pool decrease, their half-lives were identical.

### References

1. **Formation and Disappearance of \(^{14}\text{C}\) Amines in Regenerating Rat Liver after Administration of \(^{14}\text{C}\) Ornithine—Measurements with putrescine as a precursor may not provide a valid estimate of the turnover of spermidine and spermine.**

2. **Formation and Disappearance of \(^{14}\text{C}\) Amines in Regenerating Rat Liver after Administration of \(^{14}\text{C}\) Ornithine—Measurements with putrescine as a precursor may not provide a valid estimate of the turnover of spermidine and spermine.**

3. **Formation and Disappearance of \(^{14}\text{C}\) Amines in Regenerating Rat Liver after Administration of \(^{14}\text{C}\) Ornithine—Measurements with putrescine as a precursor may not provide a valid estimate of the turnover of spermidine and spermine.**

4. **Formation and Disappearance of \(^{14}\text{C}\) Amines in Regenerating Rat Liver after Administration of \(^{14}\text{C}\) Ornithine—Measurements with putrescine as a precursor may not provide a valid estimate of the turnover of spermidine and spermine.**
Fig. 2. Decline of specific activity of \(^{14}\)C-putrescine and increase of specific activity of \(^{14}\)C-spermidine in regenerating rat liver after administration of \(^{14}\)C-ornithine. Ten microcuries of \(^{14}\)C-ornithine were injected intraperitoneally 6 hours after partial heptectomy. Each point represents the mean ± S.E.M. for five rats. Inset indicates half-life of \(^{14}\)C-putrescine in regenerating rat liver.

at 24 hours. Thereafter it declined gradually with a half-life of about 5 days, similar to the decline rate of labeled spermidine in normal liver.

In contrast to the observations in liver, in which spermine labeling was not detectable until at least 24 hours after putrescine administration, \(^{3}\)H-spermine labeling in brain had reached nearly half of the peak values in 4 hours.

Fig. 3. Decline of \(^{14}\)C-spermidine specific activity and increase of \(^{14}\)C-spermine specific activity in regenerating rat liver after administration of \(^{14}\)C-ornithine. Ten microcuries of \(^{14}\)C-ornithine were injected intraperitoneally 6 hours after partial heptectomy. Each point represents the mean ± S.E.M. for five rats. Inset indicates half-life of \(^{14}\)C-spermidine specific activity in regenerating rat liver.

In some experiments the brain was dissected into five discrete areas which were assayed for \(^{3}\)H-spermidine (from 4 hours to 6 days) after \(^{3}\)H-putrescine injection (Fig. 5). Highest spermidine levels occurred in cerebellum and midbrain, areas close to the site of \(^{3}\)H-putrescine injection where, 1 day after putrescine administration, \(^{3}\)H-spermidine concentration was about 5 times greater than levels in hypothalamus, cortex, and striatum. The
H-spermidine concentration in cerebellum and midbrain fell by 6 days to one half the peak values at 1 day. In hypothalamus and corpus striatum, although there did not appear to be a decline in H-spermidine between 1 and 3 days, levels decreased at 6 days to about half the 1 or 3 day values.

DISCUSSION

In the present study radiolabeled putrescine and ornithine were used as precursors in an attempt to label endogenous pools of polyamines in rat liver and brain. For such a purpose it is important that the dose of precursor be small in relation to the endogenous pool of polyamines. The dose of HC-putrescine used in experiments with the liver was about 2.8 mmoles per g of rat. Since only about 5% of administered HC-putrescine is taken up by the liver (6) and since the liver content of putrescine is about 70 mmoles per g, it appears that HC-putrescine constitutes a tracer dose in these experiments. In a few experiments with 5 times larger doses of HC-putrescine, the same half-life for the specific activity of spermidine was obtained. In studies of brain polyamines, rats received about 14 mmoles per g (the brain weighs about 2 g) of H-putrescine intracisternally. Since the brain contains about 90 mmoles per g of endogenous putrescine, this dose is a somewhat larger percentage of the endogenous pool than in experiments with the liver. Most of the H-putrescine was taken up by the cerebellum and brainstem, which constitute only about one-half of the weight of the brain. Thus the dose of H-putrescine administered was about 30% of the endogenous brain concentration of putrescine.

In attempting to assess turnover by disappearance rate of a compound formed from exogenous precursor, it is also important that the precursor no longer be present when the disappearance rate of its product is studied. In our experiments with HC-putrescine as a precursor of hepatic polyamines in normal and regenerating rat liver, and with H-putrescine in the brain, the disappearance of spermidine was examined at times when exogenous putrescine had almost completely vanished from the organ. In experiments with HC-ornithine, at all times after 1 hour, all of the acid-soluble radioactivity could be accounted for by HC-putrescine, HC-spermidine, and HC-spermine indicating that no significant quantities of free HC-ornithine remained in the liver.

Endogenous hepatic putrescine concentration was 5 times control values by 4 hours after partial hepatectomy and then gradually declined so that 7 days after operation it was not significantly higher than basal levels. The early increase and subsequent decline in putrescine concentration confirms the observations of Jänne (6) and parallels the marked early enhancement of hepatic ornithine decarboxylase activity in regenerating rat liver (13, 14). Spermidine concentration, on the other hand, only began to increase 10 hours after the operation, and attained peak values which were double basal levels 1 day after hepatectomy, similar to findings of other workers (6, 10). Spermidine levels remained at this elevated concentration at least until 14 days following hepatectomy, suggesting that enhanced synthesis of spermidine continues even when ornithine decarboxylase levels have declined. This possibility is supported by recent findings1 that the activity of S-adenosylmethionine decarboxylase increases in regenerating rat liver and remains elevated even after ornithine decarboxylase activity has declined.

In contrast to the early increase of hepatic spermidine, spermine concentrations decreased to 57% of control levels 2 days after hepatectomy similar to the findings of Jänne (6). The decrease of spermine levels coincident with the increase of spermidine concentration suggests that spermine may be converted to spermidine during this period as proposed by Siimes (24). At 5 days, spermine had returned to normal levels, and 11 days after hepatectomy was increased 50%. If spermine is formed primarily from spermidine, the elevated spermine levels at 11 and 14 days may be attributable to continued new synthesis of spermidine.

In both normal and regenerating rat liver and in the brain, the labeling of spermine always followed that of spermidine. During time intervals examined, there was no evidence for a decline of the specific activity of spermine except in regenerating rat liver after HC-ornithine administration. In normal rat liver after HC-putrescine administration, the decline of the specific activity of spermidine approximated a straight line when plotted on semilogarithmic paper, suggesting that a single pool of spermidine was labeled. The specific activity of spermidine fell with a half-life of about 4 days, the same as found by Siimes (24) for the decline of HC-spermidine in rat liver formed from injected HC-spermine, and suggesting that spermidine formed either from administered spermine or putrescine entered the same pool. By contrast, Siimes (24) observed that exogenous HC-spermidine disappeared more quickly and in two phases, the first with a half-life of about 24 hours, and the second with a half-life of about 2 days. This suggests that exogenous spermidine may not mix with endogenous pools.

In regenerating rat liver we were able to study the disappearance of HC-spermidine after administration of either putrescine or ornithine. In both cases, after 1 day the specific activity of spermidine declined with a half-life of about 4 days, the same as had been noted in normal liver. The similarity of the results obtained with two different precursors supports the suggestion that these two procedures label endogenous pools of spermidine.

Despite the similar half-life values obtained with two different precursors, polyamine turnover estimates must be interpreted with caution. The liver consists of several different types of cellular components that could produce several polyamine pools. Both exogenous putrescine and ornithine may have entered tissue constituents that differ from the localization of endogenous precursors of the polyamines.

In regenerating rat liver the specific activity of putrescine formed from HC-ornithine fell with a half-life of 2 hours, much shorter than the half-life for spermidine decline. This suggests that, in regenerating rat liver, putrescine turns over quite rapidly. This finding might account for the rapid alterations in hepatic putrescine content in regenerating liver, since the rate at which a tissue component changes its steady state levels is determined by its turnover rate. Because we could not detect putrescine formation from exogenous ornithine in normal rat liver, no estimate of putrescine turnover could be obtained in normal rats.

One notable difference between results in regenerating and normal liver was that in regenerating liver, after either putrescine or ornithine administration, the specific activity of spermidine fell much more rapidly between 4 and 24 hours than in normal rat liver. This initial rapid decline of the specific activity of spermidine coincided with the doubling of endogenous spermidine between 4 and 24 hours after partial hepatectomy. The half-life

of specific activity of spermidine was calculated from time points after 24 hours. Since some of the major events in liver regeneration take place soon after hepatectomy, there could be alterations in the turnover of spermidine in regenerating liver that were not revealed by our experiments. In regenerating rat liver several changes in polyamine disposition may alter pool sizes. These include increases in endogenous putrescine and spermidine that may be related to increased formation of putrescine from ornithine (6, 13, 14), a possible enhanced transformation of spermidine to putrescine (24), and increased spermidine formation both from putrescine (6) and from spermine (24).

Several lines of evidence suggest a role for polyamines related to the disposition of ribosomal RNA. Polyamines can stabilize ribosomes 

in vitro (1), and relax ribosomal RNA synthesis in bacteria (27). In chick embryo, increases in RNA content parallel enhanced polyamine concentrations (11, 12) and ornithine decarboxylase activity (13). Growth hormone treatment also enhances ornithine decarboxylase activity (21, 22), and RNA synthesis (28) with similar time courses. In regenerating rat liver, enhanced RSA synthesis also parallels increases in ornithine decarboxylase activity (13, 14, 29). Recently we observed that spermidine levels in embryos of Xenopus laevis are constant from unfertilized egg to gastrulation, a period in which no new synthesis of ribosomal RNA occurs. After gastrulation the new burst of ribosomal RNA synthesis is accompanied by increased endogenous levels of spermidine (30).

In normal liver, ribosomal RNA turns over with a half-life of about 5 days, closely similar to the half-life we have described for hepatic spermidine (31, 32). Estimates of the turnover of brain RNA suggest a half-life of about 12 days (33), somewhat longer than the half-life we had observed for the disappearance of newly synthesized brain spermidine. However, there were indications that various pools had markedly shorter or longer half-lives.

REFERENCES
The Dynamics of Synthesis and Degradation of Polyamines in Normal and Regenerating Rat Liver and Brain

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