Spermidine in Regenerating Liver: Relation to Rapid Synthesis of Ribonucleic Acid

Abstract. *The spermidine in rat liver increases after partial hepatectomy, and the rate of polyamine accumulation closely approximates the increased rate of synthesis of RNA in regenerating liver. The uptake by the liver of intravenously injected putrescine and the biosynthesis of spermidine are accelerated within 2 hours after the operation. The uptake of spermidine also increases during early regeneration.*

Polyamines are growth factors for several microorganisms and for a Chinese hamster cell line in culture (*1*). The mechanism by which these compounds influence growth is not definitely established. Studies with purified enzymes or enzyme systems suggest that spermine and spermidine increase (i) RNA synthesis by RNA polymerase (*2*) and (ii) amino acid incorporation into protein (*3*). The latter effect is possibly due to the stabilization of 70*S* ribosomes by polyamines (*4*) in the presence of suboptimum concentrations of magnesium ion.

Table 1. RNA and polyamines in regenerating liver. Data in parentheses are analyses of normal liver excised by partial hepatectomy (control). Results (μmoles per gram of liver protein) are means with standard deviations; there were four to six animals in each group.

Regeneration (hr)	RNA-P (μmole)	Spermidine (μmole)	Spermine (μmole)
24	130 ± 10.0 (77.5 ± 4.0)	7.6 ± 0.9 (4.2 ± 0.3)	3.2 ± 0.3 (3.4 ± 0.5)
48	137 ± 9.2 (74.0 ± 4.0)	8.4 ± 2.2 (3.9 ± 0.9)	3.7 ± 0.5 (3.7 ± 0.8)
72	146 ± 9.0 (71.5 ± 5.5)	9.5 ± 1.6 (4.2 ± 0.6)	3.7 ± 0.8 (3.5 ± 0.3)
96	97.0 ± 5.0 (80.5 ± 7.5)	5.9 ± 1.3 (4.3 ± 0.3)	2.9 ± 0.8 (4.2 ± 0.1)

Table 2. Uptake of putrescine-H^3 and conversion to spermidine-H^3 by liver of unoperated and partially hepatectomized rats. Results are means with standard deviations (disintegrations per minute per gram of protein).

Regeneration (hr)	Animals (No.)	Putrescine-H^3 uptake (10^6 dpm)	Spermidine-H^3 synthesis (10^4 dpm)
0*	8	3.0 ± 0.7	12.2 ± 5.3
2	4	15.0 ± 6.9	20.7 ± 6.7
4	4	12.0 ± 3.9	20.1 ± 7.6
6	4	12.5 ± 0.5	29.3 ± 7.5
8	4	11.2 ± 3.2	20.0 ± 6.8
12	4	14.7 ± 4.3	20.2 ± 5.7
16	4	11.4 ± 1.1	23.1 ± 2.8
18	2	8.1 ± 0.1	20.6 ± 3.2
20	7	9.8 ± 1.8	27.8 ± 6.7
22	2	9.7 ± 2.8	28.0 ± 5.0
24	4	9.0 ± 1.5	23.9 ± 4.5

* Unoperated control.

Regenerating rat liver was selected as a means of studying the possible changes in polyamine metabolism in a growth system in which the rate of nucleic acid and protein synthesis is increased (*5*). Young male rats (80 to 120 g) were subjected to partial hepatectomy (*6*). Polyamine (*7*) and RNA (*8*) analyses were performed on the liver removed in the surgical procedure and on regenerating liver after the appropriate postoperative periods (Table 1). The increase in the spermidine concentration in regenerating liver as compared with the control tissue removed is most pronounced 48 to 72 hours after the operation. The pattern of spermidine accumulation in regenerating liver is very much like the RNA increments at 24-hour intervals during the 4-day experimental period. In contrast to the elevated spermidine in regenerating liver, the concentration of spermine is unchanged or slightly less than that of the normal liver.

The results, which suggest a functional relationship between spermidine and RNA synthesis in regenerating liver, seem to support the data on polyamine stimulation of RNA synthesis by purified preparations of RNA polymerase (*2*). Fujioka *et al.* (*9*) have shown an increase in the rate of incorporation of C^{14}-orotate into RNA within 2 hours after partial hepatectomy, with a doubling of the rate 6 hours after the operation. We have investigated the time course of spermidine biosynthesis in comparable postoperative periods and find that the rate of formation of the polyamine increases sharply during the very early regeneration process.

Fifty microcuries of putrescine-2,3-H^3 (New England Nuclear) were injected into the tail vein of rats, and the animals were held for 45 minutes before being killed by decapitation. The liver was extracted with 5 percent trichloroacetic acid, and a sample (0.1 ml) was analyzed by the liquid scintillation method to determine total uptake of labeled putrescine. The remainder of the liver extract was placed upon a chromatography column containing Dowex-50W-X2, and the spermidine fraction was eluted with hydrochloric acid (*10*). After concentration, at reduced pressure, of the HCl eluate, a sample (0.1 ml) was counted by the liquid scintillation procedure (Table 2). The uptake of labeled putrescine by regenerating liver is 3 to 5 times the rate of uptake in normal liver. The rate of conversion of putrescine to spermidine is approximately doubled. The rate of metabolism of the amines is already increased 2 hours after the operation, and both putrescine uptake and spermidine biosynthesis remain elevated throughout the 24-hour experimental period. There is no evidence that the accelerated polyamine utilization by regenerating liver is specifically linked to either DNA synthesis which is maximum at 20 to 24 hours or mitotic activity which is maximum at 22 to 26 hours (*5*). There is, however, a definite similarity in the time course of the increased rate of putrescine uptake and spermidine synthesis and the sharply elevated rate of RNA synthesis in this tissue (*5, 9*).

The time course of spermidine-H^3 uptake by normal and regenerating liver has also been studied, and the pattern is similar to that for putrescine-H^3 shown in Table 2. The rate of spermidine-H^3 uptake by the liver 2 hours after partial hepatectomy is twice that of unoperated controls. The uptake in the regenerating livers of all animals studied is maintained at 2 to 2.5 times the rate of controls during the experimental period of 2 to 24 hours after partial hepatectomy.

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Pseudouridine Formation: Evidence for RNA as an Intermediate

Abstract. *Experiments with intact or lysed spheroplasts from* Escherichia coli *indicate that conditions which impair the DNA-directed synthesis of RNA, also prevent formation of pseudouridine. Studies with uridine-labeled RNA support the concept that RNA may be a direct intermediate for pseudouridine synthesis.*

Since the description of 5-ribosyluracil (pseudouridine) as a component of cellular RNA's (*1*), several investigators have attempted to elucidate the mechanism by which this nucleoside is formed (*2*). Robbins and co-workers, using doubly labeled uridine and 5-fluorouridine, concluded that both the ribose and pyrimidine moieties of uridine are direct precursors for ψ-uridine formation in yeast (*3*). On the basis of these findings, the same authors suggested an intramolecular rearrangement of the uridine base and sugar; however, the data was insufficient to decide whether this rearrangement occurred before or after uridine incorporation into RNA.

On the other hand, several investigators have suggested that ψ-uridine might be formed by a 3,5-diribosyluracil intermediate (*4*) or by a direct condensation of uracil with ribose-5-phosphate to form ψ-UMP (*5*). The enzyme ψ-UMP synthetase which catalyzes this condensation reaction has been partially purified from extracts of *Tetrahymena pyriformis* (*5*). Although these findings indicate that ψ-uridine may be formed before nucleotide polymerization, the possibility still exists that ψ-uridine, found in the framework of cytoplasmic RNA's, is formed after polynucleotide assembly. We now report evidence that, in *Escherichia coli*, ψ-uridine formation requires the prior synthesis of RNA. Agents which inhibit ribonucleotide polymerization also prevent ψ-uridine formation.

The following experiments were conducted with either intact or lysed spheroplasts from *E. coli*. The spheroplasts were prepared as previously reported (*6*). Lysed cells were prepared by sedimenting the spheroplasts and suspending the cells in a hypotonic medium containing 0.02*M* tris, *p*H 7.5, 0.002*M* $MgCl_2$ and 0.014*M* 2-mercaptoethanol. The labeled precursors used were uracil-2-^{14}C (Schwarz BioResearch), UTP-^{14}C, or uridine-labeled cRNA-^{14}C. The UTP-^{14}C was prepared by deamination of uniformly labeled CMP (New England Nuclear) according to the procedure of Lohman (*7*), purification of the UMP product by chromatography on Dowex-1 (formate), and conversion to UTP by a yeast extract containing uridylate kinase activity (*8*). The labeled UTP was not purified from the kinase mixture but used as such after it was heated at 90°C for 3 minutes. Paper electrophoresis of this mixture indicated that more than 95 percent of the radioactivity migrated as UTP, the remainder being distributed between UMP and UDP. The above procedure was used to avoid the presence of labeled ψ-uridine nucleotides found in commercial preparations of uridine-^{14}C nucleotides. This difficulty was only partially overcome, since our preparation of UTP-^{14}C still contained some contaminant (approximately 0.05 percent) which behaved like ψ-uridine in the assay procedure and contributed to the high base lines found. The contaminant did not appear to be ψ-UTP-^{14}C since synthetic cRNA-^{14}C, prepared with the same labeled UTP, contained little radioactive ψ-uridine. Radioactive cRNA was prepared with the highly purified RNA polymerase from *Micrococcus lysodeikticus, Escherichia coli* DNA being used as template and UTP-^{14}C as the only labeled substrate (*8*). The synthetic cRNA was freed from UTP-^{14}C of the polymerase reaction mixture by repeated precipitation with ethanol and exhaustive dialysis against 0.1*M* NaCl and then against water. Pseudouridine used as carrier was isolated from human urine by the procedure of Adler and Gutman (*9*) as modified by Goldberg and Rabinowitz (*10*).

The procedure routinely used for isolating ψ-uridine was as follows: (i) The entire reaction mixture was hydrolyzed with alkali and treated with alkaline phosphatase; the total labeled nucleosides were isolated by adsorption onto Dowex-1 (acetate) and eluted with acetic acid (Table 1); (ii) carrier uridine and ψ-uridine were added to the isolated nucleoside fraction which was then concentrated and subjected to descending paper chromatography in a mixture of butanol and water (86:14); (iii) pseudouridine was eluted and passed over a column (1 by 7 cm) of Dowex-50 (H^+) in 0.05*N* HCl; the acid-eluate was neutralized and the labeled nucleoside was concentrated by treatment with Dowex-1 (acetate) as described above; (iv) the concentrate was subjected to paper chromatography in the butanol-water system a second time; (v) the ψ-uridine band was eluted and subjected to paper chromatography in a system containing isopropanol, acetic acid, and water

Table 1. Effect of actinomycin D on uracil-^{14}C incorporation into pseudouridine with *Escherichia coli* spheroplasts. The incubation mixture, final volume 10.9 ml, contained 10 ml of spheroplasts in a modified nutrient broth (*6*) (approximately 1×10^9 cells/ml) and, as labeled precursor, 0.532 μmole of uracil-2-^{14}C (64.9×10^6 count/μmole). In addition, one incubation mixture contained 250 μg of actinomycin D. After 2 hours at 37°C, the cells were sedimented by centrifugation, suspended in 2 ml of 0.4*N* KOH and held at 37°C for 18 hours. The hydrolyzate was acidified with 70 percent $HClO_4$, iced for 1 hour and centrifuged; the supernatant was exposed to the action of intestinal alkaline phosphatase (1 mg/ml) overnight at 37°C after adjustment to *p*H 9 in a final volume of 10 ml. The reaction was stopped by heat (10 minutes in a boiling-water bath, cooled, and filtered; the filtrate was made up to a volume of 100 ml and adjusted to *p*H 10.5 with NH_4OH. The ^{14}C-nucleosides were concentrated by adsorption onto a 6-cm^2 column of Dowex-1 (acetate), elution with 0.1*N* acetic acid and evaporation to dryness under vacuum. The labeled residue was dissolved in a minimum volume of water; carrier uridine and ψ-uridine were added, and the ψ-uridine was reisolated by chromatographic and Dowex-50 (H^+) treatment.

Expt. No.	Actinomycin D	Radioactivity (count/min)	
		Acid-insoluble	In ψ-uridine
1	+	0.18×10^6	775
2	−	1.54×10^6	4160